Application of whole genome sequencing to Australia-wide collections of extraintestinal pathogenic *Escherichia coli* from companion animals and commensal *E. coli* from pigs

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Abstract

Escherichia coli is one of the most important bacterial pathogens associated with extraintestinal infections in both humans and animals. In companion animals, it is associated with urinary, respiratory, gastrointestinal tract, joint, wound, skin and soft tissue infections. These infections vary significantly between cats and dogs: for example, extraintestinal infection in cats is infrequent, and they rarely have simple, uncomplicated bacterial UTIs compared to dogs. *E. coli* strains causing UTIs in both cats and dogs share pathotypic similarities and are phylogenetically related to ExPEC isolates from humans. It is, therefore, possible for humans to share pathogenic ExPEC strains with their companion animals, which suggests the possibility of both zoonotic (animal-to-human) and anthropozoonotic (human-to-animal) transmission.

Among phylogenetic group B2 FQ-susceptible cat clinical isolates from an Australia-wide collection (n = 323), it was found that ST73 was the dominant B2-associated ST, based on both WGS (23/53, 43%) and ST-specific PCR (45/221, 20%). Less dominant STs identified by WGS included ST127, ST12, and ST372 (4/53, 8% each). By contrast, among group B2 FQ-susceptible dog clinical isolates from an Australia-wide collection (n = 449), ST372, an infrequent human pathogen, was found to be the predominant B2-associated ST according to both WGS (24/77, 31%) and ST-specific PCR (53/240, 22%). The other primary STs identified by WGS included ST73 (13/77, 17%), ST12 and ST80 (5/77, 7% each).

To further compare the relatedness of ST73 isolates from dogs, cats, and humans, WGS-based phylogenetic comparison was performed. This comparison showed that there was considerable overall phylogenetic diversity among the isolates. Although most clusters were species-specific, some contained closely

related human and animal (dog>cat) isolates. These results confirmed that while there is clonal commonality among ExPEC isolated from cats, dogs and humans, there are dominant host-specific clonal groups – ST372 and ST73 in dogs and ST73 in cats – and potential for bi-directional (i.e., companion animal-human and human-companion animal) transmission of ST73 strains between host species.

A collection of FQR *E. coli* isolates from the same Australia-wide collection of isolates from extraintestinal infections in Australian cats and dogs (n = 59) was also investigated to determine the prevalence of the globally disseminated MDR pandemic clonal groups ST131 and ST1193. The main STs identified in this study were ST224 (10/59, 17%), ST744 (8/59, 14%), and ST38 (8/59, 14%). The overall prevalence of ST131 was (6/59, 10%), however, no ST1193 isolates were identified in contrast to a 5-year-earlier report which identified two dog-source ST1193 isolates closely related to human source isolates. Furthermore, the findings showed that the prevalence of ST131 as a cause of FQR infections in Australian companion animals was relatively constant between this study and the 5-year-earlier study of Platell et al. (2010) (9/125 isolates, 7.2%).

To investigate the clonal commonality of human and companion animal ST131, 20 Australian cat- and dog-source ST131 genomes were compared to 173 reference human-source ST131 genomes. This analysis revealed that the animal-sourced isolates were widely distributed throughout the ST131 phylogeny. There was also some minor sub-clustering of dog- and cat-source isolates, though most were closely related to human-source ST131 isolates. The absence of a separate clade containing only companion animal ST131 isolates combined with the higher prevalence of ST131 and ST1193 in humans reported in the literature suggests that companion animals act as spillover hosts rather than primary reservoirs for these lineages. The high degree of clonal commonality among these FQR clinical isolates from humans

and companion animals further suggests the possibility of bi-directional between-species transmission.

In addition to being both a commensal and pathogen in companion animals, *E. coli* is also the main facultative anaerobic bacteria colonising the pig gastrointestinal tract. While being a beneficial gut commensal, some *E. coli* pathotypes cause both intestinal and extraintestinal infections in pigs with AMR of increasing concern in both commensals and pathogens. On this basis the frequency of antimicrobial non-susceptibility among *E. coli* (n = 201) and *Salmonella* spp. (n = 69) isolated from rectal contents of healthy Australian finisher pigs at slaughter was determined. Only low levels of non-susceptibility to CIAs (ESCs and FQs) were identified, with non-susceptibility to both cefoxitin and ciprofloxacin observed in only 1% (2/201) of *E. coli* isolates. In addition, all *E. coli* isolates were fully susceptible to ceftiofur. None of the *Salmonella* spp. isolates showed any non-susceptibility to these CIAs.

As the use of fluoroquinolones in food-producing animals in Australia is banned, the identification of fluoroquinolone resistance in *E. coli* from Australian pigs is significant as it demonstrates that even in the absence of local antimicrobial selection pressure resistance can occur. FQR *E. coli* can enter livestock production facilities despite strict biosecurity possibly via human carriers or wild birds. Other potential sources include feed, water, rodents, and insects. The results from these studies highlight the need for further surveillance of AMR and identification of prominent resistant clonal lineages in both companion and food-producing animals in Australia.

Thesis declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed

Amanda Kidsley

Date: 21st August 2020

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times. You gave me company during several late nights and weekends in the lab. Without you, I could not have completed my PhD.

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Preamble

This thesis consists of five research articles (four published, one under review), which resulted from two collaborative research ventures. The first component of my PhD was funded by an ARC Linkage grant with Zoetis, and Australian Pork Limited as the main industry partners, and was conducted in collaboration with Associate Professor Sam Abraham and Dr Mark O'Dea from Murdoch University. I characterised Australia wide collections of *Escherichia coli* from cats, dogs and pigs and conducted a preliminary whole genome sequencing (WGS) analysis of their relationships to human isolates. The second part of my PhD, also funded by the ARC Linkage grant with Zoetis, was conducted in collaboration with Associate Professor Scott Beatson, Professor Mark Schembri and Rhys White from the University of Queensland to gain an understanding of the genetic relationships between Australian companion animal ST131 isolates and an international human ST131 collection using comparative genomics.

This thesis presents a series of research articles investigating the prevalence of extraintestinal pathogenic *E. coli* (ExPEC) associated clonal lineages in companion animals and commensal *E. coli* from pigs. Chapter 1 is a literature review, which describes the background and objectives of this thesis. The first two articles (Chapters 2 and 3) genomically analysed a collection of clinical fluoroquinolone-susceptible group B2 ExPEC from Australian dogs and cats. In the third article, a similar collection of fluoroquinolone-resistant ExPEC from Australian dogs and cats was characterised to estimate the prevalence of the globally important, *E. coli* clonal lineages ST131 and ST1193, which are infrequently isolated from companion animals (Chapter 4). Chapter 5 is a case report on malakoplakia in two dogs, a rare condition of the bladder associated with impaired intracellular killing of *E. coli* where WGS was used to

characterise the *E. coli* isolates obtained; while Chapter 6 describes the antimicrobial susceptibility of *E. coli* and *Salmonella* spp. from a national pilot study of healthy pigs and WGS analysis of isolates showing non-susceptibility to critically important antimicrobials.

List of Publications

Research articles by the author incorporated into the thesis

1. **Kidsley, A.K**., O'Dea, M., Ebrahimie, E., Mohammadi-Dehcheshmeh, M., Saputra, S., Jordan, D., Johnson, J.R., Gordon, D., Turni, C., Djordjevic, S.P., Abraham, S., Trott, D.J. (2020). Genomic analysis of fluoroquinolone-susceptible phylogenetic group B2 extraintestinal pathogenic *Escherichia coli* causing infections in cats. Vet Microbiol. 245, 108685. doi:10.1016/j.vetmic.2020.108685

2. **Kidsley, A.K**., O'Dea, M., Saputra, S., Jordan, D., Johnson, J.R., Gordon, D.M., Turni, C., Djordjevic, S.P., Abraham, S., Trott, D.J. (2020). Genomic analysis of phylogenetic group B2 extraintestinal pathogenic *E. coli* causing infections in dogs in Australia. Vet Microbiol. 248, 108783. doi.org/10.1016/j.vetmic.2020.108783

3. **Kidsley, A.K**., White, R.T., Beatson, S.A., Saputra, S., Schembri, M.A., Gordon, D., Johnson, J.R., O'Dea, M., Mollinger, J.L., Abraham, S., Trott, D.J. (2020). Companion animals are spillover hosts of the multidrug-resistant human extraintestinal *Escherichia coli* pandemic clones ST131 and ST1193. Front Microbiol. 11:1968. doi:10.3389/fmicb.2020.01968

4. **Kidsley, A.K**, Abraham, S., Bell, J.M., O'Dea, M., Laird, T.J., Jordan, D., Mitchell, P., McDevitt, C.A., Trott, D.J. (2018). Antimicrobial susceptibility of *Escherichia coli* and *Salmonella* spp. isolates from healthy pigs in Australia: Results of a pilot national survey. Front Microbiol. 9, 1207. doi:10.3389/fmicb.2018.01207

Additional research articles as a co-author but not incorporated into the thesis

5. Saputra, S., Jordan, D., Mitchell, T., San Wong, H., Abraham, R.J., **Kidsley, A.**, Turnidge, J., Trott, D.J., Abraham, S. (2017). Antimicrobial resistance in clinical *Escherichia coli* isolated from companion animals in Australia. Vet Microbiol. 211:43-50. doi:10.1016/j.vetmic.2017.09.014

Conference presentations relevant to the thesis

1. **Kidsley, A.**, Abraham, S., Saputra, S., Turnidge, J., Bell, J., Trott, D.J. The major fluoroquinolone-resistant extraintestinal pathogenic *Escherichia* coli clone B2:025b:ST131 is not undergoing epidemic spread in companion animals. Antimicrobials, 16th Annual Scientific Meeting, Australian Society for Antimicrobials, Brisbane, Australia. 26-28 February 2015. Poster presentation.

2. **Kidsley, A.**, Abraham, S., Turnidge, J., Bell, J., Gordon, D., Johnson, J., Trott, D. *Escherichia coli* O75:ST1193 remains an important clonal group among fluoroquinolone-resistant isolates from Australia. Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC), San Diego, California, United States. 17-21 September 2015. Poster presentation.

3. **Kidsley, A.**, Abraham, S., Bell, J.M., Mitchell, P., Trott, D. Antimicrobial susceptibility of *Escherichia coli* and *Salmonella* isolates from healthy pigs in Australia: Results of a pilot national survey. 4th International Conference on Responsible Use of Antibiotics in Animals, The Hague, The Netherlands, 26-28 September 2016. Poster presentation.

Conference presentations relevant to the thesis continued

4. White, R.T., Forde, B.M., Roberts, L.W., Phan, M.D., Peters, K.M., Trott, D.J., Gibson, J.S., Mollinger, J.L., Rodgers, B.A., Ben Zakour, N.L., **Kidsley, A.**, Bell, J., Turnidge, J., Schembri, M.A., Beatson, S.A. The emergence of pandemic fluoroquinolone-resistant uropathogenic *Escherichia coli* clones in Australia. BACPATH15. Swan Valley, Western Australia. 30th September - 3rd October 2019. Oral presentation.

Abbreviations

1GCs	First-generation cephalosporins
2GCs	Second-generation cephalosporins
3GC(s)	Third-generation cephalosporin(s)
4GCs	Fourth-generation cephalosporins
5GCs	Fifth-generation cephalosporins
AMR	Antimicrobial resistance
ARG-ANNOT	Antibiotic Resistance Gene ANNOTation
AST	Antimicrobial susceptibility testing
ASTAG	The Australian Strategic and Technical Advisory Group on AMR
BfT-GermVet	German resistance monitoring in veterinary medicine
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
CARD	Comprehensive Antibiotic Resistance Database
CIAs	Critically important antimicrobials
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance
CLSI	Clinical and Laboratory Standards Institute
COMPATH	Pan-European programme dedicated to the monitoring of antimicrobial susceptibility of pathogens from diseased dogs and cats
DANMAP	Danish Integrated Antimicrobial Resistance Monitoring and Research Programme
DNA	Deoxyribonucleic acid
ECOFF(s)	Epidemiological cut-off value(s)

ECOR	*Esherichia coli* reference collection
ECV(s)	Epidemiological cut-off value(s)
ESBL(s)	Extended-spectrum β-lactamase(s)
ESCs	Extended-spectrum cephalosporins
ETEC	Enterotoxigenic *Escherichia coli*
EU	European Union
EUCAST	European Committee on Antimicrobial Susceptibility Testing
ExPEC	Extraintestinal pathogenic *Escherichia coli*
FAO	Food and Agriculture Organisation
Finres-VET	Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents
FQR	Fluoroquinolone-resistant
FQ(s)	Fluoroquinolone(s)
GI	Gastrointestinal
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MARAN	Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands
MDR	Multidrug-resistant
MIC(s)	Minimum inhibitory concentration(s)
MLST	Multilocus sequence typing
NARMS	National Antimicrobial Resistance Monitoring System
NCBI	National Center for Biotechnology Information
OIE	World Organisation for Animal Health
PAIs	Pathogenicity islands
PBPs	Penicillin-binding proteins
PCR	Polymerase chain reaction

PFGE	Pulse-field gel electrophoresis
PK/PD	Pharmacokinetics/pharmacodynamics
PMQR	Plasmid-mediated quinolone resistance
PWD	Post-weaning diarrhoea
QC	Quality control
QRDR	Quinolone resistance determining region
RAPD	Random amplified polymorphic DNA
RESAPATH	French epidemiological surveillance network for monitoring trends in antimicrobial resistance in bacteria from animals
RESABO	French surveillance network for the antimicrobial resistance of pathogenic bacteria isolated from cattle
ST(s)	Sequence type(s)
SVARM	Swedish Veterinary Antimicrobial Resistance Monitoring
SvarmPat	Swedish veterinary antibiotic resistance monitoring of pathogenic bacteria
UPEC	Uropathogenic *Escherichia coli*
UTI(s)	Urinary tract infection(s)
VETCAST	Veterinary Subcommittee on Antimicrobial Susceptibility Testing
WGS	Whole genome sequencing
WHO	World Health Organisation

Chapter 1

Literature review

1. Introduction

The subject of this literature review is AMR in animals, with a particular emphasis on ExPEC in dogs and cats. In this review, the characteristics of ExPEC will be examined with particular emphasis on molecular techniques used to identify AMR and virulence genes, as well as common STs among companion animal ExPEC. As Chapter 6 comprises an AMR surveillance project involving pigs, porcine *E. coli* will be briefly mentioned. In addition, a review of current AMR surveillance studies in Australia and worldwide including food-producing and companion animals will be undertaken.

1.2. *Escherichia coli*

Escherichia coli is a Gram-negative, rod-shaped, catalase-positive, facultative anaerobe. It is also indole-positive, oxidase-negative, non-spore-forming, and motile. *E. coli* was originally classified as a member of the *Enterobacteriaceae* family; however, it has now been reclassified as a member of the order *Enterobacteriales*. In 2016, Adeolu et al. proposed the change in classification to the *Enterobacteriales* order due to advances in scientific techniques that enabled phylogenetic and molecular analysis to be used to differentiate between members of the *Enterobacteriaceae* family (Adeolu et al., 2016).

E. coli are ubiquitous colonisers of the GI-tract of both humans and animals and can be either pathogens or commensals (Bortolami et al., 2019). They are a common cause of infections in companion animals including urinary, respiratory, skin, soft tissue, ear, GI-tract, joint, and wound infections (Guardabassi et al., 2004; Zogg et al., 2018a).

Pathogenic *E. coli* are further categorised as either intestinal, causing diarrhoea, or extraintestinal, causing illness outside of the GI-tract, with each of these *E. coli* categories further differentiated into distinct pathotypes (Kaper et al., 2004) as

shown in Figure 1. *E. coli* that are pathogens of the GI-tract belong to seven pathotypes i) enteropathogenic *E. coli* (EPEC), ii) enterotoxigenic *E. coli* (ETEC), iii) enteroinvasive *E. coli* (EIEC), iv) enteroaggregative *E. coli* (EAEC), v) shiga toxin-producing *E. coli* (STEC), vi) diffusely adherent *E. coli* (DEAC), and vii) adherent invasive *E. coli* (AIEC) (Riley, 2014; Fratamico et al., 2016). Of these, only ETEC causing infection in pigs will be further discussed in Section 3.

Extraintestinal diseases such as UTIs, septicaemia and meningitis are caused by strains of *E. coli* known as ExPEC (Tapader et al., 2019) and were first called as such by Johnson and Russo in 2000 (Russo and Johnson, 2000). While ExPEC strains cause disease in non-intestinal sites they can asymptomatically colonise the intestinal tract of both humans and animals (Alhashash et al., 2016). ExPEC strains can be further broken down into uropathogenic *E. coli* (UPEC), septicaemic pathogenic *E. coli* (SPEC), neonatal septicaemic *E. coli* (NSEC), neonatal meningitis *E. coli* (NMEC) and avian pathogenic *E. coli* (APEC) (Tapader et al., 2019). Not all of these pathotypes, however, have been isolated from companion animals.

Phylogenetic analysis originally grouped *E. coli* into four main phylogroups (A, B1, B2, and D) (Whittam et al., 1983). A robust triplex PCR targeting the genes *chuA*, *yjaA*, and the DNA fragment *TSPE4.C2* was published in 2000 by Clermont et al. to quickly and easily determine these phylogenetic groups (Clermont et al., 2000). However, MLST of a large collection of isolates identified a more intricate genetic diversity within the *Escherichia* genus, with an additional three phylogenetic groups (C, E, and F), five cryptic clades and a new species delineated (Clermont et al., 2013). This required the development of a new multiplex PCR that could distinguish between these eight new phylogroups and cryptic clades. Retesting of some ExPEC strains using this new methodology grouped them into different phylogenetic groups – for

instance, some isolates previously identified as group D were reassigned into new phylogenetic groups E and F (Logue et al., 2017).

Of the eight identified phylogroups, commensal strains of *E. coli* are mostly associated with groups A, B1 and/or C, while ExPEC predominately belong to groups B2, and a lesser extent, groups D and F (Zogg et al., 2018b). In healthy animals, phylogroup B1 usually predominates in the gut followed by groups A, B2, and D, which is in contrast to commensal strains in healthy humans where group A usually predominates (Tenaillon et al., 2010). Thus, groups A, B1, B2, and D are the most commonly identified in humans and animals (Gordon and Cowling, 2003).

Prior to 2000 ExPEC were mostly susceptible to ESCs and FQs (Pitout and DeVinney, 2017), however, resistance to these antimicrobials is becoming more prevalent and is of increasing concern.

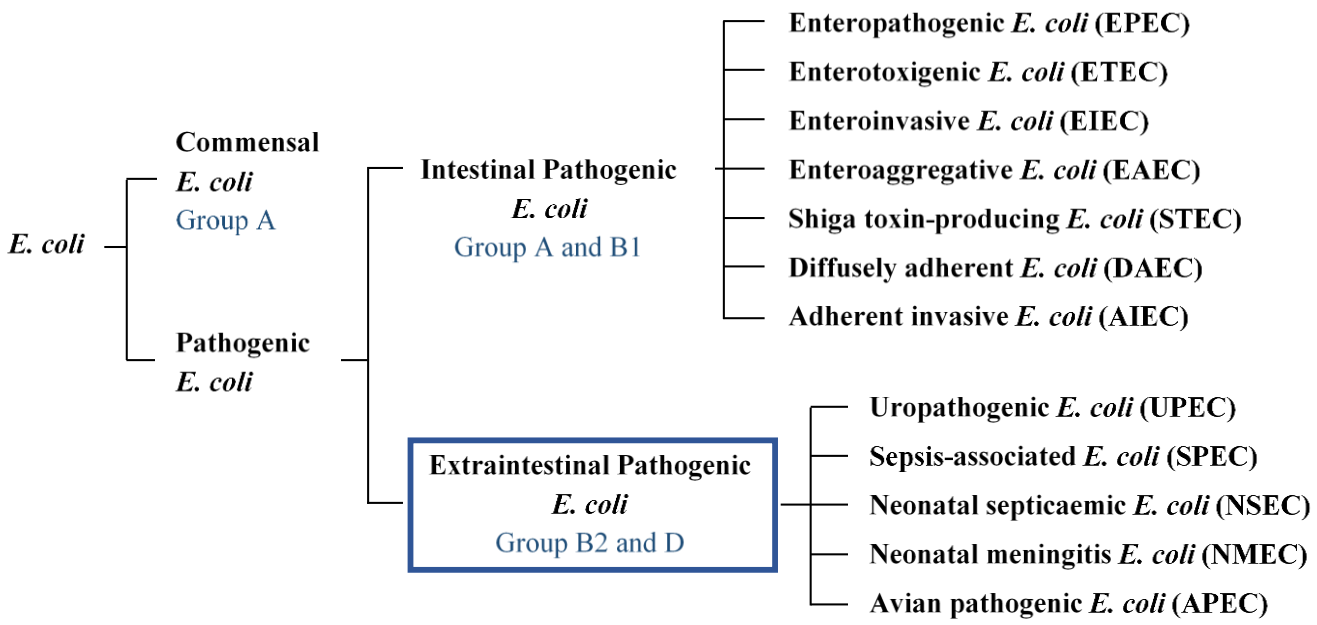

Figure 1: Diversity of *E. coli* strains

1.3. ExPEC in companion animals

ExPEC are the leading cause of UTIs in companion animals (Guo et al., 2015) and a common reason for presentation to vet practices (McMeekin et al., 2017), with clinical signs including stranguria, pollakiuria, and hematuria (Cummings et al., 2015). Throughout the lifetime of a dog, it is estimated that up to 14% will develop a UTI (Ling, 1984). Generally, using oral antimicrobial therapy, canine UTIs are uncomplicated to treat; however, some infections can persist or reoccur due to a particular pathogen or host attribute(s), such as resistance development or underlying predisposing condition(s) in the animal (Cummings et al., 2015).

Compared to dogs, cats rarely have simple uncomplicated bacterial UTI, and among those that do show clinical signs of lower urinary tract disease (dysuria, haematuria, pollakiuria, and stranguria); a large proportion has culture-negative urine (Litster et al., 2009). Among cats with clinical evidence of urinary tract disease, the overall prevalence of a positive urine culture is < 3% (Litster et al., 2011), although prevalence rates in cats with compromised urinary defence mechanisms are much higher (15-43%) (Litster et al., 2011). Similarly, the incidence of bacterial lower UTI in cats increases with age (≥ 10 years), renal disease, urinary catheterisation, perineal urethrostomy, low urine specific gravity, and diabetes mellitus (Litster et al., 2009).

1.3.1. Virulence in companion animal ExPEC

The presence of a combination of virulence factors determines the degree to which companion animal ExPEC invade the urinary tract and cause systemic infection (Thompson et al., 2011). ExPEC express a unique combination of virulence factors compared to commensal *E. coli* strains, which enable them to colonise host mucosal surfaces and avoid or subvert local, and systemic host defence mechanisms (Johnson and Russo, 2002).

ExPEC virulence factors are encoded on genes located within PAIs or on plasmids and can be separated into five primary groups: adhesins, toxins, iron acquisition systems, capsule production, and protectins/invasins (Dale and Woodford, 2015). Possession of a single virulence gene cannot result in an infection; rather it is the unique combination of genes that determines the outcome of infection (Sarowska et al., 2019). Over 57 specific *E. coli* virulence genes associated with extraintestinal infections have been identified to date (Johnson et al., 2015).

In the process of adhesion, bacterial surface structures play an important role and help increase the virulence of pathogenic *E. coli* by enabling close contact of the bacteria to the host (Sarowska et al., 2019). There are three main types of adhesins, fimbriae, afimbrial adhesins and outer membrane proteins, each of which assists the bacteria to colonise a specific niche. For instance adhesion to the epithelial cells of the intestines, kidneys and lower urinary tract is mediated by P, S, and type 1 fimbriae, respectively. In isolates from patients with UTIs, genes encoding the S fimbrial adhesin (*sfa*), F1C fimbriae (*foc*), *papC*, and *iha* are most frequently detected (Hagan and Mobley, 2007).

Siderophores assist in growth and development of the bacteria by acquiring iron, while toxins are involved in increased cytotoxicity, resistance to neutrophil phagocytosis, and the spread of the bacteria in the host tissues. The most commonly identified toxin genes in ExPEC strains include *tsh* (temperature-sensitive hemagglutinin tsh autotransporter), *hlyA*, *hlyD*, *hlyF* (α-hemolysins), *cnf1* (cytotoxic necrotising factor 1), *sat* (secreted autotransporter toxin), *pic* (protease involved in colonisation), *vat* (vacuolating autotransporter protein), *cdtB* (cytolethal distending factor), and *astA* (enteroaggregative *E. coli* toxin) (Sarowska et al., 2019).

Since the first designation of the term ExPEC in 2000, and with the genetic understanding of *E. coli* virulence genes increasing rapidly, a molecular definition of

ExPEC was devised in 2003, followed by an additional UPEC designation in 2012. Isolates can be classified as ExPEC if they contain ≥ 2 of 5 of the ExPEC defining virulence genes *papA* and/or *papC*, *sfa*/*focDE*, *afa*/*draBC*, *kpsM II*, and *iutA* (Johnson et al., 2003). Isolates can be defined as UPEC if they contain ≥ 3 of 4 of the UPEC defining virulence genes *chuA*, *fyuA*, *vat*, and *yfcV* (Spurbeck et al., 2012).

Common ExPEC genes associated with virulence include *papAH*, *papC*, *papEF*, *papG*, and *sfa*/*focDE* (adhesin-encoding genes), *hlyA*, *hlyF*, and *cnf1* (toxin-encoding genes), *iroN*, *fyuA*, *ireA*, and *iutA* (siderophores-related genes), *kpsM II*, *kpsMT III*, and *iss* (protectin/invasin-encoding genes), miscellaneous genes such as *cvaC*, *usp*, *ompT*, and *clbB* (Yair and Gophna, 2018) and type 1 fimbriae (*fimH*). These genes are rarely found in non-pathogenic strains belonging to phylogroups A, B1, and C (Johnson and Stell, 2000).

Table 1: Common ExPEC virulence genes

Category	Name	Genes	Function	Reference
Adhesin	Afimbrial adhesin	*afaA, afaB, afaC*	Encodes proteins involved in adhesion to epithelial cells	(Lalioui et al., 1999)
	Dr fimbriae	*draA, draB, draC, draD, draE, draP*	Facilitates colonisation by binding to a receptor on the surface of epithelial cells and internalisation of the bacteria to the host cells	(Sarowska et al., 2019)
	Factor adherence *E. coli*	*fdeC*	Binding to epithelial cells; kidney and bladder colonisation	(Nesta et al., 2012)
	Type 1 fimbriae	*fimA, fimB, fimC, fimD, fimE, fimF, fimG, fimH, fimI*	Colonisation factor in extraintestinal infections; biofilm formation	(Sarowska et al., 2019)
	F1C fimbriae	*focA, focC, focD, focF, focG, focH, focI*	Adhesion to renal epithelial cells and endothelial cells of the bladder and kidneys	(Sarowska et al., 2019)
	Heat-resistant agglutinin	*hra*	Putative virulence factor in UTI	(Srinivasan et al., 2003)
	Iron-regulated-gene-homologue adhesion	*iha*	Enables non-adherent laboratory *E. coli* to adhere to epithelial cells *in vitro*	(Sarowska et al., 2019)
	P fimbriae	*papA, papB, papC, papD, papE, papF, papG, papH, papI, papJ, papK*	Stimulates the production of cytokines by T lymphocytes; colonisation factor in extraintestinal infections	(Sarowska et al., 2019)
	S fimbriae	*sfaA, sfaB, sfaC, sfaD, sfaE, sfaF, sfaG, sfaH, sfaS*	Adhesion to intestinal epithelial cells, kidney, lower urinary tract cells; facilitates the penetration of bacteria into the tissues	(Sarowska et al., 2019)
	Temperature-sensitive hemagglutinin	*tsh*	Serine protease	(Johnson et al., 2008b)
	E. coli common pilus	*yagV/ecpE, yagW/ecpD, yagX/ecpC, yagY/ecpB, yagZ/ecpA, ykgk/ecpR*	Mediates the direct binding of the bacteria to the cell membrane; possibly used to by pathogenic strains to mimic commensal *E. coli*	(Rendón et al., 2007)

Table 1: Common ExPEC virulence genes

Category	Name	Genes	Function	Reference
Invasin	Putative sulfatase AslA	*aslA*	Contributes to brain microvascular endothelial cell invasion	(Kim, 2002)
	Ibes	*ibeA, ibeB, ibeC*	Cell invasion into the host tissues	(Sarowska et al., 2019)
	K1 capsule	*kpsD, kpsM, kpsT*	Encodes functions for the synthesis, activation, and polymerisation of sialic acid, as well as its translocation of to the bacterial cell surface	(Pavelka et al., 1991)
	Outer membrane protein	*ompA, ompT*	Enables intracellular survival; evasion from the body's defence mechanisms	(Sarowska et al., 2019)
	Serine protease autotransporter	*pic*	Degrades mucins; facilitates colonisation of the epithelium; damages the cell membrane	(Sarowska et al., 2019)
	Outer membrane protein	*traT*	Complement resistance gene	(Freitag et al., 2005)
Toxin	Enteroaggregative *E. coli* toxin	*astA*	Encodes the enteroaggregative *E. coli* heat-stable enterotoxin (EAST1)	(Maluta et al., 2016)
	Cytolethal distending toxin	*cdtA, cdtB, cdtC*	Suppresses cell proliferation; leads to cell death	(Tóth et al., 2009)
	Cytotoxic necrotizing factor	*cnf1, cnf2*	Prevents cytokinesis; causes multinucleated cells to form; causes membrane ruffling	(Schmidt et al., 1997)
	α-hemolysin	*hlyA, hlyB, hlyC, hlyD*	Cytotoxic to many types of cells including red and white blood cells, endothelial cells, and renal epithelial cells	(Stanley et al., 1998)
	Secreted autotransporter toxin	*sat*	Vacuolating cytotoxin; causes structural changes in the bladder and kidney epithelium; contributes to pathogenesis of urinary tract infections	(Guyer et al., 2002)
	Vacuolating autotransporter toxin	*vat*	Proteolytic toxin; induces host cell vacuolisation	(Sarowska et al., 2019)

Table 1: Common ExPEC virulence genes

Category	Name	Genes	Function	Reference
Siderophore	Heme transporter system	*chuA, chuS, chuT, chuU, chuV, chuW, chuX, chuY*	Involved in iron metabolism	(Lavigne et al., 2012)
	Ferric yersiniabactin uptake	*fyuA*	Iron-regulated outer membrane protein; receptor for the siderophore yersiniabactin; important for biofilm formation by UPEC	(Fetherston et al., 1995; Hancock et al., 2008)
	Catecholate siderophore	*ireA*	Iron-regulated putative siderophore receptor	(Freitag et al., 2005)
	Salmochelin	*iroN*	Catecholate siderophore receptor; mediates utilisation of the enterobactin	(Russo et al., 2002)
	Iron repressible protein	*irp1, irp2*	Yersiniabactin synthesis	(Sarowska et al., 2019)
	Aerobactin	*iucA, iucB, iucC, iucD*	Acquisition of iron in the host system	(Sarowska et al., 2019)
	Aerobactin	*iutA*	Aerobactin receptor	(Freitag et al., 2005)
	Yersiniabactin	*ybtA, ybtE, ybtP, ybtQ, ybtS, ybtT, ybtU, ybtX*	Scavenges iron; sequesters copper extracellularly, preventing it from entering the bacterial cell	(German et al., 2016)
Miscellaneous	Increased serum survival	*iss*	Protection factor against phagocytosis	(Sarowska et al., 2019)
	Pathogenicity island marker	*malX*	Encodes an enzyme which recognises glucose and maltose; enables *E. coli* to persist in the intestinal tract	(Tang et al., 2019)
	Uropathogenic specific protein	*usp*	Increases infection in the urinary tract by acting as a bacteriocin against competing *E. coli* strains occupying the same niche	(Parret and De Mot, 2002)
	YfC fimbria	*yfcO, yfcP, yfcQ, yfcR, yfcS, yfcT, yfcU, yfcV*	Promotes adhesion to eukaryotic epithelial cells	(Korea et al., 2010)

1.3.2. Antimicrobial resistance in companion animal ExPEC

A variety of antimicrobials have been approved for use in veterinary medicine (ASTAG, 2018), and AMR is of increasing concern. Not only does increased resistance complicate treatment by limiting available options and increasing costs, it is also a potential public health concern due to the possible transfer of bacterial strains between pets and their owners (Punia et al., 2018). To combat AMR, organisations such as the WHO, the FAO, and the OIE actively commit efforts to protect the effectiveness of antimicrobials in the face of rapidly increasing resistance (WHO, 2019).

One factor that can contribute to the selection and spread of AMR is the use of antimicrobials in animals. Veterinarians are at the forefront in helping preserve existing effective antimicrobials by carefully considering how they prescribe them. This is especially important when considering antimicrobials that are classified as critically important in human medicine as these are used in lifesaving treatment that needs to be preserved for future use. These CIAs are considered the last line treatment available for use in humans, particularly for Gram-negative infections, and include the 3GCs and 4GCs, FQs, carbapenems, and colistin (WHO, 2019). The WHO has produced a frequently updated list of all antimicrobials currently used in human medicine since 2005. This list groups the antimicrobials into three categories based on their importance to medical treatments. These categories are critically important, highly important, and important. CIAs include 3GCs, 4GCs, (and now 5GCs), macrolides, quinolones, and aminoglycosides. Other antimicrobials in the list include 1GCs and 2GCs, tetracyclines and sulfonamides (highly important) (WHO, 2019). This list is intended to aid in managing AMR, and assists in ensuring prudent usage in both human and veterinary medicine, especially of the CIAs. In Australia, to ensure antimicrobials are used appropriately in order to effectively respond to the threat of AMR, an Importance Ratings and Summary of Antibacterial Uses in Human and

Animal Health in Australia was developed by the Australian Government with expert advice from ASTAG. The Antibacterial Importance Ratings were first developed in 2002 and while they take into account the WHO guidelines, they have been adapted to reflect the situation in Australia. For instance, the macrolide erythromycin and the aminopenicillin ampicillin are listed as critically important on the WHO CIAs for Human Medicine list but as of low importance on the Australian Antibacterial Importance Ratings. Differences in the importance ratings between the WHO and Australia are two-fold: mainly, because Australia has a relatively low reliance on these antimicrobials due to the absence of widespread resistance in many human pathogens and the fact that some of the pathogens of interest to WHO are less important in Australia (ASTAG, 2018).

β-lactam antimicrobials are the most commonly prescribed class in veterinary medicine (Bortolami et al., 2019), especially for uncomplicated UTIs, with amoxicillin (1st line), amoxicillin-clavulanate (2nd line) and 1GCs (2nd line) utilised heavily. Sulfonamide/trimethoprim combinations (2nd line) are also recommended as second-line treatment options, although there are a number of side effects associated with their use in dogs and cats (Greene, 2012; Zogg et al., 2018a). FQs and 3GCs as third-line treatments, are usually recommended for severe, life-threatening infections, or where there is resistance to first- or second-line choices (Zogg et al., 2018a).

AMR can be classified as either intrinsic or acquired. Intrinsic resistance is a genus or species-specific attribute, often due to an absence of specific target structures; however, acquired resistance is strain-specific usually based on the presence of a variety of AMR mechanisms or genes (Schwarz et al., 2017). Mutations in these genes, or horizontal gene transfer of genes encoding resistant mechanisms between bacteria, can result in AMR (Toombs-Ruane et al., 2017). Mobile genetic elements including plasmids enable resistance genes to be transferred between

bacteria without cell division having to occur (Toombs-Ruane et al., 2017). Thus, they not only contribute to the spread of AMR within a bacterial species but also to other bacterial species (Mathers et al., 2015).

Antimicrobials have five broad mechanisms of action. These are cell wall synthesis inhibition (e.g. penicillins, monobactams, carbapenems, glycopeptides, and bacitracin); cytoplasmic membrane inhibition (e.g. polymyxins); bacterial protein synthesis inhibition (e.g. chloramphenicol, lincosamides, macrolides, aminoglycosides, and tetracyclines); nucleic acid synthesis blockers (e.g. quinolones, nitroimidazoles, and rifampicin), and blockade of folic acid synthesis (e.g. sulphonamides and trimethoprim) (Samaha-Kfoury and Araj, 2003). Many AMR genes have been identified in ExPEC isolates from companion animals. The most significant of these (i.e. those encoding resistance to CIAs used in companion animals) and their specific mechanisms of resistance are described below.

1.3.2.1. Fluoroquinolone resistance

The FQ class of antimicrobials is regularly used in companion animal medicine as third-line therapies in the treatment of ExPEC infections and act by inhibiting DNA gyrase and topoisomerase IV in bacterial cells (Sánchez-Céspedes et al., 2015). Resistance occurs either by chromosomal mutations in the QRDR of genes that encode the above-mentioned DNA gyrase and topoisomerase IV or the acquisition of PMQR genes (de Jong et al., 2018). Since 1997 when the first PMQR determinant *qnrA1* was discovered, many more PMQR FQ resistance genes have been identified including those encoding additional *qnr* proteins (*qnrB*, *qnrC*, *qnrD*, and *qnrS*), the dual aminoglycoside/FQ enzymatic modification gene *aac(6')-Ib-cr*, and the efflux pump genes *qepA* and *oqxAB* (Poirel et al., 2018). Mutations in the QRDR generally result in a higher level of FQ resistance than PMQRs which can only confer reduced FQ

susceptibility (Jones-Dias et al., 2016). The prevalence of FQ resistance from various studies is shown in Table 2.

Table 2: Proportion of FQR strains among clinical *E. coli* isolates from companion animals in different studies

Type of isolate	Species	Total no. isolates	Year of sample isolation	Country	% resistance	Reference
Diseased animals	Dogs and cats	307	2002-2009	6 European countries	9.1[b]	(Kroemer et al., 2014)
Urine samples	Dogs	1108 299 508	2005-2012 2005 2012	New Zealand	2.3[a] 0.3[a] 1.8[a]	(McMeekin et al., 2017)
Clinical samples	Dogs and cats	730	2007-2013	Poland	9.7b[+] 39.3[a]	(Rzewuska et al., 2015)
Cases of confirmed clinical UTI	Dogs Cats	204 107	2008- 2010	10 European countries	3.9 [a] 7.5 [a]	(Moyaert et al., 2017)
Urine of animals with suspected UTI	Dogs and cats	1512	2008-2010	USA	11.6[d]	(Liu et al., 2012)
UTI	Dogs	114	2010-2011	Taiwan	5.3[a]	(Chang et al., 2015)
Clinical specimens	Dogs and cats	1628	2015	Finland	14[a*]	(Nykäsenoja et al., 2013-2015)
Urine Samples	Dogs Cats	51 13	2012-2016	Switzerland	84.3[c] 30.8[c]	(Zogg et al., 2018b)
Diseased animals	Dogs and cats	127	2012-2017	China	54.3[a] 52.8[b] 55.1[c]	(Chen et al., 2019)

Table 2 Continued: Proportion of FQR strains among clinical *E. coli* isolates from companion animals in different studies

Type of isolate	Species	Total no. isolates	Year of sample isolation	Country	% Resistance	Reference
UTI	Dogs and cats	142	2014	Austria	12[d]	(Marques et al., 2016)
		769		Belgium	6.6[d]	
		208		Denmark	2.9[d]	
		948		France	12.8[d]	
		153		Germany	16.3[d]	
		1457		Netherlands	4.9[d]	
		69		Italy	31.9[d]	
		30		Greece	30[d]	
		31		Portugal	29[d]	
		61		Spain	29.5[d]	
		2091		Sweden	1.1[d]	
		132		Switzerland	13.6[d]	
		143		UK	11.9[d]	
From cases of UTI	Dogs and cats	45	Unknown	Brazil	16[a]	(Osugui et al., 2014)
Clinical isolates from urine	Dogs	1038	2017	Sweden	4[a]	(Swedres-Svarm, 2017)
	Cats	539			7[a]	
Urine samples	Dogs	295	2015-2016	USA	10.2[a]	(LeCuyer et al., 2018)

[+] No changes in the level of resistance to FQs over the 7 years; [*] 2011 - 2014 resistance was below 10%; [a] - resistance to enrofloxacin; [b] - resistance to marbofloxacin; [c] - resistance to ciprofloxacin; [d] - not stated

1.3.2.2. β-lactam resistance

The β-lactam class is one of the most important and frequently used groups of antimicrobials in companion animal medicine and was first described by Abraham and Chain in 1940 (Abraham and Chain, 1940). β-lactam antimicrobials are categorised based on the chemical structure within the β-lactam ring. There are six major groups: penicillins, cephalosporins, cephamycins, carbapenems, monobactams, and β-lactamase inhibitors (ur Rahman et al., 2018).

Resistance to the six major groups, especially the ESCs and carbapenems among ExPEC isolates, represents one of the major clinical challenges veterinarians encounter. Resistance to β-lactams can be caused by several factors including the inaccessibility of antimicrobials to their target (PBPs), alterations of the antimicrobial target, or inactivation of the antimicrobial by β-lactamases (Li et al., 2007). β-lactamases can disrupt β-lactam antimicrobials by hydrolysing the β-lactam ring which renders the antimicrobials ineffective (Li et al., 2007).

There are currently two classification systems for β-lactamase enzymes, the Ambler molecular classification and the Bush, Jacoby, and Medeiros functional classification. The Ambler classification is based on sequence similarity and separates β-lactamases into four classes: A, C, and D for the serine-β-lactamases and B for the metallo-β-lactamases (Ambler, 1980; Jaurin and Grundström, 1981; Ouellette et al., 1987). Class B β-lactamases can be further separated into subclasses B1, B2, and B3 using sequence conservation data (Rasmussen and Bush, 1997).

The molecular classification was formalised initially in 1980 after four β-lactamase amino acid sequences were resolved through direct protein sequencing (Bush, 2013). The molecular class A β-lactamases were defined based on the penicillinases from *Staphylococcus aureus* PC1, *Bacillus cereus* 569/H, *Bacillus licheniformis* 749/C, and *Escherichia coli* R6K, and R-TEM (Ambler, 1980). The class

A β-lactamases include such enzymes as TEM-1 and SHV-1 (Bradford, 2001). Ambler defined the second β-lactamase structural class, class B, based on a combination of a partial sequence of the zinc-containing β-lactamase II from *B. cereus*, in conjunction with its variant mechanistic properties (Ambler, 1980). Subsequent work by Jaurin and Grundstrom, following Ambler's definition of the structural classes, sequenced the AmpC gene from *E. coli* K-12 and demonstrated significant molecular variances between AmpC cephalosporinase and β-lactamases in classes A and B (Jaurin and Grundström, 1981). These molecular differences gave rise to a new class C cephalosporinase designation. The last of the four molecular classes was provided by Huovinen et al. who demonstrated that the PSE-2 serine β-lactamases, later named *bla*$_{\text{OXA-10}}$, were sufficiently different from the other three classes to warrant a new separate class designation (Huovinen et al., 1988).

The second functional classification system of Bush, Jacoby, and Medeiros is based on grouping different β-lactamases according to their substrate and inhibitor profiles. Functional β-lactamases groups were formed using the biochemical properties of the enzyme, the molecular structure, and the nucleotide sequence of the genes (Bush et al., 1995). This method is used with clinical isolates as it indicates the correlation between β-lactamase possession and the functional phenotypes.

The classification of β-lactamases into functional groups has existed since at least 1968 when Sawai et al. separated sets of species-specific chromosomal β-lactamases from Gram-negative enteric bacteria into those enzymes with cephalosporinase or penicillinase activities (Sawai et al., 1968). The most current functional classification consists of three β-lactamase groups: group one (class C) cephalosporinases; group two (classes A and D) broad-spectrum, inhibitor-resistant, ESBLs and serine carbapenemases; and lastly group three (class B) metallo-β-lactamases (Bush and Jacoby, 2010).

β-lactamases can be separated into three primary types: ESBLs (SHV, TEM, OXA, and CTX-M types), AmpC β-lactamases (e.g. CMY), and carbapenemases (e.g. metallo-β-lactamases, KPC, and the OXA-type carbapenemases) (Toombs-Ruane et al., 2017; Dandachi et al., 2018). In 1963, the first β-lactamase was described in *E. coli*, identified in an isolate in Greece. It was designated TEM-1 for TEMONEIRA after the patient (Datta and Kontomichalou, 1965). Similarly, the first *bla*$_{SHV-1}$ gene was identified in *E. coli* in the 1970s (Pitton, 1972).

1.3.2.2.1. *Extended-spectrum β-lactamases*

ESBLs are most commonly derivatives of the TEM-1, TEM-2, or SHV-1 genes. ESBLs have been reported in both commensal and pathogenic *E. coli* strains associated with healthy and diseased companion animals (Poirel et al., 2018).

The first report in veterinary medicine of an ESBL from an animal was observed in Japan in 1988. The ESBL was designated FEC-1 and was determined to be a CTX-M-type enzyme (Fujisawa *E. coli*-1). The cefotaxime-resistant *E. coli* strain was isolated from faecal microbiota obtained from a laboratory dog. The laboratory dog was used for pharmacokinetic studies of β-lactamase antimicrobials and had received β-lactams drugs (Matsumoto et al., 1988). Even though this enzyme is technically classified as a CTX-M-type enzyme the first isolation of an enzyme using the name CTX-M did not occur until 1989. The name CTX-M was derived from its extended activity against the drug cefotaxime, compared to ceftazidime (both 3GCs), and the city where it was first isolated (Munich, Germany) (Bauernfeind et al., 1990).

The first report of some ESBLs in *E. coli* isolated from both humans and companion animals is listed in Table 3. Most genes were first identified in bacteria other than *E. coli*, such as *Klebsiella* spp., before transferring to *E. coli*. The most common ESBL types are TEM, SHV, and CTX-M; however, others such as OXA and NDM are

becoming more prevalent. As of July 2020, there are 89 TEM, 236 CTX-M, 21 OXA, and 29 NDM ESBL genes in the NCBI reference gene catalogue (NCBI, 2016).

The representatives of the CTX-M type ESBL family are categorised into five main groups, group 1, group 2, group 8, group 9, and group 25 (Tyrrell et al., 2016; Aslantaş and Yilmaz, 2017). The most common CTX-M ESBLs found in humans are CTX-M-15 (group 1), followed by CTX-M-14 (group 9) (Doi et al., 2017). However, in animals, CTX-M-1 (group 1) appears to be overrepresented. Although these types are the most predominate worldwide, other CTX-M genes do tend to be more geographically limited. Some examples of this include group 2 CTX-M genes, which are more commonly reported in South America and Japan, group 8 genes that are also prevalent in South America, and group 25 genes, which are more commonly found in Israel (D'Andrea et al., 2013).

A surveillance study of diseased dogs and cats across Europe found 1.6% carried ESBL-producing *Enterobacteriaceae* in stool samples. Most of these isolates harboured *bla*$_{\text{CTX-M}}$; however, only two isolates were identified as *E. coli* ST131, a human epidemic clone associated with FQ resistance (see Section 2.1). This study also found that ESBL carriage rates were much higher in the Netherlands, with approximately a quarter of all dogs screened from the Netherlands carrying ESBL-producing *Enterobacteriaceae* in their stools (Hordijk et al., 2013).

Another similar study investigated the presence of ESBL-producing clinical *E. coli* in dogs and cats in the United States between 2009 and 2013. This study found that there was a reported prevalence of 3.8% of ESBL-producing *E. coli* found, with *bla*$_{\text{CTX-M}}$ resistance genes being the most prevalent, followed by cephalosporinase and carbapenemases *bla*$_{\text{CMY-2}}$ and *bla*$_{\text{OXA-48}}$, respectively (Liu et al., 2016b).

A study by Zogg et al. (2018) collected clinical samples from cats and dogs between 2012 and 2016 in Switzerland. This study found a high prevalence (20.8%)

of ESBL-producing *Enterobacteriaceae* in comparison with other similar studies throughout Europe - 7% in the United Kingdom, 2% in the Netherlands, and 3.7% in France (Zogg et al., 2018a).

Since the first reports of ESBL-producing *E. coli* in the 1980s and 1990s, the rates of ESBL identification in companion animals has increased rapidly (Pomba et al., 2017). A comparison of the putative first identification of various β-lactamases and ESBLs in humans versus companion animals is shown in Table 3.

Due to their physical proximity and frequent close contact with their owners, companion animals are increasingly drawing attention as a potential source or vector of ESBL-producing *Enterobacteriaceae* for humans (Doi et al., 2017).

Table 3: First report of β-lactamases and ESBLs in *E. coli* from humans and companion animals

Resistance gene	Humans			Companion Animals		
	Year	Country	Reference	Year	Country	Reference
TEM-1	1963	Greece	(Datta and Kontomichalou, 1965)	Before 2001	Portugal	(Féria et al., 2002)
TEM-30	1989	France	(Vedel et al., 1992)	2007	Netherlands	(Dierikx et al., 2012)
TEM-52	2003	Korea	(Kang et al., 2005)	2003	Portugal	(Costa et al., 2004)
FEC-1 (i.e., the first CTX-M enzyme reported)	NA	NA	NA	Before 1988	Japan	(Matsumoto et al., 1988)
CTX-M-1	1989	Germany, Italy	(Bauernfeind et al., 1990; Bauernfeind et al., 1996)	2001-2003	Portugal & Italy	(Costa et al., 2004; Carattoli et al., 2005)
CTX-M-2	1994	Argentina	(Bauernfeind et al., 1996)	2007	Netherlands	(Dierikx et al., 2012)
CTX-M-3	1996	Poland	(Gniadkowski et al., 1998)	2003-2007	China & Korea	(Tamang et al., 2012; Liao et al., 2013)
CTX-M-9	1996	Spain	(Sabaté et al., 2000)	2006-2008	China & Korea	(Deng et al., 2011; Tamang et al., 2012)
CTX-M-14	1994	France	(Saladin et al., 2002)	2003-2007	Japan & China	(Liao et al., 2013; Okubo et al., 2014)
CTX-M-15	1999	India	(Karim et al., 2001)	2004-2007	United States & China	(O'Keefe et al., 2010; Sun et al., 2010)
CTX-M-24	1999	China	(Xiong et al., 2006)	2003-2007	China & Korea	(Tamang et al., 2012; Liao et al., 2013)
CTX-M-27	2000	France	(Bonnet, 2004)	2003	Japan	(Okubo et al., 2014)

Table 3 Continued: First report of β-lactamases and ESBLs in *E. coli* from humans and companion animals

Resistance gene	Humans			Companion Animals		
	Year	Country	Reference	Year	Country	Reference
SHV-1	1972	NA	(Pitton, 1972; Matthew et al., 1979)	Before 2001	Portugal	(Féria et al., 2002)
SHV-2	1985-1987	France	(Jarlier et al., 1988)	2011	Mexico	(Rocha-Gracia et al., 2015)
SHV-5	1995	England	(M'Zali et al., 1997)	2014-2015	Spain	(Suay-García et al., 2019)
SHV-12	1993–1995	Switzerland	(Nüesch-Inderbinen et al., 1997)	1998	Spain	(Teshager et al., 2000)
CMY-2	1996	Libya	(Bauernfeind et al., 1998)	1999	USA	(Sanchez et al., 2002)
OXA-1	1977	England	(Simpson et al., 1980)	Before 2001	Portugal	(Féria et al., 2002)
OXA-48	2004	Turkey	(Gülmez et al., 2008)	2012	Germany	(Stolle et al., 2013)
NDM-1	2008	Sweden*	(Yong et al., 2009)	2008-2009	USA	(Shaheen et al., 2013)
NDM-5	2011	England	(Hornsey et al., 2011)	2014-2015	Algeria	(Yousfi et al., 2016)
IMP-type enzymes	2001-2002	Japan	(Shibata et al., 2003)	2020	Brazil	(Martins et al., 2020)
VIM-type enzymes	2001	Greece	(Miriagou et al., 2003)	2015-2016	Germany	(Boehmer et al., 2018)

*from an Indian patient transferred one day previously from a New Delhi hospital

1.3.2.2.2. *AmpC β-lactamases*

In addition to ESBLs, resistance to β-lactams can also occur via AmpC β-lactamases. Although the CTX-M family of ESBLs has been the most prevalent (mostly in isolates from humans), AmpCs are more common in isolates from animals, and are primarily of the CMY-2 type (Melo et al., 2018). The first reported clinical disease associated with AmpC-producing *E. coli* in dogs in Australia was identified in 2006 (Sidjabat et al., 2006).

1.3.2.2.3. *Carbapenemases*

Of the β-lactam antimicrobials, carbapenems are typically reserved as the last resort treatment option for serious Gram-negative infections in humans (Heffernan et al., 2014). This class of antimicrobial is not recommended for the treatment of infections in companion animals (Abraham et al., 2014b). Despite carbapenems not being registered for use in animals in any major jurisdiction, off-label veterinary use of this critical 'last-line' antimicrobial class has been reported in dogs. Usage of carbapenems in dogs has been documented for treatment of UTIs and post-operative infections caused by MDR *E. coli* (Gibson et al., 2008; Abraham et al., 2014b).

Resistance to carbapenems is achieved via carbapenemases, the main types being metallo-β-lactamases, KPC, and OXA. There have been at least nine different types of acquired metallo-β-lactamases which have been classified (Cornaglia et al., 2011). The most important of these types for epidemiological dissemination and clinical relevance are the IMP-type, VIM-type, SPM-type, and NDM-type enzymes (Cornaglia et al., 2011). Due to the high affinity for carbapenems, VIM-type enzymes are unique among the metallo-β-lactamases. However, the most recently discovered transferable molecular class B (zinc metallo-β-lactamase) is NDM-1 (New Delhi metallo-β-lactamase-1). Additionally, NDM-1 and the carbapenemase, OXA-48, have been identified in clinical extraintestinal infections in companion animals in the United States

(Shaheen et al., 2013), and Europe (Pomba et al., 2017). IMP-4 was the first and most widely reported metallo-β-lactamase in Australia; it was detected in *Pseudomonas aeruginosa* and *Enterobacteriaceae* isolated from humans. Often the only antimicrobial available for use to treat organisms that produce metallo-β-lactamases is colistin, despite it being seen as a poor choice due to its undesirable pharmacokinetics and toxic effects (Cornaglia et al., 2011).

1.3.3. Subspecific differentiation of companion animal ExPEC

The classification of *E. coli* is based on phylogenetic groups, serotypes, and sequence types, often via PCR/molecular techniques such as MLST and WGS. RAPD and PFGE analysis are often used for comparing the relatedness of strains but are becoming superseded by more rapid and cost-effective methods such as WGS.

In 1984 an *E. coli* reference collection (ECOR) was established by Ochmann and Selander to provide researchers with a tool for deciphering the population structure of commensal *E. coli* (Ochman and Selander, 1984). The collections comprised a set of 72 *E. coli* strains chosen from a collection of 2,600 *E. coli* isolates. Strains were chosen based on their allelic diversity and comprised strains isolated from the faeces of healthy humans and zoo animals as well as strains from UTIs in human patients from a variety of geographical locations. This collection has been an invaluable tool for researchers to further understand *E. coli* population dynamics; especially ExPEC and the mechanisms behind the proliferation and the spread of AMR; as well as helping researchers generate better methods for classification especially for use in surveillance. The 72 strains were thought to represent the genetic diversity that was known at that time. More recent studies have indicated there may be greater diversity in human and animal commensal *E. coli* strains than first thought and thus the ECOR collection may not be truly representative of the entire population (Tenaillon et al., 2010).

1.3.3.1. Serotyping

The outer membrane of *E. coli* is made up of lipopolysaccharides that include lipid A, core oligosaccharides, and the O-antigen, a unique polysaccharide (Fratamico et al., 2016; DebRoy et al., 2018). These O-antigens can provide important pathotypic information that is useful in the investigation of outbreaks and epidemiological studies (DebRoy et al., 2018). Due to their diversity, O-antigens have been used in the classification of *E. coli* since the 1940s. In the 1970s, a comprehensive serotyping system was developed to type *E. coli* based on the presence of three surface antigens: lipopolysaccharide O-antigens, flagellar H-antigens, and capsular K-antigens. However, few laboratories had the ability to perform K-antigen typing, thus serotyping based on the O- and H-antigens became the standard. Serotyping was performed on the basis of an agglutination reaction with specific rabbit antisera generated against each of the O-groups (Ørskov and Ørskov, 1984). However, while the test was easy to perform, it was costly, labour intensive and time-consuming, with antisera often only available in specialised laboratories. In addition, cross-reactivity of the antisera with the different serogroups could occur as well as variations between different batches of antisera (Lacher et al., 2014).

Molecular serotyping techniques were developed with the advent of cost-effective and rapid WGS. Molecular serotyping targets O-group specific genes and the H-antigen genes, found within the *E. coli* O-antigen gene clusters. Variability in the O-antigens is due to variations in the size of O-antigen gene clusters and their gene content. Variations in the sequences of the *fliC* gene that encodes the flagellar filament structural protein are used to molecularly type the H-antigen, with different H-types occurring due to differences in amino acids in the central region (Fratamico et al., 2016). To date there are 171 somatic (O), 55 flagellar (H) and 80 capsular (K) antigens recognised, with over 160 serotypes of *E. coli* identified (Sarowska et al., 2019). O- and H-antigens often relate to specific pathotypes of *E. coli*. For instance, the

serogroups O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75 are often associated with UTIs (Osugui et al., 2014; Morales-Espinosa et al., 2016).

1.3.3.2. Random amplified polymorphic DNA

RAPD analysis is a molecular fingerprinting technique, involving PCR amplification of random fragments of DNA (Kärkkäinen et al., 1996) using short primers, typically 10bp long (Meunier and Grimont, 1993). The primers used do not target specific sequences within the genome; instead, they anneal at random sites along the genome initiating DNA polymerisation (Power, 1996). Attributes of these sites include number, location, and proximity, and they produce unique DNA fingerprints or patterns, which are visible after gel electrophoresis (Kärkkäinen et al., 1996; Power, 1996).

RAPD is a simple, affordable, and rapid technique that requires very little DNA (Kärkkäinen et al., 1996). It also does not require targeted primers and instead uses generic primers, which require no prior knowledge of the bacterial genome sequence being tested (Derakhshandeh et al., 2018). This makes it ideal for screening large collections of *E. coli* before undertaking further complex and lengthy techniques such as MLST WGS (Nielsen et al., 2014).

Although RAPD analysis is an effective and useful technique with many advantageous qualities it has historically proven difficult to reproduce, especially between laboratories. If there are differences in the concentration of template DNA and/or primer, the use of different reagents or thermal cyclers, or alterations in gel electrophoresis or the technique of the operator, the PCR products amplified can be affected and therefore may result in a different DNA fingerprint being produced (Power, 1996). These issues can be overcome by standardising the techniques such as DNA preparation, consistent use of volumes and concentrations, and the usage of the same reagents, thermal cycler, and standardised procedures for performing gel

electrophoresis and DNA fingerprint visualisation. Further avenues for overcoming the difficulties of RAPD analysis reproducibility include the use of commercially available products such as pre-mixed PCR reagents as well as training for staff undertaking the analysis (Power, 1996).

Once a DNA fingerprint has been obtained via RAPD, various bioinformatics tools can be used for analysis and comparison of strains. One such tool is BioNumerics (Applied Maths) which allows analysis of gel images to compare the banding patterns of each isolate and the construction of a dendrogram to visualise the genetic relatedness of the strains. Various similarity and distance coefficients are available for different data types, as well as a number of clustering methods for calculating dendrograms from pairwise similarity values including unweighted pair grouping (UPGMA) and neighbour joining.

1.3.3.3. Multi-locus sequence typing

In the late 1990s, MLST emerged as a new technique that proved a powerful tool for bacterial population genetics (Enright and Spratt, 1999). MLST is a sequence-based genotyping method that allows for genetic comparison of bacterial strains. Using this technique, the nucleotide sequence of multiple housekeeping genes is determined (Tenaillon et al., 2010). The ST is defined based on an allelic profile which is derived from the various alleles at different loci for a bacterium.

MLST requires purified chromosomal DNA. Segments of the seven housekeeping genes are then amplified before direct sequencing is performed. Based on the sequence differences or polymorphisms identified, each unique sequence is termed an allele which contributes to the identification of a unique ST. The resulting STs can then be compared to infer further genetic relationships.

STs can be grouped into broader clonal complexes with each clonal complex containing at least three STs, each differing from the others by no more than one of

the seven alleles (Salvador et al., 2012). Each ST belongs to a particular phylogenetic group; however, each phylogroup contains multiple different STs (Johnson et al., 2017a).

There are currently three MLST schemes available for use with *E. coli* – the Michigan scheme, the Warwick scheme, and the Pasteur scheme – all named on the basis of where the databases are hosted. The schemes hosted at Michigan State University (Reid et al., 2000), the Warwick Medical School (Wirth et al., 2006), and the Pasteur Institute (Jaureguy et al., 2008) were respectively developed by Thomas Whittam, Mark Achtman, and Sylvain Brisse/Erick Denamur. Each scheme uses a different combination of genes (Tenaillon et al., 2010) and has only one gene, *icd*, in common (Clermont et al., 2015). There are currently over 9000 *E. coli* STs with new types assigned regularly (http://enterobase.warwick.ac.uk/species/index/ecoli). Despite this large number of STs only a very small percentage of these are associated with ExPEC infections (Bourne et al., 2019).

MLST profiling is a high fidelity method that can be used to differentiate between species within a genus. The method can be used to distinguish commensal *E. coli* isolates from pathogenic variants of the same genus such as serotype O157. The method is easily standardised and there are detailed protocols described for its use including a wide variety of important veterinary pathogens. Although MLST is a highly selective method, sequencing of the seven genes for each strain is labour intensive and expensive for routine testing.

1.3.3.4. Whole genome sequencing

WGS is the process of determining the complete DNA sequence of an organism's genome. Using WGS the order of bases in a genome of an organism can be determined to give a unique sequence type.

At the beginning of the genomics era, in 1997, the first *E. coli* genome was sequenced; this was a representative of the laboratory-derived strain K-12, which was originally isolated from the stool of a convalescent diphtheria patient in 1922. Later in 2002, the first genome of a UTI strain was published, this being the third *E. coli* to be fully sequenced since the original O157:H7 strain in 2001. Developments in sequencing technology now allow the sequencing of more isolates to be conducted rapidly and at a relatively low cost (Tourret and Denamur, 2016). There are now millions of bacterial whole genome sequences available in public databases such as Enterobase and NCBI.

Numerous additional typing methods have been applied to characterise *E. coli*. Of these, the most widely used technique is PFGE. Additional typing techniques are laborious, expensive, and do not always provide high enough fidelity to provide clone specific fingerprints. Advancements in WGS technology have improved how MLST is conducted and allow for highly detailed phylogenetic typing to be undertaken. Despite this, sample preparation is a laborious aspect of WGS and the technique is still too expensive for most laboratories to run on all available isolates. Due to the costs involved, when working with mixed samples with a high frequency of diverse bacteria, pre-selection is conducted for distinctive bacterial clones that will efficiently reveal the overall population structure (Nielsen et al., 2014).

One of the most common bioinformatic approaches to detecting the presence of relevant genes is achieved by comparing *de novo* assembled draft genome contigs against a reference target database using a BLAST (Schwarz et al., 2018). The number of freely available bioinformatics tools that can be used for detecting genetic determinants for AMR in WGS data is continuously increasing. A few of these tools are ARG-ANNOT, CARD, and ResFinder, an AMR gene identification tool provided by the Center for Genomic Epidemiology.

1.4. Companion animal ExPEC molecular epidemiology

Studies have shown that dogs and cats can share pathogenic *E. coli* strains with humans, including ExPEC and UPEC (Johnson et al., 2008a; Johnson et al., 2008b; Johnson et al., 2009b). In a study conducted in 64 UPEC strains from dogs and cats, 15.6% of the isolates identified as ST73 and ST131, both of which are associated with UTIs in humans (Zogg et al., 2018b). In addition, a recent study of ExPEC in dogs from the United States identified the human-associated ExPEC ST12, ST73, and ST127 (LeCuyer et al., 2018).

1.4.1. ST131

E. coli ST131 belonging to phylogenetic group B2 is commonly associated with extraintestinal infections (Schembri et al., 2015) and has become a globally disseminated MDR pandemic clonal group in humans (Rogers et al., 2011; Johnson et al., 2017b). There is some conjecture regarding the timing of the first report of ST131. Multiple studies state that ST131 was first identified in 2008 (Schembri et al., 2015; Dautzenberg et al., 2016; Johnson et al., 2017b), though the paper these studies reference states that ST131 is an established ST (Nicolas-Chanoine et al., 2008) and no other reports prior to this have been identified. Another study by Downing in 2015 (Downing, 2015) states that ST131 was first reported in 2002, though this paper does not specifically state ST131 and the strain is identified as a species of *Klebsiella*, rather than *E. coli* in the original report (Alobwede et al., 2003). There do not appear to be further studies that support the claim that the first report of ST131 was in 2002.

Despite these initial reports first identifying ST131 in 2008, a study performing PFGE analysis on historical isolates identified an ST131 isolate from 1967 (Johnson et al., 2012). The oldest sample present in Enterobase at the time of writing this review is the same strain from 1967 though there is no source or origin listed. Throughout Enterobase, there are over 3000 ST131 strains without collection data so there is the

possibility that there are yet older strains, though this is highly improbable given no papers were found referencing any older strains. Prior to the aforementioned study, the earliest report of ST131 was from a patient presenting with urosepsis in 1985 (Johnson et al., 2010). Further archival strains from 1982 and 1983 have also been described (Johnson et al., 2012).

ST131 has been identified in population genetic studies as belonging to three different clades – clade A, B, and C (Pitout and DeVinney, 2017). Each of these three clades is associated with a different type 1 fimbrial adhesin, clade A – *fimH*41, clade B – *fimH*22, and clade C – *fimH*30 (Johnson et al., 2013; Ben Zakour et al., 2016). Further studies that examine the global epidemiology of ST131 using genome sequence-based methods found that clade A was the most divergent whereas strains from clade B were very similar to those from clade C (Petty et al., 2014).

The first reported ST131 isolate of animal origin was identified in a Portuguese study that examined dog and cat isolates obtained between 2004 and 2006. This study screened 61 FQR *E. coli* isolates from dogs (n=41) and cats (n=20) for ESBLs (Pomba et al., 2009). In Australia, ST131 was first identified among human FQR clinical isolates in 2008-2009 (Sidjabat et al., 2010). A report published less than a month later further identified ST131 in human clinical samples taken from 2007-2008 (Platell et al., 2010). The same study from which this report originated also published the first report of ST131 in FQR clinical specimens from companion animals in 2007-2009 (Platell et al., 2010). These studies corroborate that ST131 was present in both humans and companion animals within Australia about the same time, however, the prevalence was much higher among human strains (202/585, 35% vs. 9/125, 7.2%) (Platell et al., 2010). The frequency of ST131 identified in studies of clinical *E. coli* isolates from companion animals is shown in Table 4. Most of these studies focused on resistant strains and there are very few studies that have investigated the prevalence of ST131

among total isolate strain collections rather than just isolates that contained resistance determinants such as ESBLs or FQ resistance (Karkaba et al., 2017; Belas et al., 2018; Zogg et al., 2018b; Valat et al., 2020). Most of the studies included in Table 4 had larger initial sample pools but only screened low numbers of resistant isolates for ST131 (i.e. ≤ 10 samples). Additional studies have identified ST131 among faecal isolates from both sick and healthy subjects but as these are not the focus of this review, they are not further described.

Table 4: Various studies that have identified ST131 in clinical samples from companion animals.

Sample type	Year of isolation	Country of isolation	Proportion of isolates ST131	Reference
CTX-M producing	1999-2014	Portugal	33.3 (4/12)	(Marques et al., 2018)
Uropathogenic *E. coli* (animals with UTI)	1999-2015	Portugal	14.5 (25/172)	(Belas et al., 2018)
CTX-M positive	2003-2010	Japan	36.4 (4/11)	(Harada et al., 2012)
qnr positive isolates from animals with UTI	2004-2006	Portugal	100 (1/1)*	(Pomba et al., 2009)
ESBL producers	2006-2010	France	5.3 (1/19)	(Dahmen et al., 2013)
Extraintestinal FQR	2007-2009	Australia	7.2 (9/125)	(Platell et al., 2010)
ESBL positive	2008-2009	Europe	7.8 (9/115)	(Ewers et al., 2010)
ESBL producers	2008-2010	Poland	25 (2/8)	(Bogaerts et al., 2015)
ESBL producers	2009-2012	United Kingdom	10.8 (4/37)	(Timofte et al., 2014)
ESBL producing MDR	2009-2013	United States	14.7 (10/68)	(Liu et al., 2016b)
Resistant isolates from urogenital infections	2010-2015	Austria & Serbia	3.6 (1/28)	(Loncaric et al., 2020)
ESC resistant	2010-2016	United Kingdom	3.1 (5/164)	(Bortolami et al., 2019)
ESC resistant B2 strains	2010-2016	France	54.9 (56/102)	(Melo et al., 2019)

Table 4 Continued: Various studies that have identified ST131 in clinical samples from companion animals.

Sample type	Year of isolation	Country of isolation	Proportion of isolates ST131	Reference
CTX-M positive	2012	Italy	14.3 (1/7)	(Nebbia et al., 2014)
Clinical isolates	2012-2013	New Zealand	6.7 (6/89)	(Karkaba et al., 2017)
ESBL producers	2012-2016	Switzerland	12.1 (7/58)	(Zogg et al., 2018a)
UPEC	2012-2016	Switzerland	7.8 (5/64)	(Zogg et al., 2018b)
CTX-M isolates	2012-2017	China	6.8 (n=44)	(Chen et al., 2019)
Non-WT susceptibility to 3GCs	2013-2014	Europe	13.6 (6/44)	(Pepin-Puget et al., 2020)
ESC resistant ExPEC	2013-2015	China	25 (10/40)	(Liu et al., 2016a)
ESBL positive	2015	Japan	35.7 (15/42)	(Kawamura et al., 2017)
CTX-M positive	2015-2016	Canada	41.7 (10/24)	(Zhang et al., 2018)
Clinical isolates	2015-2016	United States	4.1 (12/295)	(LeCuyer et al., 2018)
3GC resistant B2 strains	2016	Japan	66.7 (28/42)	(Maeyama et al., 2018)
Group B2 clinical isolates	2017	France	1.8 (9/492)	(Valat et al., 2020)

* Sixty-one strains were tested, however, only one contained a *qnr* gene and hence was the only isolate to undergo further analysis.

1.4.2. ST1193

Recently, ST1193, another lineage of FQR group B2 *E. coli*, has also emerged as an important, globally disseminated MDR human pathogen (Johnson et al., 2019). The first report of ST1193 was in Australia in 2008 where it was identified in approximately 10% of strains from a collection of phylogroup B2 clinical FQR *E. coli* isolates from humans and dogs. Further studies have since identified ST1193 among human *E. coli* strains from China (Wu et al., 2017; Xia et al., 2017), Korea (Kim et al., 2017), Norway (Jorgensen et al., 2017), Germany (Valenza et al., 2019), and the

United States (Tchesnokova et al., 2019), and in companion animals in Japan (Maeyama et al., 2018), and Canada (Zhang et al., 2018), where they have been identified in much lower numbers. In these two studies focused on clinical isolates from companion animals, ST1193 was identified among five of 18 3GC-resistant non-O25b UPEC isolates (Maeyama et al., 2018), and one of 24 CTX-M-positive isolates (Zhang et al., 2018). To the best of my knowledge, to date, no other studies have identified ST1193 in companion animals.

It is believed that ST1193 strains have arisen from a common ancestor and are likely descendants of an older clonal lineage that has recently acquired FQ resistance rather than belonging to a recently emerged clonal group. This is due to several isolates being genetically similar to a historical (mid 1980s) FQ-sensitive O75 urosepsis isolate from the United States (Platell et al., 2012).

1.4.3. Other STs

Apart from ST131 and ST1193, which are typically associated with critical antimicrobial-resistant isolates, there are several other major STs associated with non-resistant ExPEC infections that have been identified in companion animals. These include ST12, ST69, ST73, ST95, ST127, and ST372, all of which have been isolated from companion animals albeit at a lower prevalence for the majority of STs than among corresponding human ExPEC strain collections.

Studies have suggested that each host species may have a distinctive dominant ST that accounts for most isolates even though urine isolates from cats, dogs, and humans can overlap broadly by ST (LeCuyer et al., 2018). Indeed, several studies have found that UPEC isolates from dogs and cats shared genotypic and pathotypic similarities with ExPEC isolates from humans (Yuri et al., 1999; Feria et al., 2001; Johnson et al., 2001; Johnson et al., 2008a; Johnson et al., 2008b; Johnson et al., 2009a).

Studies have suggested that in dogs the dominant ExPEC clone is ST372 while in cats it is ST73. There are, however, a wide range of STs identified in ExPEC from both cats and dogs and these can vary based on the types of isolates identified i.e. whether they are general clinical strains or harbour specific resistances such as 3GC resistance mediated by ESBLs. Additional STs that have been identified in clinical isolates from both cats and dogs include ST83, ST141, ST405, ST410, ST648, and ST961.

ST73, associated with UTIs in companion animals, is a broad host range clonal group (Zogg et al., 2018b). This ST has been identified in clinical samples from dogs and cats in five main studies from three countries with only one of these specifically focusing on resistant strains. In two separate studies, one from Switzerland and one from the United States, focusing on isolates from both dog and cats, ST73 was identified in 7.8% (5/64) and 4% (3/68) of isolates, respectively (Liu et al., 2016b; Zogg et al., 2018b). As ST73 is mainly associated with antimicrobial susceptibility (Dale and Woodford, 2015; Doumith et al., 2015) this could explain the lower prevalence in the United States study as the focus was on ESBL-positive MDR isolates. However, despite this association with antimicrobial susceptibility, antimicrobial-resistant ST73 have sometimes been associated with UTI infections in companion animals (Liu et al., 2015; Liu et al., 2016b; Chen et al., 2019). In one United States study, ST73 was identified in 16% (12/74) of cat UTI isolates (Liu et al., 2015). Similarly, in a French study, 17.9% (16/89) of dog UTI isolates belonged to ST73 (Valat et al., 2020). Interestingly, in another study from the United States, only 6% (19/295) of dog UTI isolates belonged to ST73 (LeCuyer et al., 2018). In both these studies, ST73 was not the dominant strain so it is possible that the difference in prevalence is due to the much larger number of isolates investigated in the United States study.

Despite being globally disseminated, it has been suggested that ST73, unlike ST131, is not driven by the emergence and expansion of a dominant clone (Bogema et al., 2019). Unlike ST131, whose isolates have a relatively conserved genome, human ST73 isolates from the UK and Australia have been found to be genomically diverse (Alhashash et al., 2016; Bogema et al., 2019).

ST372 has been previously isolated from UTIs in both humans and dogs (Blyton and Gordon, 2017; LeCuyer et al., 2018; Valat et al., 2020), though its low prevalence (4-11%) among human clinical isolates (Adler et al., 2012; Izdebski et al., 2013; Rios et al., 2015) suggests it may not be a predominately human-associated clone. ST372 has been identified as the most common ST in studies of susceptible dog ExPEC isolates from France (18% of 89) and the United States (22% of 295) (LeCuyer et al., 2018; Valat et al., 2020). Other studies, however, have identified ST372 in *E. coli* from cats and dogs at much lower prevalence (2-5%), though most of these studies focused on resistant isolate collections (i.e. ESBL-producing/FQR) (Liu et al., 2016b; Karkaba et al., 2017; Maeyama et al., 2018; Zogg et al., 2018b; Chen et al., 2019). An Australian study in dogs did not report the presence of ST372; however, this could be due to the sampling of faeces from healthy animals rather than clinical isolates (Bourne et al., 2019).

1.5. ETEC and commensal *E. coli* in pigs

E. coli are the main aerobic bacterial population colonising the pig GI-tract. Commensal *E. coli* acquired from the sow at birth (Ahmed et al., 2017) are believed to maintain the piglet gut environment and support digestion as well as defend against enteric pathogens (Schierack et al., 2006). However, while being a beneficial gut commensal, pathogenic *E. coli* can also cause a number of diseases in pigs. These include two forms of enteric colibacillosis, neonatal diarrhoea and PWD, oedema disease, septicaemia, coliform mastitis and UTIs (Zimmerman et al., 2012). These

diseases can cause significant economic losses due to high morbidity, mortality, decreased weight gain and cost of treatment (Zimmerman et al., 2012). ETEC is the main pathotype causing enteric colibacillosis infections.

In pig production antimicrobials have, in the past, been relied upon for the treatment of enteric colibacillosis infections. AMR is thus of increasing concern, in commensal as well as pathogenic *E. coli* strains from pigs (Smith et al., 2010).

The most common antimicrobials used to treat enteric colibacillosis infections in pigs include aminoglycosides (apramycin, gentamicin, spectinomycin and neomycin), the β-lactam amoxicillin, the FQ enrofloxacin, the 3GC ceftiofur, trimethoprim/sulphonamide and colistin (Zimmerman et al., 2012). However, many countries have restrictions in place on the use of the CIAs in pigs (FQs, ceftiofur and colistin). For example, colistin is only used in Asia and Europe. In 2017, in guidelines published by the WHO on the use of CIAs in food-producing animals, it was recommended that therapeutic use be reduced overall whilst use of any antimicrobial for growth promotion and/or disease prevention prior to diagnosis should be halted entirely (WHO, 2017). In Australia, however, in contrast to many other countries worldwide, the use of CIAs has always been highly regulated (Smith et al., 2016). Australia is the only country to implement legal measures that exclude the use of FQs and gentamicin in food-producing animals (Abraham et al., 2014a). In addition, colistin has not been registered for use in Australian livestock for over 25 years (APVMA, 2017). Off-label use of ceftiofur has been reported for MDR ETEC infections in pigs (Jordan et al., 2009), however, its use has been rapidly curtailed following the emergence of resistance in commensal *E. coli* (Abraham et al., 2018). Other countries are also taking a similar approach to the use of antimicrobials in food-production. For instance, in 2002, Danish regulation restricted the use of FQs in animal production and in pig production, and in 2010 Danish farmers voluntarily stopped using cephalosporins

following the emergence of resistance in commensal *E. coli* (Agersø and Aarestrup, 2013; DANMAP, 2018).

AMR in pig production systems can occur via selection pressure (i.e. the use of antimicrobials resulting in the development of resistance), direct transfer of resistant bacteria between humans and pigs, the transfer of mobile genetic elements containing resistance genes from commensal human *E. coli* and/or via outside sources such as migratory or scavenging birds (Mukerji et al., 2019). Once inside a piggery, resistance can remain indefinitely, as demonstrated in the longitudinal study of ceftiofur-resistant commensal *E. coli* following the withdrawal of ceftiofur use (Abraham et al., 2018). In addition, antimicrobial use in pigs can potentially lead to the selection and dissemination of antimicrobial-resistant bacteria, which can then be transferred to humans via consumption and handling of contaminated food, direct contact with animals and/or through the release of resistant bacteria into the environment (Australian Government Department of Health, 2018).

A number of ESBLs have been identified in *E. coli* from food-producing animals around the world. The production of ESBLs in *E. coli* from food-producing animals is often blamed on the high use of antimicrobials as well as the inappropriate use of 3GCs (Dohmen et al., 2017). For instance, in a study investigating *E. coli* isolated from the faeces of pigs on Danish pig farms found the production of CTX-M-1 β-lactamases was associated with the use of ceftiofur (Jørgensen et al., 2007). In Australia, low numbers of ESBL and AmpC β-lactamases such as *bla*$_{\text{CMY-2}}$, *bla*$_{\text{CTX-M-14}}$ and *bla*$_{\text{CTX-M-9}}$ have been identified in 2013 from a study investigating the frequency of resistance to CIAs among *E. coli* isolates causing clinical disease in food-producing animals. The presence of these genes is likely due to the off-label use of ceftiofur, used as a last line antimicrobial to treat serious MDR infections caused by porcine ETEC (Abraham et al., 2015; Wyrsch et al., 2019).

1.6. AMR surveillance in food-producing animals

AMR is an ongoing global health issue and necessitates the need for surveillance and reporting programs. The effectiveness of antimicrobials for use in disease treatment is associated with the level of AMR, hence it is of significant importance that surveillance and monitoring is performed to gain an accurate picture of any emerging resistance and the rate of transmission of new resistance (EFSA and ECDC, 2017). The data produced from surveillance and monitoring studies can be interpreted to help detect new and emerging antimicrobial resistant phenotypes, identify susceptibility trends for certain antimicrobial agents, identify new and emerging resistance mechanisms in bacteria, and present information useful for usage guidelines (Schwarz et al., 2018). There are numerous key considerations in addressing the objectives of any long-term surveillance study, which extends to the sampling strategies utilised. Some sampling strategy considerations may include; determining if active or passive surveillance is used, what animal species should be sampled, what bacterial organism should be targeted, what location bacteria will be sampled, which species of bacteria is to be collected, and what the maximum number of isolates per location or disease related event should be used. In addition, there are other significant factors that need careful consideration prior to the commencement of a surveillance study including the selection of optimal antimicrobial agents for testing, the type of AST method to be utilised, what standardised testing method will be implemented with a nominated QC, and the type of reporting and analysis (Schwarz et al., 2018).

Commonly monitored bacteria in studies tend to include indicator bacteria of the normal enteric microflora from healthy animals such as *E. coli*. These bacteria are selected due to their high prevalence, function as a commensal, including possible role in causing a range of diseases, and effective use as an indicator for antimicrobial

selection pressure. *E. coli* also serves as an indicative early alert system for monitoring emergent resistant bacteria. Over time resistant bacteria can form reservoirs of transferable resistance genes from which AMR can spread to other bacteria; this can include those, which are responsible for infections in humans and animals.

The types of bacteria tested in surveillance programs can vary significantly, as well as other factors including what animals are sampled and the methods used to test the isolates. Although this review will focus primarily on pigs, many surveillance programs commonly cover animals such as cattle, pigs, and poultry with samples procured from the abattoir and in some cases retail meat. Many existing surveillance programs have a focus on human pathogenic bacteria, which may or may not be pathogenic to animals, while others include commensals. The WHO/OIE are mandating that surveillance should be performed in all countries, subsequently, there are numerous active surveillance programs worldwide. Of the active surveillance programs, many are committed to surveying food-producing animals in numerous countries around the world including Denmark, France, Canada, the United States, Finland, Germany, Japan, Norway, Italy, the Netherlands, Sweden, Columbia, and Spain. Only the most prominent surveillance programs will be discussed in this review. Of the studies covering multiple bacterial species, this review will only focus on *E. coli* and *Salmonella* in pigs. *Campylobacter* and *Enterococcus* will not be discussed. Although a report has been produced by the Australian Department of Agriculture which includes analysis and recommendations regarding surveillance and reporting of AMR in animals within Australia, and several ad-hoc studies have been undertaken, there is currently no continuous national surveillance program in place.

1.6.1. Canadian Integrated Program for Antimicrobial Resistance Surveillance

In 2002, CIPARS was established. CIPARS was modelled after other international initiatives such as the NARMS and DANMAP for antimicrobial monitoring and surveillance. CIPARS collects various information on antimicrobial use including use in humans and animals, and AMR. AMR information collected includes resistance in enteric pathogens isolated from humans, and AMR in enteric pathogens and commensal organisms from the agricultural and food sectors. CIPARS carries out both abattoir and farm surveillance. *E. coli* and *Salmonella* are isolated either from the caecal contents (not carcasses) of slaughtered pigs or from grower-finisher pigs. AST is carried out via MIC using CLSI guidelines. (https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars.html).

1.6.2. National Antimicrobial Resistance Monitoring System

NARMS was established in 1996 in a collaboration between federal, state, and local agencies in the United States and was initiated with the purpose of performing surveillance on AMR in enteric bacteria from humans, animals, and retail meats. In March 2013, NARMS began a program sampling caecal contents at federally inspected slaughter and processing plants. The samples were analysed for *Salmonella* and *E. coli* from both market hogs and sows. Partners of NARMS perform tests for bacterial susceptibility to a range of antimicrobial drugs including 15 antimicrobial drugs for *Salmonella* and *E. coli*. Additionally, a standard part of the NARMS surveillance includes performing WGS, to screen for resistance genes in enteric bacteria. The findings of each year's survey are published in an Annual Integrated Report summarising the most important resistance findings. (https://www.cdc.gov/narms/index.html).

1.6.3. Danish Integrated Antimicrobial Resistance Monitoring and Research Programme

DANMAP was founded in 1995 by the Danish Ministry of Food, Agriculture and Fisheries, and the Danish Ministry of Health. Similar to NARMS, DANMAP was created to monitor AMR and usage in food animals and humans. The objectives of DANMAP also extended to include identifying associations between AMR and consumption, and routes of transmission. DANMAP surveys indicator bacteria, zoonotic bacteria and animal pathogens from food animals, food and people. Samples are collected from various sources and locales but some of those of interest to this review include *Salmonella* samples taken from carcass swabs, and *Salmonella* and indicator *E. coli* from caecal samples from fattening pigs. A key part of the sampling protocols used by DANMAP is a selection criterion of only one indicatory *E. coli* isolate per flock or herd. Since 2014, isolates available for use by DANMAP have been collected in accordance with EU protocols for harmonised monitoring of AMR in zoonotic and commensal bacteria. The legislation outlines requirements such as the sampling of *Salmonella* from fattening pigs less than one year of age at slaughter in odd years (2015, 2017, 2019). DANMAP performs AST of *Salmonella* and *E. coli* isolates using MIC techniques of which, since 2007, resulting data is interpreted using EUCAST ECVs. In addition to this, specific isolation of ESBL/AmpC and carbapenemase-producing *E. coli* is also carried out. Furthermore, in additional to *Salmonella* serotyping via sequencing techniques, WGS is performed on the ESBL/AmpC and carbapenemase generating *E. coli*. Analysis on the aforementioned isolates includes MLST, detection of AMR genes, and detection of plasmid replicons. (https://www.danmap.org/).

1.6.4. French surveillance network for antimicrobial resistance in bacteria from disease animals

RESAPATH is a French epidemiological surveillance network for monitoring trends in antimicrobial resistance in bacteria from animals. The RESAPATH network was initiated in 1982 under a different name RESABO, BO indicating this was for bovines. In 2000, RESABOs scope expanded to include pigs and poultry, with this inclusion of AMR monitoring of pathogenic poultry and swine bacteria, in 2001, the surveillance network was aptly named RESAPATH. In 2007, the scope of the monitoring network was expanded to include other animal species such as ruminants, horses, and companion animals. At this time, the network's coverage was also expanded to all animal production sectors which now included animals such as sheep, goats, rabbits, horses, dogs, and cats.

RESAPATH has enjoyed a long-term cooperative effort from 71 veterinary diagnostic laboratories throughout France; laboratory membership with RESAPATH is voluntary. The primary objectives of RESAPATH are to monitor AMR in bacteria isolated from diseased animals in France, to collect resistant isolates of particular interest and to characterise their genetic background, and to provide scientific and technical support on AST methods and results interpretation to member laboratories. AST is performed using disk diffusion techniques. RESAPATH also conducts surveillance of AMR in zoonotic *Salmonella* and indicator *E. coli* bacteria collected at slaughterhouses from healthy animals in numerous livestock sectors. As part of routine activities samples are taken by veterinarians for diagnostic purposes and bacteria from these are tested for antimicrobial susceptibility by private or public veterinary laboratories throughout France.

Additional to data collection, the RESAPATH also aids in collection of isolates demonstrating AMR profiles of interest for further detailed molecular studies. All AST

data generated in public or private veterinary laboratories voluntarily participating in the RESAPATH network is collected in digital or paper form. The recorded data includes information such as sampling history, the antimicrobials tested including diameters of inhibition zones, and the context information such as laboratory conducting the analysis, chain of provenance, age of animal, pathology, and sampling type. Due to data collection being subject to the initial discretion of veterinary practitioners this does prevent this being an accurate representation of the global AMR burden of pathogenic bacteria; however, it does serve as a reliable indicator of AMR rates in field conditions. (https://resapath.anses.fr/).

1.6.5. Swedish Veterinary Antimicrobial Resistance Monitoring

The SVARM programme was established in 2000 to improve the monitoring of pathogens in farm animals. SVARM was initiated with objectives to monitor antimicrobial susceptibility of zoonotic bacteria (*Salmonella*), specific animal pathogens, and commensal enteric bacteria (*E. coli*). Additionally, it was a goal to maintain regular monitoring.

SVARMs surveillance of AMR was increased in 2005 to include bacteria causing disease in pigs, cattle, sheep, and poultry (SvarmPat). The programme uses clinical isolates from routine bacteriological examinations of clinical submissions or post-mortem examinations as well as *E. coli* from healthy animals. Isolates originating from pigs are taken from the GI-tract and for dogs and cats, it is from urine. AST is conducted using commercial MIC plates with CLSI guidelines. SVARM interpretation of MICs is performed using ECOFFs from EUCAST or values suggested by the European Food Safety Authority. Any *E. coli* isolates confirmed to be ESBL phenotypical or thought to be carbapenemase producing are subject to further scrutiny via genome sequence analysis. (https://www.sva.se/en/our-topics/antibiotics/svarm-resistance-monitoring/).

1.7. AMR surveillance in companion animals

National AMR monitoring programs in animals generally do not provide data for companion animals (Leigue et al., 2013). While most countries do not have routine surveillance programs that include isolates from companion animals there are many one-off studies in the literature. These however, only take a 'snapshot' in the form of a survey.

These one-off studies are rarely uniform and the results often do not distinguish between companion animal species and usually only contain AST data (especially the older studies). Comprehensive studies including data on AST, MLST, and the presence/absence of resistance and virulence genes are rare. Some national monitoring programs that do test samples from companion animals include RESAPATH, SVARM, COMPATH, FINRES-Vet, and BfT-GermVet.

1.8. Surveillance study techniques

1.8.1. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry

MALDI-TOF MS is now the commonly used surveillance study technique of bacterial identification. This technique has replaced numerous older techniques as it is a highly efficient and sensitive method.

Using this technique small quantities of pure bacterial growth are spot plated to a stainless-steel target and mixed with a matrix solution to co-crystallise the bacterial proteins (Wieser et al., 2012). These plates commonly accommodate between 24 to 384 samples, which are then transferred into the MALDI-TOF mass spectroscopy instrument where individual samples are exposed to short laser pulses (Christopher-Hennings et al., 2009). This exposure results in proteins becoming ionised. Ionised proteins then become separated based on their mass-to-charge ratio, which can be

plotted against signal intensity; this is then used to create a specific bacterial protein fingerprint (Randell, 2014). This fingerprint, in most cases, is unique for a given bacterial species. Once the bacterial protein fingerprint has been generated, it can be compared with reference fingerprints in the MALDI-TOF MS database to generate bacterial identification in real-time (Christopher-Hennings et al., 2009).

MALDI-TOF MS is currently replacing biochemical methods for bacterial identification; however, a successful bacterial identification using MALDI-TOF MS still relies on growing individual bacterial colonies from clinical specimens and conducting AST separately (Christopher-Hennings et al., 2009) although recent use of MALDI-TOF for AMR identification is underway with emerging publications.

1.8.2. Antimicrobial susceptibility testing

The field of medical science has included standardised test methods for determining the resistance of a bacterial culture to various antimicrobials since the 1960s (Watts et al., 2018). However, the development of rigorous AST methods for use in veterinary medicine did not commence until the early 1990s (Watts et al., 2018). Initially, the only criteria available to be interpreted for categorising isolates as susceptible or resistant were those that were developed for use with human pathogens. Veterinary laboratories routinely used these human breakpoints for similar animal pathogens regardless of the host species. Veterinary clinical breakpoints have since been created based on the pharmacokinetics of specific drugs in different animal species. These breakpoints, however, may not be suitable for AMR surveillance in healthy livestock at slaughter. The two AST methods frequently used in veterinary medicine are agar disk diffusion and broth microdilution.

1.8.2.1. Disc diffusion

Agar disc diffusion also known as the Kirby-Bauer method is a flexible and relatively inexpensive technique that is commonly used in diagnostic laboratories. This

procedure is based on CLSI protocols and is mainly used for testing rapidly growing aerobic bacteria i.e. *E. coli* or *Salmonella* spp. (CLSI, 2013).

This procedure involves streaking a suspension of pure bacterial culture onto the surface of a nutrient agar medium. Filter paper discs containing specified amounts of antibacterial agents are then placed on the surface of this uniformly seeded nutrient medium. Areas with a lack of bacterial growth, called zones of inhibition, form around the discs. These zones are then measured based on their diameter in millimetres and the resulting measurement compared with standards for interpreting zone sizes. Although the measurements are read to the nearest millimetre, these are used to determine the result which is reported as susceptible, intermediate, or resistant to the antimicrobial agent of interest. Care must be taken when taking zone measurements as zones with vague endpoints or with larger defined zones of inhibition, can contain the presence of small colonies (CLSI, 2013).

This method is highly flexible as any antimicrobial can be easily included or omitted from the testing. Conversely, there are some disadvantages associated with this method such as the inherent test and user variabilities including inoculum size used, measuring of zones, labour requirements, and the stability of discs (Watts et al., 2018).

1.8.2.2. Minimal inhibitory concentration

Unlike disk diffusion techniques, broth dilution is a quantitative test, the results of which can be expressed as a MIC. Broth dilution is performed in a microbroth format using an equal amount of bacterial suspension in each well of a 96 well MIC plate. The MIC plate is also loaded, or is pre-loaded, with varying concentrations of the antimicrobial agent of interest and the MIC plate is then incubated overnight. Resulting bacterial growth in the plate indicates that a bacterium is resistant to a particular drug concentration; conversely, a lack of bacterial growth indicates susceptibility to the drug

concentration. The MIC is a measure of the highest dilution of an antibacterial agent that inhibits the growth of an isolate (CLSI, 2013).

Unlike a disk diffusion method, the MIC method is more labour intensive, more expensive, and less flexible as it may take months before any antimicrobial changes to the commercial plate format can be made (Watts et al., 2018).

1.8.2.3. Breakpoints

CLSI is currently the only organisation that provides international available breakpoints specifically for bacteria isolated from animals. Prior to animal bacteria isolate breakpoints being defined, human infection breakpoints were used. These human infection breakpoints can be unsuitable due to differences in dosing regimens, PK/PD, and other factors in farm and companion animals compared to humans (Moyaert et al., 2017). Use of these human-based breakpoints can result in inappropriate treatments for animals.

Since this time, as data have become available, clinical breakpoints specific for veterinary compounds used in many animal hosts including dogs and cats have been developed. CLSI currently maintains the accepted worldwide standard for susceptibility testing of veterinary pathogens VET01, which also provides the breakpoints for bacteria isolated from animals. To standardise test performance the CLSI guidelines provide recommendations for testing, including preparation of bacterial suspension, media use, incubation conditions, and a listing of antimicrobial agents per animal species that may be considered for testing (Zimmerman et al., 2012). In addition to this, the CLSI standards also provide result interpretation guidelines, which are specific for bacterial species-antimicrobial agent combinations.

Presently few groups other than CLSI are developing standards for AST for veterinary pathogens. The European Society for Clinical Microbiology and Infections Disease established EUCAST in 1996. EUCAST was intended to harmonise

antimicrobial breakpoints across Europe. EUCAST was later expanded in 2014, to include veterinary pathogens with the formation of the VETCAST group (Schwarz et al., 2018).

In many studies, epidemiologic cut off values are used instead of or in conjunction with clinical breakpoints. These values, abbreviated either ECV by CLSI or ECOFF by EUCAST are used to classify bacteria as wild type or non-wild type. The wild type population are strains that are fully susceptible to a particular antimicrobial and do not contain any acquired resistance mechanisms (Simjee et al., 2018). ECVs or ECOFFs are determined by obtaining the MICs to a particular antimicrobial from hundreds of isolates to obtain a population distribution, which is then analysed using software to estimate the wild type population and develop an epidemiologic cut off value (Edelstein, 2017). Bacteria with an MIC below or equal to the ECV are likely to be from the wild type distribution whereas isolates with an MIC above the ECV for a particular antimicrobial are likely to have an acquired form of resistance. ECVs, however, are not the same as a susceptibility breakpoints and cannot be used to make clinical decisions about antimicrobial treatment (Simjee et al., 2018).

Different criteria are often used in the various surveillance studies to determine AMR. For instance in Europe RESAPATH uses French national breakpoints, Sweden (SVARM) uses a combination of epidemiological cut off values from EUCAST and CLSI breakpoints. Three different studies (DANMAP, MARAN and SWARM) all use ECVs to calculate the percentage of resistance, though each of these use different values to define resistance. On the other hand, NARMS uses both ECVs and clinical breakpoints. An example of the differences in MIC distribution between the EUCAST ECOFFs and CLSI clinical breakpoints can be seen in Figure 2 (Simjee et al., 2018), and provides a reference point for the calling of isolates as susceptible, non-susceptible/resistant, wild type and non-wild type which are terms used throughout the

remainder of this thesis. As the AST for most of the isolates used in the following chapters was performed by Saputra et al. (Saputra et al., 2017) this literature review does not extensively cover AST.

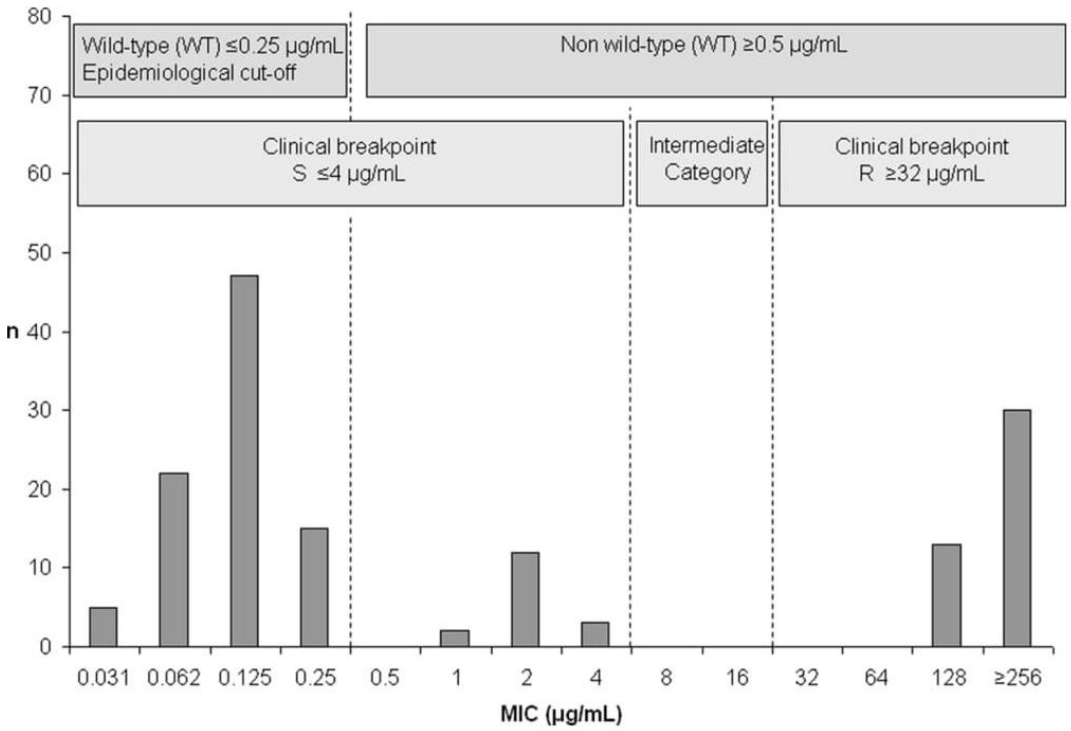

Figure 2: MIC distribution for a hypothetical bacterial species targeted in antimicrobial resistance surveillance programs. Arrows indicate the epidemiological cutoff value (ECOFF) established according to EUCAST recommendations, separating the wild type (no resistance determinants) from the non-wild type (presumed resistance determinants that could be verified by whole-genome sequencing analysis), and the clinical breakpoint. Susceptible (S), resistant (R), and intermediate value columns are indicated (image and caption are taken from (Simjee et al., 2018).

1.9. AMR surveillance in food-producing and companion animals in Australia

As mentioned previously, while there is no ongoing national surveillance programme of AMR in Australian food and companion animals, several one-off surveys have been conducted. In 2013, the first nation-wide survey of AMR in companion and food-producing animals in Australia was carried out. The study, sponsored by Zoetis, obtained clinical isolates from 22 government, private and university veterinary diagnostic laboratories throughout Australia between January 2013 and January 2014.

All isolates were accompanied by a clinical history and laboratory submission report without client details.

For companion animals, MIC testing was carried out on 883 clinical *E. coli* isolates to determine the prevalence of AMR. The study determined a low level of FQ resistance among both dog (9.3%, n = 514) and cat (5%, n = 341) isolates. Moderate (≥10%) resistance to 3GCs was observed among dog isolates (10.9%), whereas the rate was low amongst cat isolates (6.5%) and in both dogs and cats; only low prevalence of resistance (1.6%) to the CIA amikacin was identified. No resistance to carbapenems was observed (Saputra et al., 2017).

In food-producing animals, 324 clinical *E. coli* isolates underwent disc diffusion AST, with isolates resistant to CIAs undergoing further MIC testing as well as MLST, phylogenetic analysis, plasmid replicon typing/identification, resistance gene typing and virulence gene typing. In pigs, extremely low levels of resistance to FQs and the ESC ceftazidime were identified (0.9%; n = 114). Resistance to the ESC ceftiofur was slightly higher at 2.6% but still quite low especially compared to other countries that do not have the same limitations on the use of antimicrobials in food-producing animals. One ESC-resistant isolate, however, was molecularly determined to be an ETEC strain and carried the *bla*$_{\text{CMY-2}}$ AMR gene which is of potential significance in terms of animal health (Abraham et al., 2015).

1.10. Conclusion

While previous studies in companion animals have observed only low or moderate prevalence of resistance to FQs and 3GCs in Australia, it was determined that chronic and/or recurrent disease, as well as prior treatment with antimicrobials, were the main risk factors for the isolation of MDR ExPEC strains (Saputra et al., 2017). These results highlight the need for continuing AMR surveillance in companion animals as well as further characterisation of these strains to determine prevalence of AMR

genes, especially ESBLs, and if any belong to pathogenic STs of note such as ST131 or ST1193.

1.11. Aims and objectives of the present study

The aims of this thesis were; (i) to genetically characterise an Australia-wide collection of clinical *E. coli* isolates from dogs and cats; (ii) to identify relationships between STs, virulence genes and AMR genes among this nationwide collection of ExPEC isolates from dogs and cats to determine the significance of ST73, ST372 and other minor STs among FQ-susceptible strains, and ST131 and ST1193 among FQR strains; (iii) to investigate the frequency of antimicrobial non-susceptibility among commensal *E. coli* and *Salmonella* spp. isolated from the caecal contents of healthy Australian finisher pigs at slaughter. Throughout these studies, phylogenetic grouping and subspecific typing techniques (i.e. RAPD) were applied to identify genetic relationships among the larger collection of isolates, with WGS then applied to smaller representative subsets.

Chapter 2 focuses on the prevalence of ExPEC-associated STs from phylogenetic group B2 among FQ-susceptible dog clinical isolates while Chapter 3 focuses on a similar study among group B2 cat clinical ExPEC isolates. The prevalence and significance of ST131 and ST1193 as major clonal lineages among FQR isolates from cats and dogs, as determined by WGS, is reported in Chapter 4. Chapter 5 presents two cases of malakoplakia in dogs, an unusual pathology normally associated with UTI in humans, and its association with particular *E. coli* STs established by WGS. Chapter 6 focuses on antimicrobial non-susceptibility of an Australia-wide collection of commensal *E. coli* and *Salmonella* spp. isolated from healthy pigs at slaughter, with isolates resistant to CIAs subjected to WGS. The findings of these studies will provide valuable information on the presence of pandemic lineages of *E. coli* in Australian companion animals as well as the prevalence of specific virulence and AMR genes in

these populations; as well as the significance of AMR in healthy Australian pigs. These studies will also set a benchmark for future AMR surveillance studies.

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Chapter 2

Genomic analysis of fluoroquinolone-susceptible phylogenetic group B2 extraintestinal pathogenic *Escherichia coli* causing infections in cats

Statement of Authorship

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Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
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Genomic analysis of fluoroquinolone-susceptible phylogenetic group B2 extraintestinal pathogenic *Escherichia coli* causing infections in cats

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ABSTRACT

Extraintestinal pathogenic *Escherichia coli* (ExPEC) can cause urinary tract and other types of infection in cats, but the relationship of cat ExPEC to human ExPEC remains equivocal. This study investigated the prevalence of ExPEC-associated sequence types (STs) from phylogenetic group B2 among fluoroquinolone-susceptible cat clinical isolates. For this, 323 fluoroquinolone-susceptible cat clinical *E. coli* isolates from Australia underwent PCR-based phylotyping and random amplified polymorphic DNA analysis to determine clonal relatedness. Of the 274 group B2 isolates, 53 underwent whole genome sequencing (WGS), whereas 221 underwent PCR-based screening for (group B2) sequence type complexes (STc) STc12, STc73, ST131, and STc372. Group B2 was the dominant phylogenetic group (274/323, 85 %), whereas within group B2 ST73 dominated, according to both WGS (43 % of 53; followed by ST127, ST12, and ST372 [4/53, 8 % each]) and ST-specific PCR (20 % of 221). In WGS-based comparisons of cat and reference human ST73 isolates, cat isolates had a relatively conserved virulence gene profile but were phylogenetically diverse. Although in the phylogram most cat and human ST73 isolates occupied host species-specific clusters within serotype-specific clades (O2:H1, O6:H1, O25:H1, O50/O2:H1), cat and human isolates were intermingled within two serotype-specific clades: O120:H31 (3 cat and 2 human isolates) and O22:H1 (3 cat and 5 human isolates). These findings confirm the importance of human-associated group B2 lineages as a cause of urinary tract infections in cats. The close genetic relationship of some cat and human ST73 strains suggests bi-directional transmission may be possible.

1. Introduction

Escherichia coli is one of the most important bacterial pathogens associated with extraintestinal disease in both humans and animals, including urinary, respiratory, and reproductive tract infections (Zogg et al., 2018). *E. coli* isolates with an intrinsically greater ability to cause extraintestinal infections are termed extraintestinal pathogenic *E. coli* (ExPEC) and have been further divided into different sub-pathotypes (Hutton et al., 2018). Uropathogenic *E. coli* (UPEC), which possess virulence factors that enhance ability to cause disease within the host's urinary tract (Hutton et al., 2018), is the most significant sub-pathotype in both humans and animals (Liu et al., 2015).

Expression of a broad array of virulence genes – including those encoding adhesins, invasins, toxins, and siderophores – enable ExPEC to cause symptomatic urinary tract infection (UTI) (Liu et al., 2015). Over 57 specific *E. coli* virulence genes associated with extraintestinal

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infection have been identified to date (Johnson et al., 2015). Additionally, the more virulent strains of ExPEC isolated from UTIs typically belong to *E. coli* phylogenetic groups B2 and D (Litster et al., 2009). Although multiple studies have documented phylogenetic, pathotypic, and antimicrobial resistance (AMR) similarities between human and companion animal UPEC, most of these studies have focused on *E. coli* from dogs (e.g. Johnson et al., 2009a; Wedley et al., 2017).

Compared to dogs, cats rarely have simple uncomplicated bacterial UTI, and among those that do show clinical signs of lower urinary tract disease (dysuria, haematuria, pollakiuria, and stranguria) a large proportion has culture-negative urine (Litster et al., 2009). Among cats with clinical evidence of urinary tract disease the overall prevalence of a positive urine culture is < 3 % (Litster et al., 2011), although with compromised urinary defence mechanisms rates are much higher (15–43 %) (Litster et al., 2011). Similarly, the incidence of bacterial lower UTI in cats increases with age (≥ 10 years), renal disease, urinary catheterisation, perineal urethrostomy, low urine specific gravity, and diabetes mellitus (Litster et al., 2009).

In studies UPEC isolates from domestic cats shared genotypic and pathotypic similarities with ExPEC isolates from humans (Feria et al., 2001; Johnson et al., 2001, 2008; Yuri et al., 1999). Recently, Liu et al. (2015) used virulence genes, multilocus sequence typing (MLST), and resistance phenotyping to characterise a collection of cat UPEC isolates. Of the identified sequence types (STs), 50 % have known associations with human UPEC, including ST73 and ST83 (16 % and 8 % prevalence, respectively) (Liu et al., 2015). However, virulence genotyping, MLST, and resistance profiling are increasingly recognised as lacking the sensitivity and depth of whole genome sequencing (WGS) for assessing genetic relatedness among isolates from different host species.

National monitoring programmes on AMR in animals generally exclude companion animals; however, several such programmes in Europe now address this previously neglected group (Morrissey et al., 2016). In Australia, Saputra et al. (2017) tested 883 clinical *E. coli* isolates from companion animals using both human and veterinary clinical breakpoints and found a low prevalence of resistance among cat isolates to critically important antimicrobials, including fluoroquinolones (3 %) and extended-spectrum cephalosporins (ESCs) (6–7 %).

Due to the paucity of studies focusing on cat clinical *E. coli* and the low rates of resistance to fluoroquinolones identified in the Saputra et al. (2017) study, we sought here to characterise and compare fluoroquinolone-susceptible (FQS) members of the above Australia-wide collection of cat clinical *E. coli* isolates in terms of anatomical site of isolation, AMR profiles, phylogenetic grouping, and genomic profiles. Representative B2 isolates were further characterised by WGS analysis to determine STs, resistance genotypes, virulence gene profiles, and plasmid content, and to undertake comparative genomic analysis using ST-matched reference human clinical B2 isolates.

2. Materials and methods

2.1. Sample collection, isolation, and identification

The fluoroquinolone-susceptible (FQS) clinical cat *E. coli* study isolates (n = 323) and corresponding antimicrobial susceptibility data were from the first nation-wide survey of AMR in bacterial pathogens from Australian animals (Saputra et al., 2017). Isolates were obtained from 22 government, private, and university veterinary diagnostic laboratories between January 2013 and January 2014, accompanied by a clinical history and laboratory submission report, without client details. In all cases, the referring diagnostic microbiologist considered the isolate significant. Isolates were classified as multidrug-resistant (MDR) if non-susceptible to at least one antimicrobial agent in ≥ 3 antimicrobial classes, with a total of 59 isolates classified as MDR.

2.2. Molecular characterisation

DNA extraction for preliminary screening was undertaken using 6 % Chelex (Biorad) in 96-well plates (Abraham et al., 2018). *E. coli* phylogenetic group was determined using the revised Clermont multiplex PCR assay (Clermont et al., 2013). Because the focus was on phylogenetic group B2, isolates assigned provisionally to groups A, C, D, E, or F were not studied further. As such, those assigned to groups A or C were not further distinguished, so were designated A/C, and those assigned to groups D or E were designated D/E. B2 isolates were further characterised using random amplified polymorphic DNA (RAPD) analysis with arbitrary decamer 1254 (5′−CCGCAGCGAA−3′). Each PCR was carried out in a 25 µl reaction containing 5 µL of 5× buffer (supplied with Taq polymerase), 1 µL of primer, 0.2 µL of MyTaq Red DNA Polymerase (Bioline), and 2 µL of DNA. The cycling conditions were as follows: five cycles of 5 min at 94 °C, 5 min at 36 °C, and 5 min at 72 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C, followed by a final extension of 5 min at 72 °C. A RAPD profile-based similarity dendrogram was inferred by UPGMA within BioNumerics, version 7.6 (Applied Maths).

2.3. Whole genome sequence analysis

A representative isolate for each of the major sub-clusters observed in the RAPD dendrogram was selected for WGS. Additional representatives of certain clusters were selected based on differences from the index isolate according to resistance profile, anatomical site, and/or Australian state of isolation. WGS was performed on a total of 53 isolates using the Illumina Next Seq platform (Abraham et al., 2018). The resulting FASTQ files were trimmed using CLC Genomics workbench (QIAGEN Version 12), with a quality limit of 0.01. All reads with an ambiguous base were trimmed (ambiguous limit = 1). De novo assembly for each isolate also was performed using the CLC Genomics Workbench, with default settings (Rahimi et al., 2018).

For each isolate, the FASTA contigs of the de novo assembled genomes were further analysed for AMR genes, virulence genes, and plasmids by using (i) functions available via the Centre for Genomic Epidemiology (CGE), including MLST, ResFinder, VirulenceFinder, PlasmidFinder, FimTyper, and SeroType Finder (http://www.genomicepidemiology.org/), and (ii) the ABRicate function of the Galaxy web platform (https://usegalaxy.org.au/). Isolates of unknown ST per CGE were uploaded to Enterobase for further analysis (http://enterobase.warwick.ac.uk/).

A blast library was constructed for the genes *afaBC* (NCBI X76688), *draBC* (NCBI AF329316), *traT* (NCBI CP001856), *usp* (NCBI AB027193), *ompT* (NCBI AE014075), *malX* (NCBI AE014075), and *yfcV* (NCBI AE014075), and was used to search contigs using CLC Genomics Workbench. *kpsM II*, *hra*, and *intI1* were detected by performing a local blast search using the sequences in Supplementary File A.

Isolates were classified as ExPEC if they contained ≥ 2 of 5 ExPEC-defining virulence genes (*papA* and/or *papC*, *sfa/focDE*, *afa/draBC*, *kpsM II*, and *iutA*) (Johnson et al., 2003b), and as UPEC if they contained ≥ 3 of 4 UPEC-defining virulence genes (*chuA*, *fyuA*, *vat*, and *yfcV*) (Spurbeck et al., 2012).

2.4. Single nucleotide polymorphism (SNP)-based phylogenetic analysis of human and cat ST73 isolates

Genome sequences for 37 comparison human-source ST73 isolates from bacteraemia and UTI, including 21 from the United Kingdom (11 bacteraemia, 10 UTI) (Alhashash et al., 2016) and 16 from Australia (all UTI) (Bogema et al., 2019), were downloaded from the European Nucleotide Archive (ID: PRJEB9931) and Enterobase (http://enterobase.warwick.ac.uk), respectively (Supplementary Table B). Short sequence reads (FASTQ files) underwent de novo assembly and scaffolding using the genome-finishing module of the CLC Genomics Workbench, with

default settings. Genome sequences for these 37 human isolates and the 23 newly sequenced cat ST73 isolates were used to generate a SNP-based phylogenetic tree. For this, SNP analysis was performed using SNIPPY (https://github.com/tseemann/snippy), regions of recombination were identified and removed using ClonalFrameML (Didelot and Wilson, 2015), and maximum likelihood trees were constructed using RaxML (Stamatakis, 2014) under the GTRCAT model with 1000 bootstraps. Trees were visualised and curated in iTOL (Letunic and Bork, 2019).

2.5. ST-specific PCR

The remaining 221 FQS B2 cat isolates that did not undergo WGS instead underwent PCR-based detection of *pabB, dinB, aesX*, and *chuA* (Clermont et al., 2014) to screen for membership in the human ExPEC-associated sequence type complexes (STc) STc12, STc73, and STc372 (Clermont et al., 2014). Isolates identified as STc73 underwent confirmatory testing using alternative ST73-specific primers (Doumith et al., 2015). All FQS B2 isolates also were screened for ST131-associated SNPs in *mdh* and *gyrB* (Johnson et al., 2009b). STc95 was not screened for.

3. Results

3.1. Phylogenetic grouping and RAPD profiling

Among the 323 cat-source FQS *E. coli* study isolates the most frequent phylogenetic group was group B2 (274/323, 85 %), followed distantly by groups A/C and B1 (14/323 and 12/323 respectively, 4 % each), F and D/E (10/323 and 11/323 respectively, 3 % each), and undetermined (2/323, 1 %). Among the 59 isolates classified as MDR, group B2 again predominated (37/59, 63 %), followed by groups A/C (7/59, 12 %), B1 and D/E (6/59, 10 % each), and F (3/59, 5 %). A RAPD-based dendrogram for the 274 B2 isolates contained 35 main clusters (data not shown). WGS was done for at least one isolate per cluster, and for some clusters multiple isolates, selected to reflect distinctive resistance profiles, sites of isolation, and/or Australian geographic states of isolation.

3.2. Whole genome sequencing

3.2.1. Multilocus sequence typing

Based on WGS data, the 53 sequenced isolates were assigned to 18 STs, three of which were novel (ST8123, ST8238, and ST8244). Eight STs were represented by ≥ 2 isolates each, the other 10 by a single isolate each. The four main STs – ST73 (23/53, 43 %), ST127 (4/53, 8 %), ST12 (4/53, 8 %), and ST372 (4/53, 8 %) – accounted for 66 % of the 53 isolates (Fig. 1). None of the sequenced isolates represented additional major STs associated with human UTI (e.g., ST69, ST95, ST131, and ST1193).

The remaining 221 group B2 isolates underwent ST-specific PCR analysis for the three main STs identified among the 53 sequenced B2 isolates, i.e., STc12, STc73, and STc372, as well as ST131. As observed by WGS analysis, STc73 was the main PCR-detected STc (45/221, 20 %), followed by STc12 (2/221, 1 %). PCR detected no STc372 or ST131 isolates. The other 79 % (174/221) of the PCR-screened isolates remained of unknown ST.

3.2.2. Antimicrobial resistance genes

The most common AMR genes identified in the 53 sequenced isolates were $bla_{\text{TEM-1B}}$, $bla_{\text{TEM-70}}$, and $bla_{\text{TEM-105}}$ (10/53, 19 % each), followed by the aminoglycoside resistance genes *aph(3′)-Ia* (4/53, 8 %), and *aadA1* (3/53, 6 %). Less common genes included the AmpC β-lactamase gene $bla_{\text{CMY-2}}$ (2/53, 4 %), $bla_{\text{SHV-1}}$, $bla_{\text{SHV-48}}$, $bla_{\text{SHV-102}}$, and the macrolide resistance gene *mph(B)* (1/53, 2 % each). Identified AMR genes were found to correspond to the β-lactam, tetracycline and sulphonamide resistant phenotypes of the isolates (Supplementary C). However, nine isolates were identified as containing aminoglycoside resistance genes but no resistance to the antimicrobials amikacin and gentamicin was identified.

A full or partial copy of the class 1 integrase gene *intI1* was identified in four isolates (4/53, 8 %), with full gene coverage in three of the four. BLASTn hits yielded two *intI1* gene fragments in Q13-1-212 and full gene variants across the other sequences identified, with V13-2-511 and Q13-1-255 sharing a match. Three isolates (3/53, 6 %) contained the class 1 integron-associated gene *sul1*. No isolate contained CTX-M resistance genes. The most common combination of resistance genes, i.e., *aph(3′)-Ia*, $bla_{\text{TEM-1B}}$, $bla_{\text{TEM-70}}$, and $bla_{\text{TEM-105}}$, was identified in four isolates (4/53, 8 %).

3.2.3. Virulence genes

The 53 sequenced isolates contained 29 consensus virulence genes. These included the type-1 fimbriae-associated genes *fimBCDEFGHI*, the intimin-like adhesin gene *fdeC*, the outer membrane protein gene *ompA*, the enterobactin iron acquisition system genes *entABDEFS*, the ferric-enterobactin uptake genes *fepACDG*, the yersiniabactin siderophore genes *ybtAEPTUX*, the yersiniabactin receptor *fyuA*, the yersiniabactin biosynthetic protein gene *irp2*, and the pathogenicity-associated island marker *malX*. An additional 79 virulence genes were detected in 6 %–98 % of isolates each (Table 1). Most isolates (45/53, 85 %) contained one or more *pap* genes with individual genes occurring in 34–81 % of isolates (median 81 %).

Forty-two (79 %) of the 53 cat isolates qualified molecularly as ExPEC, with 41 (41/53, 98 %) of these further qualifying as UPEC. Another nine isolates qualified molecularly as UPEC, but not as ExPEC, giving 50 (50/53, 94 %) total UPEC isolates.

3.2.4. Plasmids

The 53 sequenced isolates yielded 16 distinct plasmids, mostly from the IncF group (Table 2). The most prevalent plasmids were Col156, IncFIB (AP001918), and IncFII, as identified in 26 % (14/53), 19 % (10/53), and 15 % (8/53) of isolates, respectively. Of the 16 plasmids, 12 were identified among the ST73 isolates. Five of these plasmids were ST-specific, identified in only ST73 isolates.

3.2.5. WGS analysis of cat ST73 isolates

Of the 23 sequenced ST73 isolates nine (39 %) qualified as MDR. All sequenced ST73 possessed at least two β-lactamase genes regardless of MDR status and none contained ESC resistance-associated genes. A single ST73 isolate (non-MDR) contained three β-lactamase genes ($bla_{\text{SHV-1}}$, $bla_{\text{SHV-48}}$ and $bla_{\text{SHV-102}}$). Two ST73 isolates (one MDR, one non-MDR), contained the class 1 integrase gene *intI1* and the class 1 integron-associated gene *sul1*.

Only one ST73 isolate (1/23, 4 %) contained all 17 prototypic ST73-associated virulence genes, i.e., *papC*, *papEF*, *papG*, *sfa/foc*, *fimH*, *hra*, *hlyA*, *cnf1*, *pic*, *vat*, *iroN*, *fyuA*, *kpsM II*, *usp*, and *ompT* (Riley, 2014). Fourteen isolates (14/23, 61 %) contained all of these but *papG* and one of *papE*, *cnf1*, or *ompT*. The remaining isolates had ≤ 14 of the 17 genes. The only prototypic genes common to all ST73 isolates were *sfa/foc*, *fimH*, *hra*, *pic*, *vat*, *fyuA*, *kpsM II*, and *usp*.

3.2.6. Comparison with human ST73 isolates

Core genome SNP analysis of the 23 present cat ST73 isolates (20 from UTI and one each from a vaginal swab, bile and a faecal swab) and 37 historical human ST73 isolates from Australia and the United Kingdom (11 from bacteraemia, 26 from UTI) was undertaken to produce a phylogenetic tree (Fig. 2). High diversity was observed among the cat ST73 isolates, mirroring the high diversity also observed among the human ST73 isolates, with most isolates divided into distinct serotype-specific phylogenetic clusters, as reported previously (Bogema et al., 2019). Although nine serotypes were identified, most human isolates exhibited serotype O6:H1. The 23 sequenced ST73 cat isolates

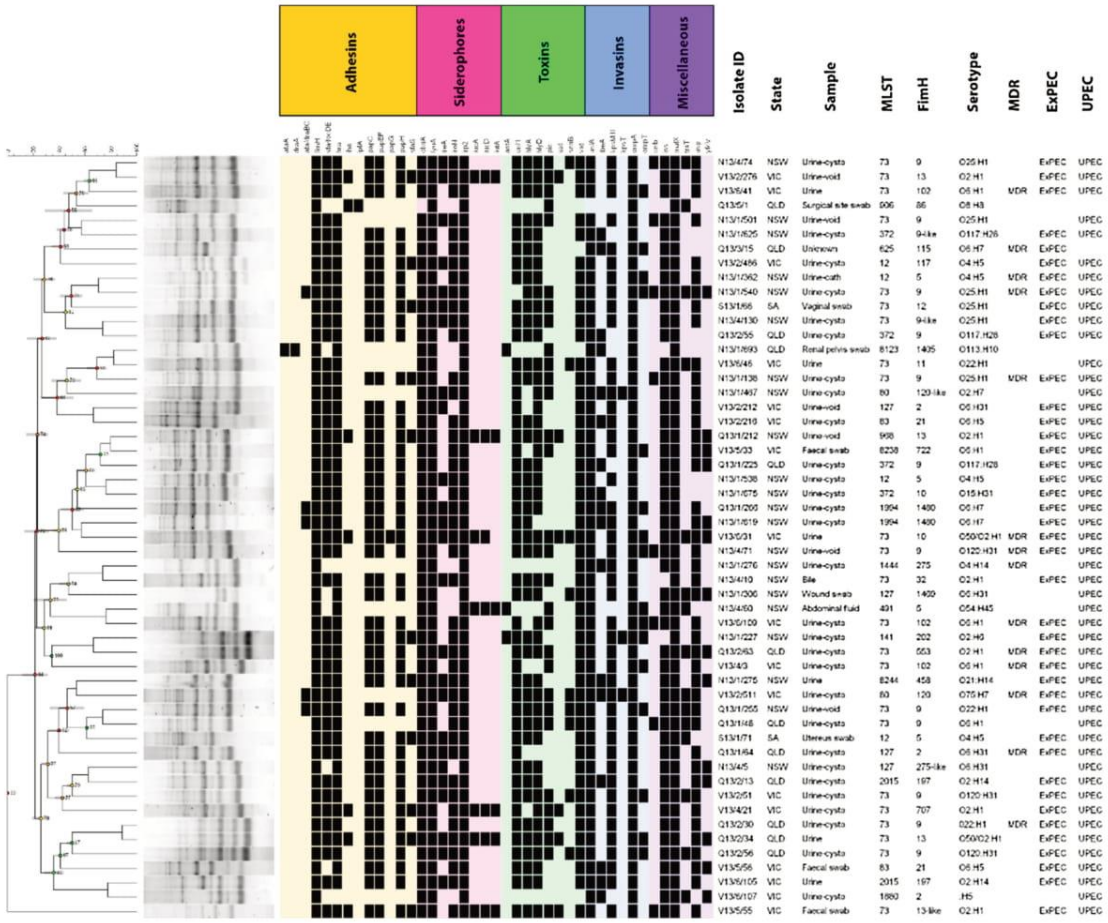

Fig. 1. Random amplified polymorphic DNA dendrogram of 53 fluoroquinolone-susceptible cat clinical *Escherichia coli* isolates. Data to the right of the dendrogram, from left to right: gel images; virulence gene profile of selected genes (black squares indicate gene presence); isolate identification number, Australian state of origin (NSW, New South Wales; QLD, Queensland; VIC, Victoria; SA, South Australia); source of isolate; sequence type; *fimH* allele; predicted serotype; multidrug-resistant (MDR) status; extraintestinal pathogenic *E. coli* (ExPEC) status; uropathogenic *E. coli* (UPEC) status.

exhibited six main O:H serotypes, including O2:H1 (5 isolates), O6:H1 (4 isolates), O22:H1 (3 isolates), O25:H1 (6 isolates), O50/O2:H1 (2 isolates), and O120:H31 (3 isolates). Within each serotype-specific phylogenetic cluster, some sub-clustering by host species (cat vs. human) and/or geographical origin (Australia vs. United Kingdom) was evident. Nonetheless, closely related cat and human isolates were sometimes intermingled within the same sub-cluster. Examples of such broad-host-range commonality included (i) within serotype O120:H31, three cat isolates and two human isolates (both from the UK); (ii) within serotype O22:H1, three cat isolates and five human isolates (three from Australia, two from the UK), and (iii) within serotype O6:H1 (the most prevalent human-associated serotype), a cat isolate – which coincidently possessed the highest number of virulence genes (80 %) – and two human isolates (both from the UK).

4. Discussion

This study, which sought to characterise genetically a large nationwide collection of clinical FQS *E. coli* isolates from extraintestinal infections in Australian cats, including a WGS-based comparison of representative isolates, led to four main conclusions. First, within a collection of isolates dominated by phylogroup B2, ST73 was the predominant ST according to both WGS and ST-specific PCR. Second, the remaining B2 isolates that underwent WGS were from diverse minor STs, including ST12 (n = 4), ST372 (n = 4), and three novel STs (1 each). Third, the cat ST73 isolates were highly diverse, and split into serotype-specific clusters in a SNP-based core genome phylogeny. Fourth, although most cat and human ST73 isolates segregated into distinct host-specific sub-clusters, some intermingling of host species within sub-clusters was apparent, suggesting the potential for bi-directional transmission.

ST73 is a broad-host-range clonal group that is associated with UTI, urosepsis, and septicaemia in humans (Mora et al., 2018) and UTI in companion animals (Zogg et al., 2018). Here, among cat-source FQS clinical isolates from Australia, ST73 was the most prevalent ST according to both WGS (43 % of 53) and PCR screening (20 % of 221). Similarly, ST73 was the most common ST (12/74, 16 %) in a prior study of cat urine *E. coli* isolates from the United States (Liu et al., 2015). By contrast, none of the present FQS Australian cat *E. coli* isolates represented the other prominent human-associated STs identified in several international studies of large collections of human ExPEC isolates, including ST69, ST95, ST131, and ST1193, in which ST131 and ST1193 were associated with antimicrobial-resistant infections (Doumith et al., 2015; Johnson et al., 2019).

Although ST73 is largely associated with antimicrobial susceptibility, especially to critically important agents such as extended-spectrum cephalosporins and fluoroquinolones (Dale and Woodford, 2015; Doumith et al., 2015), in humans antimicrobial-resistant ST73 has occasionally been implicated in UPEC infections (Zogg et al., 2018). For example, whereas in a study of human uncomplicated UTI isolates from Greece, Portugal, Sweden, and the UK all ST73 isolates were fully susceptible to the tested antimicrobials (Bengtsson et al., 2012), the

Table 1
Virulence gene prevalence by sequence type among 53 fluoroquinolone-susceptible cat *Escherichia coli* isolates that underwent whole genome sequencing.

Category	Gene	No. isolates (column %)				
		Total (n = 53)	ST12 (n = 4)	ST73 (n = 23)	ST127 (n = 4)	ST372 (n = 4)
Adhesins	*afaAC*	9	0	4	0	0
	draA-C	11	0	9	0	0
	fimA	96	100	100	100	100
	focACDFGH	76	75	100	50	100
	iha	13	0	22	0	0
	papBCDEFHIJKX	85	100	87	100	100
	sfaB-H	91	100	100	100	100
	sfaS	21	50	26	0	0
	sfaXY	89	100	100	75	100
Invasins	*aslA*	98	100	100	100	100
	ibeA	32	0	0	0	100
	kpsDT	79	25	100	100	0
	kpsM II	85	100	100	100	0
	ompT	30	25	44	0	0
Toxins	*astA*	6	0	0	0	0
	cnf1	89	100	96	100	100
	hlyA-D	91	100	100	100	100
	pic	57	0	100	0	0
	sat	11	0	22	0	0
	senB	17	0	30	25	0
	vat	93	100	100	100	100
Siderophores	*chuASTUVWXY*	98	100	100	100	100
	entC	98	100	100	100	100
	fepB	98	100	100	100	75
	ireA	47	75	52	75	0
	iroBCDEN	83	100	96	25	100
	iucA-D	13	0	22	0	0
	iutA	11	0	17	0	0
Colicin	*celb*	15	0	35	0	0
Miscellaneous	*fes*	96	100	96	75	100
	gad	94	100	96	100	100
	hra	94	100	100	100	100
	iss	92	100	96	100	100
	tcpC	30	100	22	100	0
	traT	28	25	39	25	0
	usp	81	50	100	100	25
	yfcV	26	0	17	50	25

Note: Not all genes listed from each gene cluster (e.g. pap) were present in all isolates in the percentages listed. The functions of the virulence genes listed are in Supplementary D.

Table 2
Plasmid prevalence by sequence type among 53 fluoroquinolone-susceptible cat *Escherichia coli* isolates that underwent whole genome sequencing.

Plasmids	No. isolates (column %)				
	Total (n = 53)	ST73 (n = 23)	ST12 (n = 4)	ST127 (n = 4)	ST372 (n = 4)
Col156	26	57	0	0	0
Col(MG828)	6	9	0	0	0
ColpVC	2	0	0	0	0
IncB/O/K/Z	4	0	25	0	0
IncFIB(AP001918)	19	30	25	25	0
IncFII	15	17	25	25	0
IncFII(29)	6	13	0	0	0
IncFII(29)_pUTI89	6	13	0	0	0
IncFII(pRSB107)	2	4	0	0	0
IncFII(pSE11)	2	4	0	0	0
IncI1	8	4	0	25	25
IncI1_Alpha	8	4	0	25	25
IncX1	6	4	0	0	0
IncX4	2	4	0	0	0
IncY	6	4	0	1	0
p0111	4	0	25	0	0

number of MDR ST73 human isolates containing extended-spectrum β-lactamase genes has recently risen in the UK and Australia (Alhashash et al., 2016; Bogema et al., 2019). Despite this, the present cat isolates were largely susceptible: 30 % were susceptible to all antimicrobials tested, and another 30 % to all but 1 or 2 antimicrobial classes (Saputra et al., 2017).

MLST analysis of ExPEC strains has revealed associations between specific STs and host disease syndromes (Dale and Woodford, 2015). Established UPEC clones include ST69, ST73, ST95, ST127, ST131, and ST1193, while other lineages such as highly diverse ST10 have been more recently associated with UPEC (Nüesch-Inderbinen et al., 2017). Of these major human-associated UPEC clones, only ST73 and ST127 occurred among the present cat isolates. Similarly, a study of cat UTI isolates from the United States identified ST73 (16 %) and ST127 (5%), but none of the other common human-associated UPEC clones (Liu et al., 2015). By contrast, a recent study of 138 group B2 faecal *E. coli* isolates from healthy cats in Canberra, Australia identified ST95 (17 %), in addition to ST73 (46 %) and ST127 (5%) (Bourne et al., 2019). That study's detection of ST95 could be due to the greater number of cat isolates that underwent MLST there (n = 334) vs. here (n = 53). It could also indicate that in cats, ST95 rarely causes extraintestinal infection and instead is confined predominantly to the gut, which it may enter via consumption of raw poultry (or wild birds), a common source for ST95 (Jorgensen et al., 2019).

ExPEC typically contain a diverse array of virulence genes that are responsible for initiating infection at extraintestinal sites and that enable ExPEC to be distinguished from commensal *E. coli* (Johnson et al., 2003a). ExPEC strains typically possess group II or group III capsule synthesis genes, one or more iron uptake-related genes, and at least one adhesin-related gene (Johnson et al., 2003b). Virulence genes commonly associated with ExPEC include *iha*, *sfa/sfaS*, *fimH*, *iroN*, *ireA*, *iss*, *cnf1*, *astA*, *sat*, *pic*, *vat* and *usp* (Johnson et al., 2003b). *E. coli* are often classified molecularly as ExPEC if they contain ≥ 2 of the ExPEC-associated genes [*papAH* and/or *papC*], *sfa/focDE*, *afa/draBC*, *kpsM II*, and *iutA* (Johnson et al., 2003b), although given the redundancy and co-occurrence of virulence genes in ExPEC, multiple alternative operational definitions are possible. However, according to the best-validated definition, *E. coli* qualify as UPEC if they contain ≥ 2 of *chuA*, *fyuA*, *yfcV*, and *vat* (Spurbeck et al., 2012).

Here, 77 % (41/53) of sequenced cat isolates qualified molecularly as both ExPEC and UPEC, with 51 % (27/53) represented by UPEC-associated STs (ST73 and ST127). It is not known how frequently organisms categorized as ExPEC and/or UPEC cause UTI in companion animals, compared to less virulent opportunistic strains (Hutton et al., 2018). Nonetheless, our findings suggest that most UTIs in cats are caused by virulent ExPEC/UPEC clones belonging to phylogroup B2, with ST73 predominating. Some have suggested that, by contrast with ST131, the relative abundance of ST73 in collections of ExPEC isolates is not driven by expansion of a dominant clone (Bogema et al., 2019). Indeed, human ST73 isolates from both the UK and Australia are genomically diverse, unlike ST131 isolates, which have a relatively conserved genome, despite both STs being globally disseminated; this is consistent with the concept that spatial distribution and genetic diversity may be distinct features (Alhashash et al., 2016; Bogema et al., 2019).

To elucidate potential relationships between cat and human ST73 strains, we compared the present cat isolates with 37 highly diverse (based on core genome analysis) human ST73 isolates from bacteraemia and UTI that exhibited varying levels of antimicrobial resistance (including from CTX-M extended-spectrum β-lactamases) and diverse plasmids. Although in a SNP-based phylogram the cat ST73 isolates were also genetically diverse, falling into five of the nine human ST73 serotype-specific genomic clades, most exhibited host species-specific sub-clustering. Nevertheless, several cat and human isolates were intermingled, suggesting the potential for bi-directional transmission, at least for some ST73 serotypes. Detailed within household studies would

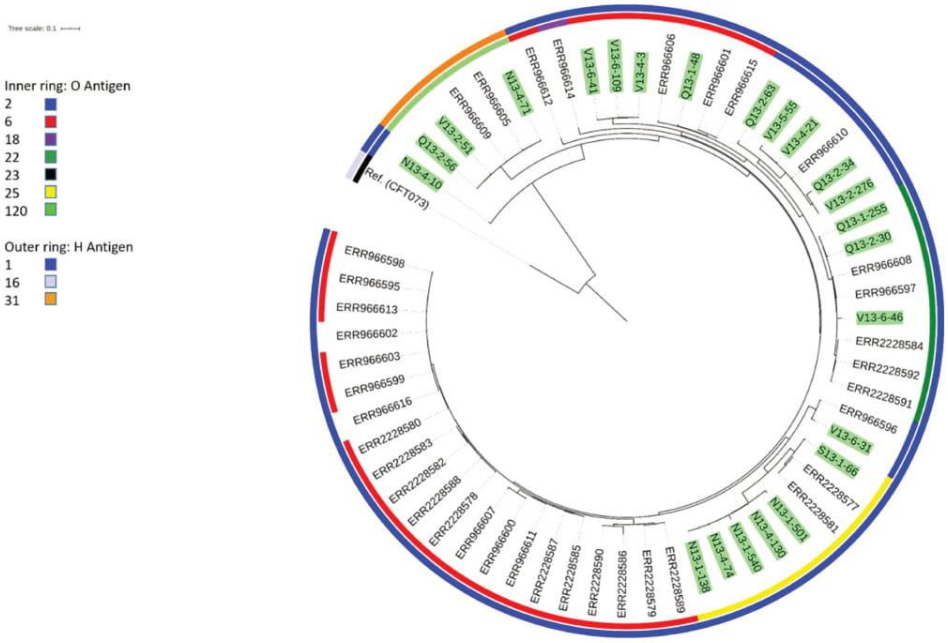

Fig. 2. Phylogenetic analysis of 23 feline and 37 human *Escherichia coli* isolates of sequence type 73 from Australia and the United Kingdom. Phylogram is based on core genome single-nucleotide polymorphisms. The final dataset contained 33, 001 sites.

now be required to confirm this.

Although *E. coli* bacteraemia is extremely rare in cats, and none of the present cat ST73 isolates was from blood, the above finding suggests that cat ST73 strains may have the same inherent capability of causing pyelonephritis and urosepsis in humans as do human ST73 strains. Because most of the cat study isolates were obtained from urine (12 collected by cystocentesis, 8 from voided or unspecified urine samples), we hypothesise that ExPEC/UPEC may require a diverse array of virulence/fitness genes to enter, persist, and initiate infection within the exceptionally highly concentrated, high osmolarity environment of the cat urinary tract (Litster et al., 2011). Underlying diseases such as diabetes and renal disorders, which are more common as cats age, reduce urine specific gravity, thereby presumably predisposing to UTI. However, the present study shows that, despite this, opportunistic *E. coli* strains of lower virulence potential, such as those from non-B2 phylogenetic groups, appear to be quite rarely represented in large collections of cat urine isolates.

To our knowledge this is the first study to use WGS to define STs, resistance genes, virulence genes, and plasmid types within a large collection of cat *E. coli* isolates. Although previous PCR-based studies have investigated the prevalence of one or more of these variables in cat *E. coli* populations (Hutton et al., 2018; Liu et al., 2015; Tramuta et al., 2011; Yuri et al., 1999; Zogg et al., 2018), none has addressed them jointly, and none using WGS-based analysis. In the present study, a large proportion of isolates (174/221, 79 %) remained of unknown identity following STc-specific PCR. These isolates belong either to STc12, STc73, or STc372 and did not amplify under the specified PCR conditions, or they represent distinct STs. We suspect the latter considering that an additional 13 STs identified among the isolates subjected to WGS were represented by just one or two isolates.

This study had some limitations. First, lack of temporal and geographical matching may have influenced the WGS comparisons of cat vs. human ST73 isolates; the human isolates derived from specific locales in both the northern (2013) and southern (2009–2011) hemispheres, whereas the cat isolates were from a year-long, nation-wide Australian study, commenced in 2013. Second, the human ST73 isolates included high proportions of MDR strains carrying CTX-M β-lactamases, which are often contained on plasmids with multiple resistance genes, whereas most cat ST73 isolates were resistant to ≤ 2 antimicrobials, did not possess CTX-M β-lactamases, and lacked plasmids with multiple resistance genes. Third, it was not possible to review the clinical histories that justified sample collection and submission to a diagnostic laboratory for culture and susceptibility testing, which may have created a selection bias.

In conclusion, in this study ST73, a broad-host-range lineage from phylogenetic group B2, was the major ST among FQS clinical *E. coli* isolates from urinary tract and other extraintestinal infections in Australian cats. Other multiply encountered human-associated clonal lineages included ST12, ST127, and ST372. Three novel STs from phylogenetic group B2 (ST8123, ST8238, and ST8244), each represented by a single isolate, were identified. The cat ST73 isolates showed high genetic and serotypic diversity and were susceptible to most tested antimicrobials. Whilst most cat ST73 isolates were genetically distinct from human ST73 exhibiting the same serotype, some were closely related, suggesting a potential for bi-directional transmission.

Author contributions

AK performed the experiments, data analysis and drafted and prepared the manuscript. MO performed the whole genome sequencing and phenogram bioinformatics analysis. EE and MM performed bioinformatics analysis. SS performed MIC testing on all isolates used in this study. CT provided training and assistance with BioNumerics analysis and manuscript reviewing. DJ, JJ, DG, SA and DT were involved in experimental design development and manuscript preparation. SA, DT and DJ were responsible for sample collection, data acquisition and coordination of the survey. SD provided access to whole genome sequence data and assisted with manuscript preparation.

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Ethics approval

Not required.

Declaration of competing interest

DJT has received research funding and undertaken consultancies for Bayer, Zoetis, Boehringer Ingelheim, Virbac, Luoda Pharma, Neoculi and IRiccorgpharm. SA has received research funding from Zoetis and Neoculi. JRJ has received research support from and/or has undertaken consultancies for Achaogen, Allergan, Crucell/Janssen, Melinta, Merck, Shionogi, Syntiron, and Tetraphase.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2020.108685.

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Supplementary A

Gene	Sequence
kspM_II_K1	ATGGCAAGAAGTGGATTTGAAGTTCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTTCGTCTGGGGTATTTGTGGGCGATT CTTGAACCCTCCGCGCATTTGCTGATACTGTTGGGAATTTTGGGTTACGTTATGCACCGCACTATGCCAGACATCTCGTTCCCGGTGTTTTTACTTAATGGCCTGATTC CCTTTTTTATCTTTAGTAGTATTAGCAAACGTTCTATTGGTGCTATTGAAGCGAACCAGGGACTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCACGT GCACTGCTTGAGACACTGATTTACGTTGCTGTTTATATTTTGCTCATGCTTATCGTCTGGATGACAGGCGAATATTTCGAAATTACAAACTTTTTACAACTTGTGCTCAC CTGGAGTTTGTTAATCATTCTTTCATGTGGCGTCGGCTTAATATTTATGGTCGTTGGTAAAACCTTTCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCTG TATTTCATCTCCTGCATCATGTTCCCTCTACACTCGATTCCAAAACAATACTGGTCATATCTACTCTGGAACCCATTAGTGCATGTTGTGGAGTTAAGCCGCGAGGCAG TTATGCCTGGCTATATCAGTGAAGGCGTGAGTCTGAACTACCTTGCAATGTTTACTCTGGTCACCCTGTTCATCGGCCTGGCATTATACCGAACGCGTGAAGAGGCAA TGCTGACATCATGA
kspM_II_K2	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATCTGTGGGCGATT CTCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATTATGCACCGCACGATGCCAGACATCTCGTTCCCGGTGTTTTTACTTAATGGCCTGATTC CCTTTTTTATCTTTAGCAGTATCAGCAAACGTTCTGTAAGTGCTATTGAAGCGAACCAGGGGCTGTTTAATTATCGCCCAGTAAAACCCATCGATACGATCATTGCGCG CGCCCTGCTTGAGACGCTGATTTACGTTTCTGTTTATATTCTGCTTATGCTCATTGTCAGGATGGCAGGCGAATATTTCGAGATAACAAATTTTTTACAACTTGTGGCTA CCTGGAGTCTGCTGATCATTCTTTCATGCAGCGTCGGCTTAATATTCATGGTCGTTGGTAAAACCTTCCCCGAAATGCAAAAGGTTCTGCCGATACTTCTTAAGCCCCT TTATTTCATCTCCTGCATCATGTTCCCTCTGCACTCAATTCCAAAGCAATACTGGTCATATCTACTCTGGAACCCACTAGTGCATGTCGTGGAGTTAAGCCGTGAGGCC GTTATGCCTGGCTATATTAGCGAAGGTGTGAGTCTGAACTACCTGGCAATGTTCACATTGATAACTCTGTTCATTGGTCTGGCGTTATATCGAACTCGTGAGGAGGCA ATGCTGACATCATGA
kspM_II_K5_kfiC_Bi8337-41	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATTTGTGGGCGATT CTCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATTATGCACCGCACGATGCCAGACATCTCATTCCCGGTGTTTTTACTTAATGGCCTGATTC CCTTTTTTATCTTTAGCAGTATCAGCAATCGTTCTGTAGGCGCTATTGAAGCGAACCAGGGGTTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCACG CGCACTGCTTGAGACGCTGATTTACGTTGCTGTTTATATATTGCTCATGCTTATCGTCTGGATGGCAGGTGAATATTTCGAGATAACAAACTTTTTACAACTAGTGCTCA CCTGGAGTTTGTTAATCATTCTTTCATGTGGCATCGGCTTAATATTCATGGTCGTTGGTAAAACCTTCCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCT GTATTTCATCTCCTGCATCATGTTCCCTCTACATTCGATTCCGAAGCAATACTGGTCATATCTACTCTGGAACCCATTAGTGCATGTCGTAGAGTTAAGCCGCGAGGCA GTTATGCCTGGCTATATCAGCGAAGGCGTGAGTCTGAACTACCTTGCAATGTTCACTCTGGTCACCCTGTTCATCGGTCTGGCGTTATACCGAACTCGTGAGGAGGCA ATGCTGACATCATGA
kspM_II_K5_kfiC	ATGAACGCAGAATATATAAATTTAGTTGAACGTAAAAAGAAATTAGGGACAAATATTGGTGCTCTTGATTTTTTATTATCAATTCATAAGGAGAAAGTTGATCTTCAACAT AAAAACTCGCCTTTAAAAGGTAACGATAACCTTATTCACAAAAGAATAAACGAATACGACAATGTACTTGAACTATCTAAGAATGTATCAGCTCAGAATTCTGGCAATGA GTTTTCTTATTTATTGGGATATGCAGATTCTCTTAGAAAAGTTGGTATGTTGGATACTTATATTAAAATTGTTTGTTATCTAACAATTCAATCTCGTTATTTTAAAAATGGC GAACGAGTTAAGCTTTTTGAACATATAAGTAACGCTCTACGGTATTCAAGGAGTGATTTTCTCATTAATCTTATTTTTGAACGATATATCGAATATATAAACCATCTAAAA TTGTCGCCCAAACAAAAAGATTTTTATTTTTGTACGAAGTTTTCAAAATTTCATGATTATACTAAAAATGGATATAAATATTTAGCATTTGATAATCAAGCCGATGCAGGG TATGGCCTGACTTTATTATTAAATGCAAACGATGATATGCAAGATAGTTATAATCTACTCCCTGAGCAAGAACTTTTTATTTGTAATGCTGTAATAGATAATATGAATATTT ATAGGAGTCAATTTAACAAATGTCTACGAAAATACGATTTATCAGAAATAACTGATATATACCCAAATAAAATTATATTGCAAGGAATTAAGTTCGATAAGAAAAAAAATG TTTATGGAAAAGATCTTGTTAGTATAATAATGTCAGTATTCAATTCAGAAGATACTATTGCATACTCATTACATTCATTGTTGAATCAAACATATGAAAATATTGAAATTCT CGTGTGCGATGATTGTTCATCGGACAAAAGCCTTGAAATAATTAAGAGCATAGCTTATTCTAGTTCAAGAGTGAAAGTATATAGCTCACGAAAAAACCAAGGCCCTTAT AATATAAGAAATGAGCTAATAAAAAAAGCACACGGTAATTTCATCACCTTTCAAGATGCAGATGATCTTTCTCATCCGGAGAGAATACAAAGACAAGTTGAGGTTCTTCG CAATAATAAGGCTGTAATCTGTATGGCTAACTGGATCCGTGTTGCGTCAAATGGAAAAATTCAATTCTTCTATGATGATAAAGCCACAAGAATGTCTGTTGTATCGTCAA TGATAAAAAAAGATATTTTTGCGACAGTTGGTGGCTATAGACAATCTTTAATTGGTGCAGATACGGAGTTTTATGAAACAGTAATAATGCGTTATGGGCGAGAAAGTATT GTAAGATTACTGCAGCCATTGATATTGGGGTTATGGGGAGACTCCGGACTTACCAGGAATAAAGGAACAGAAGCTCTACCTGATGGATATATATCACAATCTCGAAGA GAATATAGTGATATCGCGGCAAGACAACGAGTGTTAGGGAAAAGTATCGTAAGTGATAAAGATGTACGTGGTTTATTATCTCGCTATGGTTTGTTTAAAGATGTATCAG GAATAATTGAACAATAG

Supplementary A Continued

Gene	Sequence
kspM_K15	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGCCGCCGTTCATGCTCTATTTTTACGTGAGCTTAGAACTAGGTTTGGCAAATATCGCTTGGGTTATTTATGGGCTGTATT AGAACCAGCTGCTCATCTTCTAATAATGCTGGCAATTTTTGGTTTTTTTATGCATCGCACAATGCCAGATATTTCCTTTCCTGTATTTTTAATAAATGGAATAATTCCTTATT TTATATTTAGCAATATTGCTACGCGGTCTATAGGGGCAATTGAGGCAAACCAAGGCTTATTTAATTACAGGCCAGTAAGGCCTATAGATACAATTATAGCTAGAGCAATAT TAGAAGTGCTAATTTATAGCATAGTATATCTGGTTTTGATGAGTTTATTGTTAATAATAGGGGAGCAATTTAAAATATATAATATATTGCTGTTGTTTAGCGCATGGATTTTA CTGGCTTTATTTTCATGTGGAATTGGGTTGATCTTTATGGTAATTGGGAAAACTTTTCCAGAAACAGAGAAGTTTCTACCAATAATACTCAAGCCATTATATTTTGTATCAT GTATTATGCTTCCATTACATGCAATACCAAAAGGTTATTGGGGATATATATTATGGAATCCCATTGTTCATGTGGTAGAGCTTTGTCGTGAATCCGTTGTTACAGGTTATG TCTCTGAAGGGGTTAGTTTATATTACTTATTTATAAGCACGTTAATATTATTGTTTTTAGGATTGGCGCTATATTCATCTCGTGAAGAATACATGTTAACATCATGA
kpsM_II_K52	TTGGTAGCTGTTAAGCCAAGGGCGGTAGCGTACCTGAAGAGATTAGGATCACATCATCAAATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTAT TTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATTTGTGGGCGATTCTCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATT ATGCACCGCACGATGCCAGACATCTCATTCCCGGTGTTTTTACTTAATGGCCTGATTCCCTTTTTTATCTTTAGCAGTATCAGCAATCGTTCTGTAGGTGCTATTGAAGCG AATCAGGGGCTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCGCGTGCATTGCTTGAGACACTGATTTACGTTACTGTTTATATATTGCTTATGCTTATC GTCTGGATGGCAGGCGAATATTTCGAAATAACAAACTTTTTACAACTTGTGCTTACCTGGAGTTTGTTAATCATTCTTTCATGTGGCGTTGGCTTAATATTCATGGTCGTT GGTAAAACCTTCCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCTGTATTTCATCTCCTGCATCATGTTCCCTCTACACTCGATTCCGAAGCAATACTGGTC ATATCTACTCTGGAATCCATTAGTGCATGTCGTAGAGTTAAGCCGCGAGGCAGTTATGCCTGGCTATATCAGCGAAGGCGTGAGTCTGAACTACCTGGCAATGTTTACA TTGATAACTCTGTTCATTGGTCTGGCGTTATACCGAACTCGTGAGGAGGCAATGCTGACATCATGA
kspM_III	ATGGACAAACCCATTATTAGTCAAACTCCACGCACTTCTTTGCAAGTATTACGTGATGTAGTATTTGGCTTATTAATTCGTGAGCTAAAAACAAGATTTGGTAATTACCGAC TAGGTTATGCTTGGGCATTACTTGACCCATTATTAATGATTAGCCTGTTCAGCGTAGTATTTGGGATGAGAAGCCAAAGTGGCTTTGGTGGTGTCCCAGCCCAGGTTTTT ATTACTGCTGGTTATTTACCTTTCATGTTTTTCAATAAAGTTGTGACTCAGTTGAAATCTGCTGTCAATGCTAATATGGGACTTTTTTGTTATAGACAAGTGACTCCTTTTGC AACTTTTATAGCACGTTTTATGCTAGAAACAATGGTGGGCATGATTGTCGGTATCATCCTAGTACTAGGATTATTGTGGTTTGGCTTTGATGCAATACCTGCGGATCCATT GCAAGTGATCCTTGGTTATTCTCTTCTGATGCTGTTTTCTTTTTCTCTTGGTATTGTATTTTGTGTTATTTGTAACTTAGCGAAAGAGGCAGATAAATTTCTTAGCTTGTTAA TGATGCCTTTGATGTTTATCTCTTGTGTTATGTTTCCTCTTGCTACTATTCCCCCTCAATATCAGCATTGGTTTTTATGGAATCCACTTGTGCATGCTGTAGAACTAATCCG AAGGGCATGGATATCTGGTTATCGTAGTCCTGATGTAAGTTGGGCGTATCTGTCGGTTGTCACCTTATTATTGCTCACTTTTGCTATGAGTTGTTACCGATTACGGCATC GCCAATTGATTGCTAGTTAG
hra	ATGGAGATAATTGAAATGAATAAGGTTTTTGTTGTTTCAGTGGTGGCCGCAGCCTGTGTATTTGCAGTAAATGCAGGAGCAAAGGAAGGTAAAAGCGGTTTTTATCTGAC CGGTAAAGCCGGTGCCTCTGTGATGTCACTTTCAGACCAGCGTTTCCTGTCAGGAGATGAGGAAGAAACATCAAAGTATAAAGGCGGCGATGACCATGATACGGTATTC AGTGGCGGTATTGCGGTCGGTTATGATTTTTATCCGCAGTTCAGTATTCCGGTTCGTACAGAACTGGAGTTTTACGCTCGTGGAAAAGCTGATTCGAAGTATAACGTAGA TAAAGACAGCTGGTCAGGTGGTTACTGGCGTGATGACCTGAAGAATGAGGTGTCAGTCAACACACTAATGCTGAATGCGTACTATGACTTCCGGAATGACAGCGCATTC ACACCATGGGTATCCGCAGGGATTGGCTACGCCAGAATTCACCAGAAAACAACCGGTATCAGTACCTGGGATTATGAGTACGGAAGCAGTGGTCGCGAATCGTTGTCA CGTTCAGGCTCTGCTGACAACTTCGCATGGAGCCTTGGCGCGGGTGTCCGCTATGACGTAACCCCGGATATCGCTCTGGACCTCAGCTATCGCTATCTTGATGCAGGT GACAGCAGTGTGAGTTACAAGGACGAGTGGGGCGATAAATATAAGTCAGAAGTTGATGTTAAAAGTCATGACATCATGCTTGGTATGACTTATAACTTCTGA
intI1	ATGAAAACCGCCACTGCGCCGTTACCACCGCTGCGTTCGGTCAAGGTTCTGGACCAGTTGCGTGAGCGCATACGCTACTTGCATTACAGCTTACCAACCGAACAGGCT TATGTCCACTGGGTTCGTGCCTTCATCCGTTTCCACGGTGTGCGTCACCCGGCAACCTTGGGCAGCAGCGAAGTCGAGGCATTTCTGTCCTGGCTGGCGAACGAGCG CAAGGTTTCGGTCTCCACGCATCGTCAGGCATTGGCGGCCTTGCTGTTCTTCTACGGCAAGGTGCTGTGCACGGATCTGCCCTGGCTTCAGGAGATCGGAAGACCTCG GCCGTCGCGGCGCTTGCCGGTGGTGCTGACCCCGGATGAAGTGGTTCGCATCCTCGGTTTTCTGGAAGGCGAGCATCGTTTGTTCGCCCAGCTTCTGTATGGAACGG GCATGCGGATCAGTGAGGGTTTGCAACTGCGGGTCAAGGATCTGGATTTCGATCACGGCACGATCATCGTGCGGGAGGGCAAGGGCTCCAAGGATCGGGCCTTGATG TTACCCGAGAGCTTGGCACCCAGCCTGCGCGAGCAGCTGTCGCGTGCACGGGCATGGTGGCTGAAGGACCAGGCCGAGGGCCGCAGCGGCGTTGCGCTTCCCGAC GCCCTTGAGCGGAAGTATCCGCGCGCCGGGCATTCCTGGCCGTGGTTCTGGGTTTTTGCGCAGCACACGCATTCGACCGATCCACGGAGCGGTGTCGTGCGTCGCCA TCACATGTATGACCAGACCTTTCAGCGCGCCTTCAAACGTGCCGTAGAACAAGCAGGCATCACGAAGCCCGCCACACCGCACACCCTCCGCCACTCGTTCGCGACGG CCTTGCTCCGCAGCGGTTACGACATTCGAACCGTGCAGGATCTGCTCGGCCATTCCGACGTCTCTACGACGATGATTTACACGCATGTGCTGAAAGTTGGCGGTGCCG GAGTGCGCTCACCGCTTGATGCGCTGCCGCCCCTCACTAGTGAGAGGTAG

Supplementary B

Strain	Country of origin	Study
ERR966595	United Kingdom	Alhashash et al., 2016
ERR966596	United Kingdom	Alhashash et al., 2016
ERR966597	United Kingdom	Alhashash et al., 2016
ERR966598	United Kingdom	Alhashash et al., 2016
ERR966599	United Kingdom	Alhashash et al., 2016
ERR966600	United Kingdom	Alhashash et al., 2016
ERR966601	United Kingdom	Alhashash et al., 2016
ERR966602	United Kingdom	Alhashash et al., 2016
ERR966603	United Kingdom	Alhashash et al., 2016
ERR966604	United Kingdom	Alhashash et al., 2016
ERR966605	United Kingdom	Alhashash et al., 2016
ERR966606	United Kingdom	Alhashash et al., 2016
ERR966607	United Kingdom	Alhashash et al., 2016
ERR966608	United Kingdom	Alhashash et al., 2016
ERR966609	United Kingdom	Alhashash et al., 2016
ERR966610	United Kingdom	Alhashash et al., 2016
ERR966611	United Kingdom	Alhashash et al., 2016
ERR966612	United Kingdom	Alhashash et al., 2016
ERR966613	United Kingdom	Alhashash et al., 2016
ERR966614	United Kingdom	Alhashash et al., 2016
ERR966615	United Kingdom	Alhashash et al., 2016
ERR2228591	Australia	Bogema et al., 2019
ERR2228580	Australia	Bogema et al., 2019
ERR2228590	Australia	Bogema et al., 2019
ERR2228589	Australia	Bogema et al., 2019
ERR2228578	Australia	Bogema et al., 2019
ERR2228592	Australia	Bogema et al., 2019
ERR2228585	Australia	Bogema et al., 2019
ERR2228588	Australia	Bogema et al., 2019
ERR2228586	Australia	Bogema et al., 2019
ERR2228577	Australia	Bogema et al., 2019
ERR2228582	Australia	Bogema et al., 2019
ERR2228584	Australia	Bogema et al., 2019
ERR2228579	Australia	Bogema et al., 2019
ERR2228583	Australia	Bogema et al., 2019
ERR2228581	Australia	Bogema et al., 2019
ERR2228587	Australia	Bogema et al., 2019

Supplementary C

Isolate ID	State of isolation	Sample type	Sequence type	Predicted serotype	*FimH* type	Pathotype		MDR status	Antimicrobial susceptibility profiles				
						ExPEC	Other		Amikacin	Gentamicin	Amoxicillin/ clavulanate	Ampicillin	Cefoxitin
Q13/1/64	QLD	Urine-cysto	127	O6:H31	2	Y	UPEC	MDR	S	S	R	R	R
Q13/1/48	QLD	Urine-cysto	73	O6:H1	9	N	UPEC	N	S	S	S	R	S
V13/4/3	VIC	Urine-cysto	73	O6:H1	102	Y	UPEC	MDR	S	S	R	R	S
V13/4/21	VIC	Urine-cysto	73	O2:H1	707	Y	UPEC	N	S	S	S	S	S
N13/4/5	NSW	Urine-cysto	127	O6:H31	275-like	M	UPEC	N	S	S	S	S	S
Q13/2/13	QLD	Urine-cysto	2015	O2:H14	197	Y	UPEC	N	S	S	S	S	S
V13/2/51	VIC	Urine-cysto	73	O120:H31	9	Y	UPEC	N	S	S	S	S	S
Q13/2/30	QLD	Urine-cysto	73	O22:H1	9	Y	UPEC	MDR	S	S	R	R	S
Q13/2/34	QLD	Urine	73	O50/O2:H1	13	Y	UPEC	N	S	S	S	S	S
Q13/2/56	QLD	Urine-cysto	73	O120:H31	9	Y	UPEC	N	S	S	S	S	S
Q13/2/63	QLD	Urine-cysto	73	O2:H1	553	Y	UPEC	MDR	S	S	R	R	S
V13/6/31	VIC	Urine	73	O50/O2:H1	10	Y	UPEC	MDR	S	S	R	R	S
V13/6/41	VIC	Urine	73	O6:H1	102	Y	UPEC	MDR	S	S	R	R	R
V13/6/46	VIC	Urine	73	O22:H1	11	N	UPEC	N	S	S	S	S	S
N13/1/138	NSW	Urine-cysto	73	O25:H1	9	Y	UPEC	MDR	S	S	R	R	S
V13/2/212	VIC	Urine-void	127	O6:H31	2	Y	UPEC	N	S	S	S	S	R
V13/2/216	VIC	Urine-cysto	83	O6:H5	21	Y	UPEC	N	S	S	S	S	S
N13/4/60	NSW	Abdominal fluid	491	O54:H45	5	N	UPEC	N	S	S	S	S	S
Q13/1/212	NSW	Urine-catheter	968	O2:H1	13	Y	UPEC	N	S	S	R	S	S
Q13/1/225	QLD	Urine-cysto	372	O117:H28	9	Y	UPEC	N	S	S	S	S	S

Supplementary C Continued

Isolate ID	State of isolation	Sample type	Sequence type	Predicted serotype	*FimH* type	Pathotype		MDR status	Antimicrobial susceptibility profiles				
						ExPEC	Other		Amikacin	Gentamicin	Amoxicillin/ clavulanate	Ampicillin	Cefoxitin
Q13/1/255	NSW	Urine-void	73	O22:H1	9	Y	UPEC	N	S	S	S	R	S
Q13/1/266	NSW	Urine-cysto	1994	O6:H7	1480	Y	UPEC	N	S	S	S	S	S
N13/1/538	NSW	Urine-cysto	12	O4:H5	5	Y	UPEC	N	S	S	S	S	S
N13/1/540	NSW	Urine-cysto	73	O25:H1	9	Y	UPEC	MDR	S	S	R	R	S
N13/1/619	NSW	Urine-cysto	1994	O6:H7	1480	Y	UPEC	N	S	S	S	S	S
N13/1/675	NSW	Urine-cysto	372	O15:H31	10	Y	UPEC	N	S	S	S	S	S
N13/1/276	NSW	Urine-cysto	1444	O4:H14	275	N	UPEC	MDR	S	S	R	R	R
N13/1/306	NSW	Post-operative wound swab	127	O6:H31	1469	M	UPEC	N	S	S	S	S	S
N13/1/362	NSW	Urine-catheter	12	O4:H5	5	Y	UPEC	MDR	S	S	R	R	R
N13/1/467	NSW	Urine-cysto	80	O2:H7	120-like	M	UPEC	N	S	S	S	S	S
N13/1/693	QLD	Renal pelvis swab	8123	O113:H10	1405	N		N	S	S	S	S	S
N13/4/71	NSW	Urine-void	73	O120:H31	9	Y	UPEC	MDR	S	S	R	R	R
N13/4/74	NSW	Urine-cysto	73	O25:H1	9	Y	UPEC	N	S	S	S	R	S
V13/2/276	VIC	Urine-void	73	O2:H1	13	Y	UPEC	N	S	S	S	S	S
Q13/3/15	QLD	Unknown	625	O6:H7	115	Y		MDR	S	S	S	R	S
V13/5/33	VIC	Faecal swab	8238	O6:H1	722	Y	UPEC	N	S	S	S	S	S
V13/5/55	VIC	Faecal swab	73	O2:H1	13-like	Y	UPEC	N	S	S	S	S	S
V13/5/56	VIC	Faecal swab	83	O6:H5	21	Y	UPEC	N	S	S	S	S	S

Supplementary C Continued

Isolate ID	State of isolation	Sample type	Sequence type	Predicted serotype	*FimH* type	Pathotype		MDR status	Antimicrobial susceptibility profiles				
						ExPEC	Other		Amikacin	Gentamicin	Amoxicillin/ clavulanate	Ampicillin	Cefoxitin
N13/4/10	NSW	Bile	73	O2:H1	32	Y	UPEC	N	S	S	S	S	S
V13/6/105	VIC	Urine	2015	O2:H14	197	Y	UPEC	N	S	S	S	S	R
V13/6/107	VIC	Urine-cysto	1880	:H5	2	N	UPEC	N	S	S	R	S	S
V13/6/109	VIC	Urine-cysto	73	O6:H1	102	Y	UPEC	MDR	S	S	R	R	R
N13/4/130	NSW	Urine-cysto	73	O25:H1	9-like	Y	UPEC	N	S	S	S	R	S
V13/2/486	VIC	Urine-cysto	12	O4:H5	117	Y	UPEC	N	S	S	S	S	S
S13/1/66	SA	Vaginal swab	73	O25:H1	12	Y	UPEC	N	S	S	S	S	S
N13/1/501	NSW	Urine-void	73	O25:H1	9	N	UPEC	N	S	S	R	S	S
N13/1/625	NSW	Urine-cysto	372	O117:H28	9-like	Y	UPEC	N	S	S	S	S	S
N13/1/227	NSW	Urine-cysto	141	O2:H6	202	Y	UPEC	N	S	S	S	S	S
N13/1/275	NSW	Urine	8244	O21:H14	458	Y	UPEC	N	S	S	S	S	S
Q13/2/55	QLD	Urine-cysto	372	O117:H28	9	Y	UPEC	N	S	S	S	S	S
V13/2/511	VIC	Urine-cysto	80	O75:H7	120	Y	UPEC	MDR	S	S	S	R	S
Q13/5/1	QLD	Surgical site swab	906	O8:H8	86	N		N	S	S	S	S	S
S13/1/71	SA	Uterus swab	12	O4:H5	5	Y	UPEC	N	S	S	S	S	R

Supplementary C Continued

Isolate ID	Antimicrobial susceptibility profiles					Aminoglycoside resistance genes	β-lactam resistance genes	Macrolide resistance genes	Sulphonamide & tetracycline resistance genes
	Ceftriaxone	Cephalothin	Ciprofloxacin	Tetracycline	Trimethoprim/ sulfamethoxazole				
Q13/1/64	R	R	S	R	S		*AmpC2, AmpH, CMY-2, CMY-17, PBP*	*mdf(A)*	*tet(B)*
Q13/1/48	S	R	S	S	S		*AmpC2, AmpH, SHV-1, SHV-48, SHV-102, PBP*	*mdf(A)*	
V13/4/3	S	R	S	S	S		*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	
V13/4/21	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/4/5	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/2/13	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/2/51	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/2/30	S	R	S	S	S		*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	
Q13/2/34	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/2/56	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/2/63	S	R	S	S	R	*aadA1*	*AmpC2, AmpH, TEM-1, TEM-1A, TEM-150, PBP*	*mdf(A), mph(B)*	*sul1*
V13/6/31	S	R	S	S	R		*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	*sul2*
V13/6/41	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/6/46	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/138	S	R	S	S	S	*aph(3')-Ia*	*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	
V13/2/212	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/2/216	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	

Supplementary C Continued

Isolate ID	Antimicrobial susceptibility profiles					Aminoglycoside resistance genes	β-lactam resistance genes	Macrolide resistance genes	Sulphonamide & tetracycline resistance genes
	Ceftriaxone	Cephalothin	Ciprofloxacin	Tetracycline	Trimethoprim/ sulfamethoxazole				
N13/4/60	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/1/212	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/1/225	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/1/255	S	S	S	S	R	*aadA1*	*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	*sul1*
Q13/1/266	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/538	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/540	S	R	S	S	S	*aph(3')-Ia*	*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	
N13/1/619	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/675	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/276	R	R	S	S	S		*AmpC2, AmpH, CMY-2, CMY-17, PBP*	*mdf(A)*	
N13/1/306	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/362	R	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/467	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/693	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	

Supplementary C Continued

Isolate ID	Antimicrobial susceptibility profiles					Aminoglycoside resistance genes	β-lactam resistance genes	Macrolide resistance genes	Sulphonamide & tetracycline resistance genes
	Ceftriaxone	Cephalothin	Ciprofloxacin	Tetracycline	Trimethoprim/ sulfamethoxazole				
N13/4/71	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/4/74	S	R	S	S	S	*aph(3')-Ia*	*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	
V13/2/276	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/3/15	S	R	S	S	R	*aph(3'')-Ib, aph(6)-Id, strA, strB*	*AmpC2, AmpH, TEM-1, TEM-1A, TEM-150, PBP*	*mdf(A)*	*sul2*
V13/5/33	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/5/55	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/5/56	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/4/10	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/6/105	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/6/107	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/6/109	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/4/130	S	S	S	S	S	*aph(3')-Ia*	*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	
V13/2/486	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	

Supplementary C Continued

Isolate ID	Antimicrobial susceptibility profiles					Aminoglycoside resistance genes	β-lactam resistance genes	Macrolide resistance genes	Sulphonamide & tetracycline resistance genes
	Ceftriaxone	Cephalothin	Ciprofloxacin	Tetracycline	Trimethoprim/ sulfamethoxazole				
S13/1/66	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/501	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/625	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/227	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
N13/1/275	S	S	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
Q13/2/55	S	R	S	S	S		*AmpC2, AmpH, PBP*	*mdf(A)*	
V13/2/511	S	R	S	S	R	*aadA1*	*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	*sul1*
Q13/5/1	S	S	S	S	S		*AmpC1, AmpC2, AmpH, PBP*	*mdf(A)*	
S13/1/71	S	R	S	S	S	*aph(3'')-Ib, aph(6)-Id, strA, strB*	*AmpC2, AmpH, TEM-1B, TEM-70, TEM-105, PBP*	*mdf(A)*	*sul2*

Supplementary D

Virulence gene functions- *afaAC*: afimbrial adhesin; *draA-C*: Dr-binding adhesin; *fimA*: type-1 fimbrial protein; *focACDFGH*: F1C fimbriae; *iha*: iron-regulated-gene-homologue; *papBCDEFHIJKX*: P fimbriae; *sfaB-H*: S fimbriae; *sfaS*: S fimbriae; *sfaXY*: S fimbriae; *aslA*: putative arylsulfatase; *ibeA*: invasin protein; *kpsDT*: K1 capsule; *kpsM II*: group 2 capsule; *ompT*: outer membrane protease; *astA*: heat-stable enterotoxin 1; *cnf1*: cytotoxic necrotizing factor 1; *hlyA-D*: hemolysin; *pic*: serine protease autotransporter; *sat*: secreted autotransporter toxin; *senB*: plasmid encoded enterotoxin; *vat*: vacuolating autotransporter toxin; *chuASTUVWXY*: heme binding protein; *entC*: isochorismate synthase 1; *febB*: ferrienterobactin ABC transporter periplasmic binding protein; *ireA*: siderophores receptor; *iroBCDEN*: encode the salmochelin siderophore system; *iucA-D*: aerobactin siderophore biosynthesis protein; *iutA*: aerobactin receptor; *celb*: permease IIC component; *fes*: enterobactin/ferric enterobactin esterase; *gad*: glutamate decarboxylase; *hra*: heat-resistant hemagglutinin; *iss*: increased serum survival; *tcpC*: *tir* domain containing protein; *traT*: conjugal transfer surface exclusion protein; *usp*: uropathogenic specific protein; *yfcV*: fimbria A protein.

Chapter 3

Genomic analysis of phylogenetic group B2 extraintestinal pathogenic *Escherichia coli* causing infections in dogs in Australia

Statement of Authorship

Title of Paper	Genomic analysis of phylogenetic group B2 extraintestinal pathogenic *E. coli* causing infections in dogs in Australia
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Principal Author

Name of Principal Author (Candidate)	Amanda K. Kidsley		
Contribution to the Paper	Performed laboratory work, analysis, interpreted data, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	17/4/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Supervised development of work, helped to evaluate and edit the manuscript		
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Contribution to the Paper	Supervised development of work, helped to evaluate and edit the manuscript		
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Genomic analysis of phylogenetic group B2 extraintestinal pathogenic *E. coli* causing infections in dogs in Australia

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ABSTRACT

This study investigated the prevalence of extraintestinal pathogenic *E. coli* (ExPEC)-associated sequence types (STs) from phylogenetic group B2 among 449 fluoroquinolone-susceptible dog clinical isolates from Australia. Isolates underwent PCR-based phylotyping and random amplified polymorphic DNA analysis to determine clonal relatedness. Of the 317 so-identified group B2 isolates, 77 underwent whole genome sequencing (WGS), whereas the remainder underwent PCR-based screening for ST complexes (STc) STc12, STc73, STc372, and ST131. The predominant ST was ST372 according to both WGS (31 % of 77) and ST-specific PCR (22 % of 240), followed by (per WGS) ST73 (17 %), ST12 (7 %), and ST80 (7 %). A WGS-based phylogenetic comparison of ST73 isolates from dogs, cats, and humans showed considerable overall phylogenetic diversity. Although most clusters were species-specific, some contained closely related human and animal (dog > cat) isolates. For dogs in Australia these findings both confirm ST372 as the predominant *E. coli* clonal lineage causing extraintestinal infections and clarify the importance of human-associated group B2 lineage ST73 as a cause of UTI, with some strains possibly being capable of bi-directional (i.e., dog-human and human-dog) transmission.

1. Introduction

Escherichia coli is commonly isolated from infections in dogs, including urinary, respiratory, skin and soft tissue, gastrointestinal tract, joint, and wound infections (Guardabassi et al., 2004; Zogg et al., 2018a). Urinary tract infections (UTIs) reportedly are the most frequently diagnosed infectious disease of dogs; approximately 14 % of all dogs experience at least one bacterial UTI episode during their lifetime (McMeekin et al., 2017). Clinical syndromes associated with UTI in dogs include acute cystitis, pyelonephritis, and urosepsis (Qekwana et al., 2018).

E. coli isolates with an intrinsically greater ability to cause extraintestinal infections are termed extraintestinal pathogenic *E. coli* (ExPEC). These strains differ from intestinal *E. coli* strains by possessing multiple virulence factors, such as siderophores, type 1 and P fimbriae, hemolysin, and cytotoxic necrotizing factor (Sykes, 2014). The virulence genes of uropathogenic *E. coli* (UPEC), a sub-pathotype of ExPEC and the main cause of UTIs, enable a successful transition from the intestinal tract to the urinary tract (Qekwana et al., 2018) and invasion of and persistence within bladder epithelial cells (Sykes, 2014). Some of the *E. coli* strains that cause UTIs in dogs are phylogenetically related to human-source ExPEC strains (Guardabassi et al., 2004), and humans can share these pathogenic strains with their companion animals, which suggests the possibility of both zoonotic (animal-to-human) and anthropozoonotic (human-to-animal) transmission (Johnson et al., 2008a, b).

Although many previous studies have characterised companion animal-source ExPEC resistant to critically important antimicrobials (e.g., extended-spectrum β–lactamase (ESBL)-producing and/or fluoroquinolone-resistant (FQR) isolates), comparatively fewer have focused

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on susceptible isolates. Examples of the latter include a recent study from the USA that used multilocus sequence typing (MLST), resistance phenotyping, and virulence genotyping to analyse a collection of 295 *E. coli* isolates from canine urine samples acquired from laboratories in five states (LeCuyer et al., 2018). Sequence type (ST) 372, an infrequent human pathogen, was the predominant ST (22 % of isolates). ESBL-producing isolates with bla_{CTX-M} were uncommon, but often represented human-associated STs. Likewise, a study from Switzerland assessed antimicrobial resistance (AMR), phylogenetic background, and virulence profiles of 51 ESBL producing, and non-ESBL producing UPEC isolated from dogs admitted to a veterinary clinic. Among the 20 different STs identified the most common were ST410 (31 %), ST361 (8 %), and (ExPEC-associated) ST131 (10 %) (Zogg et al., 2018b). Similarly, a recent study in France of 618 dog-source ExPEC isolates identified both ST372 (21 %) and ST73 (20 %) as major ExPEC clonal lineages (Valat et al., 2020).

Characterisation of large collections of ExPEC isolates can overcome selection bias inherent in such smaller studies. Therefore, Saputra et al. (2017) tested 883 clinical *E. coli* isolates from companion animals in Australia and found a low rate of fluoroquinolone resistance among both cat (5 %, n = 341) and dog isolates (9.3 %, n = 514). Due to the limited number of studies focusing on susceptible dog clinical *E. coli*, we sought here to compare fluoroquinolone-susceptible (FQS) members of this Australia-wide collection of dog clinical *E. coli* isolates according to anatomical site of isolation, AMR profiles, phylogenetic grouping, and genomic profiles. Representative group B2 isolates were further characterised by WGS analysis to determine STs, resistance genotypes, and virulence gene profiles, and to allow phylogenetic comparisons with ST-matched reference human and cat clinical group B2 isolates.

2. Materials and methods

2.1. Sample collection, isolation, and identification

The FQS clinical dog *E. coli* study isolates (n = 449) and corresponding antimicrobial susceptibility data were from the first nationwide survey of AMR in bacterial pathogens from Australian animals (Saputra et al., 2017). For that study, isolates had been obtained from 22 government, private, and university veterinary diagnostic laboratories between January 2013 and January 2014, accompanied by a clinical history and laboratory submission report, without client details. In all cases, the referring diagnostic microbiologist considered the isolate the significant cause of morbidity in the affected animal. Isolates were classified as multidrug-resistant (MDR) if non-susceptible to at least one antimicrobial agent in three or more antimicrobial classes.

2.2. Molecular characterisation

DNA extraction for preliminary screening was undertaken using 6 % Chelex (Biorad) in 96-well plates (Abraham et al., 2018). *E. coli* phylogenetic group was determined using the revised Clermont multiplex PCR assay (Clermont et al., 2013). Because the focus was on phylogenetic group B2, isolates assigned provisionally to groups A, C, D, E, F, or clade 1 were not studied further. Consequently, those assigned to groups A or C were not further distinguished, so were designated A/C, those assigned to groups D or E were designated D/E, and those assigned to group E or clade 1 were designated E/clade 1. Group B2 isolates were further characterised using random amplified polymorphic DNA (RAPD) analysis with arbitrary decamer 1254 (5′−CCG CAGCGAA-3′). Each PCR was carried out in a 25-µl reaction containing 5 µL of 5× buffer (supplied with Taq polymerase), 1 µL of primer, 0.2 µL of MyTaq Red DNA Polymerase (Bioline), and 2 µL of DNA. The cycling conditions were as follows: five cycles of 5 min at 94 °C, 5 min at 36 °C, and 5 min at 72 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C, followed by a final extension of 5 min at 72 °C. A RAPD profile-based similarity dendrogram was inferred by UPGMA within BioNumerics, version 7.6 (Applied Maths).

2.3. Whole genome sequencing

A representative isolate for each of the major sub-clusters observed in the RAPD dendrogram was selected for WGS. Additional representatives of certain clusters were selected based on differences from the index isolate according to resistance profile, anatomical site, and/or Australian state of isolation. WGS was performed using the Illumina Next Seq platform (Abraham et al., 2018). The resulting FASTQ files were trimmed using CLC Genomics workbench (QIAGEN Version 12), with a quality limit of 0.01, and reads with an ambiguous base were trimmed (ambiguous limit = 1). De novo assembly for each isolate also was performed using the CLC Genomics Workbench, with default settings (Rahimi et al., 2018).

For each isolate, the FASTA contigs of the de novo assembled genomes were further analysed by using (i) functions available via the Centre for Genomic Epidemiology (CGE), including MLST, ResFinder, VirulenceFinder, FimTyper, and SeroType Finder (http://www.genomicepidemiology.org/), and (ii) the ABRicate function of the Galaxy web platform (https://usegalaxy.org.au/). Isolates of unknown ST per CGE were uploaded to Enterobase for further analysis (http://enterobase.warwick.ac.uk/).

A blast library was constructed for the genes *afaBC* (NCBI X76688), *draBC* (NCBI AF329316), *traT* (NCBI CP001856), *usp* (NCBI AB027193), *ompT* (NCBI AE014075), *malX* (NCBI AE014075), and *yfcV* (NCBI AE014075), and was used to search contigs using CLC Genomics Workbench. The genes *kpsM II*, *hra*, and *intI1* were detected by performing a local blast search using the sequences in Supplementary File A.

Isolates were classified operationally as ExPEC if they contained at least two of the five ExPEC-defining virulence genes (*papA* and/or *papC*, *sfa/focDE*, *afa/draBC*, *kpsM II*, and *iutA*) (Johnson et al., 2003), and as UPEC if they contained at least three of the four UPEC-defining virulence genes (*chuA*, *fyuA*, *vat*, and *yfcV*) (Spurbeck et al., 2012).

2.4. Single nucleotide polymorphism (SNP)-based phylogenetic analysis

Genome sequences for 37 comparison human-source ST73 isolates, including 21 from the United Kingdom (Alhashash et al., 2016) and 16 from Australia (Bogema et al., 2019), were downloaded from the European Nucleotide Archive (ID: PRJEB9931) and Enterobase (http://enterobase.warwick.ac.uk), respectively (Supplementary Table B). Short sequence reads (FASTQ files) underwent de novo assembly and scaffolding using the genome-finishing module of the CLC Genomics Workbench, with default settings. Genome sequences for these 37 human isolates, plus 23 cat ST73 isolates (Kidsley et al., 2020a) and 13 newly sequenced dog ST73 isolates, were used to generate a SNP-based phylogenetic tree. For this, SNP analysis was performed using SNIPPY (https://github.com/tseemann/snippy), regions of recombination were identified and removed using ClonalFrameML (Didelot and Wilson, 2015), and maximum likelihood trees were constructed using RaxML (Stamatakis, 2014) under the GTRCAT model with 1000 bootstraps. Trees were visualised and curated in iTOL (Letunic and Bork, 2019).

2.5. ST-specific PCR

The 240 FQS group B2 dog isolates that were not selected for WGS instead underwent PCR-based detection of *pabB, dinB, aesX*, and *chuA* (Clermont et al., 2014) to screen for membership in (human ExPEC-associated) ST complex (STc) 12, STc73, and STc372 (Clermont et al., 2014). Isolates identified as STc73 underwent confirmatory testing using alternative ST73-specific primers (Doumith et al., 2015). All FQS group B2 isolates also were screened for ST131-associated SNPs in *mdh* and *gyrB* (Johnson et al., 2009). STc95 was not screened for.

3. Results

3.1. Phylogenetic grouping and RAPD profiling

Among the 449 clinical dog FQS *E. coli* isolates the most frequent phylogenetic group was group B2 (317, 71 %), followed by groups B1 (13 %), D/E (8 %), A/C (5 %), F (2 %), E/clade 1 (0.2 %), and undetermined (1 %). Among the 89 isolates (20 %) that qualified as MDR, group B2 again predominated (47 %), followed by groups B1 (21 %), D/E (16 %), A/C (7 %), F (7 %), and unknown (2 %). A RAPD-based dendrogram for the 317 group B2 isolates suggested the presence of 35 main clusters (data not shown). For each cluster, WGS was done for at least one isolate, and in some instances multiple isolates, selected arbitrarily to reflect distinctive resistance profiles, sites of isolation, and/or Australian geographic states of isolation.

3.2. WGS

3.2.1. Multilocus sequence typing

Based on WGS data, the 77 sequenced dog isolates were assigned to 24 STs, two of which were novel (ST8336 and ST9023). Ten STs comprised ≥ 2 isolates each, the other 14 a single isolate each. Collectively, the four main STs – ST372 (31 %), ST73 (17 %), ST12 (7 %), and ST80 (7 %) – accounted for 61 % of the 77 isolates (Fig. 1). None of the sequenced isolates represented additional major STs associated with human UTIs (e.g., ST69, ST131, and ST1193).

The remaining 240 group B2 isolates underwent ST-specific PCR analysis for ST131 and three of the four main STs identified among the 77 sequenced group B2 isolates, i.e., STc12, STc73, and STc372. As observed by WGS analysis, STc372 was the main PCR-detected ST (22 %), followed by STc73 (12 %), and STc12 (3 %). ST131 accounted for five isolates (2 %), but because these isolates were characterised within another study (unpublished data) they did not undergo genomic analysis here and hence are not included in the following sections. The remaining 60 % of the PCR-screened isolates were of unknown ST.

3.2.2. Resistance genes

The most common AMR genes identified in the 77 sequenced isolates were $bla_{\text{TEM-1B}}$, $bla_{\text{TEM-70}}$, $bla_{\text{TEM-105}}$ (14 % each), and $bla_{\text{CMY-2}}$ (8 %), followed by the aminoglycoside resistance genes *aph(3′)-Ib*, *aph(6)-Id*, *strA*, and *strB* (5 % each). Additionally, a full or partial copy of the class 1 integrase gene *intI1* was identified in eight isolates (10 %), with full gene coverage in six of these, whereas the class 1 integron-associated gene *sul1* was identified in five isolates (7 %). By contrast, no isolate contained $bla_{\text{CTX-M}}$.

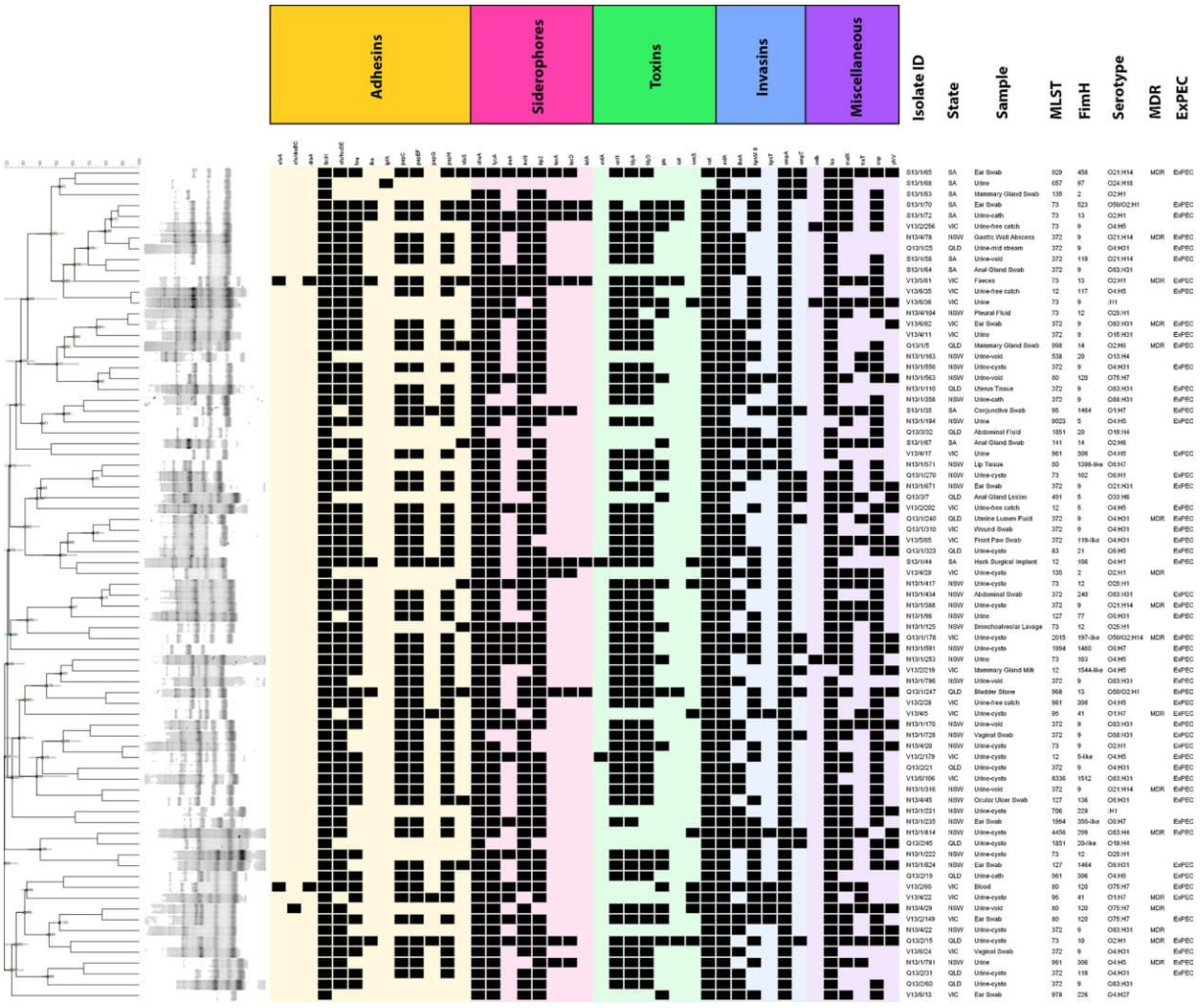

Fig. 1. Random amplified polymorphic DNA dendrogram of 77 fluoroquinolone-susceptible dog clinical *Escherichia coli* isolates. Data to the right of the dendrogram, from left to right: gel images; virulence gene profile of selected genes (black squares indicate gene presence); isolate identification number; Australian state of origin (NSW, New South Wales; QLD, Queensland; VIC, Victoria; SA, South Australia); source of isolate; sequence type; *fimH* allele; predicted serotype; multidrug-resistant (MDR) status; extraintestinal pathogenic *E. coli* (ExPEC) status.

Table 1
Virulence gene prevalence by sequence type (ST) among 77 genome-sequenced fluoroquinolone-susceptible dog *Escherichia coli*.

		Percent of Isolates				
Category	Gene	Total (n = 77)	ST12 (n = 5)	ST73 (n = 13)	ST80 (n = 5)	ST372 (n = 24)
Adhesins	*afa/draAC*	4	0	8	40	0
	fimA	92	100	100	80	100
	focACDFG	55	20	77	0	100
	focH	58	100	54	0	100
	focI	4	0	15	0	4
	iha	8	20	31	0	0
	papBCDFHJK	71	100	54	40	88
	papEIX	75	80	77	0	88
	sfaBCD	84	100	62	100	100
	sfaE-G	49	100	69	100	4
	sfaH	17	0	3	100	0
	sfaS	14	0	23	0	0
	sfaX	51	40	62	60	67
Invasins	*sfaY*	83	100	100	100	100
	ibeA	58	0	0	100	100
	kpsD	64	80	100	100	8
	kpsT	13	0	0	100	0
	kpsM II	64	80	100	100	8
	ompT	21	20	15	0	13
Toxins	*astA*	3	40	0	0	0
	cnf1	78	100	100	80	92
	hlyA-D	78	100	100	80	92
	pic	30	0	100	100	0
	sat	7	0	31	0	0
	senB	12	20	23	40	0
	vat	99	100	100	100	100
Siderophores	*chuASX*	99	100	100	100	100
	entABE	99	100	100	100	100
	fepD	97	100	100	80	100
	fyuA	94	0	100	100	92
	ireA	33	60	77	40	8
	iroBCDEN	91	100	92	100	100
	iucA-D	13	20	31	0	0
	iutA	7	20	23	0	0
Colicin	*celb*	4	0	23	0	0
Miscellaneous	*gad*	77	60	92	80	71
	hra	77	100	92	80	88
	iss	96	80	100	80	100
	malX	64	60	92	100	33
	tcpC	21	80	15	0	0
	traT	29	0	31	40	21
	usp	78	80	100	80	67
	yfcV	29	40	23	40	21

Note: Not all genes listed from each gene cluster (e.g. pap) were present in all isolates in the percentages listed. Virulence gene functions- *afaAC*: afimbrial adhesin; *draAC*: Dr-binding adhesin; *fimA*: type-1 fimbrial protein; *focACDFGHI*: F1C fimbriae; *iha*: iron-regulated-gene-homologue; *papBCDEFHIJKX*: P fimbriae; *sfaB-H*: S fimbriae; *sfaS*: S fimbriae; *sfaXY*: S fimbriae; *ibeA*: invasin protein; *kpsDT*: K1 capsule; *kpsM II*: group 2 capsule; *ompT*: outer membrane protease; *astA*: heat-stable enterotoxin 1; *cnf1*: cytotoxic necrotizing factor 1; *hlyA-D*: hemolysin; *pic*: serine protease autotransporter; *sat*: secreted autotransporter toxin; *senB*: plasmid encoded enterotoxin; *vat*: vacuolating autotransporter toxin; *chuASX*: heme binding protein; *entABE*: isochorismate synthase 1; *fepD*: ferrienterobactin ABC transporter periplasmic binding protein; *fyuA*: pesticin/yersiniabactin receptor protein; *ireA*: siderophores receptor; *iroBCDEN*: encode the salmochelin siderophore system; *iucA-D*: aerobactin siderophore biosynthesis protein; *iutA*: aerobactin receptor; *celb*: permease IIC component; *gad*: glutamate decarboxylase; *hra*: heat-resistant haemagglutinin; *iss*: increased serum survival; *malX*: maltodextrin-binding protein; *tcpC*: tir domain-containing protein; *traT*: conjugal transfer surface exclusion protein; *usp*: uropathogenic-specific protein; *yfcV*: encodes the major subunit of a putative chaperone-usher fimbria.

3.2.3. Virulence genes

Thirty virulence genes were detected in all 77 sequenced isolates. These 30 consensus genes included the type-1 fimbriae-associated genes *fimBCDEFGHI*, the intimin-like adhesin gene *fdeC*, the outer membrane protein gene *ompA*, the enterobactin iron acquisition system genes *entCDFS*, and the ferric-enterobactin uptake genes *fepABCG*, *chuTUVWY*, *fes*, and *aslA*. An additional 76 virulence genes were variably present, detected in 3 %–99 % of isolates each (Table 1). Most isolates (81 %) contained one or more *pap* adhesin genes, which are prevalent in *E. coli* strains associated with ascending UTIs in humans – with individual genes occurring in 7–71 % of isolates each (median 71 %).

Of the 77 dog isolates, 55 (71 %) qualified molecularly as ExPEC, and of these, 53 (96 %) further qualified as UPEC. Another 20 isolates qualified molecularly as UPEC but not ExPEC, yielding 73 (95 %) total UPEC isolates.

3.2.4. WGS analysis of ST73 isolates

Of the 13 present sequenced ST73 isolates, all possessed at least two β-lactamase genes, but no ESC resistance-associated genes. Two isolates (15 %) qualified as MDR, but neither contained the class 1 integron-associated gene *sul1*.

Only one ST73 isolate (7 %) contained all 17 ST73-associated prototypic virulence genes, i.e., *papC*, *papEF*, *papG*, *sfa/foc*, *fimH*, *hra*, *hlyA*, *cnf1*, *pic*, *vat*, *iroN*, *fyuA*, *kpsM II*, *usp*, and *ompT* (Riley, 2014). The remaining isolates each had ≤ 14 of these 17 genes. The only prototypic genes common to all ST73 isolates were *sfa/foc*, *fimH*, *cnf1*, *pic*, *vat*, *fyuA*, *kpsM II*, and *usp*.

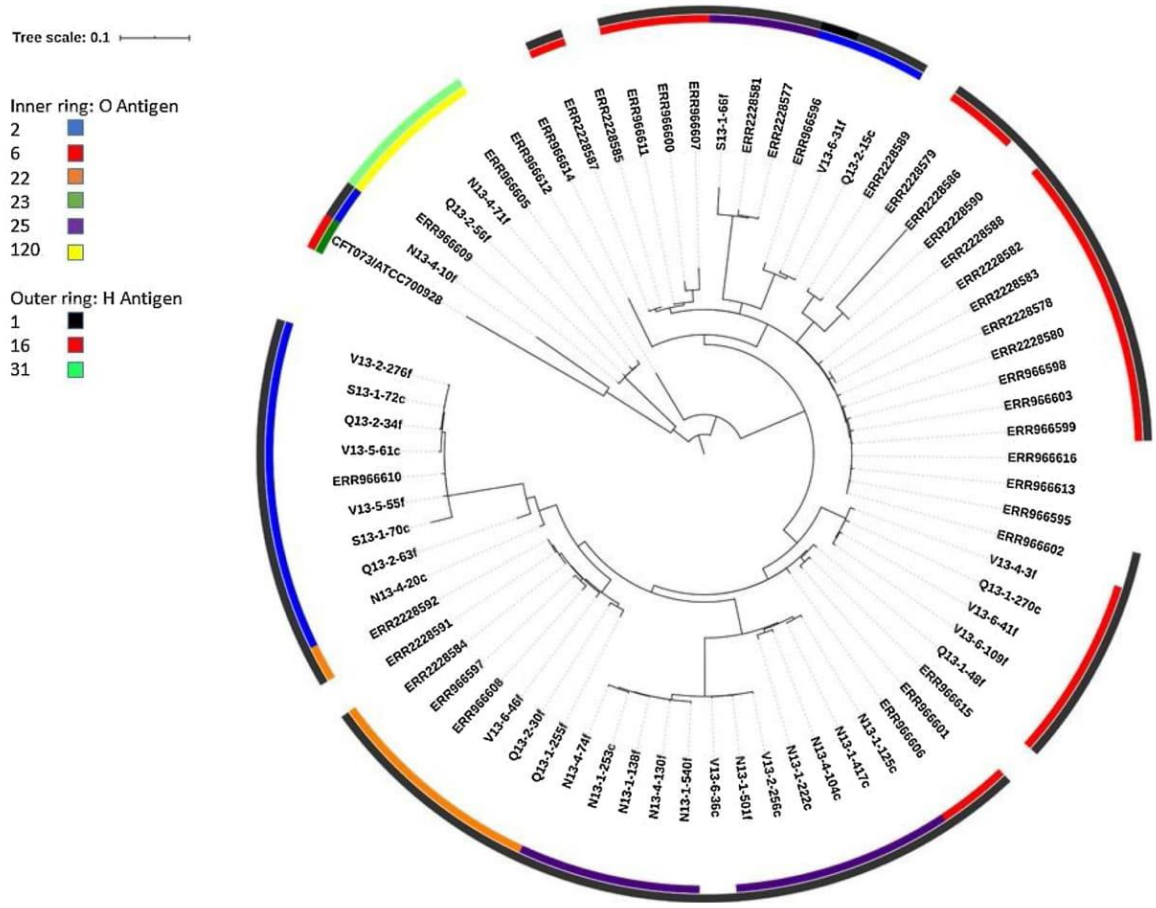

Fig. 2. Phylogenetic analysis of 13 dog, 23 cat, and 37 human *Escherichia coli* isolates of sequence type 73 from Australia and the United Kingdom. Phylogram is based on core genome single-nucleotide polymorphisms. The final dataset contained 40,634 sites.

3.2.5. Comparison with human and cat ST73 isolates

Core genome SNP analysis of the 13 present dog ST73 isolates, 23 cat ST73 isolates from another study (Kidsley et al., 2020a), and 37 historical human ST73 isolates from Australia and the United Kingdom (Alhashash et al., 2016; Bogema et al., 2019) was used to produce a phylogenetic tree (Fig. 2). The dog ST73 isolates exhibited extensive phylogenetic diversity, mirroring the extensive diversity also observed among the cat and human ST73 isolates, with most isolates occurring within distinct serotype and host-specific phylogenetic clusters, as reported previously (Bogema et al., 2019).

The 13 dog ST73 isolates exhibited four of the eight identified serotypes. Specifically, six dog isolates exhibited serotype O25:H1 (five isolates) or Ont:H1 (1 isolate) and formed an animal-specific cluster that included five closely related O25:H1 cat isolates. Likewise, serotype O2:H1 dog and cat isolates (4 each), plus a single, closely related human O2:H1 isolate, also clustered together, whilst single dog, cat, and human isolates formed a second O2:H1-specific cluster. Finally, a single dog isolate exhibited serotype O6:H1 and formed another animal-specific cluster that included three feline O6:H1 isolates and was distinct from the four main clusters of human O6:H1 isolates (n = 16).

4. Discussion

This study, which sought to genetically characterise a large nationwide collection of clinical FQS *E. coli* isolates from extraintestinal infections in Australian dogs, including via a WGS-based comparison of selected representative isolates, led to four main conclusions. First, ST372 (from phylogroup B2, which predominated here) was the predominant ST according to both WGS (31 %) and ST-specific PCR (22 %). Second, the remaining group B2 isolates were mainly from ST73, but also represented diverse minor STs, including ST12 (n = 5), ST80 (n = 5), and two novel STs (one of each). Third, in a SNP-based core genome phylogeny the dog ST73 isolates were highly diverse, and separated into serotype-specific clusters. Fourth, although most ST73 isolates segregated into distinct animal-specific and human-specific clusters, some animal and human isolates were intermingled within the same cluster, suggesting possible bi-directional transmission.

ST372 has been previously implicated in UTIs in both humans and dogs (Blyton and Gordon, 2017; LeCuyer et al., 2018; Valat et al., 2020). For human clinical isolates, three main studies – from Israel, Spain, France, and Italy – have identified ST372 at low frequency (4–11 %) (Adler et al., 2012; Izdebski et al., 2013; Rios et al., 2015), suggesting that ST372 is an uncommon cause of ExPEC infections in humans, as compared to other more prominent STs such as ST73, ST95, and ST131. By contrast, here among the dog FQS clinical isolates from Australia, ST372 was the most common ST according to both WGS (31 % of 77) and ST-specific PCR (22 % of 240). Similarly, ST372 was the most common ST in prior studies of dog ExPEC isolates from France (21 % of 618) and the United States (22 % of 295), both of which also focused on susceptible isolates (Valat et al., 2020; LeCuyer et al., 2018). By contrast, other studies have identified ST372 in *E. coli* isolates from cats and dogs in much lower proportions (2–5 %) (Chen et al., 2019; Karkaba et al., 2017; Liu et al., 2016; Maeyama et al., 2018; Zogg et al., 2018b). However, many of these studies were biased towards resistant isolates (i.e., ESBL-producing or FQR strains).

Previous investigators suggested that urine isolates from cats, dogs, and humans overlap broadly by ST, despite the fact that for each host species distinctive dominant STs may account for most isolates (LeCuyer et al., 2018). This across-host-species overlap of STs is apparent in both the present study and a recent similar study investigating

FQS *E. coli* from Australian cats by WGS (Kidsley et al., 2020a). In both studies the three most prevalent STs identified were ST12, ST73, and ST372; however, ST73 predominated in cats (43 % of cat isolates vs 17 % of dog isolates), whereas ST372 predominated in dogs (31 % of dog isolates vs 4 % of cat isolates). Similarly, in a comparative study of faecal *E. coli* from healthy cats and dogs in Canberra, Australia, ST73 accounted for fully 19 % (64/334) of cat isolates, vs. only 3.9 % (8/203) of dog isolates (Bourne et al., 2019).

ST12, ST73, ST95, ST127, and ST372 have been previously linked with human extraintestinal disease; indeed, ST73 and ST95 represent two of the major clonal lineages identified among human clinical isolates (Bogema et al., 2019). By contrast, among the present FQS *E. coli* isolates from Australian dogs, ST12 (7 %), ST95 (4 %), and ST127 (4 %) represented only minor STs. In the recent study of faecal *E. coli* from healthy dogs in Canberra, 31 % of cat isolates belonged to one of the human-associated STs – ST69, ST73, ST95, ST127, and ST131 – compared to just 10 % of the dog isolates (Bourne et al., 2019). Among the dog isolates, ST73 was the predominant ST, followed by ST95 and ST127, whereas ST372 was not identified (Bourne et al., 2019). However, in that study only 52 group B2 isolates were characterised, and STs were detected by PCR rather than WGS (Bourne et al., 2019); thus, making it difficult to correlate ST distributions with the present study. However, it is also possible that ST73 could be more prevalent among clinical rather than gut commensal dog isolates, as appears to be the case for ST372.

Our hypothesis at the commencement of this study, based on previous research (Johnson et al., 2008a, 2001), was that the dog *E. coli* isolates would closely resemble human ExPEC strains belonging to the same ST, consistent with frequent dog-human or human-dog transfer of strains, especially within households. Here, the only ST with sufficient representation for a comparison of dog, human, and cat isolates was ST73, and the phylogenetic comparison of dog- and human-source isolates by WGS did not support widespread across-host-species clonal commonality. As previously demonstrated for cat ST73 isolates, dog ST73 isolates were also highly diverse, and were distributed among four mostly serotype-specific clusters. Others have suggested that, unlike ST131 (Johnson et al., 2013), ST73 epidemiology is not characterised by emergence and expansion of a single dominant clone, given that human ST73 isolates from the UK and Australia are also phylogenetically diverse (Alhashash et al., 2016; Bogema et al., 2019). The present study confirms that dog ST73 isolates are just as diverse as human ST73 isolates.

To resolve relationships between cat, dog, and human ST73 strains the present dog isolates were compared with 23 cat and 37 human ST73 isolates by WGS. In a SNP-based phylogram the dog ST73 isolates were more closely related to cat rather than human ST73 isolates. Nevertheless, several dog, cat, and human isolates were intermingled in the tree, suggesting some potential for bi-directional transmission. Interestingly, the present cat and dog isolates from Australia were more closely related to human isolates from the UK than to those from Australia. This is most likely due to the existence of a reservoir or transmission network of circulating ST73 strains that has yet to be defined.

Study strengths included the availability of a national collection of *E. coli* isolates from Australian companion animals and the corresponding clinical data, as obtained over a one-year period, and the acquisition of existing human-source ST73 whole genome sequence data for comparison. Study limitations include the minimal temporal and geographical matching of dog and cat vs. human ST73 isolates, which may have influenced the WGS comparisons; the human isolates derived from specific locales in both the northern (2013) and southern (2009–2011) hemispheres, whereas the dog and cat isolates were obtained in Australia in 2013. The future availability of a temporally matched Australia-wide collection of human ST73 isolates could further support or refute the hypothesis of bi-directional transmission potential.

In conclusion, ST372, which is uncommonly associated with human disease, was the leading ST identified among FQS clinical dog *E. coli* isolates from Australia. Other human-associated clonal lineages identified included ST12, ST73, ST95, and ST127. Whilst most dog ST73 isolates were genetically distinct from human ST73 isolates, some were more closely related to both cat-source and human-source isolates, suggesting a potential for bi-directional transmission. Despite this, the results indicate that the overwhelming majority of clinical *E. coli* isolates associated with extraintestinal infections in dogs are host-specific.

Author contributions

AK performed the experiments, data analysis and drafted and prepared the manuscript. MO performed the whole genome sequencing and phenogram bioinformatics analysis. SS performed MIC testing on all isolates used in this study. CT provided training and assistance with BioNumerics analysis and manuscript reviewing. DJ, JJ, DG, SA and DT were involved in experimental design development and manuscript preparation. SA, DT and DJ were responsible for sample collection, data acquisition and co-ordination of the survey. SD provided access to whole genome sequence data and assisted with manuscript preparation.

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Ethics approval

Not required

Declaration of competing interest

DJT has received research funding and undertaken consultancies for Bayer, Zoetis, Boehringer Ingelheim, Virbac, Luoda Pharma, Neoculi, and IRiccorgpharm. SA has received research funding from Zoetis and Neoculi. JRJ has received research support from and/or has undertaken consultancies for Achaogen, Allergan, Crucell/Janssen, Melinta, Merck, Shionogi, Syntiron, and Tetraphase.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2020.108783.

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Supplementary A

Gene	Sequence
kspM_II_K1	ATGGCAAGAAGTGGATTTGAAGTTCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTTCGTCTGGGGTATTTGTGGGCGATTC TTGAACCCTCCGCGCATTTGCTGATACTGTTGGGAATTTTGGGTTACGTTATGCACCGCACTATGCCAGACATCTCGTTCCCGGTGTTTTTACTTAATGGCCTGATTCCC TTTTTTATCTTTAGTAGTATTAGCAAACGTTCTATTGGTGCTATTGAAGCGAACCAGGGACTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCACGTGCA CTGCTTGAGACACTGATTTACGTTGCTGTTTATATTTTGCTCATGCTTATCGTCTGGATGACAGGCGAATATTTCGAAATTACAAACTTTTTACAACTTGTGCTCACCTGG AGTTTGTTAATCATTCTTTCATGTGGCGTCGGCTTAATATTTATGGTCGTTGGTAAAACCTTTCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCTGTATTTC ATCTCCTGCATCATGTTCCCTCTACACTCGATTCCAAAACAATACTGGTCATATCTACTCTGGAACCCATTAGTGCATGTTGTGGAGTTAAGCCGCGAGGCAGTTATGCC TGGCTATATCAGTGAAGGCGTGAGTCTGAACTACCTTGCAATGTTTACTCTGGTCACCCTGTTCATCGGCCTGGCATTATACCGAACGCGTGAAGAGGCAATGCTGAC ATCATGA
kspM_II_K2	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATCTGTGGGCGATTC TCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATTATGCACCGCACGATGCCAGACATCTCGTTCCCGGTGTTTTTACTTAATGGCCTGATTCCC TTTTTTATCTTTAGCAGTATCAGCAAACGTTCTGTAAGTGCTATTGAAGCGAACCAGGGGCTGTTTAATTATCGCCCAGTAAAACCCATCGATACGATCATTGCGCGCGC CCTGCTTGAGACGCTGATTTACGTTTCTGTTTATATTCTGCTTATGCTCATTGTCAGGATGGCAGGCGAATATTTCGAGATAACAAATTTTTTACAACTTGTGGCTACCTG GAGTCTGCTGATCATTCTTTCATGCAGCGTCGGCTTAATATTCATGGTCGTTGGTAAAACCTTCCCCGAAATGCAAAAGGTTCTGCCGATACTTCTTAAGCCCCTTTATT TCATCTCCTGCATCATGTTCCCTCTGCACTCAATTCCAAAGCAATACTGGTCATATCTACTCTGGAACCCACTAGTGCATGTCGTGGAGTTAAGCCGTGAGGCCGTTAT GCCTGGCTATATTAGCGAAGGTGTGAGTCTGAACTACCTGGCAATGTTCACATTGATAACTCTGTTCATTGGTCTGGCGTTATATCGAACTCGTGAGGAGGCAATGCTG ACATCATGA
kspM_II_K5_kfiC_Bi8337-41	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATTTGTGGGCGATTC TCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATTATGCACCGCACGATGCCAGACATCTCATTCCCGGTGTTTTTACTTAATGGCCTGATTCCC TTTTTTATCTTTAGCAGTATCAGCAATCGTTCTGTAGGCGCTATTGAAGCGAACCAGGGGTTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCACGCGC ACTGCTTGAGACGCTGATTTACGTTGCTGTTTATATATTGCTCATGCTTATCGTCTGGATGGCAGGTGAATATTTCGAGATAACAAACTTTTTACAACTAGTGCTCACCTG GAGTTTGTTAATCATTCTTTCATGTGGCATCGGCTTAATATTCATGGTCGTTGGTAAAACCTTCCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCTGTATT TCATCTCCTGCATCATGTTCCCTCTACATTCGATTCCGAAGCAATACTGGTCATATCTACTCTGGAACCCATTAGTGCATGTCGTAGAGTTAAGCCGCGAGGCAGTTATG CCTGGCTATATCAGCGAAGGCGTGAGTCTGAACTACCTTGCAATGTTCACTCTGGTCACCCTGTTCATCGGTCTGGCGTTATACCGAACTCGTGAGGAGGCAATGCTG ACATCATGA
kspM_II_K5_kfiC	ATGAACGCAGAATATATAAATTTAGTTGAACGTAAAAAGAAATTAGGGACAAATATTGGTGCTCTTGATTTTTTATTATCAATTCATAAGGAGAAAGTTGATCTTCAACATA AAAACTCGCCTTTAAAAGGTAACGATAACCTTATTCACAAAAGAATAAACGAATACGACAATGTACTTGAACTATCTAAGAATGTATCAGCTCAGAATTCTGGCAATGAGT TTTCTTATTTATTGGGATATGCAGATTCTCTTAGAAAAGTTGGTATGTTGGATACTTATATTAAAATTGTTTGTTATCTAACAATTCAATCTCGTTATTTTAAAAATGGCGAA CGAGTTAAGCTTTTTGAACATATAAGTAACGCTCTACGGTATTCAAGGAGTGATTTTCTCATTAATCTTATTTTTGAACGATATATCGAATATATAAACCATCTAAAATTGT CGCCCAAACAAAAAGATTTTTATTTTTGTACGAAGTTTTCAAAATTTCATGATTATACTAAAAATGGATATAAATATTTAGCATTTGATAATCAAGCCGATGCAGGGTATGG CCTGACTTTATTATTAAATGCAAACGATGATATGCAAGATAGTTATAATCTACTCCCTGAGCAAGAACTTTTTATTTGTAATGCTGTAATAGATAATATGAATATTTATAGG AGTCAATTTAACAAATGTCTACGAAAATACGATTTATCAGAAATAACTGATATATACCCAAATAAAATTATATTGCAAGGAATTAAGTTCGATAAGAAAAAAAATGTTTATG GAAAAGATCTTGTTAGTATAATAATGTCAGTATTCAATTCAGAAGATACTATTGCATACTCATTACATTCATTGTTGAATCAAACATATGAAAATATTGAAATTCTCGTGTG CGATGATTGTTCATCGGACAAAAGCCTTGAAATAATTAAGAGCATAGCTTATTCTAGTTCAAGAGTGAAAGTATATAGCTCACGAAAAAACCAAGGCCCTTATAATATAA GAAATGAGCTAATAAAAAAAGCACACGGTAATTTCATCACCTTTCAAGATGCAGATGATCTTTCTCATCCGGAGAGAATACAAAGACAAGTTGAGGTTCTTCGCAATAAT AAGGCTGTAATCTGTATGGCTAACTGGATCCGTGTTGCGTCAAATGGAAAAATTCAATTCTTCTATGATGATAAAGCCACAAGAATGTCTGTTGTATCGTCAATGATAAA AAAAGATATTTTTGCGACAGTTGGTGGCTATAGACAATCTTTAATTGGTGCAGATACGGAGTTTTATGAAACAGTAATAATGCGTTATGGGCGAGAAAGTATTGTAAGAT TACTGCAGCCATTGATATTGGGGTTATGGGGAGACTCCGGACTTACCAGGAATAAAGGAACAGAAGCTCTACCTGATGGATATATATCACAATCTCGAAGAGAATATAG TGATATCGCGGCAAGACAACGAGTGTTAGGGAAAAGTATCGTAAGTGATAAAGATGTACGTGGTTTATTATCTCGCTATGGTTTGTTTAAAGATGTATCAGGAATAATTG AACAATAG

Supplementary A Continued

Gene	Sequence
kspM_K15	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGCCGCCGTTCATGCTCTATTTTTACGTGAGCTTAGAACTAGGTTTGGCAAATATCGCTTGGGTTATTTATGGGCTGTATT AGAACCAGCTGCTCATCTTCTAATAATGCTGGCAATTTTTGGTTTTTTTATGCATCGCACAATGCCAGATATTTCCTTTCCTGTATTTTTAATAAATGGAATAATTCCTTATT TTATATTTAGCAATATTGCTACGCGGTCTATAGGGGCAATTGAGGCAAACCAAGGCTTATTTAATTACAGGCCAGTAAGGCCTATAGATACAATTATAGCTAGAGCAATAT TAGAAGTGCTAATTTATAGCATAGTATATCTGGTTTTGATGAGTTTATTGTTAATAATAGGGGAGCAATTTAAAATATATAATATATTGCTGTTGTTTAGCGCATGGATTTTA CTGGCTTTATTTTCATGTGGAATTGGGTTGATCTTTATGGTAATTGGGAAAACTTTTCCAGAAACAGAGAAGTTTCTACCAATAATACTCAAGCCATTATATTTTGTATCAT GTATTATGCTTCCATTACATGCAATACCAAAAGGTTATTGGGGATATATATTATGGAATCCCATTGTTCATGTGGTAGAGCTTTGTCGTGAATCCGTTGTTACAGGTTATG TCTCTGAAGGGGTTAGTTTATATTACTTATTTATAAGCACGTTAATATTATTGTTTTTAGGATTGGCGCTATATTCATCTCGTGAAGAATACATGTTAACATCATGA
kpsM_II_K52	TTGGTAGCTGTTAAGCCAAGGGCGGTAGCGTACCTGAAGAGATTAGGATCACATCATCAAATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTAT TTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATTTGTGGGCGATTCTCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATT ATGCACCGCACGATGCCAGACATCTCATTCCCGGTGTTTTTACTTAATGGCCTGATTCCCTTTTTTATCTTTAGCAGTATCAGCAATCGTTCTGTAGGTGCTATTGAAGCG AATCAGGGGCTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCGCGTGCATTGCTTGAGACACTGATTTACGTTACTGTTTATATATTGCTTATGCTTATC GTCTGGATGGCAGGCGAATATTTCGAAATAACAAACTTTTTACAACTTGTGCTTACCTGGAGTTTGTTAATCATTCTTTCATGTGGCGTTGGCTTAATATTCATGGTCGTT GGTAAAACCTTCCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCTGTATTTCATCTCCTGCATCATGTTCCCTCTACACTCGATTCCGAAGCAATACTGGTC ATATCTACTCTGGAATCCATTAGTGCATGTCGTAGAGTTAAGCCGCGAGGCAGTTATGCCTGGCTATATCAGCGAAGGCGTGAGTCTGAACTACCTGGCAATGTTTACA TTGATAACTCTGTTCATTGGTCTGGCGTTATACCGAACTCGTGAGGAGGCAATGCTGACATCATGA
kspM_III	ATGGACAAACCCATTATTAGTCAAACTCCACGCACTTCTTTGCAAGTATTACGTGATGTAGTATTTGGCTTATTAATTCGTGAGCTAAAAACAAGATTTGGTAATTACCGA CTAGGTTATGCTTGGGCATTACTTGACCCATTATTAATGATTAGCCTGTTCAGCGTAGTATTTGGGATGAGAAGCCAAAGTGGCTTTGGTGGTGTCCCAGCCCAGGTTTT TATTACTGCTGGTTATTTACCTTTCATGTTTTTCAATAAAGTTGTGACTCAGTTGAAATCTGCTGTCAATGCTAATATGGGACTTTTTTGTTATAGACAAGTGACTCCTTTTG CAACTTTTATAGCACGTTTTATGCTAGAAACAATGGTGGGCATGATTGTCGGTATCATCCTAGTACTAGGATTATTGTGGTTTGGCTTTGATGCAATACCTGCGGATCCAT TGCAAGTGATCCTTGGTTATTCTCTTCTGATGCTGTTTTCTTTTTCTCTTGGTATTGTATTTTGTGTTATTTGTAACTTAGCGAAAGAGGCAGATAAATTTCTTAGCTTGTTA ATGATGCCTTTGATGTTTATCTCTTGTGTTATGTTTCCTCTTGCTACTATTCCCCCTCAATATCAGCATTGGTTTTTATGGAATCCACTTGTGCATGCTGTAGAACTAATCC GAAGGGCATGGATATCTGGTTATCGTAGTCCTGATGTAAGTTGGGCGTATCTGTCGGTTGTCACCTTATTATTGCTCACTTTTGCTATGAGTTGTTACCGATTACGGCAT CGCCAATTGATTGCTAGTTAG
hra	ATGGAGATAATTGAAATGAATAAGGTTTTTGTTGTTTCAGTGGTGGCCGCAGCCTGTGTATTTGCAGTAAATGCAGGAGCAAAGGAAGGTAAAAGCGGTTTTTATCTGAC CGGTAAAGCCGGTGCCTCTGTGATGTCACTTTCAGACCAGCGTTTCCTGTCAGGAGATGAGGAAGAAACATCAAAGTATAAAGGCGGCGATGACCATGATACGGTATT CAGTGGCGGTATTGCGGTCGGTTATGATTTTTATCCGCAGTTCAGTATTCCGGTTCGTACAGAACTGGAGTTTTACGCTCGTGGAAAAGCTGATTCGAAGTATAACGTA GATAAAGACAGCTGGTCAGGTGGTTACTGGCGTGATGACCTGAAGAATGAGGTGTCAGTCAACACACTAATGCTGAATGCGTACTATGACTTCCGGAATGACAGCGCA TTCACACCATGGGTATCCGCAGGGATTGGCTACGCCAGAATTCACCAGAAAACAACCGGTATCAGTACCTGGGATTATGAGTACGGAAGCAGTGGTCGCGAATCGTTG TCACGTTCAGGCTCTGCTGACAACTTCGCATGGAGCCTTGGCGCGGGTGTCCGCTATGACGTAACCCCGGATATCGCTCTGGACCTCAGCTATCGCTATCTTGATGCA GGTGACAGCAGTGTGAGTTACAAGGACGAGTGGGGCGATAAATATAAGTCAGAAGTTGATGTTAAAAGTCATGACATCATGCTTGGTATGACTTATAACTTCTGA
intI1	ATGAAAACCGCCACTGCGCCGTTACCACCGCTGCGTTCGGTCAAGGTTCTGGACCAGTTGCGTGAGCGCATACGCTACTTGCATTACAGCTTACCAACCGAACAGGCT TATGTCCACTGGGTTCGTGCCTTCATCCGTTTCCACGGTGTGCGTCACCCGGCAACCTTGGGCAGCAGCGAAGTCGAGGCATTTCTGTCCTGGCTGGCGAACGAGCG CAAGGTTTCGGTCTCCACGCATCGTCAGGCATTGGCGGCCTTGCTGTTCTTCTACGGCAAGGTGCTGTGCACGGATCTGCCCTGGCTTCAGGAGATCGGAAGACCTC GGCCGTCGCGGCGCTTGCCGGTGGTGCTGACCCCGGATGAAGTGGTTCGCATCCTCGGTTTTCTGGAAGGCGAGCATCGTTTGTTCGCCCAGCTTCTGTATGGAACG GGCATGCGGATCAGTGAGGGTTTGCAACTGCGGGTCAAGGATCTGGATTTCGATCACGGCACGATCATCGTGCGGGAGGGCAAGGGCTCCAAGGATCGGGCCTTGA TGTTACCCGAGAGCTTGGCACCCAGCCTGCGCGAGCAGCTGTCGCGTGCACGGGCATGGTGGCTGAAGGACCAGGCCGAGGGCCGCAGCGGCGTTGCGCTTCCCG ACGCCCTTGAGCGGAAGTATCCGCGCGCCGGGCATTCCTGGCCGTGGTTCTGGGTTTTTGCGCAGCACACGCATTCGACCGATCCACGGAGCGGTGTCGTGCGTCG CCATCACATGTATGACCAGACCTTTCAGCGCGCCTTCAAACGTGCCGTAGAACAAGCAGGCATCACGAAGCCCGCCACACCGCACACCCTCCGCCACTCGTTCGCGA CGGCCTTGCTCCGCAGCGGTTACGACATTCGAACCGTGCAGGATCTGCTCGGCCATTCCGACGTCTCTACGACGATGATTTACACGCATGTGCTGAAAGTTGGCGGTG CCGGAGTGCGCTCACCGCTTGATGCGCTGCCGCCCCTCACTAGTGAGAGGTAG

Supplementary B

Strain	Country of origin	Study
ERR966595	United Kingdom	Alhashash et al., 2016
ERR966596	United Kingdom	Alhashash et al., 2016
ERR966597	United Kingdom	Alhashash et al., 2016
ERR966598	United Kingdom	Alhashash et al., 2016
ERR966599	United Kingdom	Alhashash et al., 2016
ERR966600	United Kingdom	Alhashash et al., 2016
ERR966601	United Kingdom	Alhashash et al., 2016
ERR966602	United Kingdom	Alhashash et al., 2016
ERR966603	United Kingdom	Alhashash et al., 2016
ERR966604	United Kingdom	Alhashash et al., 2016
ERR966605	United Kingdom	Alhashash et al., 2016
ERR966606	United Kingdom	Alhashash et al., 2016
ERR966607	United Kingdom	Alhashash et al., 2016
ERR966608	United Kingdom	Alhashash et al., 2016
ERR966609	United Kingdom	Alhashash et al., 2016
ERR966610	United Kingdom	Alhashash et al., 2016
ERR966611	United Kingdom	Alhashash et al., 2016
ERR966612	United Kingdom	Alhashash et al., 2016
ERR966613	United Kingdom	Alhashash et al., 2016
ERR966614	United Kingdom	Alhashash et al., 2016
ERR966615	United Kingdom	Alhashash et al., 2016
ERR2228591	Australia	Bogema et al., 2019
ERR2228580	Australia	Bogema et al., 2019
ERR2228590	Australia	Bogema et al., 2019
ERR2228589	Australia	Bogema et al., 2019
ERR2228578	Australia	Bogema et al., 2019
ERR2228592	Australia	Bogema et al., 2019
ERR2228585	Australia	Bogema et al., 2019
ERR2228588	Australia	Bogema et al., 2019
ERR2228586	Australia	Bogema et al., 2019
ERR2228577	Australia	Bogema et al., 2019
ERR2228582	Australia	Bogema et al., 2019
ERR2228584	Australia	Bogema et al., 2019
ERR2228579	Australia	Bogema et al., 2019
ERR2228583	Australia	Bogema et al., 2019
ERR2228581	Australia	Bogema et al., 2019
ERR2228587	Australia	Bogema et al., 2019

Chapter 4

Companion animals are spillover hosts of the multidrug-resistant human extraintestinal *Escherichia coli* pandemic clones ST131 and ST1193

Statement of Authorship

Title of Paper	Companion animals are spillover hosts of the multidrug-resistant human extraintestinal *Escherichia coli* pandemic clones ST131 and ST1193
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Principal Author

Name of Principal Author (Candidate)	Amanda K. Kidsley		
Contribution to the Paper	Performed laboratory work, analysis, interpreted data, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	2/5/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Supervised development of work, helped to evaluate and edit the manuscript		
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Contribution to the Paper	Supervised development of work, helped to evaluate and edit the manuscript		
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Companion Animals Are Spillover Hosts of the Multidrug-Resistant Human Extraintestinal *Escherichia coli* Pandemic Clones ST131 and ST1193

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Escherichia coli sequence types 131 (ST131) and 1193 are multidrug-resistant extraintestinal pathogens that have recently spread epidemically among humans and are occasionally isolated from companion animals. This study characterized a nationwide collection of fluoroquinolone-resistant (FQ^R) *E. coli* isolates from extraintestinal infections in Australian cats and dogs. For this, 59 cat and dog FQ^R clinical *E. coli* isolates (representing 6.9% of an 855-isolate collection) underwent PCR-based phylotyping and whole-genome sequencing (WGS). Isolates from commensal-associated phylogenetic groups A (14/59, 24%) and B1 (18/59, 31%) were dominant, with ST224 (10/59, 17%), and ST744 (8/59, 14%) predominating. Less prevalent were phylogenetic groups D (12/59, 20%), with ST38 (8/59, 14%) predominating, and virulence-associated phylogenetic group B2 (7/59, 12%), with ST131 predominating (6/7, 86%) and no ST1193 isolates identified. In a WGS-based comparison of 20 cat and dog-source ST131 isolates with 188 reference human and animal ST131 isolates, the cat and dog-source isolates were phylogenetically diverse. Although cat and dog-source ST131 isolates exhibited some minor sub-clustering, most were closely related to human-source ST131 strains. Furthermore, the prevalence of ST131 as a cause of FQ^R infections in Australian companion animals was relatively constant between this study and the 5-year-earlier study of Platell et al. (2010) (9/125 isolates, 7.2%). Thus, although the high degree of clonal commonality among FQ^R clinical isolates from humans vs. companion animals suggests the possibility of bi-directional between-species transmission, the much higher reported prevalence of ST131 and ST1193 among FQ^R clinical isolates from humans as compared to companion animals suggests that companion animals are spillover hosts rather than being a primary reservoir for these lineages.

Keywords: *Escherichia coli*, companion animals, ST131, genomics, virulence genes

INTRODUCTION

Extraintestinal pathogenic *E. coli* (ExPEC) strains have an enhanced ability to traverse from their usual gut environment to normally sterile extraintestinal body sites and cause disease (Johnson et al., 2016). Before the 2000s most ExPEC strains were susceptible to critically important antimicrobials (CIAs) such as fluoroquinolones (FQs) and extended-spectrum cephalosporins (ESCs) (Pitout, 2012), however, multidrug resistance is increasingly common globally (Nicolas-Chanoine et al., 2014). Resistance to FQs, ESCs, and carbapenems is of special concern due these drugs' critical role in humans for treating life-threatening infections such as urosepsis (Matsumura et al., 2017).

Emerging FQ resistance is due mostly to the expansion and spread of sequence type (ST) ST131, a clonal lineage from *E. coli* phylogenetic group B2, first identified in 2008 on three continents (Coque et al., 2008; Lau et al., 2008; Nicolas-Chanoine et al., 2008). Genomic epidemiological analysis using Bayesian estimation predicts that FQ-resistance-conferring point mutations in chromosomal genes *gyrA* and *parC* occurred around 1987 in North America, coinciding with the first clinical use of FQs in the USA in 1986 (Ben Zakour et al., 2016; Stoesser et al., 2016). Most FQ-resistant (FQ^R) ST131 strains derive from a single sub-ST clade, designated clade C (Petty et al., 2014), or *H*30R (Price et al., 2013) Clade C/*H*30R in turn exhibits a prominent sub-lineage, referred to as clade C2 (Petty et al., 2014) or *H*30Rx (Johnson et al., 2016), members of which typically exhibit ESC resistance, mediated by the CTX-M-15 extended-spectrum β-lactamase (ESBL). ST131 (and, specifically, clade C/*H*30R) has become the most prevalent CIA-resistant human ExPEC lineage globally (Dautzenberg et al., 2016), causing millions of antimicrobial-resistant (AMR) infections annually (Pitout and DeVinney, 2017).

Recently, ST1193, another lineage of FQ^R group B2 *E. coli*, has also emerged as an important multidrug-resistant (MDR) human pathogen (Johnson et al., 2019; Tchesnokova et al., 2019). ST1193 was identified first in Australia as an emerging FQ^R clonal group (Platell et al., 2012), and has now been reported worldwide, including in Asia (Kim et al., 2017; Wu et al., 2017; Xia et al., 2017), North America (Tchesnokova et al., 2019), and Europe (Jorgensen et al., 2017; Valenza et al., 2019). In a recent multi-center study (United States, 2016–2017), ST1193 represented one-quarter of all FQ^R human clinical urine isolates (Tchesnokova et al., 2019).

The cause of such widespread dissemination of AMR ExPEC lineages is unclear. Host-to-host transmission, including between animals and humans, likely contributes, in view of documented colonization of multiple members of the same household with the same ExPEC strain (Johnson et al., 2016) and the occurrence of (human-associated) pandemic lineages such as ST131 and ST1193 in dogs and cats (Johnson et al., 2009c; Pomba et al., 2009; Platell et al., 2012). The first reported case of an *E. coli* ST131 infection in an animal involved a dog with a UTI in Portugal. Within-household ST131 transmission between humans and pets (particularly dogs and cats) has also been documented. Additionally, in the first description of FQ^R ST1193 as a potential pandemic clone, two isolates were from dogs. As such, companion animals may be an important reservoir of these resistant clonal lineages for acquisition by humans, and humans may be a reservoir of pathogens for their pets (Timofte et al., 2014). Notably, however, in the few relevant clinical and/or ecological studies (apart from the initial report involving ST1193), both ST131 and ST1193 have been relatively rare among companion animal ExPEC isolates (Platell et al., 2012; Pitout and DeVinney, 2017), consistent with the human reservoir being primary. Additionally, both clonal lineages have been reported recently in fecal samples from wild scavenging birds (Mukerji et al., 2019; Zurfluh et al., 2019), which suggests another potential zoonotic reservoir and/or transmission pathway.

Relevant to the human-companion animal interface, previous studies have documented the emergence of FQ^R ST131 in Australian companion animals (Platell et al., 2010, 2011a; Guo et al., 2015), as both an extraintestinal pathogen and a gastrointestinal colonizer of hospitalized dogs. Whereas Platell et al. and Guo et al. investigated companion animal-source FQ^R *E. coli* from only one region in Australia, Saputra et al. (2017) later surveyed companion animal-source *E. coli* on an Australia-wide level; they found that 9.3% of 514 dog isolates and 5% of 341 cat isolates were FQ^R. Based on these findings, the present study aimed to determine the prevalence of ST131 and ST1193 among both FQ^R (both ST131 and ST1193) and FQ-susceptible (FQ^S) (ST131 only) members of this Australia-wide *E. coli* isolate collection. An additional aim was to compare any identified companion animal ST131 isolates with members of a multi-national collection of human and animal ST131 isolates using whole-genome sequencing (WGS).

MATERIALS AND METHODS

Study Isolates

This study utilized three different sources of isolates. First, 855 clinical *E. coli* isolates from dogs and cats (January 2013–January 2014), accompanied by their antimicrobial susceptibility data, were obtained from the above-mentioned nation-wide survey of antimicrobial resistance in bacterial pathogens from Australian animals (Saputra et al., 2017). The isolates had been collected from 22 government, private, and university veterinary diagnostic laboratories between January 2013 and January 2014, accompanied by a brief clinical history and laboratory submission report, without client details. In all cases, the referring diagnostic microbiologist considered the isolate significant. Of the isolates, 59 (6.9%) were FQ^R (48 dog, 11 cat) and 796 (93%) were FQ^S: 445 dog, 351 cat) (**Table 1**). Isolates were classified as multidrug-resistant (MDR) if they showed non-susceptibility to at least one antimicrobial agent in three or more antimicrobial classes. WGS was done on these 59 FQ^R cat and dog-source *E. coli* isolates as described in section "WGS of the 59 Cat and Dog-Source FQR *E. coli* Isolates" below.

Second, we established an Australian cat and dog-source ST131 collection (n = 20). This collection included 11 ST131 isolates [six FQ^R (4 dog, 2 cat), five FQ^S (all dog)] identified here within the above-mentioned nation-wide collection of Saputra

TABLE 1 | Isolates used in this study.

Study	Year	Country	FQ status	No. isolates	No. of these ST131	Total no. ST131 isolates
Present Study	Jan 2013–Jan 2014	Australia	FQ^R	59	6	11
			FQ^S	796	5	
Platell et al., 2010	2007–2009	Australia	FQ^R	9	9	9
Ben Zakour et al., 2016	1967–2011	Various	FQ^R	62	62	184
			FQ^S	38	38	
			Unknown	84	84	
Reference Strains	2005–2008	United States, United Kingdom	FQ^R	3	3	4
	Unknown	Japan	Unknown	1	1	

et al. (2017). It also included nine FQ^R ST131 isolates (2007–2009; 8 dog, 1 cat) from a previous study of ST131 prevalence among human and companion animal FQ^R *E. coli* from eastern Australia (Platell et al., 2010, 2011a). A more detailed WGS and phylogenetic analysis were done on these 20 Australian cat and dog-source ST131 isolates, as described in section "WGS of the 20 Australian Cat and Dog-Source ST131 Isolates" below.

Third, the above 20 Australian cat and dog-source ST131 genomes were placed into a broader context by comparing them with 188 published diverse-source ST131 genomes (Ben Zakour et al., 2016), including the complete genomes of four established ST131 reference strains: EC958 (Totsika et al., 2011) (GenBank: HG941718), SE15 (GenBank: AP009378), JJ1886 (GenBank: CP006784), and JJ1887 (GenBank: CP014316). The final multi-national dataset included the genomes of 208 ST131 isolates, as collected between 1967 and 2014 in nine countries: The United States (n = 83); The United Kingdom (n = 27); Australia (n = 44); Spain (n = 20); Canada (n = 19); New Zealand (n = 6); India (n = 5); Portugal (n = 1); Japan (n = 1); and Korea (n = 1). In addition to the 20 Australian cat and dog-source ST131 study isolates, the multi-national ST131 genome dataset included isolates from cats (n = 3), dogs (n = 3), avian species (n = 7), a dolphin (n = 1), and a primate (n = 1). Single-locus variants of ST131 were counted as ST131. Isolate names and available metadata are summarized in **Supplementary Table S1**.

PCR-Based Phylogenetic Grouping and ST131 Status

E. coli phylogenetic group was determined for the present 855 clinical *E. coli* study isolates from Australian dogs and cats (59 FQ^R, 796 FQ^S) by using the revised Clermont multiplex PCR assay (Clermont et al., 2013). This identified 594 isolates (7 FQ^R, 587 FQ^S) as belonging to group B2. The B2 isolates were then screened for ST131-specific single-nucleotide polymorphisms (SNPs) in *mdh* and *gyrB* (Johnson et al., 2009b), which identified six (86% of 7) FQ^R and five (0.9% of 587) FQ^S B2 isolates as ST131.

WGS of the 59 Cat and Dog-Source FQ^R *E. coli* Isolates

DNA extraction and WGS was done on the present 59 cat and dog-source FQ^R *E. coli* study isolates (48 dog, 11 cat) using the Illumina Next Seq platform as described (Abraham et al., 2018). The resulting FASTQ files were trimmed using CLC Genomics Workbench (QIAGEN Version 12), with a quality limit of 0.01, and reads with an ambiguous base were trimmed (ambiguous limit = 1). *De novo* assembly for each isolate was also performed using the CLC Genomics Workbench, using the default settings (Rahimi et al., 2018). WGS analysis was undertaken using web-based tools available at the Centre for Genome Epidemiology (CGE) and custom BLAST databases implemented in the CLC Genomics Workbench (see **Supplementary Material B**).

Isolates were classified provisionally as ExPEC if they contained $\geq$ 2 of 5 hallmark ExPEC-associated VGs, i.e., *papA* and/or *papC*, *sfa/focDE*, *afa/draBC*, *kpsM II*, and *iutA* (Johnson et al., 2003), and as UPEC if they contained $\geq$ 3 of 4 hallmark UPEC-associated VGs (Virulance Genes), i.e., *chuA, fyuA, vat, and yfcV* (Spurbeck et al., 2012). Additionally, isolates classified as ST131 were analyzed to determine the presence of 12 prototypic ST131-associated VGs, i.e., *iha*, *fimH*, *sat*, *fyuA/irp2*, *iutA/iucD*, *kpsM II*, *usp*, *traT*, *ompT*, and *malX* (Riley, 2014).

ST131 Maximum-Likelihood Analysis and Phylogenetic Tree Construction

WGS of the 20 Australian Cat and Dog-Source ST131 Isolates

For WGS of the 20 Australian cat and dog-source ST131 isolates, genomic DNA was extracted from overnight LB broth cultures using MoBio UltraClean Microbial DNA isolation kit (QIAGEN), per the manufacturer's instructions. Sequencing was done using the Illumina NextSeq 500 platform (San Diego, CA, United States) at the Australian Centre for Ecogenomics[1]. For this, NextSeq DNA libraries were prepared using the Nextera XT Library Preparation Kit (Illumina) with the Nextera XT Index Kit (Illumina) and were sequenced using a NextSeq 500 2 × 150 bp High-Output v2 kit (Illumina). WGS generated a median of 3.65 million reads per sample (IQR: 2.87–4.30 million reads; range: 2.33–5.11 million reads) (**Supplementary Table S2**). Sequence read data have been submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject PRJNA627752.

Multi-Continental ST131 Sequence Data

The publicly available sequence read data, which had passed previously defined quality controls (Ben Zakour

[1]http://ecogenomic.org/services

et al., 2016), were downloaded from the NCBI SRA using the "prefetch" and "FASTQ-dump" tools within the SRA Toolkit v2.9.0-mac64[2] (**Supplementary Table S3**). The methods used for quality control, MLST and *de novo* assembly for this dataset are available in **Supplementary Material B**.

High-Resolution Phylogenetic Reconstruction of ST131

Strain EC958 was used as the template reference genome for generating a SNP-based phylogeny for 208 multi-national ST131 isolate genomes, including the 20 newly sequenced cat and dog-source ST131 isolates from Australia and the 188 published diverse-source genomes (Price et al., 2013; Petty et al., 2014) (**Supplementary Table S4**). For this, the coordinates of prophage and genomic island elements in the complete chromosome of EC958 were masked (**Supplementary Table S5**) using the bedtools (Quinlan and Hall, 2010) v2.27.1 "maskfasta" function. The trimmed paired-end reads from the 208 ST131 isolates were mapped onto the EC958-masked chromosome using the Bowtie 2 v2.3.4.2 read aligner within the Nesoni v0.132 pipeline under default settings[3]. Pseudo-genomes were created for each strain by integrating strain-specific SNPs into the backbone of EC958, which were then aligned to generate a multiple-genome alignment (Petty et al., 2014).

All polymorphic substitution sites were concatenated into a multi-FASTA alignment that was filtered for recombination by using the Gubbins algorithm (default settings, "raxml mode" with the General Time Reversible (GTR) GAMMA correction). Removal of putatively recombined regions left 7,527 total non-recombinant core-genome SNPs. The recombination-filtered SNP alignment was imported into jModelTest v2.1.10 (Guindon and Gascuel, 2003; Darriba et al., 2012) and 12 candidate models were tested from three substitution schemes, with the base tree for likelihood calculations optimized for ML and parameters " + F," " + I," " + G (nCat = 4)." The GTR nucleotide substitution model with GAMMA distribution was determined as the best-fit evolutionary model based on the lowest Akaike and Bayesian Information Criterion (AIC and BIC) scores.

The evolutionary history was inferred by importing this alignment into RAxML (Stamatakis, 2014) v8.2.10 (GTR-GAMMA correction) and using the ML method thorough optimization of the 20 distinct randomized Maximum Parsimony trees, before adding 1,000 bootstrap replicates. FigTree visualized the ML phylogenetic tree outputs and exported the trees in NEXUS format. NEXUS trees were visualized in EvolView v2 (Zhang et al., 2012; He et al., 2016) for final output generation. The recombination-filtered SNP alignment also was converted into a pairwise SNP distance matrix using snp-dists v0.2[4].

[2]http://ncbi.github.io/sra-tools

[3]https://github.com/Victorian-Bioinformatics-Consortium/nesoni

[4]https://github.com/tseemann/snp-dists

RESULTS

Phylogenetic Grouping of FQ^R Isolates

The present study's 59 Australian cat and dog-source FQ^R *E. coli* isolates were distributed broadly by phylogenetic group (in order of descending prevalence) as follows: B1 (31%), A (24%), D (20%), B2 (12%), F (10%), and C (3%). Nearly all isolates qualified as MDR (56/59, 95%), and MDR isolates exhibited a similar phylogenetic group distribution as did the total population of 59 isolates (not shown).

Whole Genome Sequenced-Based Analyses

STs, ARGs, and VGs identified for each isolate by *in silico* analysis are shown in **Supplementary Table S6**.

In silico MLST

According to *in silico* MLST the 59 Australian cat and dog-source FQ^R isolates represented 18 STs, including one novel ST (ST8242; phylogroup C). Ten STs accounted for $\geq$ 2 isolates each, the other eight (including ST8242) for a single isolate each. The five most prevalent STs – ST38 (eight isolates), ST131 [six isolates (these strains had already been identified as ST131 by SNP PCR)], ST224 (10 isolates), ST744 (eight isolates), and ST2179 (six isolates) – accounted for 64% of the 59 FQ^R isolates (**Figure 1**). Six STs occurred among both cat and dog-source isolates (ST38, ST131, ST224, ST354, ST744, and ST2179). None of the isolates represented other major STs associated with FQ^R human UTI isolates, e.g., ST1193.

The six phylogroup F isolates from STs 354 (5 isolates) and 648 (1 isolate) were characterized previously (Vangchhia et al., 2016). Consequently, they did not undergo further genomic analysis here and are not included in the following sections, which are limited to the remaining 53 sequenced FQ^R isolates.

ARGs

The 53 further-analyzed FQ^R isolates contained two PMQR genes, including *aac(6')-lb-cr* (25%) and *qnrS1* (2%). The most common CTX-M type ESBL genes were $bla_{CTX-M-15}$ (25%) and $bla_{CTX-M-14}$ (6%). The AmpC β-lactamase gene bla_{CMY-2} was identified in 12 isolates (23%) and bla_{OXA-1} in 13 isolates (25%). The ESBL genes $bla_{CTX-M-15}$, $bla_{CTX-M-14}$ were both identified in isolates belonging to ST38 and ST131. Isolates containing $bla_{CTX-M-15}$ were also assigned to ST405, ST410, and ST627, while $bla_{\mathrm{CTX-M-14}}$-containing isolates also belonged to ST224. The AmpC β-lactamase gene bla_{CMY-2} was identified in ST38, ST224, ST617, ST744, and ST2179, whereas $bla_{\mathrm{OXA-1}}$ was identified in ST38, ST131, ST405, ST410, and ST617.

VGs

The 53 further-analyzed FQ^R isolates contained 160 distinct VGs (representing 51 operons). Of the 160 VGs, 16 (10%) occurred in all 53 isolates; these included *ompA* (outer membrane protein), *entABCDEFS* (enterobactin siderophore system), *fepACDG* (ferric enterobactin uptake), and *ecpABCDE* (*E. coli* common pilus).

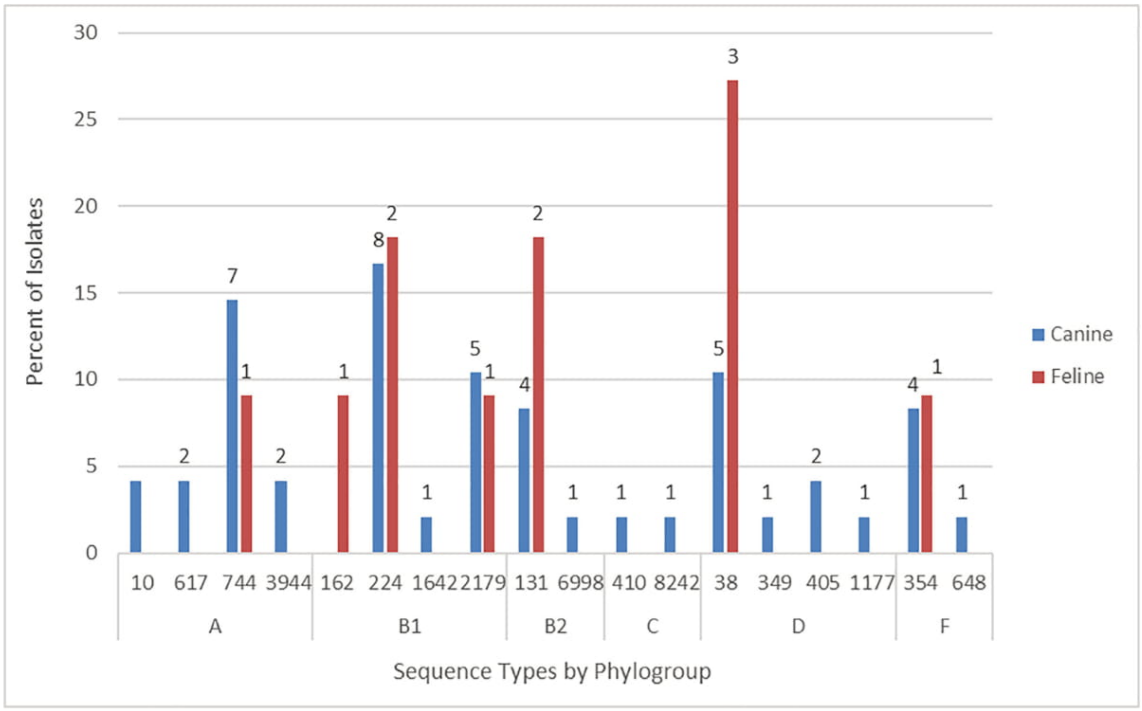

FIGURE 1 | Sequence type frequency among 59 fluoroquinolone-resistant cat and dog clinical *Escherichia coli* isolates that underwent whole genome sequencing.

The number of VGs per isolate differed by phylogroup, and within a given phylogroup differed by ST. By phylogroup, isolates from groups B2 and D contained the most VGs per isolate (medians, 80 and 88, respectively), followed by those from phylogroups B1 (median 61.5), C (median 58), and A (median 43.5) (**Supplementary Table S6**). By ST, isolates from ST131 (phylogroup B2) and ST38 (phylogroup D) contained the most VGs per isolate (medians 81 and 92.5, respectively), whereas those from ST744 (phylogroup A) and ST224 (group B1) contained the fewest (medians 39 and 54.5, respectively).

Nine of the 53 FQ^R isolates qualified molecularly as ExPEC (but not UPEC), one as UPEC (but not ExPEC), and one as both ExPEC and UPEC, giving 10 total ExPEC isolates (19%) and two UPEC isolates (4%). Of the 10 ExPEC isolates, nine (90%) belonged to phylogroups B2 (n = 3; all from ST131) or D (n = 6; 5 from ST38, 1 from ST349); the sole exception was from phylogroup A (ST744).

Serotypes, *fimH* Alleles, ARGs, and VGs in the FQ^R vs. FQ^S ST131 Isolates From Cats and Dogs in Australia

Regarding O:H serotypes and *fimH* alleles, the six FQ^R ST131 isolates exhibited two predicted combinations, O16:H5 *fimH*41 (n = 1) and O25:H4 *fimH*30 (n = 5; representing the C/*H*30R subclone). Regarding resistance characteristics, all six isolates were MDR and possessed at least two β-lactamase genes, including $bla_{CTX-M-15}$ (three of five C/*H*30R isolates) and $bla_{CTX-M-14}$ and $bla_{CTX-M-27}$ (one isolate each). Four isolates were MDR; two of these contained, respectively, *aac(6')-Ib-cr* or *qnrB4*. The five FQ^S ST131 strains also exhibited two predicted serotype-*fimH* allele combinations, O16:H5 *fimH41* (n = 2) and O25:H4 *fimH22* (n = 3). Four isolates were MDR, and one each contained *aac(6')-Ib-cr* or *qnrB4*.

Regarding virulence genotypes of FQ^R vs. FQ^S companion animal-source ST131 isolates, the six dog or cat-source FQ^R ST131 isolates each contained 72–94 putative VGs (median 81 VGs). Although none of the six had all 12 prototypic ST131-associated VGs, i.e., *iha*, *fimH*, *sat*, *fyuA*/*irp2*, *iutA*/*iucD*, *kpsM II*, *usp*, *traT*, *ompT*, and *malX* (Riley, 2014), three contained all 12 of these genes except *iutA* and *ompT*, while the other three additionally lacked only *usp*. Three of the six FQ^R ST131 isolates qualified as ExPEC but none as UPEC. By comparison, the five FQ^S dog-source ST131 isolates each contained 71-100 VGs (median 79 VGs). Like the FQ ST131 strains, none contained all 12 prototypic ST131-associated VGs, however, one isolate contained all of these except *iutA* and *ompT*, whereas the rest contained $\leq$ 9 of the 12 genes each. One dog-source FQ^S ST131 isolate qualified as both ExPEC and UPEC, one as ExPEC only, and one as UPEC only. Thus, FQ^R and FQ^S companion animal ST131 isolates from Australia possess similar virulence characteristics.

Phylogenetic Relationships Among 208 Multi-National ST131 Isolates, Including 20 From Australian Cats and Dogs and 188 From Diverse Host Species in Other Locales

Mapping of Illumina reads from the 208 diverse-source ST131 isolates to the EC958 ST131 reference chromosome identified

7,527 non-recombinant core-genome SNPs (**Figure 2**). After masking of SNPs within the 577,661 bp of identified prophage and genomic island sequence (11.3% of the EC958 genome) a ML phylogeny was inferred. The phylogeny had strong bootstrap support for its topology, which matched that of previous reports (Price et al., 2013; Ben Zakour et al., 2016), including resolution of the three major ST131 clades (A, B, and C, corresponding to the *H*41, *H*22 and *H*30 *fimH* fimbrial types). Most of the 20 Australian cat and dog-source ST131 isolates fell within Clade C/*H*30 (13/20, 65%; 8/13, 62% in C1/*H*30R1 and 5/13, 38% in C2/*H*30Rx), followed distantly by Clades A/*H*41 (4/20, 20%) and B/*H*22 (3/20, 15%) (**Figure 2**).

Notwithstanding the absence of an obvious animal-only clade, seven of the 20 Australian cat and dog-source ST131 isolates clustered closely within three sub-clades (**Figure 2**). (i) C1/*H*30R1 strains MS10902 and MS10903, which differed by only six SNPs, were dog-source urine isolates from Victoria (Platell et al., 2010). (ii) C2/*H*30Rx strains N13/4/101 and N13/1/351, which differed by only 20 SNPs, were dog urine isolates from New South Wales that clustered with strain N13/4/38 (dog trachea biopsy isolate, also from New South Wales) in another sub-clade (Saputra et al., 2017). These differed by only 42–72 SNPs from two human isolates collected in 2011 in the United Kingdom (strains S118EC and S119EC). Strain JJ2008, a cat isolate collected in the United States, formed an outgroup to this mixed human/animal sub-clade (**Figure 2**). (iii) The remaining two isolates, *H*22/B strains N13/1/75 and S13/1/53 (dog-source urine and ear swab isolates from New South Wales and South Australia, respectively) (Saputra et al., 2017), formed a sub-clade and differed from one another by only 96 SNPs (**Figure 2**).

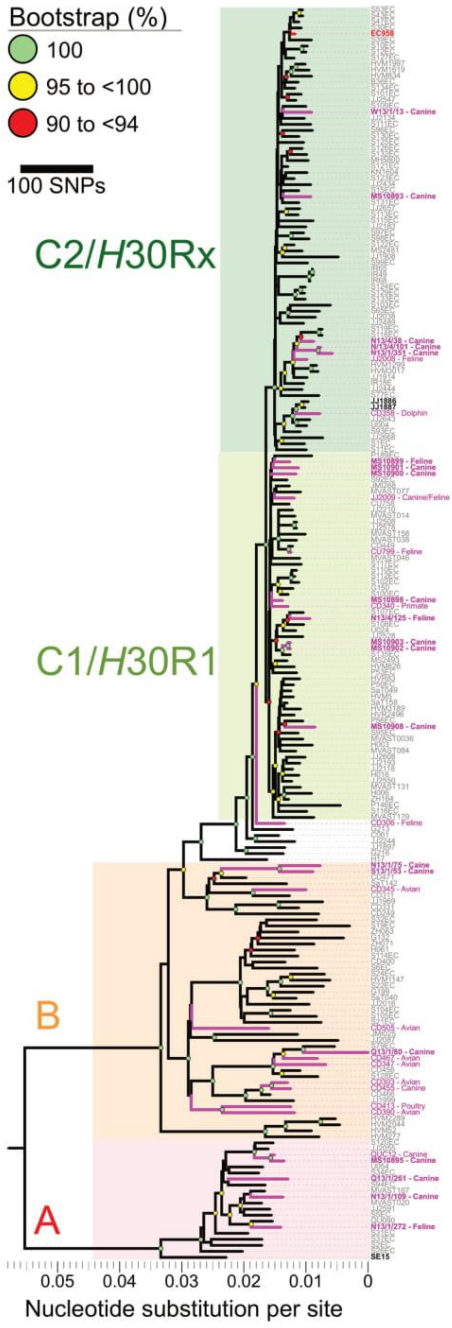

FIGURE 2 | Core-genome single nucleotide polymorphisms (SNPs) phylogeny of 208 *Escherichia coli* ST131 isolates from humans, cats, dogs, and other animals. The Maximum Likelihood tree was built using 7,527 non-recombinant core-genome single nucleotide polymorphisms (SNPs), as called by mapping sequencing reads to the chromosome of EC958 (GenBank: HG941718.1) with masked genomic islands and prophage regions. Colored groupings represent three labeled clades. *H*41/A (red), *H*22/B (orange), and C1/C2/*H*30 (green). Branch lengths are drawn to scale and represent the number of nucleotide substitutions per site (scalebar) or number of SNPs (key). Pink labels represent isolates collected from animals. Boldface pink labels with an asterisk represent the 20 cat and dog-source ST131 isolates sequenced as part of this study (11 from the Saputra et al. (2017) study, and nine [indicated by the MS prefix] from the Platell et al. (2010) study).

DISCUSSION

This study, which sought to characterize genetically a nationwide collection of clinical FQ^R *E. coli* isolates from extraintestinal infections in Australian dogs and cats (January 2013–January 2014) – including a WGS-based phylogenetic comparison across host species and geographical regions – led to four main conclusions. First, most (58%) FQ^R dog and cat isolates represented commensal phylogenetic groups A (24%), B1 (31%), and C (3%), rather than the group B2-derived pandemic lineages (ST131 and ST1193) that predominate among human FQ^R isolates. Second, the prevalence of ST131 as a cause of FQ^R *E. coli* infections in Australian companion animals was similarly low here among comparatively recent isolates (January 2013–January 2014) (6/59, 10%) as that noted previously by Platell et al. among historical isolates (2007–2009) (9/125, 7.2%), suggesting stability over time. Third, in a SNP-based phylogeny, the ST131 isolates from companion animals and humans were intermingled throughout the tree, rather than forming host-specific clades. This, together with the comparatively low prevalence of ST131 among isolates from companion animals, supports the hypothesis that companion animals in Australia are spillover hosts for ST131. Fourth, the absence of ST1193 among the present companion animal-source FQ^R *E. coli* isolates and its rarity in

other companion animal-source isolate collections suggests that the same spillover hypothesis likely applies also to this clonal group, which is emerging among humans but not in animals.

Regarding our first main conclusion, according to most studies ExPEC derive mainly from phylogroups B2, D, and F, and non-pathogenic commensal *E. coli* from phylogroups A and B1 (Johnson and Stell, 2000; Vangchhia et al., 2016). Today, resistance to FQs and ESCs among human-source clinical *E. coli* is due mainly to the group B2-derived lineages ST131 and ST1193, most members of which qualify as ExPEC (Johnson et al., 2019; Tchesnokova et al., 2019). By contrast, prior to these two clonal groups' recent emergence and expansion, most FQ^R human-source clinical isolates belonged to groups A and B1, and typically contained fewer VGs than did FQ^S isolates (Clermont et al., 2000).

The present study confirms that this historic pattern of phylogroup distribution persists among recent dog and cat-source FQ^R clinical *E. coli* isolates from Australia. Whilst the overall prevalence of FQ resistance in the Australia-wide collection of companion animal *E. coli* is low (< 10%) by international standards (Saputra et al., 2017), the FQ^R isolates belonged predominantly to groups A and B1 and possessed fewer VGs than group B2 and D isolates, with only seven of 53 (13%) FQ^R non-ST131 isolates qualifying molecularly as ExPEC. These findings echo a comparable study conducted over 10 years ago on *E. coli* isolates from North American dogs with UTI, in which FQ^R differed significantly in VG content (fewer) and phylogenetic background (mostly non-group B2) from FQS isolates (more VGs, mostly B2) (Johnson et al., 2009a).

Regarding our second main conclusion, comparison of the present findings with those of the regionally based study of Platell et al. (2010) confirmed that the prevalence of ST131 as a cause of FQ^R clinical infections in Australian companion animals did not increase significantly over the 5 year between-study interval. Two recent studies documented ST131 as a cause of extraintestinal infections in companion animals in Europe, but identified different proportions of C1/*H*30R1 vs. C2/*H*30Rx among cat and dog-source isolates (Belas et al., 2018; Melo et al., 2019). However, neither included a detailed genomic comparison with human ST131 isolates. Specifically, among urine *E. coli* isolates from cats and dogs in Portugal (1999–2015), 15% (25/172) of group B2 isolates were ST131, of which seven (28%) represented C/*H*30. Three (43%) of the C/*H*30 isolates represented the C2/*H*30Rx subclade, and two of these possessed $bla_{CTX-M-15}$ (Belas et al., 2018). Likewise, among more recent ESC-resistant *E. coli* isolates from cats and dogs in France (2010–16), most ST131 isolates represented C/*H*30 (50/56, 89%), 32 (64%) of which represented the C2/*H*30Rx subclade, and several possessed $bla_{CTX-M-27}$ (Melo et al., 2019). By contrast, 36% (4/11) of the present cat and dog ST131 isolates (one of which was identified as carrying the $bla_{CTX-M-27}$ gene), and only 11% (1/9) of those studied by Platell et al. (2010), represented the C2/*H*30Rx subclade. Taken together, these four studies suggest that the C2/*H*30Rx clade is becoming more common as a cause of extraintestinal infection in companion animals. A larger multicenter study involving genomic comparison of more recent companion animal ST131 isolates is needed to confirm this hypothesis.

Regarding human-companion animal ST131 clonal commonality, our comparison of 20 Australian cat and dog-source ST131 genomes with 173 human-source ST131 genomes (Ben Zakour et al., 2016) confirmed that the animal-source isolates were widely distributed throughout the ST131 phylogeny, in many cases placed closely to human ST131 strains. The absence of a separate clade containing only companion animal ST131 strains suggests a spillover model for ST131 occurrence in companion animals, as hypothesized previously (Bourne et al., 2019). Indeed, some animal ST131 strains shared very high identity with human strains. For example, MS10899, an Australian cat urine isolate (2007–2009), differed from P189EC, a Spanish human fecal isolate (2011), by only 66 non-recombinant core-genome SNPs (**Figure 2**). Although we cannot exclude that an individual from Spain came into contact with a cat in Australia in 2005, a more plausible explanation is the existence of an as-yet undefined reservoir or transmission network of circulating ST131 strains that links Australia and Spain (and, likely, much of the rest of the world).

Regarding our third main conclusion, human-animal clonal commonality implies interspecies transmission potential (of indeterminate direction), whereas the comparative prevalence of ST131 among human vs. animal clinical isolates implicates humans rather than companion animals as the main reservoir. Combined with the fact that the relative prevalence of ST131 among clinical isolates from dogs in Australia was fairly stable between 2007 and 2009 (Platell et al., 2010) and 2013 (this study), this adds further support for the hypothesis that ST131 causes infections predominantly in humans, with cats and dogs representing occasional spillover hosts (Melo et al., 2019). It is plausible, however, that pets may carry significant numbers of ST131 strains in the gut and could facilitate interspecies transfer to humans, who may simply be more susceptible than pets to developing extraintestinal infection due to ST131. By contrast, pets may be predisposed to developing infection due to other *E. coli* STs such as ST372, which is comparatively rarer among humans (LeCuyer et al., 2018; Kidsley et al., 2020; Valat et al., 2020).

Despite the overall genetic diversity of the Australian cat and dog-source ST131 isolates, some sub-clustering was apparent within the reference strain ST131 phylogeny. Based on the geographical, temporal, and host species metadata and minimal SNP diversity, it is likely that a single epidemiological source (per cluster) gave rise to each of the three clusters of closely related companion animal isolates within ST131 clades B, C1/*H*30R1, and C2/*H*30Rx. Given the distribution of the 34 animal ST131 isolates within the SNP-based phylogeny and the spillover hypothesis, further work will be required to characterize the reservoirs and transmission pathways that facilitate the spread of ST131 beyond the human population (Platell et al., 2011b).

Regarding our fourth main conclusion, ST1193 – another emerging clonal lineage of FQ^R *E. coli* within phylogroup B2 (Johnson et al., 2019; Tchesnokova et al., 2019; Valenza et al., 2019) — accounted for none of the 53 present FQ^R cat and dog isolates, despite having accounted for two FQ^R dog clinical isolates from Australia in the earlier Platell et al. study (Platell et al., 2012). To our knowledge only two other studies have

recovered ST1193 from companion animals. Maeyama et al. (2018) identified five ESC resistant ST1193 isolates among 381 clinical *E. coli* isolates from companion animals in Japan in 2016 (Maeyama et al., 2018); while Zhang et al. (2018) identified one (n = 24) CTX-M-positive ST1193 of clinical origin in Canada (Zhang et al., 2018). ST1193's rarity of isolation from companion animals suggests that ST1193, like ST131, is likely is predominantly a human-source clone, for which dogs are mainly spillover hosts. Regarding possible alternate transmission pathways for ST1193, in a recent Australian study a high proportion of silver gulls carried ESC-resistant and FQ^R *E. coli* in their feces. Whilst the most commonly identified ST was ST131 (17%), a substantial proportion of isolates represented ST1193 (6%), and these were closely related to human isolates (Mukerji et al., 2019).

This study has relevant limitations. First, selection bias may have influenced the observed distribution of FQ^R STs, since the study isolates came from veterinary diagnostic laboratories, which preferentially receive specimens from complicated cases and hosts with prior antimicrobial therapy (unpublished data). Second, detailed clinical data were lacking. Third, the clinical impact of the observed *in vitro* and genotypically predicted antimicrobial resistance is unknown. The study also has strengths: these include the large sample size (n = 855, making it the largest such study to date), the "snapshot" 1-year study period, the broad geographical sampling across Australia, and the genomic comparison with a large multi-national collection of (predominantly human-source) reference ST131 genomes.

CONCLUSION

In conclusion, we found that despite ST131 being the most prevalent ExPEC strain reported globally among humans, it occurred only at comparatively low frequency among FQ^R and FQ^S *E. coli* clinical isolates from Australian cats and dogs over a 1-year period in 2013–2014 (10%), with little change since 2007–2009 (7.2%). Phylogenomic comparisons of ST131 isolates from humans, cats, and dogs in Australia showed that despite some sub-clustering, the animal isolates were widely distributed throughout the ST131 phylogeny, often closely resembling human isolates. By contrast with ST131, ST1193, although identified first as an emerging clonal group among Australian FQ^R human and animal-source *E. coli* from 2007–2009 (Platell et al., 2012), was absent from the present FQ^R dog and cat isolate collection.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: Illumina sequence data for all 20 isolates have been deposited to the SRA under the accession numbers SRR11608154–SRR11608173 (BioProject PRJNA627752).

AUTHOR CONTRIBUTIONS

AK performed the experiments and data analysis, and drafted and prepared the manuscript. RW performed the whole genome sequencing and built and analyzed the phylogenetic tree. SS performed the initial antimicrobial susceptibility testing. JM provided the additional ST131 isolates. SB, MS, MO'D, DG, JJ, SA, and DT were involved in the experimental design development and manuscript preparation. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.01968/full#supplementary-material

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Conflict of Interest: DT has received research funding and undertaken consultancies for Bayer, Zoetis, Boehringer Ingelheim, Virbac, Luoda Pharma, Neoculi, and IRiccorgpharm. SA has received research funding from Zoetis and Neoculi. JJ has received research support from and/or has undertaken consultancies for Achaogen, Allergan, Crucell/Janssen, Melinta, Merck, Shionogi, Syntiron, and Tetraphase.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Table S1. Whole genome sequences of 20 Australian ST131 isolates from domesticated animals used in this investigation

Strain	ID	Species	MLST	Continent	Country	State	Collection date	Source	Sample type	Bio Project ID	Project ID	BioSample	SRA
MS10667	Q13/1/80	*Escherichia coli*	ST131	Oceania	Australia	QLD	2013	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686254	SRR11608173
MS10669	W13/1/13	*Escherichia coli*	ST131	Oceania	Australia	WA	2013	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686255	SRR11608172
MS10670	N13/1/75	*Escherichia coli*	ST131	Oceania	Australia	NSW	2013	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686256	SRR11608161
MS10671	N13/1/109	*Escherichia coli*	ST131	Oceania	Australia	NSW	2013	*Canus lumpus familiaris*	Swab	PRJNA627752	SRP258456	SAMN14686257	SRR11608160
MS10672	N13/4/38	*Escherichia coli*	ST131	Oceania	Australia	NSW	2013	*Canus lumpus familiaris*	Biopsy	PRJNA627752	SRP258456	SAMN14686258	SRR11608159
MS10673	N13/1/351	*Escherichia coli*	ST131	Oceania	Australia	NSW	2013	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686259	SRR11608158
MS10674	S13/1/53	*Escherichia coli*	ST131	Oceania	Australia	SA	2013	*Canus lumpus familiaris*	Swab	PRJNA627752	SRP258456	SAMN14686260	SRR11608157
MS10675	N13/4/125	*Escherichia coli*	ST131	Oceania	Australia	NSW	2013	*Felis catus*	Urine	PRJNA627752	SRP258456	SAMN14686261	SRR11608156
MS10677	N13/4/101	*Escherichia coli*	ST131	Oceania	Australia	NSW	2013	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686262	SRR11608155
MS10678	N13/1/272	*Escherichia coli*	ST131	Oceania	Australia	NSW	2013	*Felis catus*	Urine	PRJNA627752	SRP258456	SAMN14686263	SRR11608154
MS10893	QUC02	*Escherichia coli*	ST131	Oceania	Australia	QLD	2008	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686264	SRR11608171
MS10895	QUC12	*Escherichia coli*	ST2639*	Oceania	Australia	NSW	2009	*Canus lumpus familiaris*	Wound swab	PRJNA627752	SRP258456	SAMN14686265	SRR11608170
MS10898	QUC01	*Escherichia coli*	ST131	Oceania	Australia	QLD	2007	*Canus lumpus familiaris*	Anal gland sinus	PRJNA627752	SRP258456	SAMN14686266	SRR11608169
MS10899	QUC03	*Escherichia coli*	ST131	Oceania	Australia	QLD	2009	*Felis catus*	Urine	PRJNA627752	SRP258456	SAMN14686267	SRR11608168
MS10900	QUC04	*Escherichia coli*	ST131	Oceania	Australia	NSW	2008	*Canus lumpus familiaris*	Stomach wall swab	PRJNA627752	SRP258456	SAMN14686268	SRR11608167
MS10901	QUC08	*Escherichia coli*	ST131	Oceania	Australia	QLD	2007	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686269	SRR11608166
MS10902	QUC09	*Escherichia coli*	ST131	Oceania	Australia	VIC	2005	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686270	SRR11608165
MS10903	QUC10	*Escherichia coli*	ST131	Oceania	Australia	VIC	2005	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686271	SRR11608164
MS10908	QUC13	*Escherichia coli*	ST131	Oceania	Australia	QLD	2009	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686272	SRR11608163
Q13/1/261	Q13/1/261	*Escherichia coli*	ST131	Oceania	Australia	NSW	2013	*Canus lumpus familiaris*	Urine	PRJNA627752	SRP258456	SAMN14686315	SRR11608162

**adk*53, *fumC*347, *gyrB*47, *icd13*, *mdh36*, *purA*28, *recA*29. MS10895 is the original QUC12 strain (Price et al. 2013)

Table S2. Quality control and assembly metrics for 20 Australian isolates

Strain	Raw data								
	Median read length	Total reads (pairs)	No. reads unclassified (%)	No. reads species #1 (%)	Species #1	No. reads species #2 (%)	Species #2	No. reads species #3 (%)	Species #3
MS10667	151 (35 to 151)	2,634,522	14885 (0.56)	2006130 (76.15)	*Escherichia coli*	9716 (0.37)	*Salmonella enterica*	2975 (0.11)	*Escherichia albertii*
MS10669	151 (35 to 151)	4,336,046	11875 (0.27)	3475940 (80.16)	*Escherichia coli*	6860 (0.16)	*Morganella morganii*	5248 (0.12)	*Salmonella enterica*
MS10670	151 (35 to 151)	4,273,742	12188 (0.29)	3612524 (84.53)	*Escherichia coli*	5611 (0.13)	*Salmonella enterica*	5402 (0.13)	*Escherichia albertii*
MS10671	151 (35 to 151)	4,475,160	13790 (0.31)	3631035 (81.14)	*Escherichia coli*	6034 (0.13)	*Salmonella enterica*	4831 (0.11)	*Escherichia albertii*
MS10672	151 (35 to 151)	3,706,930	10080 (0.27)	2982635 (80.46)	*Escherichia coli*	6134 (0.17)	*Salmonella enterica*	4160 (0.11)	*Escherichia albertii*
MS10673	151 (35 to 151)	3,054,136	6377 (0.21)	2490076 (81.53)	*Escherichia coli*	3297 (0.11)	*Salmonella enterica*	3058 (0.1)	*Escherichia albertii*
MS10674	151 (35 to 151)	2,520,677	6362 (0.25)	2129736 (84.49)	*Escherichia coli*	4471 (0.18)	*Salmonella enterica*	2975 (0.12)	*Escherichia albertii*
MS10675	151 (35 to 151)	2,378,585	5428 (0.23)	1954650 (82.18)	*Escherichia coli*	2989 (0.13)	*Escherichia albertii*	2580 (0.11)	*Salmonella enterica*
MS10677	151 (35 to 151)	2,560,511	6052 (0.24)	2091617 (81.69)	*Escherichia coli*	3213 (0.13)	*Salmonella enterica*	3134 (0.12)	*Escherichia albertii*
MS10678	151 (35 to 151)	2,332,622	11603 (0.5)	1845554 (79.12)	*Escherichia coli*	4527 (0.19)	*Salmonella enterica*	3473 (0.15)	*Escherichia albertii*
MS10893	151 (35 to 151)	3,109,599	6038 (0.19)	2564511 (82.47)	*Escherichia coli*	3371 (0.11)	*Salmonella enterica*	3022 (0.1)	*Escherichia albertii*
MS10895	151 (35 to 151)	4,283,652	42402 (0.99)	3753992 (87.64)	*Escherichia coli*	3850 (0.09)	*Shigella sonnei*	3758 (0.09)	*Escherichia fergusonii*
MS10898	151 (35 to 151)	4,260,202	13403 (0.31)	3436747 (80.67)	*Escherichia coli*	7355 (0.17)	*Salmonella enterica*	3820 (0.09)	*Escherichia albertii*
MS10899	151 (35 to 151)	3,007,240	5388 (0.18)	2495335 (82.98)	*Escherichia coli*	7505 (0.25)	*Enterobacter hormaechei*	3286 (0.11)	*Salmonella enterica*
MS10900	151 (35 to 151)	4,428,958	8148 (0.18)	3559818 (80.38)	*Escherichia coli*	12024 (0.27)	*Klebsiella pneumoniae*	6178 (0.14)	*Salmonella enterica*
MS10901	151 (35 to 151)	3,819,464	21906 (0.57)	3132404 (82.01)	*Escherichia coli*	7991 (0.21)	*Salmonella enterica*	3154 (0.08)	*Escherichia albertii*
MS10902	151 (35 to 151)	3,600,161	5865 (0.16)	2846340 (79.06)	*Escherichia coli*	4842 (0.13)	*Salmonella enterica*	3222 (0.09)	*Escherichia albertii*
MS10903	151 (35 to 151)	4,557,445	7721 (0.17)	3592943 (78.84)	*Escherichia coli*	6346 (0.14)	*Salmonella enterica*	4120 (0.09)	*Escherichia albertii*
MS10908	151 (35 to 151)	5,114,690	28850 (0.56)	4085207 (79.87)	*Escherichia coli*	8561 (0.17)	*Salmonella enterica*	4058 (0.08)	*Escherichia albertii*
Q13/1/261	151 (35 to 151)	2,944,921	12841 (0.44)	2164454 (73.50)	*Escherichia coli*	2942 (0.10)	*Escherichia alberti*	2899 (0.10)	*Salmonella enterica*

Table S2 Continued. Quality control and assembly metrics for 20 Australian isolates

Strain	Quality control				*de novo* assembly			
	Total reads (pairs) after trimming	Median (range) read length (base pair)	Total mapped reads (%)	Estimated coverage (-fold)	No. contigs (min=1kbp)	Total length (base pairs)	GC (%)	N50
MS10667	2,621,088	141 (40 to 141)	3832152 (72.7%)	102.0	116	5,546,001	50.38	230,256
MS10669	4,311,394	141 (40 to 141)	6757967 (77.9%)	180.9	54	5,221,408	50.75	235,715
MS10670	4,254,023	141 (40 to 141)	6897306 (80.7%)	184.9	26	5,024,015	50.64	332,128
MS10671	4,455,446	141 (40 to 141)	7077871 (79.1%)	189.4	74	5,151,974	50.76	247,208
MS10672	3,689,701	141 (40 to 141)	5815934 (78.4%)	155.8	82	5,429,515	50.66	208,069
MS10673	3,043,555	141 (40 to 141)	4877020 (79.8%)	130.9	89	5,412,603	50.71	226,639
MS10674	2,514,575	141 (40 to 141)	4143336 (82.2%)	111.9	38	5,041,008	50.64	303,060
MS10675	2,371,583	141 (40 to 141)	4013453 (84.4%)	108.2	62	5,050,511	50.74	192,455
MS10677	2,555,169	141 (40 to 141)	4198131 (82%)	113.3	104	5,413,093	50.72	156,514
MS10678	2,322,439	141 (40 to 141)	3690596 (79.1%)	98.8	102	5,041,402	50.68	191,083
MS10893	3,095,997	141 (40 to 141)	5217886 (83.9%)	140.2	51	5,189,766	50.78	235,548
MS10895	4,272,179	141 (40 to 141)	6683365 (78%)	178.8	553	6,386,927	50.32	52,076
MS10898	4,246,819	141 (40 to 141)	6754772 (79.3%)	182.0	56	5,103,730	50.75	340,215
MS10899	2,990,696	141 (40 to 141)	4931881 (82%)	132.0	53	5,036,376	50.77	245,963
MS10900	4,409,168	141 (40 to 141)	7163850 (80.9%)	191.5	53	5,171,835	50.76	235,785
MS10901	3,799,159	141 (40 to 141)	6012205 (78.7%)	160.8	59	5,086,896	50.92	229,597
MS10902	3,588,631	141 (40 to 141)	5576990 (77.5%)	149.8	74	5,204,970	50.77	229,637
MS10903	4,543,584	141 (40 to 141)	6850253 (75.2%)	184.1	72	5,189,747	50.78	370,428
MS10908	5,089,348	141 (40 to 141)	7774222 (76%)	208.1	72	5,209,506	50.74	222,092
Q13/1/261	2,811,267	141 (40 to 141)		141.0	66	4,900,997	50.72	222,896

Table S3. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
C001	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228452	SRS456854	24345742
CD249	*Escherichia coli*	North America	United States	2005	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228453	SRS456855	24345742
CD303	*Escherichia coli*	North America	United States	2001	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228509	SRS456911	24345742
CD306	*Escherichia coli*	North America	United States	2002	*Felis catus*	Faeces	-	-	PRJNA211153	SRP027327	SAMN02228510	SRS456912	24345742
CD311	*Escherichia coli*	North America	United States	2002	Food	-	-	-	PRJNA211153	SRP027327	SAMN02228511	SRS456913	24345742
CD331	*Escherichia coli*	North America	United States	2004	Water	-	-	-	PRJNA211153	SRP027327	SAMN02228512	SRS456914	24345742
CD340	*Escherichia coli*	North America	United States	2005	Monkey	-	-	-	PRJNA211153	SRP027327	SAMN02228513	SRS456915	24345742
CD345	*Escherichia coli*	North America	United States	2005	Avian	Faeces	-	-	PRJNA211153	SRP027327	SAMN02228514	SRS456916	24345742
CD347	*Escherichia coli*	North America	United States	2006	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228515	SRS456918	24345742
CD358	*Escherichia coli*	North America	United States	2007	Dolphin	-	-	-	PRJNA211153	SRP027327	SAMN02228516	SRS456917	24345742
CD390	*Escherichia coli*	North America	United States	1990	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228517	SRS456919	24345742
CD400	*Escherichia coli*	North America	United States	1992	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228455	SRS456857	24345742
CD413	*Escherichia coli*	North America	United States	1995	Chicken	-	-	-	PRJNA211153	SRP027327	SAMN02228518	SRS456920	24345742
CD449	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228519	SRS456921	24345742
CD455	*Escherichia coli*	North America	United States	2008	*Canus lumpus familiaris*	-	-	-	PRJNA211153	SRP027327	SAMN02228520	SRS456922	24345742
CD456	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228521	SRS456923	24345742
CD466	*Escherichia coli*	North America	United States	1990	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228456	SRS456858	24345742
CD467	*Escherichia coli*	North America	United States	2009	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228457	SRS456859	24345742

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
CD471	*Escherichia coli*	North America	United States	1967	-	-	-	-	PRJNA211153	SRP027327	SAMN02228458	SRS456860	24345742
CD505	*Escherichia coli*	North America	United States	1983	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228459	SRS456861	24345742
CU758	*Escherichia coli*	North America	United States	2009	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228460	SRS456862	24345742
CU799	*Escherichia coli*	North America	United States	2008	*Felis catus*	Faeces	-	-	PRJNA211153	SRP027327	SAMN02228522	SRS456924	24345742
G132	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228461	SRS456863	24345742
G150	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228462	SRS456864	24345742
G199	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228463	SRS456865	24345742
G213	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228464	SRS456866	24345742
G216	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228465	SRS456867	24345742
H003	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228466	SRS456868	24345742
H006	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228467	SRS456869	24345742
H016	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228468	SRS456870	24345742
H061	*Escherichia coli*	North America	United States	2011	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228469	SRS456871	24345742
H17	*Escherichia coli*	North America	United States	1985	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228470	SRS456872	24345742
JJ1897	*Escherichia coli*	North America	United States	2004	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228471	SRS456873	24345742
JJ1908	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228482	SRS456884	24345742
JJ1914	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228525	SRS456927	24345742
JJ1969	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228526	SRS456928	24345742

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
JJ1999	*Escherichia coli*	Asia	India	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228454	SRS456856	24345742
JJ2008	*Escherichia coli*	North America	United States	2007	*Felis catus*	-	-	-	PRJNA211153	SRP027327	SAMN02228529	SRS456931	24345742
JJ2009	*Escherichia coli*	North America	United States	2005	canine/ feline	-	-	-	PRJNA211153	SRP027327	SAMN02228530	SRS456932	24345742
JJ2016	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228483	SRS456885	24345742
JJ2038	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228484	SRS456886	24345742
JJ2055	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228450	SRS456852	24345742
JJ2087	*Escherichia coli*	North America	United States	2003	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228486	SRS456888	24345742
JJ2118	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228451	SRS456853	24345742
JJ2134	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228449	SRS456850	24345742
JJ2183	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228487	SRS456889	24345742
JJ2193	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228488	SRS456890	24345742
JJ2210	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228532	SRS456934	24345742
JJ2244	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228534	SRS456936	24345742
JJ2434	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228535	SRS456937	24345742
JJ2444	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228536	SRS456938	24345742
JJ2489	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228552	SRS456954	24345742
JJ2508	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228537	SRS456939	24345742

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
JJ2528	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228538	SRS456940	24345742
JJ2547	*Escherichia coli*	North America	United States	2009	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228473	SRS456875	24345742
JJ2550	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228539	SRS456941	24345742
JJ2578	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228541	SRS456943	24345742
JJ2591	*Escherichia coli*	North America	United States	2006	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228542	SRS456944	24345742
JJ2608	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228543	SRS456945	24345742
JJ2643	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228489	SRS456891	24345742
JJ2657	*Escherichia coli*	North America	United States	2009	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228544	SRS456946	24345742
JJ2668	*Escherichia coli*	North America	United States	2009	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228545	SRS456947	24345742
JMI025	*Escherichia coli*	North America	United States	2000	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228474	SRS456876	24345742
JMI268	*Escherichia coli*	North America	United States	2006	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228475	SRS456877	24345742
KN1604	*Escherichia coli*	Asia	Korea	2003	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228547	SRS456949	24345742
MH5800	*Escherichia coli*	Europe	Portugal	2005	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228546	SRS456948	24345742
MVAST0036	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228448	SRS456851	24345742
MVAST014	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228476	SRS456878	-
MVAST020	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228477	SRS456879	-
MVAST038	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228478	SRS456880	-

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
MVAST046	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228479	SRS456881	-
MVAST077	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228480	SRS456882	-
MVAST084	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228491	SRS456893	-
MVAST131	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228492	SRS456894	-
MVAST158	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228493	SRS456895	-
MVAST167	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228494	SRS456896	-
MVAST179	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228495	SRS456897	-
QU090	*Escherichia coli*	Oceania	Australia	2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228490	SRS456892	24345742
QUC12	*Escherichia coli*	Oceania	Australia	2008	*Canus lumpus familiaris*	-	-	-	PRJNA211153	SRP027327	SAMN02228550	SRS456952	24345742
SaT040	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228496	SRS456898	24345742
SaT049	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228497	SRS456899	24345742
SaT142	*Escherichia coli*	North America	United States	2003	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228498	SRS456900	24345742
SaT158	*Escherichia coli*	North America	United States	2003	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228499	SRS456901	24345742
U004	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228500	SRS456902	24345742
U024	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228501	SRS456903	24345742
U054	*Escherichia coli*	North America	United States	2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228502	SRS456904	24345742
ZH063	*Escherichia coli*	North America	Canada	2002	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228503	SRS456905	-

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
ZH071	*Escherichia coli*	North America	Canada	2002	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228504	SRS456906	-
ZH164	*Escherichia coli*	North America	Canada	2004	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228505	SRS456907	24345742
B36EC	*Escherichia coli*	Oceania	Australia	2007	*Homo sapiens*	Blood	21	Female	PRJEB2968	ERP001354	SAMEA1486609	ERR161254	24706808
MS2481	*Escherichia coli*	Oceania	Australia	2007	*Homo sapiens*	Blood	51	Male	PRJEB2968	ERP001354	SAMEA1486653	ERR161252	24706808
MS2493	*Escherichia coli*	Oceania	Australia	2007	*Homo sapiens*	Blood	61	Male	PRJEB2968	ERP001354	SAMEA1486625	ERR161253	24706808
S100EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Faeces	50	Female	PRJEB2968	ERP001354	SAMEA1486600	ERR161263	24706808
S101EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Faeces	59	Female	PRJEB2968	ERP001354	SAMEA1486580	ERR161264	24706808
S102EC	*Escherichia coli*	Oceania	Australia	2010	*Homo sapiens*	Urine	85	Female	PRJEB2968	ERP001354	SAMEA1486606	ERR161265	24706808
S103EC	*Escherichia coli*	Oceania	Australia	2010	*Homo sapiens*	Urine	28	Female	PRJEB2968	ERP001354	SAMEA1486630	ERR161266	24706808
S104EC	*Escherichia coli*	Oceania	Australia	2008	*Homo sapiens*	Urine	91	Female	PRJEB2968	ERP001354	SAMEA1486667	ERR161267	24706808
S105EC	*Escherichia coli*	Oceania	Australia	2008	*Homo sapiens*	Urine	94	Female	PRJEB2968	ERP001354	SAMEA1486587	ERR161268	24706808
S107EC	*Escherichia coli*	Oceania	Australia	2010	*Homo sapiens*	Urine	80	Male	PRJEB2968	ERP001354	SAMEA1486616	ERR161270	24706808
S108EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Blood	45	Female	PRJEB2968	ERP001354	SAMEA1486644	ERR161271	24706808
S109EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Urine	29	Female	PRJEB2968	ERP001354	SAMEA1486668	ERR161272	24706808
S110EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Urine	81	Female	PRJEB2968	ERP001354	SAMEA1486658	ERR161273	24706808
S111EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Urine	19	Female	PRJEB2968	ERP001354	SAMEA1486637	ERR161274	24706808
S112EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Urine	-	-	PRJEB2968	ERP001354	SAMEA1486640	ERR161275	24706808

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S113EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Faeces	19	Male	PRJEB2968	ERP001354	SAMEA1486629	ERR161276	24706808
S114EC	*Escherichia coli*	Oceania	Australia	2011	*Homo sapiens*	Urine	16	Female	PRJEB2968	ERP001354	SAMEA1486661	ERR161277	24706808
S115EC	*Escherichia coli*	Oceania	Australia	2011	*Homo sapiens*	Urine	87	Female	PRJEB2968	ERP001354	SAMEA1486593	ERR161278	24706808
S65EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Urine	83	Female	PRJEB2968	ERP001354	SAMEA1486581	ERR161303	24706808
S77EC	*Escherichia coli*	Oceania	Australia	2010	*Homo sapiens*	Urine	52	Female	PRJEB2968	ERP001354	SAMEA1486657	ERR161304	24706808
S79EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Urine	16	Male	PRJEB2968	ERP001354	SAMEA1486627	ERR161305	24706808
S98EC	*Escherichia coli*	Oceania	Australia	2008	*Homo sapiens*	Faeces	47	Male	PRJEB2968	ERP001354	SAMEA1486648	ERR161261	24706808
S99EC	*Escherichia coli*	Oceania	Australia	2009	*Homo sapiens*	Faeces	44	Female	PRJEB2968	ERP001354	SAMEA1486651	ERR161262	24706808
S120EC	*Escherichia coli*	North America	Canada	2009	*Homo sapiens*	Blood	93	Female	PRJEB2968	ERP001354	SAMEA1486584	ERR161283	24706808
S121EC	*Escherichia coli*	North America	Canada	2000	*Homo sapiens*	Urine	57	Female	PRJEB2968	ERP001354	SAMEA1486669	ERR161284	24706808
S122EC	*Escherichia coli*	North America	Canada	2003	*Homo sapiens*	Urine	62	Male	PRJEB2968	ERP001354	SAMEA1486638	ERR161285	24706808
S123EC	*Escherichia coli*	North America	Canada	2001	*Homo sapiens*	Urine	46	Male	PRJEB2968	ERP001354	SAMEA1486635	ERR161286	24706808
S124EC	*Escherichia coli*	North America	Canada	2003	*Homo sapiens*	Urine	70	Male	PRJEB2968	ERP001354	SAMEA1486656	ERR161287	24706808
S125EC	*Escherichia coli*	North America	Canada	2002	*Homo sapiens*	Surgical wound	72	Male	PRJEB2968	ERP001354	SAMEA1486665	ERR161288	24706808
S126EC	*Escherichia coli*	North America	Canada	2002	*Homo sapiens*	Urine	68	Female	PRJEB2968	ERP001354	SAMEA1486594	ERR161289	24706808
S127EC	*Escherichia coli*	North America	Canada	2002	*Homo sapiens*	Urine	70	Male	PRJEB2968	ERP001354	SAMEA1486619	ERR161290	24706808
S128EC	*Escherichia coli*	North America	Canada	2004	*Homo sapiens*	Blood	81	Female	PRJEB2968	ERP001354	SAMEA1486636	ERR161291	24706808

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S129EC	*Escherichia coli*	North America	Canada	2004	*Homo sapiens*	Urine	9	Female	PRJEB2968	ERP001354	SAMEA1486654	ERR161292	24706808
S130EC	*Escherichia coli*	North America	Canada	2004	*Homo sapiens*	Urine	67	Male	PRJEB2968	ERP001354	SAMEA1486595	ERR161293	24706808
S131EC	*Escherichia coli*	North America	Canada	2002	*Homo sapiens*	Urine	78	Female	PRJEB2968	ERP001354	SAMEA1486655	ERR161294	24706808
S132EC	*Escherichia coli*	North America	Canada	2005	*Homo sapiens*	Urine	90	Female	PRJEB2968	ERP001354	SAMEA1486639	ERR161295	24706808
S133EC	*Escherichia coli*	North America	Canada	2005	*Homo sapiens*	Blood	73	Male	PRJEB2968	ERP001354	SAMEA1486615	ERR161296	24706808
S134EC	*Escherichia coli*	North America	Canada	2005	*Homo sapiens*	Urine	70	Female	PRJEB2968	ERP001354	SAMEA1486598	ERR161297	24706808
S135EC	*Escherichia coli*	North America	Canada	2005	*Homo sapiens*	Blood	32	Female	PRJEB2968	ERP001354	SAMEA1486666	ERR161298	24706808
IR18E	*Escherichia coli*	Asia	India	2009	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA2400646	ERR458470	24706808
IR49	*Escherichia coli*	Asia	India	2009	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA2400647	ERR458471	24706808
IR65	*Escherichia coli*	Asia	India	2009	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA2400648	ERR458472	24706808
IR68	*Escherichia coli*	Asia	India	2009	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA2400649	ERR458473	24706808
SE15	*Escherichia coli*	Asia	Japan	-	*Homo sapiens*	Faeces	-	-	PRJNA224116	-	SAMD00060923	-	20008064
S92EC	*Escherichia coli*	Oceania	New Zealand	2009	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486582	ERR161255	24706808
S93EC	*Escherichia coli*	Oceania	New Zealand	2009	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486671	ERR161256	24706808
S94EC	*Escherichia coli*	Oceania	New Zealand	2009	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486649	ERR161257	24706808
S95EC	*Escherichia coli*	Oceania	New Zealand	2009	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486613	ERR161258	24706808
S96EC	*Escherichia coli*	Oceania	New Zealand	2010	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486610	ERR161259	24706808

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S97EC	*Escherichia coli*	Oceania	New Zealand	2010	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486585	ERR161260	24706808
HVM1147	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Peritoneal fluid	28	Female	PRJEB2968	ERP001354	SAMEA1486670	ERR161318	24706808
HVM1299	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Abdominal abscess	66	Male	PRJEB2968	ERP001354	SAMEA1486617	ERR161320	24706808
HVM1619	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Surgical wound	79	Male	PRJEB2968	ERP001354	SAMEA1486603	ERR161321	24706808
HVM1997	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Urine	91	Female	PRJEB2968	ERP001354	SAMEA1486586	ERR161322	24706808
HVM2044	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Blood	72	Female	PRJEB2968	ERP001354	SAMEA1486626	ERR161323	24706808
HVM2289	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Urine	86	Female	PRJEB2968	ERP001354	SAMEA1486663	ERR161325	24706808
HVM277	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Urine	87	Female	PRJEB2968	ERP001354	SAMEA1486672	ERR161315	24706808
HVM3017	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Urine	95	Male	PRJEB2968	ERP001354	SAMEA1486643	ERR161328	24706808
HVM3189	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Urine	52	Female	PRJEB2968	ERP001354	SAMEA1486675	ERR161329	24706808
HVM5	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Blood	74	Male	PRJEB2968	ERP001354	SAMEA1486614	ERR161306	24706808
HVM52	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Urine	17	Female	PRJEB2968	ERP001354	SAMEA1486674	ERR161308	24706808
HVM826	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Blood	81	Male	PRJEB2968	ERP001354	SAMEA1486589	ERR161316	24706808
HVM834	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Urine	86	Female	PRJEB2968	ERP001354	SAMEA1486607	ERR161317	24706808
HVR2496	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Blood	80	Male	PRJEB2968	ERP001354	SAMEA1486583	ERR161326	24706808
HVR83	*Escherichia coli*	Europe	Spain	2010	*Homo sapiens*	Blood	74	Female	PRJEB2968	ERP001354	SAMEA1486611	ERR161311	24706808
P146EC	*Escherichia coli*	Europe	Spain	2011	*Homo sapiens*	Faeces	82	Male	PRJEB2968	ERP001354	SAMEA1486623	ERR161313	24706808

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
P189EC	*Escherichia coli*	Europe	Spain	2011	*Homo sapiens*	Faeces	91	Male	PRJEB2968	ERP001354	SAMEA1486647	ERR161314	24706808
P50EC	*Escherichia coli*	Europe	Spain	2011	*Homo sapiens*	Faeces	81	Female	PRJEB2968	ERP001354	SAMEA1486591	ERR161307	24706808
P53EC	*Escherichia coli*	Europe	Spain	2011	*Homo sapiens*	Faeces	81	Female	PRJEB2968	ERP001354	SAMEA1486645	ERR161309	24706808
P56EC	*Escherichia coli*	Europe	Spain	2011	*Homo sapiens*	Faeces	88	Female	PRJEB2968	ERP001354	SAMEA1486621	ERR161310	24706808
EC958	*Escherichia coli*	Europe	United Kingdom	2005	*Homo sapiens*	Urine	8	Female	PRJNA224116	-	SAMEA2272019	-	25126841
S10EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	92	Male	PRJEB2968	ERP001354	SAMEA1486642	ERR161237	24706808
S116EC	*Escherichia coli*	Europe	United Kingdom	2011	*Homo sapiens*	Unknown	88	Male	PRJEB2968	ERP001354	SAMEA1486612	ERR161279	24706808
S117EC	*Escherichia coli*	Europe	United Kingdom	2011	*Homo sapiens*	Unknown	94	Female	PRJEB2968	ERP001354	SAMEA1486641	ERR161280	24706808
S118EC	*Escherichia coli*	Europe	United Kingdom	2011	*Homo sapiens*	Urine	70	Male	PRJEB2968	ERP001354	SAMEA1486628	ERR161281	24706808
S119EC	*Escherichia coli*	Europe	United Kingdom	2011	*Homo sapiens*	Blood	70	Male	PRJEB2968	ERP001354	SAMEA1486659	ERR161282	24706808
S11EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	90	Male	PRJEB2968	ERP001354	SAMEA1486664	ERR161238	24706808
S12EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	92	Male	PRJEB2968	ERP001354	SAMEA1486596	ERR161239	24706808
S15EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	46	Female	PRJEB2968	ERP001354	SAMEA1486602	ERR161240	24706808
S19EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	40	Male	PRJEB2968	ERP001354	SAMEA1486631	ERR161241	24706808
S1EC	*Escherichia coli*	Europe	United Kingdom	2007	*Homo sapiens*	Urine	69	Female	PRJEB2968	ERP001354	SAMEA1486597	ERR161234	24706808
S21EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	35	Male	PRJEB2968	ERP001354	SAMEA1486650	ERR161242	24706808
S22EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	63	Female	PRJEB2968	ERP001354	SAMEA1486633	ERR161243	24706808

Table S3 Continued. Whole genome sequences of 188 global ST131 isolates used in this investigation

Strain	Species	Continent	Country	Collection date	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S24EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	16	Female	PRJEB2968	ERP001354	SAMEA1486588	ERR161244	24706808
S26EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	72	Female	PRJEB2968	ERP001354	SAMEA1486608	ERR161245	24706808
S2EC	*Escherichia coli*	Europe	United Kingdom	2007	*Homo sapiens*	Urine	68	Female	PRJEB2968	ERP001354	SAMEA1486620	ERR161235	24706808
S30EC	*Escherichia coli*	Europe	United Kingdom	2007	*Homo sapiens*	Urine	86	Female	PRJEB2968	ERP001354	SAMEA1486634	ERR161246	24706808
S31EC	*Escherichia coli*	Europe	United Kingdom	2007	*Homo sapiens*	Urine	41	Female	PRJEB2968	ERP001354	SAMEA1486624	ERR161300	24706808
S32EC	*Escherichia coli*	Europe	United Kingdom	2007	*Homo sapiens*	Urine	51	Female	PRJEB2968	ERP001354	SAMEA1486601	ERR161301	24706808
S34EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	64	Female	PRJEB2968	ERP001354	SAMEA1486652	ERR161247	24706808
S37EC	*Escherichia coli*	Europe	United Kingdom	2009	*Homo sapiens*	Urine	34	Female	PRJEB2968	ERP001354	SAMEA1486599	ERR161302	24706808
S39EC	*Escherichia coli*	Europe	United Kingdom	2004	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA1486673	ERR161248	24706808
S43EC	*Escherichia coli*	Europe	United Kingdom	2004	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA1486590	ERR161249	24706808
S47EC	*Escherichia coli*	Europe	United Kingdom	2004	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA1486618	ERR161250	24706808
S53EC	*Escherichia coli*	Europe	United Kingdom	2004	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA1486622	ERR161251	24706808
S5EC	*Escherichia coli*	Europe	United Kingdom	2007	*Homo sapiens*	Urine	27	Female	PRJEB2968	ERP001354	SAMEA1486632	ERR161236	24706808
S6EC	*Escherichia coli*	Europe	United Kingdom	2007	*Homo sapiens*	Urine	49	Female	PRJEB2968	ERP001354	SAMEA1486662	ERR161299	24706808
JJ1886	*Escherichia coli*	North America	United States	2008	*Homo sapiens*	Blood	-	Female	PRJNA218163	-	SAMN02603887	-	24309736
JJ1887	*Escherichia coli*	North America	United States	2007	*Homo sapiens*	Urine	-	Female	PRJNA311313	-	SAMN04481707	-	27174264

Table S4. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S39EC	1	C2	*Escherichia coli*	ST131	United Kingdom		2004	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA1486673	ERR161248	24706808
S30EC	2	C2	*Escherichia coli*	ST131	United Kingdom		2007	*Homo sapiens*	Urine	86	Female	PRJEB2968	ERP001354	SAMEA1486634	ERR161246	24706808
EC958	3	C2	*Escherichia coli*	ST131	United Kingdom		2005	*Homo sapiens*	Urine	8	Female	PRJNA224116	-	SAMEA2272019	-	25126841
S53EC	4	C2	*Escherichia coli*	ST131	United Kingdom		2004	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA1486622	ERR161251	24706808
S43EC	5	C2	*Escherichia coli*	ST131	United Kingdom		2004	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA1486590	ERR161249	24706808
S47EC	6	C2	*Escherichia coli*	ST131	United Kingdom		2004	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA1486618	ERR161250	24706808
S12EC	7	C2	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	92	Male	PRJEB2968	ERP001354	SAMEA1486596	ERR161239	24706808
S10EC	8	C2	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	92	Male	PRJEB2968	ERP001354	SAMEA1486642	ERR161237	24706808
S127EC	9	C2	*Escherichia coli*	ST131	Canada		2002	*Homo sapiens*	Urine	70	Male	PRJEB2968	ERP001354	SAMEA1486619	ERR161290	24706808
HVM1997	10	C2	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Urine	91	Female	PRJEB2968	ERP001354	SAMEA1486586	ERR161322	24706808
HVM1619	11	C2	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Surgical wound	79	Male	PRJEB2968	ERP001354	SAMEA1486603	ERR161321	24706808
HVM834	12	C2	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Urine	86	Female	PRJEB2968	ERP001354	SAMEA1486607	ERR161317	24706808
B36EC	13	C2	*Escherichia coli*	ST131	Australia		2007	*Homo sapiens*	Blood	21	Female	PRJEB2968	ERP001354	SAMEA1486609	ERR161254	24706808
S134EC	14	C2	*Escherichia coli*	ST131	Canada		2005	*Homo sapiens*	Urine	70	Female	PRJEB2968	ERP001354	SAMEA1486598	ERR161297	24706808
JJ2547	15	C2	*Escherichia coli*	ST131	United States		2009	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228473	SRS456875	24345742
S101EC	16	C2	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Faeces	59	Female	PRJEB2968	ERP001354	SAMEA1486580	ERR161264	24706808
MS10669	17	C2	*Escherichia coli*	ST131	Australia	WA	2013	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686255	SRR11608172	
S109EC	18	C2	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Urine	29	Female	PRJEB2968	ERP001354	SAMEA1486668	ERR161272	24706808
JJ2134	19	C2	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228449	SRS456850	24345742

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S111EC	20	C2	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Urine	19	Female	PRJEB2968	ERP001354	SAMEA1486637	ERR161274	24706808
S126EC	21	C2	*Escherichia coli*	ST131	Canada		2002	*Homo sapiens*	Urine	68	Female	PRJEB2968	ERP001354	SAMEA1486594	ERR161289	24706808
S132EC	22	C2	*Escherichia coli*	ST131	Canada		2005	*Homo sapiens*	Urine	90	Female	PRJEB2968	ERP001354	SAMEA1486639	ERR161295	24706808
MH5800	23	C2	*Escherichia coli*	ST131	Portugal		2005	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228546	SRS456948	24345742
S121EC	24	C2	*Escherichia coli*	ST131	Canada		2000	*Homo sapiens*	Urine	57	Female	PRJEB2968	ERP001354	SAMEA1486669	ERR161284	24706808
KN1604	25	C2	*Escherichia coli*	ST131	Korea		2003	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228547	SRS456949	24345742
S123EC	26	C2	*Escherichia coli*	ST131	Canada		2001	*Homo sapiens*	Urine	46	Male	PRJEB2968	ERP001354	SAMEA1486635	ERR161286	24706808
JJ2434	27	C2	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228535	SRS456937	24345742
S15EC	28	C2	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	46	Female	PRJEB2968	ERP001354	SAMEA1486602	ERR161240	24706808
MS10893	29	C2	*Escherichia coli*	ST131	Australia	QLD	2008	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686264	SRR11608171	
S131EC	30	C2	*Escherichia coli*	ST131	Canada		2002	*Homo sapiens*	Urine	78	Female	PRJEB2968	ERP001354	SAMEA1486655	ERR161294	24706808
S96EC	31	C2	*Escherichia coli*	ST131	New Zealand		2010	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486610	ERR161259	24706808
S130EC	32	C2	*Escherichia coli*	ST131	Canada		2004	*Homo sapiens*	Urine	67	Male	PRJEB2968	ERP001354	SAMEA1486595	ERR161293	24706808
S125EC	33	C2	*Escherichia coli*	ST131	Canada		2002	*Homo sapiens*	Surgical wound	72	Male	PRJEB2968	ERP001354	SAMEA1486665	ERR161288	24706808
S113EC	34	C2	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Faeces	19	Male	PRJEB2968	ERP001354	SAMEA1486629	ERR161276	24706808
JJ2657	35	C2	*Escherichia coli*	ST131	United States		2009	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228544	SRS456946	24345742
S115EC	36	C2	*Escherichia coli*	ST131	Australia		2011	*Homo sapiens*	Urine	87	Female	PRJEB2968	ERP001354	SAMEA1486593	ERR161278	24706808

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
JJ2183	37	C2	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228487	SRS456889	24345742
S98EC	38	C2	*Escherichia coli*	ST131	Australia		2008	*Homo sapiens*	Faeces	47	Male	PRJEB2968	ERP001354	SAMEA1486648	ERR161261	24706808
S97EC	39	C2	*Escherichia coli*	ST131	New Zealand		2010	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486585	ERR161260	24706808
S122EC	40	C2	*Escherichia coli*	ST131	Canada		2003	*Homo sapiens*	Urine	62	Male	PRJEB2968	ERP001354	SAMEA1486638	ERR161285	24706808
MS2481	41	C2	*Escherichia coli*	ST131	Australia		2007	*Homo sapiens*	Blood	51	Male	PRJEB2968	ERP001354	SAMEA1486653	ERR161252	24706808
JJ1908	42	C2	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228482	SRS456884	24345742
S99EC	43	C2	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Faeces	44	Female	PRJEB2968	ERP001354	SAMEA1486651	ERR161262	24706808
S124EC	44	C2	*Escherichia coli*	ST131	Canada		2003	*Homo sapiens*	Urine	70	Male	PRJEB2968	ERP001354	SAMEA1486656	ERR161287	24706808
S129EC	45	C2	*Escherichia coli*	ST131	Canada		2004	*Homo sapiens*	Urine	9	Female	PRJEB2968	ERP001354	SAMEA1486654	ERR161292	24706808
S133EC	46	C2	*Escherichia coli*	ST131	Canada		2005	*Homo sapiens*	Blood	73	Male	PRJEB2968	ERP001354	SAMEA1486615	ERR161296	24706808
IR49	47	C2	*Escherichia coli*	ST131	India		2009	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA2400647	ERR458471	24706808
IR65	48	C2	*Escherichia coli*	ST131	India		2009	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA2400648	ERR458472	24706808
IR68	49	C2	*Escherichia coli*	ST131	India		2009	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA2400649	ERR458473	24706808
S65EC	50	C2	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Urine	83	Female	PRJEB2968	ERP001354	SAMEA1486581	ERR161303	24706808
S103EC	51	C2	*Escherichia coli*	ST131	Australia		2010	*Homo sapiens*	Urine	28	Female	PRJEB2968	ERP001354	SAMEA1486630	ERR161266	24706808
JJ2038	52	C2	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228484	SRS456886	24345742
JJ2489	53	C2	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228552	SRS456954	24345742
S119EC	54	C2	*Escherichia coli*	ST131	United Kingdom		2011	*Homo sapiens*	Blood	70	Male	PRJEB2968	ERP001354	SAMEA1486659	ERR161282	24706808

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S118EC	55	C2	*Escherichia coli*	ST131	United Kingdom		2011	*Homo sapiens*	Urine	70	Male	PRJEB2968	ERP001354	SAMEA1486628	ERR161281	24706808
MS10672	56	C2	*Escherichia coli*	ST131	Australia	NSW	2013	*Canus lumpus familiaris*	Biopsy	-	-	PRJNA627752	SRP258456	SAMN14686258	SRR11608159	
MS10677	57	C2	*Escherichia coli*	ST131	Australia	NSW	2013	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686262	SRR11608155	
MS10673	58	C2	*Escherichia coli*	ST131	Australia	NSW	2013	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686259	SRR11608158	
JJ2008	59	C2	*Escherichia coli*	ST131	United States		2007	*Felis catus*	-	-	-	PRJNA211153	SRP027327	SAMN02228529	SRS456931	24345742
HVM3017	60	C2	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Urine	95	Male	PRJEB2968	ERP001354	SAMEA1486643	ERR161328	24706808
HVM1299	61	C2	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Abdominal abscess	66	Male	PRJEB2968	ERP001354	SAMEA1486617	ERR161320	24706808
JJ1914	62	C2	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228525	SRS456927	24345742
IR18E	63	C2	*Escherichia coli*	ST131	India		2009	*Homo sapiens*	Unknown	-	-	PRJEB2968	ERP001354	SAMEA2400646	ERR458470	24706808
JJ2444	64	C2	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228536	SRS456938	24345742
S77EC	65	C2	*Escherichia coli*	ST131	Australia		2010	*Homo sapiens*	Urine	52	Female	PRJEB2968	ERP001354	SAMEA1486657	ERR161304	24706808
JJ1887	66	C2	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	Urine	-	Female	PRJNA311313	-	SAMN04481707	-	27174264
JJ1886	67	C2	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	Blood	-	Female	PRJNA218163	-	SAMN02603887	-	24309736
CD358	68	C2	*Escherichia coli*	ST131	United States		2007	Dolphin	-	-	-	PRJNA211153	SRP027327	SAMN02228516	SRS456917	24345742
U004	69	C2	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228500	SRS456902	24345742
JJ2643	70	C2	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228489	SRS456891	24345742
S93EC	71	C2	*Escherichia coli*	ST131	New Zealand		2009	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486671	ERR161256	24706808
S1EC	72	C2	*Escherichia coli*	ST131	United Kingdom		2007	*Homo sapiens*	Urine	69	Female	PRJEB2968	ERP001354	SAMEA1486597	ERR161234	24706808

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
JJ2668	73	C2	*Escherichia coli*	ST131	United States		2009	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228545	SRS456947	24345742
S11EC	74	C2	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	90	Male	PRJEB2968	ERP001354	SAMEA1486664	ERR161238	24706808
MS10899	75	C1	*Escherichia coli*	ST131	Australia	QLD	2009	*Felis catus*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686267	SRR11608168	
P189EC	76	C1	*Escherichia coli*	ST131	Spain		2011	*Homo sapiens*	Faeces	91	Male	PRJEB2968	ERP001354	SAMEA1486647	ERR161314	24706808
MS10901	77	C1	*Escherichia coli*	ST131	Australia	QLD	2007	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686269	SRR11608166	
MS10900	78	C1	*Escherichia coli*	ST131	Australia	NSW	2008	*Canus lumpus familiaris*	Stomach wall swab	-	-	PRJNA627752	SRP258456	SAMN14686268	SRR11608167	
S117EC	79	C1	*Escherichia coli*	ST131	United Kingdom		2011	*Homo sapiens*	Unknown	94	Female	PRJEB2968	ERP001354	SAMEA1486641	ERR161280	24706808
S92EC	80	C1	*Escherichia coli*	ST131	New Zealand		2009	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486582	ERR161255	24706808
MVAST077	81	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228480	SRS456882	-
JMI268	82	C1	*Escherichia coli*	ST131	United States		2006	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228475	SRS456877	24345742
JJ2009	83	C1	*Escherichia coli*	ST131	United States		2005	canine/feline	-	-	-	PRJNA211153	SRP027327	SAMN02228530	SRS456932	24345742
CU758	84	C1	*Escherichia coli*	ST131	United States		2009	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228460	SRS456862	24345742
MVAST158	85	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228493	SRS456895	-
MVAST038	86	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228478	SRS456880	-
JJ2210	87	C1	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228532	SRS456934	24345742
CD449	88	C1	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228519	SRS456921	24345742
CU799	89	C1	*Escherichia coli*	ST131	United States		2008	*Felis catus*	Faeces	-	-	PRJNA211153	SRP027327	SAMN02228522	SRS456924	24345742

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
MVAST014	90	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228476	SRS456878	-
JJ2578	91	C1	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228541	SRS456943	24345742
JJ2508	92	C1	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228537	SRS456939	24345742
MVAST046	93	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228479	SRS456881	-
S107EC	94	C1	*Escherichia coli*	ST131	Australia		2010	*Homo sapiens*	Urine	80	Male	PRJEB2968	ERP001354	SAMEA1486616	ERR161270	24706808
MS10675	95	C1	*Escherichia coli*	ST131	Australia	NSW	2013	*Felis catus*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686261	SRR11608156	
S108EC	96	C1	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Blood	45	Female	PRJEB2968	ERP001354	SAMEA1486644	ERR161271	24706808
U024	97	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228501	SRS456903	24345742
JJ2528	98	C1	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228538	SRS456940	24345742
MS10903	99	C1	*Escherichia coli*	ST131	Australia	VIC	2005	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686271	SRR11608164	
MS10902	100	C1	*Escherichia coli*	ST131	Australia	VIC	2005	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686270	SRR11608165	
S135EC	101	C1	*Escherichia coli*	ST131	Canada		2005	*Homo sapiens*	Blood	32	Female	PRJEB2968	ERP001354	SAMEA1486666	ERR161298	24706808
MS2493	102	C1	*Escherichia coli*	ST131	Australia		2007	*Homo sapiens*	Blood	61	Male	PRJEB2968	ERP001354	SAMEA1486625	ERR161253	24706808
HVM826	103	C1	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Blood	81	Male	PRJEB2968	ERP001354	SAMEA1486589	ERR161316	24706808
P53EC	104	C1	*Escherichia coli*	ST131	Spain		2011	*Homo sapiens*	Faeces	81	Female	PRJEB2968	ERP001354	SAMEA1486645	ERR161309	24706808
S110EC	105	C1	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Urine	81	Female	PRJEB2968	ERP001354	SAMEA1486658	ERR161273	24706808

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S112EC	106	C1	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Urine	-	-	PRJEB2968	ERP001354	SAMEA1486640	ERR161275	24706808
S102EC	107	C1	*Escherichia coli*	ST131	Australia		2010	*Homo sapiens*	Urine	85	Female	PRJEB2968	ERP001354	SAMEA1486606	ERR161265	24706808
G150	108	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228462	SRS456864	24345742
S100EC	109	C1	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Faeces	50	Female	PRJEB2968	ERP001354	SAMEA1486600	ERR161263	24706808
CD340	110	C1	*Escherichia coli*	ST131	United States		2005	Monkey	-	-	-	PRJNA211153	SRP027327	SAMN02228513	SRS456915	24345742
MS10898	111	C1	*Escherichia coli*	ST131	Australia	QLD	2007	*Canus lumpus familiaris*	Anal gland sinus	-	-	PRJNA627752	SRP258456	SAMN14686266	SRR11608169	
P50EC	112	C1	*Escherichia coli*	ST131	Spain		2011	*Homo sapiens*	Faeces	81	Female	PRJEB2968	ERP001354	SAMEA1486591	ERR161307	24706808
HVR83	113	C1	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Blood	74	Female	PRJEB2968	ERP001354	SAMEA1486611	ERR161311	24706808
HVM5	114	C1	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Blood	74	Male	PRJEB2968	ERP001354	SAMEA1486614	ERR161306	24706808
SaT049	115	C1	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228497	SRS456899	24345742
SaT158	116	C1	*Escherichia coli*	ST131	United States		2003	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228499	SRS456901	24345742
HVM3189	117	C1	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Urine	52	Female	PRJEB2968	ERP001354	SAMEA1486675	ERR161329	24706808
HVR2496	118	C1	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Blood	80	Male	PRJEB2968	ERP001354	SAMEA1486583	ERR161326	24706808
S95EC	119	C1	*Escherichia coli*	ST131	New Zealand		2009	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486613	ERR161258	24706808
MS10908	120	C1	*Escherichia coli*	ST131	Australia	QLD	2009	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686272	SRR11608163	
P56EC	121	C1	*Escherichia coli*	ST131	Spain		2011	*Homo sapiens*	Faeces	88	Female	PRJEB2968	ERP001354	SAMEA1486621	ERR161310	24706808
MVAST084	122	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228491	SRS456893	-

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
MVAST0036	123	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228448	SRS456851	24345742
H003	124	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228466	SRS456868	24345742
JJ2608	125	C1	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228543	SRS456945	24345742
JJ2193	126	C1	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228488	SRS456890	24345742
JJ2118	127	C1	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228451	SRS456853	24345742
H016	128	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228468	SRS456870	24345742
JJ2550	129	C1	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228539	SRS456941	24345742
S116EC	130	C1	*Escherichia coli*	ST131	United Kingdom		2011	*Homo sapiens*	Unknown	88	Male	PRJEB2968	ERP001354	SAMEA1486612	ERR161279	24706808
H006	131	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228467	SRS456869	24345742
MVAST131	132	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228492	SRS456894	-
ZH164	133	C1	*Escherichia coli*	ST131	Canada		2004	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228505	SRS456907	24345742
P146EC	134	C1	*Escherichia coli*	ST131	Spain		2011	*Homo sapiens*	Faeces	82	Male	PRJEB2968	ERP001354	SAMEA1486623	ERR161313	24706808
MVAST179	135	C1	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228495	SRS456897	-
CD306	136	C0	*Escherichia coli*	ST131	United States		2002	*Felis catus*	Faeces	-	-	PRJNA211153	SRP027327	SAMN02228510	SRS456912	24345742
G213	137	C0	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228464	SRS456866	24345742
C001	138	C0	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228452	SRS456854	24345742
JJ2244	139	C0	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228534	SRS456936	24345742
JJ1897	140	B0	*Escherichia coli*	ST131	United States		2004	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228471	SRS456873	24345742

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
G216	141	B0	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228465	SRS456867	24345742
H17	142	B0	*Escherichia coli*	ST131	United States		1985	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228470	SRS456872	24345742
JJ1969	143	B	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228526	SRS456928	24345742
CD331	144	B	*Escherichia coli*	ST131	United States		2004	Water	-	-	-	PRJNA211153	SRP027327	SAMN02228512	SRS456914	24345742
CD249	145	B	*Escherichia coli*	ST131	United States		2005	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228453	SRS456855	24345742
CD311	146	B	*Escherichia coli*	ST131	United States		2002	Food	-	-	-	PRJNA211153	SRP027327	SAMN02228511	SRS456913	24345742
CD345	147	B	*Escherichia coli*	ST131	United States		2005	Avian	Faeces	-	-	PRJNA211153	SRP027327	SAMN02228514	SRS456916	24345742
MS10670	148	B	*Escherichia coli*	ST131	Australia	NSW	2013	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686256	SRR11608161	
MS10674	149	B	*Escherichia coli*	ST131	Australia	SA	2013	*Canus lumpus familiaris*	Swab	-	-	PRJNA627752	SRP258456	SAMN14686260	SRR11608157	
CD471	150	B	*Escherichia coli*	ST131	United States		1967	-	-	-	-	PRJNA211153	SRP027327	SAMN02228458	SRS456860	24345742
SaT142	151	B	*Escherichia coli*	ST131	United States		2003	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228498	SRS456900	24345742
S19EC	152	B	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	40	Male	PRJEB2968	ERP001354	SAMEA1486631	ERR161241	24706808
S32EC	153	B	*Escherichia coli*	ST131	United Kingdom		2007	*Homo sapiens*	Urine	51	Female	PRJEB2968	ERP001354	SAMEA1486601	ERR161301	24706808
ZH063	154	B	*Escherichia coli*	ST131	Canada		2002	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228503	SRS456905	-
G132	155	B	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228461	SRS456863	24345742
ZH071	156	B	*Escherichia coli*	ST131	Canada		2002	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228504	SRS456906	-
H061	157	B	*Escherichia coli*	ST131	United States		2011	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228469	SRS456871	24345742

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
S114EC	158	B	*Escherichia coli*	ST131	Australia		2011	*Homo sapiens*	Urine	16	Female	PRJEB2968	ERP001354	SAMEA1486661	ERR161277	24706808
CD400	159	B	*Escherichia coli*	ST131	United States		1992	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228455	SRS456857	24345742
S6EC	160	B	*Escherichia coli*	ST131	United Kingdom		2007	*Homo sapiens*	Urine	49	Female	PRJEB2968	ERP001354	SAMEA1486662	ERR161299	24706808
HVM1147	161	B	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Peritoneal fluid	28	Female	PRJEB2968	ERP001354	SAMEA1486670	ERR161318	24706808
S24EC	162	B	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	16	Female	PRJEB2968	ERP001354	SAMEA1486588	ERR161244	24706808
S22EC	163	B	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	63	Female	PRJEB2968	ERP001354	SAMEA1486633	ERR161243	24706808
G199	164	B	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228463	SRS456865	24345742
SaT040	165	B	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228496	SRS456898	24345742
JJ2016	166	B	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228483	SRS456885	24345742
S104EC	167	B	*Escherichia coli*	ST131	Australia		2008	*Homo sapiens*	Urine	91	Female	PRJEB2968	ERP001354	SAMEA1486667	ERR161267	24706808
S105EC	168	B	*Escherichia coli*	ST131	Australia		2008	*Homo sapiens*	Urine	94	Female	PRJEB2968	ERP001354	SAMEA1486587	ERR161268	24706808
S21EC	169	B	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	35	Male	PRJEB2968	ERP001354	SAMEA1486650	ERR161242	24706808
JMI025	170	B	*Escherichia coli*	ST131	United States		2000	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228474	SRS456876	24345742
JJ2087	171	B	*Escherichia coli*	ST131	United States		2003	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228486	SRS456888	24345742
CD505	172	B	*Escherichia coli*	ST131	United States		1983	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228459	SRS456861	24345742
S79EC	173	B	*Escherichia coli*	ST131	Australia		2009	*Homo sapiens*	Urine	16	Male	PRJEB2968	ERP001354	SAMEA1486627	ERR161305	24706808
MS10667	174	B	*Escherichia coli*	ST131	Australia	QLD	2013	*Canus lumpus familiaris*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686254	SRR11608173	

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
CD467	175	B	*Escherichia coli*	ST131	United States		2009	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228457	SRS456859	24345742
CD347	176	B	*Escherichia coli*	ST131	United States		2006	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228515	SRS456918	24345742
S128EC	177	B	*Escherichia coli*	ST131	Canada		2004	*Homo sapiens*	Blood	81	Female	PRJEB2968	ERP001354	SAMEA1486636	ERR161291	24706808
CD456	178	B	*Escherichia coli*	ST131	United States		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228521	SRS456923	24345742
CD455	179	B	*Escherichia coli*	ST131	United States		2008	*Canus lumpus familiaris*	-	-	-	PRJNA211153	SRP027327	SAMN02228520	SRS456922	24345742
CD303	180	B	*Escherichia coli*	ST131	United States		2001	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228509	SRS456911	24345742
CD466	181	B	*Escherichia coli*	ST131	United States		1990	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228456	SRS456858	24345742
JJ1999	182	B	*Escherichia coli*	ST131	India		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228454	SRS456856	24345742
CD413	183	B	*Escherichia coli*	ST131	United States		1995	Chicken	-	-	-	PRJNA211153	SRP027327	SAMN02228518	SRS456920	24345742
CD390	184	B	*Escherichia coli*	ST131	United States		1990	Avian	-	-	-	PRJNA211153	SRP027327	SAMN02228517	SRS456919	24345742
HVM2289	185	B	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Urine	86	Female	PRJEB2968	ERP001354	SAMEA1486663	ERR161325	24706808
HVM2044	186	B	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Blood	72	Female	PRJEB2968	ERP001354	SAMEA1486626	ERR161323	24706808
HVM52	187	B	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Urine	17	Female	PRJEB2968	ERP001354	SAMEA1486674	ERR161308	24706808
HVM277	188	B	*Escherichia coli*	ST131	Spain		2010	*Homo sapiens*	Urine	87	Female	PRJEB2968	ERP001354	SAMEA1486672	ERR161315	24706808
MVAST167	189	A	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228494	SRS456896	-
MS10671	190	A	*Escherichia coli*	ST131	Australia	NSW	2013	*Canus lumpus familiaris*	Swab	-	-	PRJNA627752	SRP258456	SAMN14686257	SRR11608160	

Table S4 Continued. Whole genome sequences of 208 isolates used in this investigation: Metadata and public database accession numbers

Strain	Position	Clade	Species	MLST	Country	State	Year	Source	Sample type	Age	Sex	Bio Project ID	Project ID	BioSample	SRA	PubMed ID
MVAST020	191	A	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228477	SRS456879	-
S5EC	192	A	*Escherichia coli*	ST131	United Kingdom		2007	*Homo sapiens*	Urine	27	Female	PRJEB2968	ERP001354	SAMEA1486632	ERR161236	24706808
JJ2591	193	A	*Escherichia coli*	ST131	United States		2006	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228542	SRS456944	24345742
QU090	194	A	*Escherichia coli*	ST131	Australia		2008	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228490	SRS456892	24345742
MS10678	195	A	*Escherichia coli*	ST131	Australia	NSW	2013	*Felis catus*	Urine	-	-	PRJNA627752	SRP258456	SAMN14686263	SRR11608154	
S94EC	196	A	*Escherichia coli*	ST131	New Zealand		2009	*Homo sapiens*	Blood	-	-	PRJEB2968	ERP001354	SAMEA1486649	ERR161257	24706808
JJ2055	197	A	*Escherichia coli*	ST131	United States		2007	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228450	SRS456852	24345742
S120EC	198	A	*Escherichia coli*	ST131	Canada		2009	*Homo sapiens*	Blood	93	Female	PRJEB2968	ERP001354	SAMEA1486584	ERR161283	24706808
QUC12	199	A	*Escherichia coli*	ST2639*	Australia	NSW	2009	*Canus lumpus familiaris*	Wound swab	-	-	PRJNA211153	SRP027327	SAMN02228550	SRS456952	24345742
MS10895	200	A	*Escherichia coli*	ST2639*	Australia	NSW	2009	*Canus lumpus familiaris*	Wound swab	-	-	PRJNA627752	SRP258456	SAMN14686265	SRR11608170	
S34EC	201	A	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	64	Female	PRJEB2968	ERP001354	SAMEA1486652	ERR161247	24706808
U054	202	A	*Escherichia coli*	ST131	United States		2010	*Homo sapiens*	-	-	-	PRJNA211153	SRP027327	SAMN02228502	SRS456904	24345742
S31EC	203	A	*Escherichia coli*	ST131	United Kingdom		2007	*Homo sapiens*	Urine	41	Female	PRJEB2968	ERP001354	SAMEA1486624	ERR161300	24706808
S2EC	204	A	*Escherichia coli*	ST131	United Kingdom		2007	*Homo sapiens*	Urine	68	Female	PRJEB2968	ERP001354	SAMEA1486620	ERR161235	24706808
S37EC	205	A	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	34	Female	PRJEB2968	ERP001354	SAMEA1486599	ERR161302	24706808
S26EC	206	A	*Escherichia coli*	ST131	United Kingdom		2009	*Homo sapiens*	Urine	72	Female	PRJEB2968	ERP001354	SAMEA1486608	ERR161245	24706808
SE15	207	A	*Escherichia coli*	ST131	Japan		-	*Homo sapiens*	Faeces	-	-	PRJNA224116	-	SAMD00060923	-	20008064

**adk*53, *fumC*347, *gyrB*47, *icd13*, *mdh36*, *purA*28, *recA*29

Table S5. Mobile genetic elements identified & masked in EC958

ID	Genome coordinates	Size (bp)	GC%	Insertion site
GI-*thrW*	317,502 to 328,310	10,809	49.9	tRNA-*thrW*
Phi1	949,891 to 988,545	38,655	51.9	d/s of *serS*
Phi2	1,004,823 to 1,041,887	37,065	54.8	d/s of *aroA*
Phi3	1,256,754 to 1,304,683	47,930	50.5	d/s of *icdA*
ROD2	1,348,912 to 1,374,958	26,047	47.4	u/s of *ycgT*
Phi4	1,436,674 to 1,490,889	54,216	51.8	d/s of *ompW*
Phi5	2,043,305 to 2,085,906	42,602	48.0	tRNA-*leuZ*
Phi6	2,138,328 to 2,179,641	41,314	49.7	tRNA-*ser*U
GI-a*sn*(HPI)	2,180,800 to 2,212,247	31,448	57.7	tRNA-*asn*
Phi7	2,656,878 to 2,667,927	11,050	44.31	tRNA-*argW*
GI-pheV	3,284,884 to 3,359,938	75,055	48.3	tRNA-*phe*V
GI-selC	4,131,132 to 4,199,117	67,986	50.0	tRNA-*selC*
GI-leuX	4,913,861 to 5,007,344	93,484	48.9	tRNA-*leuX*

d/s = downstream u/s = upstream

Table S6: Virulence gene prevalence by phylogenetic group and sequence type among 59 FQR and five FQS Australian cat and dog-source clinical *Escherichia coli*

									Antimicrobial resistance genes																				
									Aminoglycoside resistance																	β-lactamases				
Ref lab ID	Species	State	Phylogroup	ST	FQ status	MDR	ExPEC	UPEC	*aac6-Ib*	*aac(3)-IIa*	*aac(6')-Ib-cr*	*aac(3)-IId*	*aadA1*	*aadA2*	*aadA5*	*aph(3')-Ia*	*aph(3')-Ic*	*Aph3-Ia*	*aph(3'')-Ib*	*aph(6)-Id*	*Sat-2A*	*strA*	*strB*	*ampC1*	*ampC2*	*ampH*	*bla*$_{CMY-2}$	*bla*$_{CMY-17}$	*bla*$_{CMY-22}$
Q13/1/288	Canine	WA	A	10	FQR										■				■	■		■	■	■	■	■			
W13/1/6	Canine	WA	A	10	FQR	■						■							■	■		■	■	■	■	■			
W13/1/10	Canine	WA	A	617	FQR	■					■				■									■	■	■	■	■	
W13/1/8	Canine	WA	A	617	FQR	■				■	■				■				■	■		■	■	■	■	■			
Q13/2/52-2	Feline	QLD	A	744	FQR	■							■		■	■		■	■	■		■	■	■	■	■			
N13/1/733	Canine	NSW	A	744	FQR	■									■			■	■	■		■	■	■	■	■	■	■	
N13/1/274	Canine	NSW	A	744	FQR	■	■								■			■	■	■		■	■	■	■	■			
V13/5/59	Canine	VIC	A	744	FQR	■									■			■	■	■		■	■	■	■	■			
S13/1/48	Canine	SA	A	744	FQR	■												■						■	■	■			
S13/1/61	Canine	SA	A	744	FQR																			■	■	■			
S13/1/84	Canine	SA	A	744	FQR	■										■								■	■	■			
S13/1/85	Canine	SA	A	744	FQR	■							■		■	■	■	■	■			■	■	■	■	■			
Q13/2/49	Canine	QLD	A	3944	FQR																			■	■	■			
Q13/2/87	Canine	QLD	A	3944	FQR																			■	■	■			
N13/1/707	Feline	NSW	B1	162	FQR	■									■	■		■	■	■		■	■	■	■	■			
Q13/2/28	Feline	QLD	B1	224	FQR	■													■	■		■	■	■	■	■			
Q13/2/85	Feline	QLD	B1	224	FQR	■													■	■		■	■	■	■	■			
Q13/2/9	Canine	QLD	B1	224	FQR	■				■			■	■				■	■	■		■	■	■	■	■	■	■	
Q13/2/29	Canine	QLD	B1	224	FQR	■				■			■	■				■	■	■		■	■	■	■	■	■		■
Q13/1/89	Canine	QLD	B1	224	FQR	■						■		■					■	■		■	■	■	■	■			
N13/1/378	Canine	QLD	B1	224	FQR	■				■			■	■				■	■	■		■	■	■	■	■			
N13/1/795	Canine	NSW	B1	224	FQR	■						■		■					■	■		■	■	■	■	■			
N13/4/97	Canine	NSW	B1	224	FQR	■							■	■	■									■	■	■	■	■	
V13/4/28	Canine	VIC	B1	224	FQR	■																		■	■	■	■	■	
S13/1/69	Canine	SA	B1	224	FQR								■	■										■	■	■			
V13/2/170	Canine	VIC	B1	1642	FQR	■						■			■			■	■	■		■	■	■	■	■			
Q13/1/229	Feline	NSW	B1	2179	FQR	■									■				■	■		■	■	■	■	■	■		■
Q13/1/272	Canine	NSW	B1	2179	FQR	■									■				■	■		■	■	■	■	■			
N13/1/6	Canine	NSW	B1	2179	FQR	■									■				■	■		■	■	■	■	■	■	■	

Table S6 Continued: Virulence gene prevalence by phylogenetic group and sequence type among 59 FQR and five FQS Australian cat and dog-source clinical *Escherichia coli*

									Antimicrobial resistance genes																					
									Aminoglycoside resistance																	β-lactamases					
Ref lab ID	**Species**	**State**	**Phylogroup**	**ST**	**FQ status**	**MDR**	**ExPEC**	**UPEC**	*aac6-Ib*	*aac(3)-IIa*	*aac(6')-Ib-cr*	*aac(3)-IId*	*aadA1*	*aadA2*	*aadA5*	*aph(3')-Ia*	*aph(3')-Ic*	*Aph3-Ia*	*aph(3'')-Ib*	*aph(6)-Id*	*Sat-2A*	*strA*	*strB*	*ampC1*	*ampC2*	*ampH*	bla_{CMY-2}	bla_{CMY-17}	bla_{CMY-22}
N13/1/34	Canine	NSW	B1	2179	FQR	■													■	■		■	■	■	■	■	■	■	■	
N13/1/133	Canine	NSW	B1	2179	FQR	■									■				■	■		■	■	■	■	■	■	■	■	
N13/1/709	Canine	NSW	B1	2179	FQR	■									■				■	■		■	■	■	■	■	■	■	■	
N13/1/109	Canine	QLD	B2	131	FQS	■		■	■		■								■	■		■	■		■	■				
N13/1/75	Canine	NSW	B2	131	FQS																				■	■				
Q13/1/261	Canine	NSW	B2	131	FQS	■	■	■											■	■		■	■		■	■				
S13/1/53	Canine	SA	B2	131	FQS	■																			■	■				
Q13/1/80	Canine	SA	B2	131	FQS	■	■							■	■	■			■	■		■	■	■	■	■				
N13/1/272	Feline	NSW	B2	131	FQR	■									■				■	■		■	■		■	■				
N13/4/125	Feline	NSW	B2	131	FQR	■									■				■	■		■	■		■	■				
N13/1/351	Canine	NSW	B2	131	FQR	■	■			■	■				■										■	■				
N13/4/38	Canine	NSW	B2	131	FQR	■	■			■	■				■				■	■		■	■		■	■				
N13/4/101	Canine	NSW	B2	131	FQR	■	■	■		■	■				■										■	■				
W13/1/13	Canine	WA	B2	131	FQR	■					■				■										■	■				
S13/1/48-2	Canine	SA	B2	6998	FQR	■		■																	■	■				
Q13/1/281	Canine	NSW	C	410	FQR	■					■				■									■	■	■				
V13/2/449	Canine	VIC	C	8242	FQR	■												■	■	■		■	■	■	■	■				
Q13/2/40	Feline	QLD	D	38	FQR	■				■	■		■						■	■	■	■	■	■	■	■				
Q13/2/52	Feline	QLD	D	38	FQR	■				■	■		■						■	■	■	■	■	■	■	■				
N13/1/284	Feline	QLD	D	38	FQR	■				■	■		■						■	■	■	■	■	■	■	■				
N13/4/131	Canine	NSW	D	38	FQR	■	■											■	■	■		■	■	■		■				
Q13/1/297	Canine	VIC	D	38	FQR	■	■					■			■				■	■		■	■	■	■	■	■	■		
V13/6/2	Canine	VIC	D	38	FQR	■	■				■	■			■				■	■		■	■	■	■					
V13/5/4	Canine	VIC	D	38	FQR	■	■								■				■			■		■	■	■				
W13/1/2	Canine	WA	D	38	FQR	■	■			■	■		■						■	■	■	■	■	■	■	■				
W13/1/7	Canine	WA	D	349	FQR	■	■						■					■	■	■		■	■		■	■				
N13/4/17	Canine	NSW	D	405	FQR	■				■	■				■									■	■	■				
N13/4/72	Canine	NSW	D	405	FQR	■				■					■									■	■	■				
N13/1/97	Canine	NSW	D	1177	FQR	■																		■	■	■				

Table S6 Continued: Virulence gene prevalence by phylogenetic group and sequence type among 59 FQR and five FQS Australian cat and dog-source clinical *Escherichia coli*

	Antimicrobial resistance genes																												
	β-lactamases													FQ		MLS		Phenicol						Sulphonamide			Tetracycline			
Ref lab ID	*bla*$_{CTX-M-15}$	*bla*$_{CTX-M-14}$	*bla*$_{CTX-M-27}$	*bla*$_{CTX-M-71}$	*bla*$_{CTX-M-103}$	*bla*$_{TEM-1B}$	*bla*$_{TEM-70}$	*bla*$_{TEM-104}$	*bla*$_{TEM-105}$	*bla*$_{TEM-122}$	*bla*$_{TEM-141}$	*bla*$_{OXA-1}$	*bla*$_{PBP}$	*qnrB4*	*qnrS1*	*mdf(A)*	*mph(A)*	*catA1*	*catA2*	*catB3*	*catB4*	*cml*	*cmlA1*	*sul1*	*sul2*	*sul3*	*tetA*	*tetB*	*tetD*	*tetR*
Q13/1/288						■	■		■				■			■		■						■	■			■		
W13/1/6						■	■		■				■			■		■							■			■		
W13/1/10	■											■	■			■	■			■	■			■				■		
W13/1/8	■											■	■			■	■			■	■			■	■			■		
Q13/2/52-2						■	■		■				■			■	■	■						■	■			■		
N13/1/733						■	■		■				■			■	■	■						■	■			■		
N13/1/274													■			■		■						■	■			■		
V13/5/59						■	■		■				■			■	■	■						■	■			■		
S13/1/48													■			■	■	■										■		
S13/1/61													■			■		■										■		
S13/1/84													■			■	■	■										■		
S13/1/85						■	■		■				■			■	■	■						■	■			■		
Q13/2/49													■			■														
Q13/2/87													■			■														
N13/1/707						■	■		■				■			■		■							■			■		
Q13/2/28						■	■		■				■			■			■						■			■		
Q13/2/85						■					■		■			■			■						■			■		
Q13/2/9						■	■		■				■			■			■				■		■	■	■	■			■
Q13/2/29						■	■		■				■			■			■				■		■	■	■	■			■
Q13/1/89						■	■		■				■			■									■			■			
N13/1/378						■	■		■				■			■			■				■		■	■	■	■			■
N13/1/795						■	■		■				■			■							■			■	■				■
N13/4/97													■			■														
V13/4/28													■			■							■			■	■				■
S13/1/69						■	■		■				■			■	■							■	■			■		
V13/2/170						■	■		■				■			■		■						■	■			■		
Q13/1/229						■	■		■				■			■		■						■	■			■		
Q13/1/272						■	■		■				■			■		■						■	■			■		
N13/1/6						■	■		■				■			■		■							■			■		

Table S6 Continued: Virulence gene prevalence by phylogenetic group and sequence type among 59 FQR and five FQS Australian cat and dog-source clinical *Escherichia coli*

	Antimicrobial resistance genes																											
	β-lactamases													FQ		MLS		Phenicol						Sulphonamide			Tetracycline			
Ref lab ID	*bla*$_{CTX-M-15}$	*bla*$_{CTX-M-14}$	*bla*$_{CTX-M-27}$	*bla*$_{CTX-M-71}$	*bla*$_{CTX-M-103}$	*bla*$_{TEM-1B}$	*bla*$_{TEM-70}$	*bla*$_{TEM-104}$	*bla*$_{TEM-105}$	*bla*$_{TEM-122}$	*bla*$_{TEM-141}$	*bla*$_{OXA-1}$	*bla*$_{PBP}$	*qnrB4*	*qnrS1*	*mdf(A)*	*mph(A)*	*catA1*	*catA2*	*catB3*	*catB4*	*cml*	*cmlA1*	*sul1*	*sul2*	*sul3*	*tetA*	*tetB*	*tetD*	*tetR*
N13/1/34						■	■		■				■			■		■						■	■			■		
N13/1/133						■	■		■				■			■		■						■	■			■		
N13/1/709						■				■			■			■									■					
N13/1/109													■			■														
N13/1/75						■	■		■				■			■		■							■			■		
Q13/1/261													■			■														
S13/1/53						■	■		■				■	■		■	■	■				■		■	■	■	■	■		■
Q13/1/80		■											■			■	■							■	■		■			■
N13/1/272			■										■			■	■							■	■		■			■
N13/4/125	■											■	■			■	■			■	■			■			■			■
N13/1/351	■											■	■			■				■	■			■			■			■
N13/4/38	■											■	■			■	■			■	■			■			■			■
N13/4/101												■	■			■	■			■	■			■			■			■
W13/1/13													■			■														
S13/1/48-2	■											■	■			■	■			■	■			■			■			■
Q13/1/281													■			■														
V13/2/449	■											■	■			■				■	■				■					
Q13/2/40	■											■	■			■				■	■				■					
Q13/2/52	■											■	■			■				■	■				■					
N13/1/284		■											■			■								■	■		■	■		■
N13/4/131						■	■		■				■			■	■							■	■		■			■
Q13/1/297	■			■	■	■		■				■	■			■	■			■	■			■	■		■	■		■
V13/6/2													■			■														
V13/5/4	■					■	■		■			■	■			■		■		■	■				■		■		■	■
W13/1/2						■	■		■				■		■	■									■	■				
W13/1/7	■											■	■			■	■			■	■			■				■		
N13/4/17	■												■			■	■							■				■		
N13/4/72						■	■		■				■			■														
N13/1/97						■	■		■				■			■							■			■	■			■

Table S6 Continued: Virulence gene prevalence by phylogenetic group and sequence type among 59 FQR and five FQS Australian cat and dog-source clinical *Escherichia coli*

	Antimicrobial resistance genes					Virulence genes																														
	Trimethoprim					Adhesins																												Invasins					
Ref lab ID	*dfrA1*	*dfrA5*	*dfrA12*	*dfrA14*	*dfrA17*	*afaA*	*afaB*	*afaC*	*afaD*	*afaF*	*draA*	*draB*	*draC*	*draD*	*fimA*	*fimBCDEFG*	*fimH*	*fimI*	*iha*	*iptA*	*papB*	*papC*	*papD*	*papH*	*papI*	*papFGJK*	*papX*	*sfaBDEGY*	*sfaC*	*aslA*	*ibeA*	*kpsD*	*kspM II*	*kpsT*	*ompA*	*ompT*
Q13/1/288					■										■	■	■	■												■						■	
W13/1/6		■													■	■	■	■												■						■	■
W13/1/10					■																									■						■	
W13/1/8					■											■	■	■		■										■		■	■			■	
Q13/2/52-2					■											■	■	■												■						■	
N13/1/733					■											■	■	■												■						■	
N13/1/274					■											■	■	■						■			■	■	■	■	■		■			■	■
V13/5/59					■											■	■	■												■						■	
S13/1/48																■	■	■												■						■	
S13/1/61																■	■	■												■						■	
S13/1/84																■	■	■												■						■	
S13/1/85					■											■	■	■												■						■	
Q13/2/49																■	■	■												■						■	
Q13/2/87																■	■	■												■						■	
N13/1/707					■										■	■	■	■																		■	
Q13/2/28				■											■	■	■	■																		■	
Q13/2/85				■											■	■	■	■																		■	
Q13/2/9				■											■	■	■	■		■																■	
Q13/2/29				■											■	■	■	■		■																■	
Q13/1/89			■												■	■	■	■		■																■	
N13/1/378				■											■	■	■	■		■																■	
N13/1/795			■												■	■	■	■		■																■	
N13/4/97			■												■	■	■	■		■	■															■	
V13/4/28															■	■	■	■		■																■	
S13/1/69			■												■	■	■	■		■																■	
V13/2/170					■										■	■	■	■		■																■	
Q13/1/229					■										■	■	■	■																		■	
Q13/1/272					■										■	■	■	■		■																■	

Table S6 Continued: Virulence gene prevalence by phylogenetic group and sequence type among 59 FQR and five FQS Australian cat and dog-source clinical *Escherichia coli*

	Antimicrobial resistance genes					Virulence genes																														
	Trimethoprim					Adhesins																												Invasins						
Ref lab ID	*dfrA1*	*dfrA5*	*dfrA12*	*dfrA14*	*dfrA17*	*afaA*	*afaB*	*afaC*	*afaD*	*afaF*	*draA*	*draB*	*draC*	*draD*	*fimA*	*fimBCDEFG*	*fimH*	*fimI*	*iha*	*ipfA*	*papB*	*papC*	*papD*	*papH*	*papI*	*papFGJK*	*papX*	*sfaBDEGY*	*sfaC*	*aslA*	*ibeA*	*kpsD*	*kspM II*	*kpsT*	*ompA*	*ompT*
N13/1/6					■										■	■	■	■		■																■	
N13/1/34															■	■	■	■		■																■	■
N13/1/133					■										■	■	■	■		■																■	■
N13/1/709					■										■	■	■	■		■																■	■
N13/1/109															■	■	■	■	■		■		■		■		■			■		■	■		■	
N13/1/75															■	■	■	■												■	■	■	■		■	
Q13/1/261					■	■	■	■	■				■		■	■	■	■	■		■				■		■			■		■	■		■	
S13/1/53															■	■	■	■												■	■	■	■		■	
Q13/1/80		■	■	■	■					■					■	■	■			■	■		■	■		■				■	■	■	■	■	■	■
N13/1/272					■										■	■	■	■	■		■				■		■			■		■	■		■	
N13/4/125					■										■	■	■	■	■		■				■		■			■		■	■		■	
N13/1/351					■										■	■	■	■	■		■	■	■	■	■	■	■			■		■	■		■	
N13/4/38							■	■		■		■	■		■	■	■	■	■		■	■	■	■	■	■	■		■	■		■	■		■	
N13/4/101					■										■	■	■	■	■		■	■	■	■		■	■			■		■	■		■	
W13/1/13					■										■	■	■	■	■		■				■		■			■		■	■		■	
S13/1/48-2																■	■	■												■	■	■	■		■	
Q13/1/281					■											■	■	■		■															■	
V13/2/449	■														■	■	■	■		■		■		■	■										■	
Q13/2/40	■														■	■	■	■												■		■	■		■	
Q13/2/52	■														■	■	■	■												■		■	■		■	
N13/1/284	■														■	■	■	■												■		■	■		■	
N13/4/131	■							■					■		■	■	■	■		■		■		■	■		■			■		■	■		■	
Q13/1/297					■		■	■			■		■	■	■	■	■	■	■		■				■		■			■		■	■		■	
V13/6/2					■		■	■		■	■		■	■	■	■	■	■	■		■				■		■			■		■	■		■	
V13/5/4					■		■	■			■		■	■	■	■	■	■	■		■				■		■			■		■	■		■	
W13/1/2	■							■	■				■	■	■	■	■	■							■					■		■	■		■	
W13/1/7				■				■					■			■	■	■												■		■	■	■	■	■
N13/4/17					■										■	■	■	■									■			■		■	■		■	
N13/4/72					■										■	■	■	■									■			■		■	■		■	
N13/1/97															■	■	■	■	■		■				■		■			■		■	■		■	■

Table S6 Continued: Virulence gene prevalence by phylogenetic group and sequence type among 59 FQR and five FQS Australian cat and dog-source clinical *Escherichia coli*

	Virulence genes																										
	Siderophores												Toxins									Other					
Ref lab ID	*chuA*	*chuS*	*chuT*	*chuUVW*	*chuX*	*chuY*	*fyuA*	*ironN*	*irp1/2*	*iucA*	*iucBD*	*iucC*	*astA*	*cba*	*cma*	*cnf1*	*hlyABD*	*hlyC*	*sat*	*senB*	*vat*	*CapU*	*malX*	*traT*	*usp*	*yfcV*	*iss*
Q13/1/288							■	■	■	■	■	■	■										■				■
W13/1/6										■	■	■											■				
W13/1/10							■		■	■	■	■											■	■			■
W13/1/8				■						■	■	■											■	■			■
Q13/2/52-2																							■				
N13/1/733																							■			■	
N13/1/274		■		■	■	■											■						■		■	■	
V13/5/59																							■			■	
S13/1/48																							■			■	
S13/1/61																							■			■	
S13/1/84																							■			■	
S13/1/85																							■			■	
Q13/2/49							■		■														■				
Q13/2/87							■		■														■				
N13/1/707							■	■	■	■	■	■											■				■
Q13/2/28																											
Q13/2/85															■								■				
Q13/2/9															■								■				
Q13/2/29															■								■				
Q13/1/89							■		■					■	■								■				
N13/1/378															■								■				
N13/1/795							■		■														■				
N13/4/97					■	■					■							■					■	■	■			
V13/4/28							■		■														■				■
S13/1/69							■		■					■	■								■	■			
V13/2/170										■	■	■											■				
Q13/1/229							■	■	■	■	■	■											■				■
Q13/1/272							■	■	■	■	■	■											■	■			■
N13/1/6							■	■	■	■	■	■											■	■			■
N13/1/34							■	■	■	■	■	■											■	■			■
N13/1/133							■	■	■	■	■	■											■	■		■	

Table S6 Continued: Virulence gene prevalence by phylogenetic group and sequence type among 59 FQR and five FQS Australian cat and dog-source clinical *Escherichia coli*

	Virulence genes																									
	Siderophores												Toxins									Other					
Ref lab ID	*chuA*	*chuS*	*chuT*	*chuUVW*	*chuX*	*chuY*	*fyuA*	*iroN*	*irp1/2*	*iucA*	*iucBD*	*iucC*	*astA*	*cba*	*cma*	*cnf1*	*hlyABD*	*hlyC*	*sat*	*senB*	*vat*	*CapU*	*malX*	*traT*	*usp*	*yfcV*	*iss*
N13/1/709							■	■	■	■	■	■											■	■		■	
N13/1/109	■	■	■	■	■	■	■		■	■	■	■							■	■			■	■	■	■	
N13/1/75	■	■	■	■	■	■	■		■											■			■	■	■		■
Q13/1/261	■	■	■	■	■	■	■		■											■				■	■	■	
S13/1/53	■	■	■	■	■	■	■		■											■			■	■	■		■
Q13/1/80	■	■	■	■	■	■	■		■		■	■	■										■	■	■		■
N13/1/272	■	■	■	■	■	■	■		■	■	■	■							■	■			■	■			
N13/4/125	■	■	■	■	■	■	■		■	■	■	■							■				■	■			■
N13/1/351	■	■	■	■	■	■	■		■	■	■	■				■	■	■	■	■			■	■	■		■
N13/4/38	■	■	■	■	■	■	■		■	■	■	■				■	■	■	■	■			■	■			■
N13/4/101	■	■	■	■	■	■	■		■	■	■	■				■	■	■	■	■			■	■	■	■	■
W13/1/13	■	■	■	■	■	■	■		■	■	■	■							■				■	■	■		■
S13/1/48-2	■	■	■	■	■	■	■		■												■		■		■		■
Q13/1/281							■		■	■	■	■								■			■				
V13/2/449							■		■	■	■	■											■				
Q13/2/40		■		■		■	■		■													■	■				■
Q13/2/52		■		■		■	■		■													■	■				■
N13/1/284		■		■		■	■		■													■	■				■
N13/4/131		■		■		■	■		■	■	■		■										■			■	
Q13/1/297		■		■		■	■		■	■	■	■							■				■	■			■
V13/6/2		■		■		■	■		■	■	■	■							■	■			■	■		■	■
V13/5/4		■		■		■	■		■	■	■	■							■	■			■	■			■
W13/1/2		■		■		■							■									■	■	■			■
W13/1/7				■		■	■		■		■												■	■		■	■
N13/4/17		■		■		■	■		■														■	■			
N13/4/72		■		■		■	■		■	■	■	■											■	■			
N13/1/97		■		■		■	■		■	■	■	■							■				■	■			■

Blue squares indicate gene presence

Supplementary materials and methods

WGS analysis of 59 cat and dog-source FQ^R *E. coli* isolates

For each isolate, assembled contigs were analyzed for sequence type (ST), antimicrobial resistance genes (ARGs), virulence genes (VGs), plasmids, *fimH* alleles, and O:H serotypes by using web-services at the Centre for Genome Epidemiology (CGE, http://www.genomicepidemiology.org/), and the ABRicate function of the Galaxy web platform (https://usegalaxy.org.au/). Isolates of unknown ST per CGE were uploaded to Enterobase for further analysis (http://enterobase.warwick.ac.uk/).

A BLAST library was constructed for *afaBC* (NCBI X76688), *draBC* (NCBI AF329316), *traT* (NCBI CP001856), *usp* (NCBI AB027193), *ompT* (NCBI AE014075), *malX* (NCBI AE014075), and *yfcV* (NCBI AE014075), and was used to search contigs using CLC Genomics Workbench. *kpsM II* and *hra* were detected by performing a local blastn search using the sequences in Supplementary File A.

Additional methods supporting ST131 phylogenetic tree construction

Quality control and MLST

DNA contamination was screened within the Illumina sequence data by using Kraken v2.0.7-beta (Wood and Salzberg, 2014) against the NCBI Reference Sequence (RefSeq) database (Sayers et al., 2009). Next the quality of the paired-end reads was assessed by using the FastQC package v0.11.8 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) by aggregating all reports into a single report and visualizing them using MultiQC v1.7 (Ewels et al., 2016). Low-quality bases, read-pairs and Illumina adaptor sequences were removed using Trimmomatic v0.36 (Bolger et al., 2014) (settings: LEADING:10 TRAILING:10 MINLEN:50 HEADCROP:10). Quality filtering with Trimmomatic retained 99.6% of read pairs (Table S2). SRST2 v0.2.0 (Inouye et al., 2014) (default settings) was used

to screen for MLST genes by querying all genomes against the *E. coli* MLST allelic profiles hosted on PubMLST (Wirth et al., 2006; Larsen et al., 2012).

De novo assembly of Australian cat and dog-source ST131 draft genomes

Using the chromosome of ST131 reference strain EC958, a reference-assisted approach was taken to generate *de novo* assemblies of the 20 newly sequenced Australian cat and dog-source ST131 isolate genomes using MGAP (https://github.com/dsarov/MGAP Microbial-Genome-Assembler-Pipeline). By comparing draft genome assemblies to the chromosome of EC958, assembly statistics were generated using QUAST v4.5 (Gurevich et al., 2013). The 20 Australian draft genomes had a median total length of 5.18 Mbp (IQR: 5.05 to 5.27 Mbp; range: 5.02 Mbp to 6.39 Mbp), a median GC content of 50.7% (IQR: 50.7% to 50.8%; range: 50.3% to 50.9%), and a median N50 statistic of 229.9 Kbp (IQR: 218.6 to 246.3 Kbp; range: 52.1 to 370.4 Kbp) (Table S2).

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Supplementary A

Gene	Sequence
kspM_II_K1	ATGGCAAGAAGTGGATTTGAAGTTCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTTCGTCTGGGGTATTTGTGGGCGATTC TTGAACCCTCCGCGCATTTGCTGATACTGTTGGGAATTTTGGGTTACGTTATGCACCGCACTATGCCAGACATCTCGTTCCCGGTGTTTTTACTTAATGGCCTGATTCCC TTTTTTATCTTTAGTAGTATTAGCAAACGTTCTATTGGTGCTATTGAAGCGAACCAGGGACTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCACGTGCA CTGCTTGAGACACTGATTTACGTTGCTGTTTATATTTTGCTCATGCTTATCGTCTGGATGACAGGCGAATATTTCGAAATTACAAACTTTTTACAACTTGTGCTCACCTGG AGTTTGTTAATCATTCTTTCATGTGGCGTCGGCTTAATATTTATGGTCGTTGGTAAAACCTTTCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCTGTATTTC ATCTCCTGCATCATGTTCCCTCTACACTCGATTCCAAAACAATACTGGTCATATCTACTCTGGAACCCATTAGTGCATGTTGTGGAGTTAAGCCGCGAGGCAGTTATGCC TGGCTATATCAGTGAAGGCGTGAGTCTGAACTACCTTGCAATGTTTACTCTGGTCACCCTGTTCATCGGCCTGGCATTATACCGAACGCGTGAAGAGGCAATGCTGAC ATCATGA
kspM_II_K2	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATCTGTGGGCGATTC TCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATTATGCACCGCACGATGCCAGACATCTCGTTCCCGGTGTTTTTACTTAATGGCCTGATTCCC TTTTTTATCTTTAGCAGTATCAGCAAACGTTCTGTAAGTGCTATTGAAGCGAACCAGGGGCTGTTTAATTATCGCCCAGTAAAACCCATCGATACGATCATTGCGCGCGC CCTGCTTGAGACGCTGATTTACGTTTCTGTTTATATTCTGCTTATGCTCATTGTCAGGATGGCAGGCGAATATTTCGAGATAACAAATTTTTTACAACTTGTGGCTACCTG GAGTCTGCTGATCATTCTTTCATGCAGCGTCGGCTTAATATTCATGGTCGTTGGTAAAACCTTCCCCGAAATGCAAAAGGTTCTGCCGATACTTCTTAAGCCCCTTTATT TCATCTCCTGCATCATGTTCCCTCTGCACTCAATTCCAAAGCAATACTGGTCATATCTACTCTGGAACCCACTAGTGCATGTCGTGGAGTTAAGCCGTGAGGCCGTTAT GCCTGGCTATATTAGCGAAGGTGTGAGTCTGAACTACCTGGCAATGTTCACATTGATAACTCTGTTCATTGGTCTGGCGTTATATCGAACTCGTGAGGAGGCAATGCTG ACATCATGA
kspM_II_K5_kfiC_Bi8337-41	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTATTTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATTTGTGGGCGATTC TCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATTATGCACCGCACGATGCCAGACATCTCATTCCCGGTGTTTTTACTTAATGGCCTGATTCCC TTTTTTATCTTTAGCAGTATCAGCAATCGTTCTGTAGGCGCTATTGAAGCGAACCAGGGGTTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCACGCGC ACTGCTTGAGACGCTGATTTACGTTGCTGTTTATATATTGCTCATGCTTATCGTCTGGATGGCAGGTGAATATTTCGAGATAACAAACTTTTTACAACTAGTGCTCACCTG GAGTTTGTTAATCATTCTTTCATGTGGCATCGGCTTAATATTCATGGTCGTTGGTAAAACCTTCCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCTGTATT TCATCTCCTGCATCATGTTCCCTCTACATTCGATTCCGAAGCAATACTGGTCATATCTACTCTGGAACCCATTAGTGCATGTCGTAGAGTTAAGCCGCGAGGCAGTTATG CCTGGCTATATCAGCGAAGGCGTGAGTCTGAACTACCTTGCAATGTTCACTCTGGTCACCCTGTTCATCGGTCTGGCGTTATACCGAACTCGTGAGGAGGCAATGCTG ACATCATGA
kspM_II_K5_kfiC	ATGAACGCAGAATATATAAATTTAGTTGAACGTAAAAAGAAATTAGGGACAAATATTGGTGCTCTTGATTTTTTATTATCAATTCATAAGGAGAAAGTTGATCTTCAACATA AAAACTCGCCTTTAAAAGGTAACGATAACCTTATTCACAAAAGAATAAACGAATACGACAATGTACTTGAACTATCTAAGAATGTATCAGCTCAGAATTCTGGCAATGAGT TTTCTTATTTATTGGGATATGCAGATTCTCTTAGAAAAGTTGGTATGTTGGATACTTATATTAAAATTGTTTGTTATCTAACAATTCAATCTCGTTATTTTAAAAATGGCGAA CGAGTTAAGCTTTTTGAACATATAAGTAACGCTCTACGGTATTCAAGGAGTGATTTTCTCATTAATCTTATTTTTGAACGATATATCGAATATATAAACCATCTAAAATTGT CGCCCAAACAAAAAGATTTTTATTTTTGTACGAAGTTTTCAAAATTTCATGATTATACTAAAAATGGATATAAATATTTAGCATTTGATAATCAAGCCGATGCAGGGTATGG CCTGACTTTATTATTAAATGCAAACGATGATATGCAAGATAGTTATAATCTACTCCCTGAGCAAGAACTTTTTATTTGTAATGCTGTAATAGATAATATGAATATTTATAGG AGTCAATTTAACAAATGTCTACGAAAATACGATTTATCAGAAATAACTGATATATACCCAAATAAAATTATATTGCAAGGAATTAAGTTCGATAAGAAAAAAAATGTTTATG GAAAAGATCTTGTTAGTATAATAATGTCAGTATTCAATTCAGAAGATACTATTGCATACTCATTACATTCATTGTTGAATCAAACATATGAAAATATTGAAATTCTCGTGTG CGATGATTGTTCATCGGACAAAAGCCTTGAAATAATTAAGAGCATAGCTTATTCTAGTTCAAGAGTGAAAGTATATAGCTCACGAAAAAACCAAGGCCCTTATAATATAA GAAATGAGCTAATAAAAAAAGCACACGGTAATTTCATCACCTTTCAAGATGCAGATGATCTTTCTCATCCGGAGAGAATACAAAGACAAGTTGAGGTTCTTCGCAATAAT AAGGCTGTAATCTGTATGGCTAACTGGATCCGTGTTGCGTCAAATGGAAAAATTCAATTCTTCTATGATGATAAAGCCACAAGAATGTCTGTTGTATCGTCAATGATAAA AAAAGATATTTTTGCGACAGTTGGTGGCTATAGACAATCTTTAATTGGTGCAGATACGGAGTTTTATGAAACAGTAATAATGCGTTATGGGCGAGAAAGTATTGTAAGAT TACTGCAGCCATTGATATTGGGGTTATGGGGAGACTCCGGACTTACCAGGAATAAAGGAACAGAAGCTCTACCTGATGGATATATATCACAATCTCGAAGAGAATATAG TGATATCGCGGCAAGACAACGAGTGTTAGGGAAAAGTATCGTAAGTGATAAAGATGTACGTGGTTTATTATCTCGCTATGGTTTGTTTAAAGATGTATCAGGAATAATTG AACAATAG

Supplementary A Continued

Gene	Sequence
kspM_K15	ATGGCAAGAAGTGGATTTGAAGTCCAGAAAGCCGCCGTTCATGCTCTATTTTTACGTGAGCTTAGAACTAGGTTTGGCAAATATCGCTTGGGTTATTTATGGGCTGTATT AGAACCAGCTGCTCATCTTCTAATAATGCTGGCAATTTTTGGTTTTTTTATGCATCGCACAATGCCAGATATTTCCTTTCCTGTATTTTTAATAAATGGAATAATTCCTTATT TTATATTTAGCAATATTGCTACGCGGTCTATAGGGGCAATTGAGGCAAACCAAGGCTTATTTAATTACAGGCCAGTAAGGCCTATAGATACAATTATAGCTAGAGCAATAT TAGAAGTGCTAATTTATAGCATAGTATATCTGGTTTTGATGAGTTTATTGTTAATAATAGGGGAGCAATTTAAAATATATAATATATTGCTGTTGTTTAGCGCATGGATTTTA CTGGCTTTATTTTCATGTGGAATTGGGTTGATCTTTATGGTAATTGGGAAAACTTTTCCAGAAACAGAGAAGTTTCTACCAATAATACTCAAGCCATTATATTTTGTATCAT GTATTATGCTTCCATTACATGCAATACCAAAAGGTTATTGGGGATATATATTATGGAATCCCATTGTTCATGTGGTAGAGCTTTGTCGTGAATCCGTTGTTACAGGTTATG TCTCTGAAGGGGTTAGTTTATATTACTTATTTATAAGCACGTTAATATTATTGTTTTTAGGATTGGCGCTATATTCATCTCGTGAAGAATACATGTTAACATCATGA
kpsM_II_K52	TTGGTAGCTGTTAAGCCAAGGGCGGTAGCGTACCTGAAGAGATTAGGATCACATCATCAAATGGCAAGAAGTGGATTTGAAGTCCAGAAAGTCACCGTAGAGGCATTAT TTCTACGAGAAATACGAACACGCTTTGGTAAGTTCCGTCTGGGATATTTGTGGGCGATTCTCGAACCCTCTGCGCATTTGCTGATACTGTTGGGCATTTTTGGTTACATT ATGCACCGCACGATGCCAGACATCTCATTCCCGGTGTTTTTACTTAATGGCCTGATTCCCTTTTTTATCTTTAGCAGTATCAGCAATCGTTCTGTAGGTGCTATTGAAGCG AATCAGGGGCTGTTTAATTATCGACCAGTAAAACCCATCGATACGATCATTGCGCGTGCATTGCTTGAGACACTGATTTACGTTACTGTTTATATATTGCTTATGCTTATC GTCTGGATGGCAGGCGAATATTTCGAAATAACAAACTTTTTACAACTTGTGCTTACCTGGAGTTTGTTAATCATTCTTTCATGTGGCGTTGGCTTAATATTCATGGTCGTT GGTAAAACCTTCCCTGAAATGCAAAAGGTCCTGCCGATACTGCTTAAGCCGCTGTATTTCATCTCCTGCATCATGTTCCCTCTACACTCGATTCCGAAGCAATACTGGTC ATATCTACTCTGGAATCCATTAGTGCATGTCGTAGAGTTAAGCCGCGAGGCAGTTATGCCTGGCTATATCAGCGAAGGCGTGAGTCTGAACTACCTGGCAATGTTTACA TTGATAACTCTGTTCATTGGTCTGGCGTTATACCGAACTCGTGAGGAGGCAATGCTGACATCATGA
kspM_III	ATGGACAAACCCATTATTAGTCAAACTCCACGCACTTCTTTGCAAGTATTACGTGATGTAGTATTTGGCTTATTAATTCGTGAGCTAAAAACAAGATTTGGTAATTACCGA CTAGGTTATGCTTGGGCATTACTTGACCCATTATTAATGATTAGCCTGTTCAGCGTAGTATTTGGGATGAGAAGCCAAAGTGGCTTTGGTGGTGTCCCAGCCCAGGTTTT TATTACTGCTGGTTATTTACCTTTCATGTTTTTCAATAAAGTTGTGACTCAGTTGAAATCTGCTGTCAATGCTAATATGGGACTTTTTTGTTATAGACAAGTGACTCCTTTTG CAACTTTTATAGCACGTTTTATGCTAGAAACAATGGTGGGCATGATTGTCGGTATCATCCTAGTACTAGGATTATTGTGGTTTGGCTTTGATGCAATACCTGCGGATCCAT TGCAAGTGATCCTTGGTTATTCTCTTCTGATGCTGTTTTCTTTTTCTCTTGGTATTGTATTTTGTGTTATTTGTAACTTAGCGAAAGAGGCAGATAAATTTCTTAGCTTGTTA ATGATGCCTTTGATGTTTATCTCTTGTGTTATGTTTCCTCTTGCTACTATTCCCCCTCAATATCAGCATTGGTTTTTATGGAATCCACTTGTGCATGCTGTAGAACTAATCC GAAGGGCATGGATATCTGGTTATCGTAGTCCTGATGTAAGTTGGGCGTATCTGTCGGTTGTCACCTTATTATTGCTCACTTTTGCTATGAGTTGTTACCGATTACGGCAT CGCCAATTGATTGCTAGTTAG
hra	ATGGAGATAATTGAAATGAATAAGGTTTTTGTTGTTTCAGTGGTGGCCGCAGCCTGTGTATTTGCAGTAAATGCAGGAGCAAAGGAAGGTAAAAGCGGTTTTTATCTGAC CGGTAAAGCCGGTGCCTCTGTGATGTCACTTTCAGACCAGCGTTTCCTGTCAGGAGATGAGGAAGAAACATCAAAGTATAAAGGCGGCGATGACCATGATACGGTATT CAGTGGCGGTATTGCGGTCGGTTATGATTTTTATCCGCAGTTCAGTATTCCGGTTCGTACAGAACTGGAGTTTTACGCTCGTGGAAAAGCTGATTCGAAGTATAACGTA GATAAAGACAGCTGGTCAGGTGGTTACTGGCGTGATGACCTGAAGAATGAGGTGTCAGTCAACACACTAATGCTGAATGCGTACTATGACTTCCGGAATGACAGCGCA TTCACACCATGGGTATCCGCAGGGATTGGCTACGCCAGAATTCACCAGAAAACAACCGGTATCAGTACCTGGGATTATGAGTACGGAAGCAGTGGTCGCGAATCGTTG TCACGTTCAGGCTCTGCTGACAACTTCGCATGGAGCCTTGGCGCGGGTGTCCGCTATGACGTAACCCCGGATATCGCTCTGGACCTCAGCTATCGCTATCTTGATGCA GGTGACAGCAGTGTGAGTTACAAGGACGAGTGGGGCGATAAATATAAGTCAGAAGTTGATGTTAAAAGTCATGACATCATGCTTGGTATGACTTATAACTTCTGA
intI1	ATGAAAACCGCCACTGCGCCGTTACCACCGCTGCGTTCGGTCAAGGTTCTGGACCAGTTGCGTGAGCGCATACGCTACTTGCATTACAGCTTACCAACCGAACAGGCT TATGTCCACTGGGTTCGTGCCTTCATCCGTTTCCACGGTGTGCGTCACCCGGCAACCTTGGGCAGCAGCGAAGTCGAGGCATTTCTGTCCTGGCTGGCGAACGAGCG CAAGGTTTCGGTCTCCACGCATCGTCAGGCATTGGCGGCCTTGCTGTTCTTCTACGGCAAGGTGCTGTGCACGGATCTGCCCTGGCTTCAGGAGATCGGAAGACCTC GGCCGTCGCGGCGCTTGCCGGTGGTGCTGACCCCGGATGAAGTGGTTCGCATCCTCGGTTTTCTGGAAGGCGAGCATCGTTTGTTCGCCCAGCTTCTGTATGGAACG GGCATGCGGATCAGTGAGGGTTTGCAACTGCGGGTCAAGGATCTGGATTTCGATCACGGCACGATCATCGTGCGGGAGGGCAAGGGCTCCAAGGATCGGGCCTTGA TGTTACCCGAGAGCTTGGCACCCAGCCTGCGCGAGCAGCTGTCGCGTGCACGGGCATGGTGGCTGAAGGACCAGGCCGAGGGCCGCAGCGGCGTTGCGCTTCCCG ACGCCCTTGAGCGGAAGTATCCGCGCGCCGGGCATTCCTGGCCGTGGTTCTGGGTTTTTGCGCAGCACACGCATTCGACCGATCCACGGAGCGGTGTCGTGCGTCG CCATCACATGTATGACCAGACCTTTCAGCGCGCCTTCAAACGTGCCGTAGAACAAGCAGGCATCACGAAGCCCGCCACACCGCACACCCTCCGCCACTCGTTCGCGA CGGCCTTGCTCCGCAGCGGTTACGACATTCGAACCGTGCAGGATCTGCTCGGCCATTCCGACGTCTCTACGACGATGATTTACACGCATGTGCTGAAAGTTGGCGGTG CCGGAGTGCGCTCACCGCTTGATGCGCTGCCGCCCCTCACTAGTGAGAGGTAG

Chapter 5

Malakoplakia of the urinary bladder in two dogs

Statement of Authorship

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Principal Author

Name of Principal Author (Candidate)	Amanda K. Kidsley		
Contribution to the Paper	Performed laboratory work, analysis, interpreted data, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	37.5%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	17/4/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Assisted with analysis, helped to evaluate and edit the manuscript		
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Contribution to the Paper	Helped to evaluate and edit the manuscript		
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Name of Co-Author	Sam Abraham		
Contribution to the Paper	Helped to evaluate and edit the manuscript		
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Name of Co-Author	Mark O'Dea		
Contribution to the Paper	Assisted with laboratory work, helped to evaluate and edit the manuscript		
Signature		Date	21/4/2020

Name of Co-Author	Kenneth W. Simpson		
Contribution to the Paper	Performed FISH analysis, guided genetic analysis of AIEC, and helped to evaluate and edit the manuscript		
Signature		Date	7/21/2020

Name of Co-Author	Belgin Dogan		
Contribution to the Paper	Assisted with analysis, helped to evaluate and edit the manuscript		
Signature		Date	21/7/2020

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Contribution to the Paper	Supervised development of work, helped to evaluate and edit the manuscript		
Signature		Date	07/08/2020

Malakoplakia of the Urinary Bladder in Two Dogs

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Keywords: cystitis, dog, fluorescent *in situ* hybridization, granulomatous, multidrug-resistant *Escherichia coli*, urinary tract infection

Abbreviations: AIEC, adherent-invasive *Escherichia coli*; AMR, antimicrobial resistance; ARG, antimicrobial resistance genes; ExPEC, extra-intestinal pathogenic *Escherichia coli*; FISH, fluorescent in situ hybridization; GC, granulomatous colitis; HAC, hyperadrenocorticism; MDREC, multidrug-resistant *Escherichia coli*; MG, Michaelis-Gutmann; MIC, minimum inhibitory concentration; PAS, periodic acid-Schiff; RBC/HPF, red blood cells per high-power field; ST, sequence type; TMS, trimethoprim-sulfadiazine; UBM, urinary bladder malakoplakia; UTI, urinary tract infection; VG, virulence genes; WBC/HPF, white blood cells per high-power field

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Conflict of Interest Declaration

DJT has received funding from Zoetis, Bayer, Boehringer Ingelheim and Virbac

Off-label Antimicrobial Declaration

Authors declare no off-label use of antimicrobials.

Institutional Animal Care and Use Committee (IACUC) or Other Approval Declaration

Authors declare no IACUC or other approval was needed

Human Ethics Approval Declaration

Authors declare human ethics approval was not needed for this study

Abstract

Rationale: Malakoplakia is a chronic granulomatous inflammatory condition associated with bacterial infections of the urinary bladder of immunocompromised humans. One case has been reported previously in dogs.

Clinical Findings: Bladder biopsies from a Boxer dog and a Cocker Spaniel with *Escherichia coli* urinary tract infections and multiple bladder nodules disclosed periodic acid-Schiff-positive macrophages and intracellular *E. coli*. The Boxer had concurrent *E. coli*-associated granulomatous colitis.

Pertinent Interventions: Antibacterial selection was guided by *in vitro* susceptibility and pharmacokinetics. *E. coli* isolates were evaluated for phylogroup, sequence type, antimicrobial resistance and virulence genes.

Outcome: Cure was attained in the Boxer dog. Multidrug resistance developed in the other, but spontaneous resolution ensued. *E. coli* isolates represented phylogroups A, B2 (identified as ST131) and D. All isolates possessed virulence gene profiles consistent with typical ExPEC strains.

Clinical Relevance: Urinary bladder malakoplakia can arise from *E. coli* infection in susceptible dogs. Successful treatment is possible.

1. Introduction

Malakoplakia in humans is a rare chronic granulomatous inflammatory condition, most commonly affecting the urinary bladder[1-5]. Human urinary bladder malakoplakia (UBM) is characterized by irritative voiding, hematuria and soft yellow-brown centrally umbilicated mucosal plaques[3,4,6,7]. Histological characteristics of malakoplakia are dense infiltrates of “von Hansemann” cells (macrophages containing abundant eosinophilic granular cytoplasm) and “Michaelis-Gutmann” (MG) bodies (periodic acid-Schiff (PAS), von Kossa and Perls’-positive mineralized residual phagolysosomes)[1,2,4,5,7,8]. There is persistent coliform urinary tract infection (UTI) in up to 90 percent of cases[1,5,7,8] and systemic immunosuppression or urinary bladder neoplasia underlie approximately 40 percent of cases[1,2,4,5,7]. Defective intracellular bacterial killing is suspected[1-4,6,7] and current treatment recommendations entail intracellularly-penetrating antibacterial agents such as ciprofloxacin or trimethoprim, with surgical debridement in obstructive or refractory cases[1,3,5-7].

There are only three published reports of UBM in veterinary literature: two in kittens[9,10] and one recent report in a four-month-old puppy[11]. In canine medicine, mucosal infiltration by PAS-positive macrophages is the defining characteristic of granulomatous colitis (GC), typically reported in young Boxers and French Bulldogs[12]. Fluorescent *in situ* hybridization (FISH) studies of dogs with PAS-positive GC demonstrate intracellular *Escherichia coli* whose phenotype, phylogeny and virulence genotypes resemble extra-intestinal pathogenic *E. coli* (ExPEC)[13]. *E. coli*-associated GC can be cured by prolonged administration of an antibacterial agent with *in vitro* activity against *E. coli* and which attains therapeutic intracellular concentration[14].

This report describes two cases of canine UBM with intracellular *E. coli* infection, the genomic profiling of *E. coli* isolates, successful treatment of one dog and spontaneous resolution in the other.

2. Materials and methods

2.1 Case 1

A 5-month-old intact female Boxer presented with a 3-month history of malodorous urine, hematuria, pollakiuria, urinary frequency and incontinence persisting after two courses of amoxycillin-clavulanate (Amoxyclav 250 tablets, Apex Laboratories, Somersby NSW Australia; 10.5 mg/kg PO q12h for 7 and 21 days). *E. coli* susceptible to amoxycillin-clavulanate was isolated from urine prior to the second course. Hematochezia had been noted in the preceding 2 weeks. Physical examination, hematology and serum biochemistry findings were normal. Urinalysis and culture revealed hematuria (25 red blood cells per high-power field (RBC/HPF)), pyuria (15 white blood cells per high-power field (WBC/HPF)) and *E. coli* susceptible *in vitro* to all antibacterials tested including amoxycillin-clavulanate. Sonography revealed thickened urinary bladder mucosa with irregular isoechoic polypoid nodules cranioventrally. On vaginourethrocystoscopy there was mild vaginitis and ulcerated polypoid nodules in the cranioventral bladder. Colonoscopy showed mild multifocal ulceration. Histology revealed diffuse infiltration of the urinary bladder submucosa by macrophages containing abundant eosinophilic cytoplasm comprising PAS-positive granules (Figure 1A, 1B) and similar multifocal PAS-positive macrophage infiltration of the colonic mucosa. FISH demonstrated invasive clusters of *E. coli* in the bladder (Figure 1C) and colonic mucosa. *E. coli* isolates from homogenized tissue cultures were susceptible to trimethoprim-sulfadiazine (TMS; Tribrissen 80 tablets, Jurox, Rutherford NSW Australia; 20 mg/kg PO q12h). Clinical signs of UTI resolved by Week 2 of TMS therapy and there was only one subsequent incident of

hematochezia. Urine cultures were negative in Weeks 2 and 5. Repeat ultrasound showed mild bladder thickening and on cystoscopy nodules were decreased in size and number. PAS-positive macrophages persisted in bladder biopsies but tissue culture and FISH were negative. After 12 weeks of TMS therapy, *Enterococcus faecalis* was isolated from urine and after TMS withdrawal in Week 21 amoxycillin (Amoxycillin-400 tablets, Apex Laboratories, Somersby NSW Australia; 9.5 mg/kg PO q8h) was administered for two weeks. In Week 30, no abnormalities were identified on bladder ultrasound, cystoscopy or colonoscopy. Colonoscopic biopsies showed mild lymphoplasmacytic inflammation. Bladder biopsy taken during ovariohysterectomy (Week 32) revealed marked reduction of the submucosal cellular infiltrate, with only single or paired PAS-positive macrophages remaining (Figure 1D). Bacterial culture and FISH (Figure 1E) were negative. In Weeks 40, 68 and 94 urine cultures remained negative and there were no clinical signs of UTI or colitis.

2.2 Case 2

An 8-year-old spayed female Cocker Spaniel-Poodle mixed-breed dog presented with a 1-month history of hematuria, pollakiuria and nocturia unresponsive to amoxycillin-clavulanate (Noroclav 250 mg Tablets for Dogs, Norbrook Laboratories, Tullamarine VIC Australia; 20 mg/kg PO q12h). Blood tests showed neutrophilia (13,900/µL, normal 3,500-12,000), lymphopenia (800/µL, normal 900-3,500), increased serum alkaline phosphatase (558 U/L, normal 1-150) and decreased total thyroxine (< .78 ug/dL, normal 1.01-4.04). Ultrasound revealed markedly thickened irregular urinary bladder mucosa. The dog had been diagnosed with progressive retinal atrophy nine months previously but had no other reported health problems. No abnormalities were noted on physical examination. Antibacterial therapy was withdrawn and subsequent urinalysis and culture revealed urine specific gravity of 1.010, hematuria (> 100 RBC/HPF), moderate pyuria and *E. coli* susceptible to all

antibacterials tested including amoxycillin-clavulanate and enrofloxacin. Vaginourethrocystoscopy revealed vestibular lymphoid follicles and multiple yellow-pink-red polypoid nodules in the urinary bladder. Formalin-fixed bladder biopsies showed PAS-positive macrophages diffusely infiltrating the submucosa with fewer lymphocytes, plasma cells, and neutrophils. FISH demonstrated multifocal mucosal/submucosal clusters of intracellular *E. coli*.

Enrofloxacin (Baytril 150 mg tablets, Bayer, Pymble NSW Australia; 6 mg/kg PO q24h) was commenced. At Week 6 urine remained discolored and *E. coli* susceptible to all antibacterials tested, including enrofloxacin, was cultured. Bethanechol chloride (Urocarb 10 mg tablets, Mayne Pharma, Salisbury South SA Australia; 0.4 mg/kg PO q12h) was commenced. In Week 8 multidrug-resistant *E. coli* (MDREC) was cultured. Marbofloxacin (Zeniquin 25 mg tablets, Zoetis, Rhodes NSW Australia; 3 mg/kg PO q24h) commenced but the isolate was marbofloxacin-resistant on further testing. Hematuria, sonographic bladder lesions, and MDREC UTI persisted in Week 11. A low-dose dexamethasone suppression test was consistent with pituitary-dependent hyperadrenocorticism (HAC; baseline cortisol 7.79 ug/dL, normal .91-2.72 ug/dL; 4-hour cortisol 1.88 ug/dL, normal <.91 ug/dL; 8-hour cortisol 5.47 ug/dL, normal < .91 ug/dL) however no clinical signs of HAC were evident, including two separate water intake measurements of approximately 40 mL/kg/day. A subsequent ACTH stimulation test was not supportive of HAC (baseline cortisol 7.07 ug/dL normal .91-2.72 ug/dL; 1-hour post-ACTH cortisol 8.41 ug/dL, normal 7.25-14.5 ug/dL). Total thyroxine was normal (1.94 ug/dL) when repeated in Week 15.

The *E. coli* isolate was susceptible to chloramphenicol (Chlor-B 500 mg tablets, CEVA Animal Health Pty Ltd, Glenorie NSW Australia; 40mg/kg PO q8h) which commenced in Week 12. In Weeks 15 and 19, there were no clinical signs of UTI, no pyuria and negative urine cultures. In Week 22 pyuria (5-10 WBC/HPF) and MDREC

UTI recurred; UBM lesions persisted on ultrasound, cystoscopy (Figure 2), and histology. Based on *in vitro* susceptibility testing chloramphenicol was withdrawn and marbofloxacin recommenced, withdrawn later due to acquired resistance. Bethanechol chloride was withdrawn. From Weeks 29 to 65, despite persistent MDREC UTI and sonographic UBM lesions, there were no clinical signs or pyuria. In Week 52 the *E. coli* isolate became susceptible to TMS (20.8 mg/kg PO q12h) but as resistance developed in Week 60 TMS was withdrawn.

Twenty-eight months after withdrawal of antibacterial therapy the dog presented to its referring veterinarian with generalized lymphadenomegaly, having remained free from clinical signs of UTI. Prednisolone (Macrolone 20 mg tablets, Mavlab, Logan City QLD Australia; 1 mg/kg PO q12h for 5 days then q24h) commenced for presumed lymphoma. Euthanasia was performed 11 weeks later after clinical deterioration. Necropsy examination confirmed high-grade lymphoma in spleen, lymph nodes, lung and urinary bladder. Additional bladder lesions comprised hyperplasia, vacuolation and erosion of the uroepithelium, fibrosis, neovascularization, hemorrhage and macrophages containing hemosiderin and occasional PAS-positive granules. FISH showed colonization and invasion of bladder epithelium by mixed bacteria, with no *E. coli* or bacterial clusters. Urine and bladder tissue culture disclosed *Proteus mirabilis*.

2.3 Antimicrobial susceptibility testing of available *E. coli* isolates

Six isolates of *E. coli* from bladder, colon (Case 1, Isolates 1, 2) and urine (Case 2, Isolates 3-6) underwent antimicrobial susceptibility testing by micro-broth dilution[15]. Minimum inhibitory concentration (MIC) interpretation followed Clinical and Laboratory Standards Institute M100S guidelines given it is a more accurate reflection of the presence of antimicrobial resistance genes (ARG) in the isolate for some antimicrobials[15,16]. Isolates with MIC above the susceptible breakpoint were

classified as non-susceptible. Isolates were classified as MDREC if non-susceptible to ≥1 antimicrobial agents in ≥3 antimicrobial classes[17].

2.4 Whole genome sequencing

Whole-genome sequencing was performed on the six isolates using Illumina MiSeq. Resulting sequences were screened for sequence type (ST), virulence genes (VG) and ARG as previously described[18]. Additional genes were identified by performing a local blast using the CLC Genomics Workbench, as well as the search for motifs tool using Geneious Prime.

Isolates were classified as ExPEC if they contained ≥ 2 of five ExPEC-defining VGs (*papA* and/or *papC*, *sfa/focDE*, *afa/draBC*, *kpsM II*, *iutA*)[19].

3. Results

E. coli isolates from Case 1 (Isolate 1 from the bladder, Isolate 2 from the colon) belonged to phylogroup D but differing STs (ST963 and ST335; Table 1). Urine cystocentesis samples from Case 2 yielded multiple MDREC isolates (Table 2) belonging to phylogroups B2 (Isolate 3, Week 22) and A (Isolates 4 and 5, Week 35; Isolate 6, Week 52) with sequence types ST131 and ST744, respectively (Table 1). Isolate 1 possessed ExPEC-associated genes *aslA*, *fimH*, *ireA*, *irp1*, *irp2*, *kpsM II*, *fyuA*, *kpsD*, *malX, ompA,* entero-invasive *E. coli* gene *senB*, and iron acquisition/transport gene *fepC* (Table 3). Isolate 2 possessed ExPEC-associated genes *aslA*, *astA*, *fimH*, *malX, ompA*, and the enterohemorrhagic *E. coli* genes *efa1* and *fepC*. The ST131 isolate was fluoroquinolone-susceptible (Table 1) but contained the fluoroquinolone ARG *qnrB4* (Table 2) and the prototypic VG *fimH*, *fyuA*, *irp2*, *iucD*, *kpsM II*, *malX*, *ompT* and *traT* as well as the genes *aslA*, *chuA*, *colV*, *ibeA*, *iroN*, *iss*, *IpfA-154,* and *ompA*. ST744 isolates possessed the ExPEC-associated *aslA, fimH, malX,* and *ompA* (Table 3).

4. Discussion

Published reports of malakoplakia in dogs are confined to a one 4-month-old Staffordshire bull terrier puppy with UBM[11]. The two cases of canine UBM described here add to the limited information on this rare disease and reveal by FISH the presence of intralesional *E. coli*, as previously documented in cats[9,10] but not in dogs[11] or humans. Targeted antibacterial therapy achieved cure in one dog while development of MDREC subclinical bacteriuria followed by spontaneous resolution occurred in the other. Phylogenetic and genotypic characterization of infecting *E. coli* strains revealed diverse ST, ARG and VG profiles.

There are only a few published case reports of animal malakoplakia in urogenital or other organs: three pigs[20-22], three cats[9,10,23] and one cynomolgus monkey[24]. In humans UBM occurs most frequently in 50-60 year-old women[1,4,5,7], with sporadic reports in children and adolescents[5,25-27]. A female predisposition to UTI is also known in dogs[28,29], while increasing age may be protective[28]. In this report, both dogs were female; one was mature (8-year-old) and the other juvenile (5-month-old), suggesting differing predisposing factors for development of chronic intracellular infection.

Genomic studies in Boxers and French Bulldogs with PAS-positive *E. coli*-associated GC identified a 200 kB region on chromosome 38 containing signalling lymphocytic activation molecule genes[30], encoding proteins linked to intracellular sensing and killing of *E. coli* and human inflammatory bowel disease[30-34]. The concurrence of *E. coli* associated UBM and GC in the Boxer reported here suggests a similar inherited defect(s) in innate immunity, although genomic testing was not undertaken. Although not described as malakoplakia, PAS-positive *E. coli*-associated GC and nephritis in another young Boxer has recently been reported[35]. The mixed-breed dog had laboratory findings suggestive of HAC, however confirmatory tests were discordant

198 and as overt clinical signs of HAC such as polydipsia or alopecia did not develop, no
199 treatment for HAC was administered. Immunosuppressive effects of HAC might have
200 facilitated UTI[36] and chronic intracellular infection in this case. Treatment for HAC
201 may have improved its outcome[28]. This dog eventually succumbed to lymphoma
202 which may have indicated another underlying immunosuppressive condition.

203 When selecting an antibacterial agent integration of its pharmacokinetic properties is
204 essential for therapeutic success. As for malakoplakia in humans[3,6,7,37], *E.coli*-
205 associated malakoplakia in cats[9,10,23] and GC in dogs[12,14,38] successful treatment of
206 UBM in the Boxer was obtained via prolonged antibacterial therapy (TMS) selected
207 by *in vitro* susceptibility and capacity for intracellular concentration. In humans with
208 malakoplakia, bethanechol chloride normalizes monocyte guanosine monophosphate
209 concentration, lysosomal granule morphology and bactericidal activity and is
210 recommended treatment[1,5,7,37], so after initial treatment failure it was commenced as
211 adjunctive therapy in the mixed-breed dog. Although this approach was
212 unsuccessful, subclinical bacteriuria developed over time and antibacterial therapy
213 was withdrawn. When re-evaluated 28 months later the mixed-breed dog was free of
214 *E. coli* UTI and malakoplakia. Spontaneous resolution of human urinary tract
215 malakoplakia is also documented[39].

216 MG bodies are regarded as pathognomonic for human malakoplakia however were
217 not identified in the previous case of canine UBM[11] or the cases reported here.
218 Similarly, MG bodies are reported in human ulcerative colitis[40] but not in canine
219 PAS-positive GC[41]. Reports of MG bodies in human and animal malakoplakia are
220 inconsistent and their absence does not preclude this diagnosis[2,23,40,42].

221 *E. coli* phylogroup D strain ST963 was isolated from bladder tissue of the Boxer and
222 has been previously isolated from the stools of human diarrhea patients[43], a dog with
223 UTI and the rectum of a healthy dog[44]. MDREC belonging to phylogenetic groups B2

(ST131, Isolate 3) and A (ST744, Isolates 4-6) were isolated from the urine of the mixed-breed dog during treatment. ST131 is the most common fluoroquinolone-resistant ExPEC lineage isolated from humans but is infrequently isolated from dogs[45-47]. Adherent-invasive *E. coli* (AIEC) belonging to ST131 has been previously identified in three human patients: one UTI and two intestinal infections, including one patient with ulcerative colitis[48]. Isolate 3 was fluoroquinolone-susceptible *in vitro* despite possessing the fluoroquinolone resistance gene *qnrB4*, which imparts reduced fluoroquinolone susceptibility but not resistance when point mutations in chromosomal target genes are absent[49].

After unsuccessful treatment and a period of subclinical bacteriuria, the ST131 Isolate 3 was replaced by phylogroup A ST744 strains of comparatively lower virulence and variable MDR phenotype and ARG content. ExPEC-associated VG identified in ST744 strains included *fimH, malX,* and the endothelial cell invasin-associated *ompA* and *aslA*[50]. The only adhesin/invasin VG common to all isolates were *aslA*, *fimH,* and *ompA*. Further investigation of their role in the pathogenesis of malakoplakia may be warranted.

All six *E. coli* isolates possessed ExPEC-related genes while none contained diarrheagenic *E. coli* VG (*LT*, *STa*, *STb*, *stx1*, *stx2*, *cnf1)*, consistent with previously-reported GC-associated strains[13]. ST131 Isolate 3 contained genes previously associated with GC and the Crohn's disease-associated *E. coli* strain LF82 including *irp1*, *irp2*, *fyuA*, *chuA*, *fepC*, *iroN*, *malX*, *iss* and *ibeA*[13] (Table 3) and conformed to the molecular definition of an ExPEC strain, containing at least two of the ExPEC-defining VG *papA*/*papC*, *sfa/focDE*, *afa/draBC*, *kpsM II* and *iutA*[19]. No other isolates contained all of these genes previously associated with GC, however, genes related to AIEC in Boxers such as *pduC* and *ratA* were not present in any isolates.

Malakoplakia is a rare but possibly under-recognized condition in veterinary medicine. Our report of two cases of canine UBM implicates invasive *E.coli* in the pathogenesis in dogs, and supports treatment directed at eradicating bacterial infection. To the authors' knowledge, this is the first association of ST131, the global pandemic clonal lineage of highly virulent MDREC, and malakoplakia in any species.

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Table 1: Phylogenetic characterization of *Escherichia coli* isolates from two cases of canine urinary bladder malakoplakia.

Case	Isolate	Sample type	Site of isolation	Phylogroup	MLST	Resistance phenotype
1	1	Tissue	Bladder	D	963	KF
	2	Tissue	Colon	D	335	KF
2	3	Urine cystocentesis	Bladder	B2	131	AMP, TET, SXT
	4	Urine cystocentesis	Bladder	A	744	AMP, CIP, TET, SXT
	5	Urine cystocentesis	Bladder	A	744	AMP, KF, CIP, TET, SXT
	6	Urine cystocentesis	Bladder	A	744	CIP, TET

MLST: multilocus sequence type
AMP, ampicillin; CIP, ciprofloxacin; KF, cephalothin; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline

Table 2: *Escherichia coli* antimicrobial resistance genes identified in two cases of canine urinary bladder malakoplakia.

Case	Isolate	Phylogroup	MLST	Antimicrobial Resistance Genes																														
				aadA1	*aadA2*	*aadA5*	*aph(3')-Ia*	*aph3''Ia*	*aph(3'')-Ib*	*aph(3')-Ic*	*aph(6)-Id*	*strA*	*strB*	*blaTEM-1B*	*blaTEM-70*	*blaTEM-105*	*blaPBP*	*AmpC1*	*AmpC2*	*AmpH*	*qnrB4*	*mdf(A)*	*mph(A)*	*CatA1*	*cml*	*sul1*	*sul2*	*sul3*	*tet(A)*	*tet(B)*	*tet(R)*	*dfrA5*	*dfrA12*	*dfrA14*	*dfrA17*
1	1	D	963														■	■	■	■		■													
	2	D	335														■	■	■	■		■													
2	3	B2	131		■	■	■		■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	4	A	744				■										■	■	■	■		■	■	■						■					
	5	A	744	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■		■	■			■					■
	6	A	744														■	■	■	■		■		■						■					

MLST: multilocus sequence type
Note: black squares indicate gene presence

383 Table 3: *Escherichia coli* virulence genes identified in two cases of canine urinary

384 bladder malakoplakia.

Case	Isolate	Phylogroup	MLST	Virulence Genes																																		
				*aslA**	*astA**	*chuA**	*colV*	*efa1*	*entA-F*	*entS*	*fepA-D*	*fepG*	*fimA*	*fimB-G*	*fimH**	*fyuA**	*gad*	*gspF*	*hra**	*ibeA**	*ireA**	*ironN**	*irp1-2**	*iss**	*iucD**	*kpsD**	*kpsM II**	*kpsT*	*lpfA-154*	*malX**	*ompA**	*ompT**	*papE-G**	*senB*	*traT**	*tsh**	*ybtP*	*ybtQ*
1	1	D	963	■					■	■	■	■	■	■	■	■	■	■	■		■		■			■	■			■	■	■		■	■		■	■
	2	D	335	■	■			■	■	■	■		■	■	■		■	■						■						■	■	■			■			
2	3	B2	131	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	4	A	744	■					■	■	■	■		■	■		■													■	■							
	5	A	744	■					■	■	■	■		■	■		■													■	■							
	6	A	744	■					■	■	■	■		■	■		■													■	■							

385 *MLST: multilocus sequence type*

386 *Note: black squares indicate gene presence. Asterisk (*) denotes specific genes*

387 *identified in ExPEC strains.*

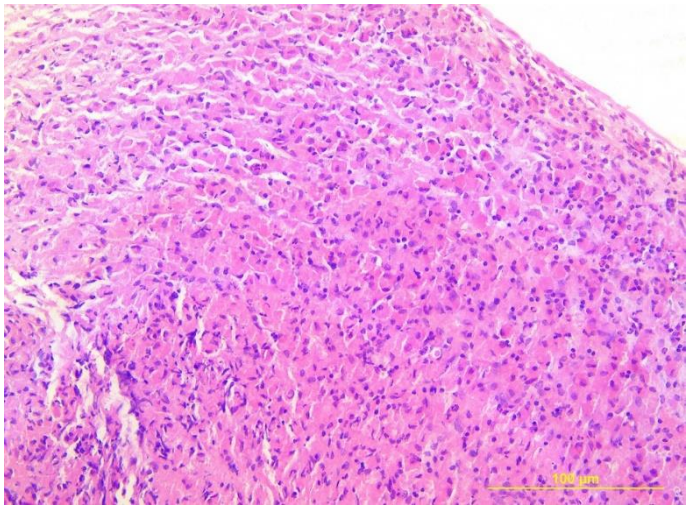

Figure 1A: Cystoscopic biopsy from Case 1 showing dense sheets of macrophages containing abundant eosinophilic granular cytoplasm ("von Hansemann" cells) expanding the urinary bladder submucosa. Haematoxylin and eosin, X 20.

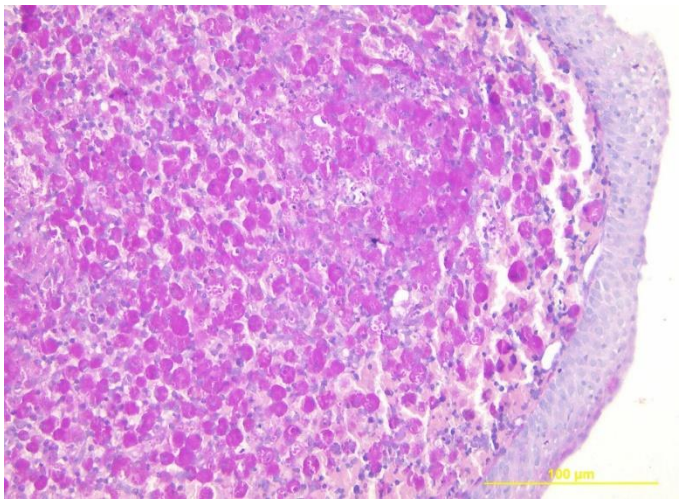

Figure 1B: Cystoscopic biopsy from Case 1 showing macrophages are filled with periodic acid–Schiff-positive granules. PAS, X 20.

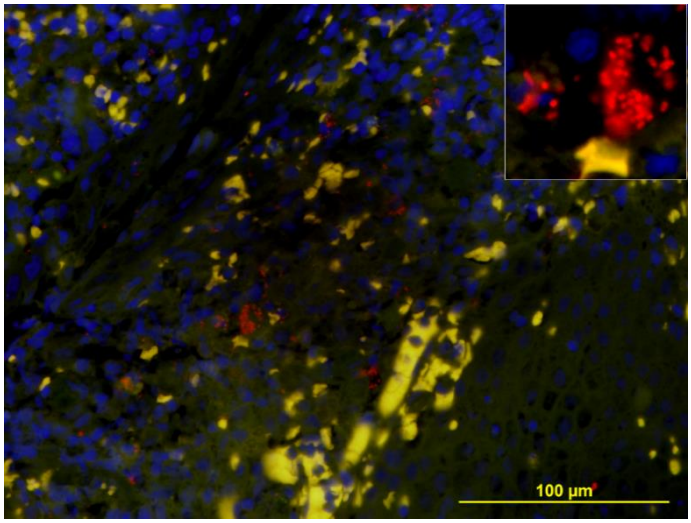

Figure 1C: Cystoscopic biopsy from Case 1. Fluorescent *in situ* hybridization analysis of urinary bladder pre-treatment reveals intralesional clusters of intracellular *Escherichia coli* bacteria within the submucosa. Intracellular bacteria (Cy3) are red (see insert). Nuclei (4′,6-diamidino-2-phenylindole) are blue.

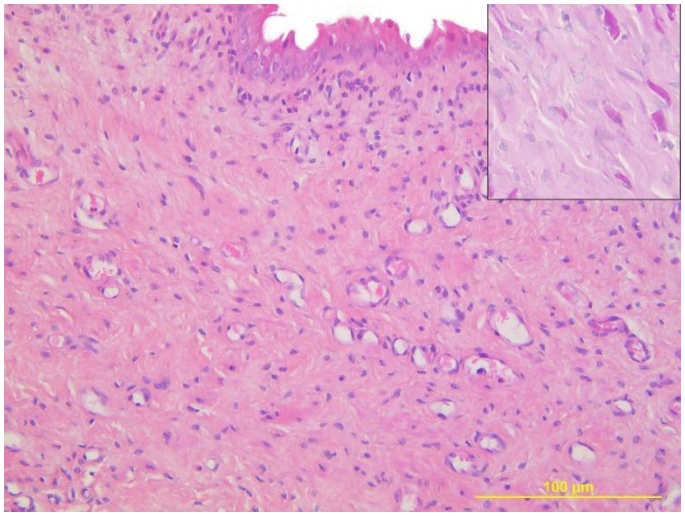

Figure 1D: Surgical biopsy of urinary bladder of Case 1 after treatment with TMS. The submucosal histiocytic infiltrate has largely resolved, with only a few residual individual PAS-positive macrophages (see insert). Haematoxylin and eosin, X 20.

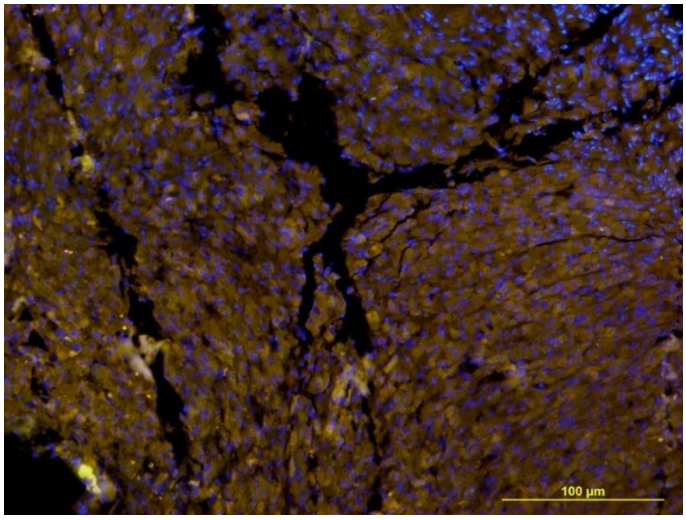

Figure 1E: Surgical biopsy of urinary bladder of Case 1 after treatment with TMS. Fluorescent *in situ* hybridization analysis of urinary bladder post-treatment is negative for intracellular bacteria

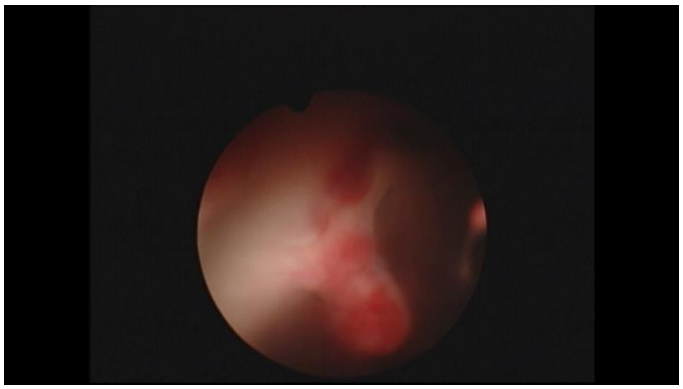

Figure 2: Cystoscopic image from Case 2 (Week 22) showing nodular proliferation and hemorrhage of urinary bladder mucosa associated with malakoplakia.

Chapter 6

Antimicrobial susceptibility of *Escherichia coli* and *Salmonella* spp. isolates from healthy pigs in Australia: Results of a pilot national survey

Statement of Authorship

Title of Paper	Antimicrobial susceptibility of *Escherichia coli* and *Salmonella* spp. isolates from healthy pigs in Australia: Results of a pilot national survey
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Principal Author

Name of Principal Author (Candidate)	Amanda K. Kidsley		
Contribution to the Paper	Performed laboratory work, analysis, interpreted data, wrote manuscript and acted as corresponding author		
Overall percentage (%)	75%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	7/6/19

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Antimicrobial Susceptibility of *Escherichia coli* and *Salmonella* spp. Isolates From Healthy Pigs in Australia: Results of a Pilot National Survey

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This study investigated the frequency of antimicrobial non-susceptibility (defined as the frequency of isolates with minimum inhibitory concentrations above the CLSI susceptible clinical breakpoint) among *E. coli* and *Salmonella* spp. isolated from healthy Australian finisher pigs. *E. coli* ($n = 201$) and *Salmonella* spp. ($n = 69$) were isolated from cecal contents of slaughter-age pigs, originating from 19 farms distributed throughout Australia during July-December 2015. Isolates underwent minimum inhibitory concentration (MIC) susceptibility testing to 11 antimicrobials. The highest frequencies of non-susceptibility among respective isolates of *E. coli* and *Salmonella* spp. were to ampicillin (60.2 and 20.3%), tetracycline (68.2 and 26.1%), chloramphenicol (47.8 and 7.3%), and trimethoprim/sulfamethoxazole (33.8 and 11.6%). Four *E. coli* isolates had MICs above the wild-type epidemiological cut-off value for ciprofloxacin, with two isolates from the same farm classified as clinically resistant (MICs of > 4 μg/ml), a noteworthy finding given that fluoroquinolones (FQs) are not legally available for use in Australian food-producing animals. Three of these four *E. coli* isolates belonged to the sequence type (ST) 10, which has been isolated from both humans and production animals, whilst one isolate belonged to a new ST (7573) and possessed *qnrS1*. This study shows that non-susceptibility to first line antimicrobials is common among *E. coli* and *Salmonella* spp. isolates from healthy slaughter age pigs in Australia. However, very low levels of non-susceptibility to critically important antimicrobials (CIAs), namely third generation cephalosporins and fluoroquinolones were observed. Nevertheless, the isolation of two ciprofloxacin-resistant *E. coli* isolates from Australian pigs demonstrates that even in the absence of local antimicrobial selection pressure, fluoroquinolone-resistant *E. coli* clonal lineages may enter livestock production facilities despite strict biosecurity.

Keywords: antimicrobial resistance, *Escherichia coli*, food-producing animals, fluoroquinolones, critically important antimicrobials

INTRODUCTION

Escherichia coli and *Salmonella* spp. while common commensals in many animals, are also known to be the causative agents of a number of production limiting diseases in pigs (Quinn et al., 2011). *E. coli* can cause pre-weaning scours and septicemia in piglets, post-weaning diarrhea and edema disease in weaners, and mastitis and cystitis in sows (Zimmerman et al., 2012). Enterotoxigenic *E. coli* (ETEC), the main agent associated with post-weaning enteric colibacillosis, is among the most significant bacterial pathogens in Australian pig production and is commonly resistant to multiple antimicrobial agents (Smith et al., 2016).

Salmonella enterica, subspecies *enterica* one of the primary subspecies of *Salmonella* associated with foodborne disease and a well-known zoonotic pathogen, is commonly carried by pigs and other food-producing animals (Abraham et al., 2014b). High rates of multidrug resistance have been found in *Salmonella* iolates from food-producing animals in several countries. For example, 54.5–55.6% of *Salmonella* spp. isolates from bovine carcasses in Croatia and Spain (European Food Safety Authority (EFSA), and European Centre for Disease Prevention and Control (ECDC), 2017); 66% of *Salmonella* spp. isolates from poultry and swine in Thailand (Van et al., 2012); and 41% of turkey, 8.3% of chicken and 17% of cattle *Salmonella* spp. isolates in the United States (Centers for Disease Control and Prevention (CDC), U.S. Department of Agriculture(USDA), and Food and Drug Administration (FDA), 2014) are multidrug-resistant (MDR). A recent Australian study found a high proportion (66.1%) of clinical *Salmonella* spp. isolates from food-producing animals, the majority obtained from bovine sources, were susceptible to all antimicrobials tested, including to critically important antimicrobials (CIAs), namely extended-spectrum cephalosporins (ESCs) and fluoroquinolones (FQs) (Abraham et al., 2014a). This low rate of resistance among bovine origin *Salmonella* spp. isolates was also confirmed in a study of *Salmonella* carriage in healthy cattle at slaughter (Barlow et al., 2015). However, ESC-resistant *Salmonella* spp. strains have recently been isolated from Australian dairy cattle in Gippsland, Victoria (Sparham et al., 2017). Although Australian pigs have previously been considered to have low rates of *Salmonella* spp. infection, since 2011 increasing numbers of clinical cases have been reported (Hamilton et al., 2015). Despite this, there have been no published studies on the estimated prevalence of antimicrobial resistance (AMR) in *Salmonella* spp. isolated from healthy Australian pigs at slaughter.

Antimicrobial agents are vital for the treatment and control of many bacterial diseases in pig production (Smith et al., 2016), but widespread use is often associated with the selection of AMR (Smith et al., 2016). MDR pathogens in humans, companion, and food-producing animals are a potential threat to animal health through the loss of antibiotic effectiveness to treat diseases and also to human health via direct cross-infection or foodborne transmission of organisms such as *Salmonella* spp. or indirectly through the transfer of mobile genetic elements, such as plasmids, between bacteria (Jordan et al., 2009; Mukerji et al., 2017). The reported use of antimicrobials and associated resistance in food-producing animals differs throughout the world. European AMR surveillance data show large differences between countries in both their antimicrobial use and frequency of AMR in key indicator bacteria (Österberg et al., 2016). For example, in respective studies undertaken in Italy and Poland, 12% ($n = 125$) (Österberg et al., 2016) and 11.1% ($n = 190$) (Wasyl et al., 2013) of *E. coli* isolated from the feces of healthy pigs close to slaughter weight were resistant or non-wild type to ciprofloxacin respectively. In contrast, similar studies undertaken in Canada during 2013 and Sweden in 2015, reported frequencies of resistance and non-wild type to ciprofloxacin of 2.4% ($n = 171$) and 2.5% ($n = 200$), respectively (Government of Canada, 2015; Swedish Veterinary Antimicrobial Resistance Monitoring (SVARM), 2015). In France, the frequency of ciprofloxacin resistance in *E. coli* isolated from porcine colonic contents at slaughter was 4.3% ($n = 94$). Interestingly, no ciprofloxacin resistance was reported from isolates collected during a contemporaneous study in Denmark ($n = 52$) (Österberg et al., 2016).

Australia was recently ranked the 5th lowest user of antimicrobials in livestock (mg/kg) in the world (O'Neill, 2015), which may in part be due to its heavy reliance on extensive grazing systems, but could also be related to other factors. Antimicrobials approved in Australia for the treatment of infections in pigs cover a broad range of classes and include sulphonamide-trimethoprim combinations, tetracyclines, β-lactams, and aminoglycosides (neomycin, apramycin, and spectinomycin) (Smith et al., 2016). Other antimicrobials, such as the ESC ceftiofur and the phenicol florfenicol, can be used by Australian veterinarians "off label" for individual cases of porcine colibacillosis as they are only approved for use for respiratory infections in cattle (ceftiofur), and both cattle and pigs (florfenicol) (Smith et al., 2016). In contrast to several other countries, the use of CIAs in Australian livestock is highly regulated (Smith et al., 2016; Mukerji et al., 2017). Australia is the only country to implement legal measures that exclude the use of FQs and gentamicin in food-producing animals (Abraham et al., 2014a). Further, no product containing colistin has been registered for use in Australian livestock for over 25 years (Australian Pesticides and Veterinary Medicine Authority (APVMA), 2017). In addition, by international comparison, the label constraints on the use of ESCs in Australian livestock are strict, while the ESC cefquinome is not registered for use. However, in a 2006 study, off label use of ceftiofur was reported to have occurred on 25% of Australian piggeries (Jordan et al., 2009).

Minimum inhibitory concentration (MIC) testing of commensal bacteria from healthy animals is commonly used to evaluate the occurrence of AMR in animal populations and farms and is the basis for mandatory monitoring of food production animals in the European Union (EU) (Österberg et al., 2016). Importantly, commensal bacteria such as *E. coli* can be reservoirs of plasmid-associated resistance genes of public health significance (Trott, 2013). While proof of concept national AMR surveys in the various livestock sectors have commenced (Shaban et al., 2014), a number of opportunistic surveys conducted in recent years have

confirmed a low public health risk in the Australian food animal sector in relation to resistance to CIAs, such as FQs and ESCs (Abraham et al., 2012, 2014a, 2015; Barlow et al., 2015). However, given the critical differences between the antimicrobial use in the Australian pig industry and elsewhere, and the lack of contemporary information on the occurrence of resistance, the aim of this pilot study was to investigate the occurrence of AMR among commensal *E. coli* and *Salmonella* spp. isolated from cecal contents of Australian finisher pigs at slaughter. The frequency of isolates with MICs classified as non-susceptible based on Clinical Laboratory Standards Institute (CLSI) and National Antimicrobial Resistance Monitoring System (NARMS) clinical breakpoints was determined. In addition, isolates with MICs above the wild-type epidemiological cut off values (ECOFFs) for CIAs were further characterized by whole genome sequencing analysis.

MATERIALS AND METHODS

Sample Collection, Isolation, and Identification

All cecal specimens were obtained using a systematic-random sampling method from healthy pigs at slaughter originating from 19 farms distributed throughout Australia between July and December 2015. Abattoirs were identified based on their eligibility criteria (e.g., export abattoirs processing finishing pigs where a Department of Agriculture on-plant veterinarian was present) and then randomly selected. The number of animals sampled from each abattoir was proportional to the output of that establishment, and calculated in advance. A systematic-random method of sampling was used with samples collected at regular intervals along the chain throughout the day. The interval between collections of individual samples for each plant was calculated (approx.) as a function of chain speed, daily throughput and shift length. A total of 201 pigs were sampled with one sample per pig obtained after slaughter and scalding when the gastrointestinal tract was removed. Samples were stored at 2–4°C before being packed and shipped with samples arriving at the primary laboratory within 24 h of collection.

A 10 g sample of fecal material was suspended in 7 ml of 0.1% sterile buffered peptone water (BPW) and thoroughly mixed, before 1 ml of the fecal mixture was extracted and centrifuged. The homogenate was plated on to MacConkey agar (Oxoid, Thermofisher Scientific) and incubated at 37°C for 18–24 h. Several lactose positive presumptive *E. coli* colonies were sub-cultured onto sheep blood agar (SBA) (Oxoid, Thermofisher Scientific) and incubated at 37°C for 24 h. One colony identified as *E. coli* using standard biochemical tests (Markey et al., 2013) was used for further analysis. For *Salmonella* isolation, the remaining fecal sample in BPW was incubated at 37°C for 18–24 h. Following incubation 10 ml of Rappaport-Vassiliadis broth (Micromedia, Edwards) was inoculated with 0.1 ml of the incubated buffered peptone water and incubated at 42°C for 18 h. An aliquot was then streak plated onto *Salmonella* Brilliance agar (Oxoid, Thermofisher Scientific) and XLD agar (Micromedia, Edwards) to select for single colonies and incubated at 37°C for 24 h. Well isolated single colonies were sub-cultured onto SBA and incubated at 37°C for 24 h. These presumptive *Salmonella* spp. isolates were then confirmed biochemically (Markey et al., 2013), with one isolate per sample selected for further analysis. The identity of each bacterial isolate to species (*E. coli* and *Salmonella* spp.) level was confirmed using mass-spectrometry (MALDI-TOF) prior to antimicrobial susceptibility testing (AST).

Antimicrobial Susceptibility Testing

AST was performed by micro-broth dilution using commercially prepared dryform panels (Sensititre CMV3AGNF, NARMS; Trek Diagnostic Systems, Thermofisher Scientific). Inoculation and incubation was carried out as per the manufacturer's guidelines, with quality control strains *E. coli* ATCC 35218, *E. coli* ATCC 25922, *Enterococcus fecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 used throughout the study. The antimicrobials tested were ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, tetracycline and trimethoprim/sulfamethoxazole and were selected based on consultation with industry and their widespread use in international antimicrobial resistance surveillance programmes (Shaban et al., 2014). MICs were interpreted using CLSI VET01S (Clinical Laboratory Standard Institute, 2015) guidelines or NARMS guidelines (Centers for Disease Control and Prevention (CDC), U.S. Department of Agriculture(USDA), and Food and Drug Administration (FDA), 2014) where no interpretative criteria were available (**Table 1**). In addition, CLSI M100S (Clinical Laboratory Standards Institute, 2016) breakpoints were used where animal species specific breakpoints were not available. Isolates with MICs above the susceptible breakpoint (i.e., in the intermediate or resistant category) were classified as non-susceptible (Clinical Laboratory Standards Institute, 2011). Resistance profiles were generated, with isolates classified as MDR if they showed non-susceptibility to one antimicrobial agent in three or more antimicrobial classes (Magiorakos et al., 2012). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) ECOFFs were used to select isolates with an MIC value above the wild-type for ESCs and FQs.

Whole Genome Sequencing

Whole genome sequencing was performed on eight isolates that had an ECOFF value above the wild-type for either ESCs or FQs, using Illumina MiSeq as described by Worthing et al. (2017). Briefly, samples underwent library preparation using the Nextera XT DNA library preparation kit according to the manufacturer's instructions, and sequencing was performed on a MiSeq V3 2x300 flow cell. The Nullarbor pipeline v1.01 (https://github.com/tseemann/nullarbor) was used to assemble the eight Illumina sequenced strains. The resulting FASTA files were analyzed using the ResFinder, VirulenceFinder and PlasmidFinder functions of the Centre for Genomic Epidemiology database (http://www.genomicepidemiology.org/).

TABLE 1 | Breakpoints used for AST testing of *E. coli* and *Salmonella* spp. isolates.

Antimicrobial class	Antimicrobial agent	Range (μg/ml)	ECOFF[a]		CLSI[b] or NARMS[c]		
			E. coli	*Salmonella* spp.	S	I	R
Aminoglycosides	Gentamicin	0.25–16	2	2	≤4	8	≥16
	Streptomycin	2–64	16	16	≤32	–	> 32
β-lactam / β-lactam inhibitor combinations	Amoxicillin-clavulanate	1–32	–[d]	–	≤8	16	≥32
Cephems	Cefoxitin	0.5–32	8	8	≤8	16	≥32
	Ceftiofur	0.12–8	1	2	≤2 [e]	4	≥8
	Ceftriaxone	0.25–64	0.12	–	≤1	2	≥4
Fluoroquinolones	Ciprofloxacin (*E. coli*)	0.015–4	0.06	0.06	≤1	2	≥4
	Ciprofloxacin (*Salmonella* spp.)	0.015–4	0.06	0.06	≤0.06	0.12–0.5	≥1
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	0.12–4	1	1	≤2	–	≥4
Penicillins	Ampicillin	1–32	8	8	≤8	16	≥32
Phenicols	Chloramphenicol	2–32	16	16	≤8	16	≥32
Tetracyclines	Tetracycline	4–32	8	8	≤4	8	≥16

[a] *EUCAST epidemiological cut-off values (μg/ml).*
[b] *CLSI VET01S, or M100S breakpoints (μg/ml), S = sensitive; I = intermediate; R = resistant.*
[c] *NARMS breakpoints (μg/ml) (in blue).*
[d] *not defined.*
[e] *E. coli only.*

RESULTS

Culture Results

E. coli was isolated from all porcine cecal samples collected ($n = 201$). In contrast, *Salmonella* spp. were only recovered from cecal samples from 14 of the 19 (73.7%) farms sampled ($n = 69$ isolates).

Phenotypic Antimicrobial Resistance Characterization

The 201 *E. coli* isolates showed the highest levels of non-susceptibility to ampicillin (60.2%), tetracycline (68.2%), chloramphenicol (47.8%) and trimethoprim/sulfamethoxazole (34.3%) (**Figure 1**). By contrast, although the 69 *Salmonella* spp. isolates also showed the highest levels of non-susceptibility to ampicillin and tetracycline, these had a lower frequency of occurrence (20.3 and 26.1%, respectively). Furthermore, *Salmonella* spp. isolates had lower levels of non-susceptibility to trimethoprim/sulfamethoxazole (11.6%) and chloramphenicol (7.3%). Fifty-one percent of *E. coli* and 21.7% of *Salmonella* spp. isolates were classified as MDR.

Low levels of non-susceptibility were observed among isolates of both species to amoxicillin/clavulanate (*E. coli* 9.5%; *Salmonella* spp. 2.9%) and gentamicin (*E. coli* 0.5%; *Salmonella* spp. 2.9%). Overall, low levels of non-susceptibility were detected to antimicrobials classified as critically important to human health (ESCs and FQs). Ceftiofur non-susceptibility was not observed for either *E. coli* or *Salmonella* spp. However, two *Salmonella* spp. isolates (2.9%) were found to have MIC values above the wild-type ECOFF (**Table 2**). Four *E. coli* isolates (2.0%) had ciprofloxacin MICs above 0.25 μg/ml, which is also above the wild-type ECOFF (MIC $>$ 0.06 μg/ml). However, only two of these isolates, both obtained from the same farm (farm Q), had MICs above the CLSI resistant clinical breakpoint (MICs of $>$ 4 μg/ml), despite no reported usage of FQs on this farm according to the Australian Pork Industry Quality Assurance Program (APIQ) audits. In addition, two *E. coli* isolates (1.0%) had cefoxitin MICs above the wild-type ECOFF and were classified as non-susceptible on the basis of CLSI clinical breakpoints. All isolates from farm Q ($n = 12$) were also classified as MDR. One *E. coli* isolate (MIC above the cefoxitin wild-type ECOFF) and one *Salmonella* spp. isolate (MIC above the ceftiofur wild-type ECOFF) were isolated from the same farm (farm D). All other isolates showing non-susceptibility to ESCs and/or FQs were obtained from different farms [farms H, K, R, and X ($n = 1$ for all farms)]. One of the *Salmonella* spp. isolates with a MIC value above the wild-type ECOFF for ceftiofur showed susceptibility to all other antimicrobials tested (farm H). The other seven isolates of interest were classified as MDR.

The percentage of MDR *E. coli* isolates was further broken down and analyzed on a per farm basis (**Figure 2**). Although the sample size per farm is limited, there is sufficient evidence to suggest there is a large variation between farms in the underlying proportion of *E. coli* that are MDR. The *Salmonella* spp. were unable to be analyzed by farm due to the lower number of isolates obtained. As shown in **Table 3** the most common MDR profile was non-susceptibility to β-lactam/β-lactam inhibitor combinations, phenicols, aminoglycosides, tetracyclines and folate pathway inhibitors; followed by non-susceptibility to β-lactam/β-lactam inhibitors, phenicols and tetracyclines.

Molecular Characterization

Eight isolates were selected for whole genome sequencing based on having MICs at or above the ECOFF for CIAs. These comprised the two *E. coli* isolates that were clinically resistant to FQs from farm Q (ciprofloxacin MICs $>$ 4 μg/ml), two *E. coli*

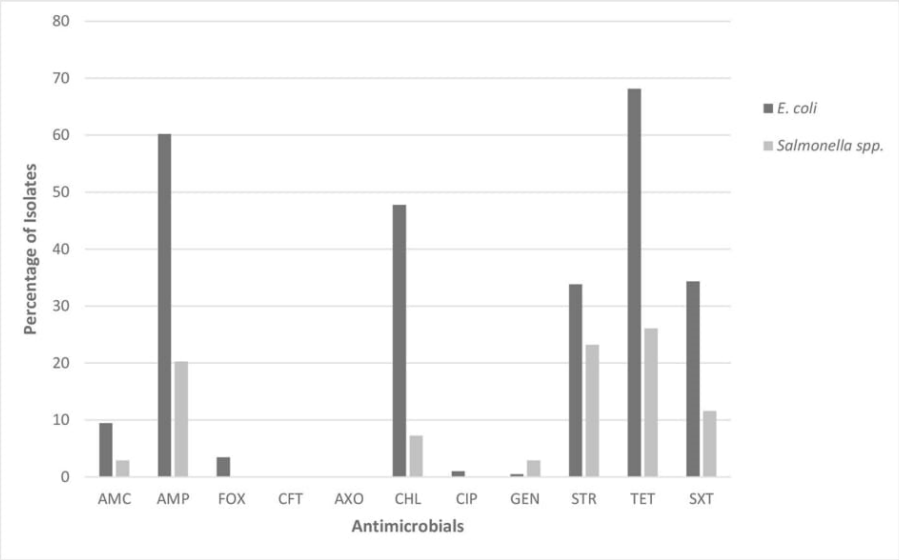

FIGURE 1 | Percentage of *E. coli* ($n = 201$) and *Salmonella* spp. ($n = 69$) isolates showing non-susceptibility to 11 selected antimicrobials. AMC, amoxicillin/clavulanic acid; AMP, ampicillin; FOX, cefoxitin; CFT, ceftiofur; AXO, ceftriaxone; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; STR, streptomycin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole.

isolates with ciprofloxacin MICs of 0.25 μg/ml and 0.5 μg/ml, two *E. coli* isolates with cefoxitin MICs of 32 μg/ml, and two *Salmonella* isolates with ceftiofur MICs of 2 μg/ml (**Table 4**).

Four of the six *E. coli* isolates belonged to *E. coli* sequence type (ST) 10, which belongs to phylogenetic group A and is commonly isolated from a range of animal species as well as humans. All *E. coli* ST10 isolates were classified as MDR and possessed at least one β-lactamase gene, but no ESC resistance-associated genes were identified in these isolates. The three ST10 isolates with the highest ciprofloxacin MICs had similar amino acid substitutions in the quinolone resistance-determining regions (QRDRs) of DNA gyrase A subunit (GyrA) and topoisomerase IV A subunit (ParC). The main substitutions of note were S83L and D87N in GyrA and S80I in ParC. Although other point mutations were identified in GyrB (A185G) and ParE (I136V), these are not typically associated with quinolone resistance. Whilst it is possible that additional mechanisms of FQ resistance, such as overexpression of efflux pumps may be present in the two ST10 isolates with ciprofloxacin MICs $>$ 4.0 μg/ml, their genomes were not interrogated further.

The remaining *E. coli* isolates belonged to a new sequence type, designated ST7573, and ST4417 (**Table 4**). The isolate belonging to ST7573 was the only isolate that contained a plasmid-mediated quinolone resistance (PMQR) gene (*qnrS1*). However, this isolate was still classified as ciprofloxacin-susceptible according to CLSI guidelines, and did not possess any identifiable chromosomally-encoded FQ resistance mechanisms. Further, this isolate was MDR and contained the extraintestinal pathogenic *E. coli* (ExPEC) virulence factor gene *iss*, which encodes for increased serum survival, in addition to a range of microcin-associated genes, the EAST-1 toxin (*astA*) and long polar flagella genes (*ipfA*) (**Table 4**). However, this particular combination of *E. coli* virulence genes does not classify the ST7573 isolate as belonging to any particular *E. coli* pathotype.

The *iss* virulence associated gene was also identified in two *E. coli* isolates belonging to ST10 together with *gad*, a glutamate decarboxylase gene involved in acid tolerance. Apart from the cellobiose utilization gene *celB* being identified in one ST10 isolate and the single ST4417 isolate, no other ExPEC-associated virulence genes were identified in any of the isolates subjected to whole genome sequence analysis.

The two *Salmonella* spp. isolates belonged to ST469 (serotype Rissen), a commonly distributed serotype previously associated with pig production, and ST515 (serotype Johannesburg).

Isolate sequences were deposited in Enterobase with the accession numbers: traces-0GpondC, traces-0GQRdxI, traces-0YZcnKW, traces-0ILjJIq, traces-0bhIgfw, and traces-0fwHIrT (*E. coli* isolates 1, 2, 3, 4, 5, and 6 respectively) and traces-0OMUTNy and traces-0LIahFC (*Salmonella* spp. isolates 1 and 2).

DISCUSSION

The main aims of this study were to investigate the occurrence of AMR among *E. coli* and *Salmonella* spp. isolated from healthy Australian finisher pigs at slaughter and further characterize any isolates found to be non-susceptible to CIAs (ESCs and FQs) using whole genome sequencing. The major findings from this study are: (1) Low levels of non-susceptibility to CIAs were detected among both *E. coli* and *Salmonella* spp. isolates; (2) Of the eight isolates with MICs above the wild-type for either ciprofloxacin, cefoxitin or ceftiofur, four *E. coli* isolates belonged

TABLE 2 | MIC distribution frequency of *E. coli* ($n = 201$) and *Salmonella* spp. ($n = 69$) isolates.

Number and percentage of isolates with MICs (μg/ml) at:[a]																
Antimicrobial Agent	**Species**	**0.008**	**0.015**	**0.03**	**0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**
Ampicillin	*E. coli*								7 3.48	29 14.43	41 20.40	3 1.49	1 0.50	0 -	120 59.70	
	Salmonella spp.								51 73.91	4 5.80	0 -	0 -	0 -	0 -	14 20.29	
Amoxicillin / clavulanate	*E. coli*								1 0.50	18 8.96	64 31.84	99 49.25	19 9.45	0 0.50		
	Salmonella spp.								53 76.81	2 2.90	0 -	12 17.39	2 2.90	0 -		
Ceftiofur	*E. coli*					0 -	63 31.34	127 63.18	11 5.47	0 -	0 -	0 -				
	Salmonella spp.					0 -	0 -	3 4.35	64 92.75	2 2.90	0 -	0 -				
Cefoxitin	*E. coli*							0 -	0 -	19 9.45	126 62.69	49 24.38	5 2.49	2 1.0		
	Salmonella spp.							0 -	0 -	17 24.64	50 72.46	2 2.90	0	0		
Ceftriaxone	*E. coli*						201 100	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	
	Salmonella spp.						69 100	0 -	0 -	0 -	0 -	0 -	0 -	0 -	0 -	
Chloramphenicol	*E. coli*									5 2.49	30 14.93	70 34.83	12 5.97	39 19.4	45 22.39	
	Salmonella spp.									0 -	7 10.15	57 82.61	0 -	0 -	5 7.25	
Ciprofloxacin	*E. coli*		184 91.54	13 6.47	0 -	0 -	1 0.50	1 0.50	0 -	0 -	0 -	2 1.0				
	Salmonella spp.		46 66.67	23 33.33	0 -	0 -	0 -	0 -	0 -	0 -	0 -					
Gentamicin	*E. coli*						10 4.98	114 56.72	70 34.83	6 2.99	0 -	1 0.50	0 -			
	Salmonella spp.						1 1.45	62 89.86	4 5.80	0 -	0 -	0 -	0 -	2 2.90		
Streptomycin	*E. coli*									1 0.50	30 14.93	49 24.38	23 11.44	30 14.93	34 16.92	34 16.92
	Salmonella spp.									0 -	6 8.70	13 18.84	33 47.83	1 1.45	5 7.25	11 15.94
Tetracycline	*E. coli*										64 31.84	1 0.50	0 -	6 2.99	130 64.68	
	Salmonella spp.										51 73.91	0 -	0 -	0 -	18 26.09	
Trimethoprim / sulfamethoxazole	*E. coli*					112 55.72	13 6.47	6 2.99	1 0.50	0 -	0 -	69 34.33				
	Salmonella spp.					58 84.06	1 1.45	0 -	0 -	2 2.90	2 2.90	6 8.70				

[a] *Unshaded areas indicate MIC range for each agent available on the Sensititre CMV3AGNF card. MICs > than highest concentration available are indicated in the shaded region. Vertical blue lines indicate EUCAST ECOFF values; CLSI susceptible (green) and resistant (red) breakpoints; and NARMS breakpoints (red dashes).*

to ST10 including two isolates that were clinically resistant to ciprofloxacin and one *Salmonella* spp. isolate belonged to the internationally distributed ST469 associated with serotype Rissen; and (3) High frequencies of non-susceptibility were observed to antimicrobial classes with a lower importance rating (Australian Strategic and Technical Advisory Group on AMR (ASTAG), 2015; Australian Veterinary Association, 2015) that are registered for use in pigs in Australia (i.e., tetracyclines, aminopenicillins and sulphonamide/trimethoprim combinations).

Ceftiofur resistance was previously reported in porcine commensal *E. coli* isolated from 1.8% of pooled fecal samples from finisher pigs at Australian piggeries. However, none of the isolates possessed plasmid-mediated AmpC or extended-spectrum β-lactamases (ESBLs) (Smith et al., 2016). The first detection of ESC resistance associated with ESBLs in *E. coli* from Australian food-producing animals was reported in clinical isolates in 2015. A national survey of clinical isolates from diseased pigs obtained from veterinary diagnostic laboratories identified three porcine *E. coli* isolates (2.6%) as resistant to ceftiofur, with one isolate, identified as an ST774 strain, also exhibiting resistance to ciprofloxacin (Abraham et al., 2015). The frequency of ciprofloxacin non-susceptibility observed in the present study was also low (1%) with two isolates from farm Q both exhibiting ciprofloxacin MICs above the resistant clinical breakpoint (indicating that FQ-R ST10 was the dominant *E. coli* present in the gut of slaughter age pigs on this farm). This is a significant finding as it indicates the presence of resistance to FQs in commensal *E. coli* from Australian pigs despite the absence of direct on farm selection pressure. This correlates to data from a recent Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) report where 1% of *E. coli* isolated from cecal samples randomly collected from healthy pigs at slaughter were identified as being ciprofloxacin non-wild-type (Høg et al., 2015). FQ resistance has previously been strongly correlated with the quantity of

antimicrobials used in the treatment of pigs (Barton, 2014), so given the legal constraints on FQ use in Australian pigs it is possible that these isolates did not develop FQ resistance on farm and were likely introduced from an extraneous source. One hypothesis is that they may have been introduced via human carriers or wild birds, as suggested by Abraham and co-workers (Abraham et al., 2015), but other potential sources of transmission could also be considered such as feed, water, rodents and insects. This highlights the need for emphasis of biosecurity measures and their widespread application and extension to non-animal sources of AMR transmission, such as in-contact humans.

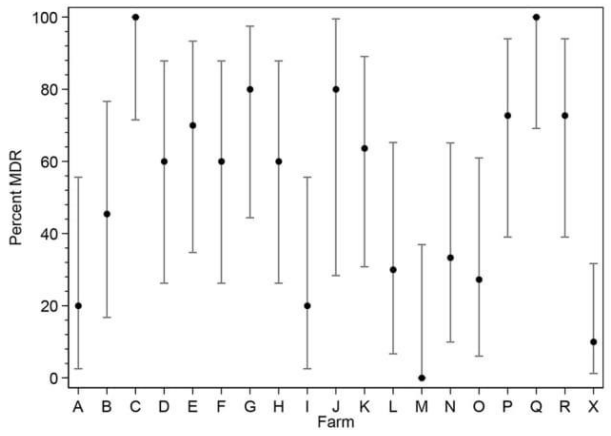

FIGURE 2 | Point estimates and 95% confidence interval for the percent of commensal *E. coli* colonies expressing MDR phenotype within each of 19 Australian pig herds.

Resistance to ceftiofur in commensal bacteria (*Salmonella* and *E. coli*) isolated from pigs was first reported in 2002 in south-east Asia (Hanson et al., 2002). Other reports soon followed (Barton, 2014). A recent Australian regional survey of fecal samples from 22 Australian commercial pig farms found 5.2% of *E. coli* isolates were resistant to ceftiofur (van Breda et al., 2018). The emergence of some level of non-susceptibility to ceftiofur in Australian herds is not unexpected, given previous reporting of "off-label" use in individual pigs, which is assumed to be for the treatment of ETEC infection (Jordan et al., 2009), raising concerns of the potential for cross-transfer of ceftiofur non-susceptibility to humans via the food chain. However, the results of the current Australia-wide survey suggest that ceftiofur-resistant commensal *E. coli* are not dominant coliforms in the gut of slaughter age pigs that are likely to be isolated during AMR surveillance programs. Nevertheless, the emergence and recent detection of ceftiofur-resistant *E. coli* containing AmpC and ESBLs (Abraham et al., 2015; van Breda et al., 2018) confirms that off-label use of ESCs should be more critically evaluated by the Australian industry, as has recently occurred in Denmark (Agersø and Aarestrup, 2013). The Danish Agriculture and Food Council recommended a voluntary ban on the use of ESCs in pigs following increased detection of ESBL resistance genes in swine production facilities (Agersø and Aarestrup, 2013). It is noted that FQs are registered for use in food-producing animals in many European countries, such as Denmark, although restrictions on their use were introduced by the Danish Veterinary and Food Administration (DVFA) in 2002 (Høg et al., 2015).

Multilocus sequence type (ST10) is an extremely diverse *E. coli* lineage found in many different host species, belonging

TABLE 3 | Resistant and MDR profiles with the highest frequency in *E. coli* ($n = 201$) and *Salmonella* spp. ($n = 69$).

Number of antimicrobial classes	Total no. of isolates (%)		Resistance pattern (no. of isolates)	
	E. coli	***Salmonella* spp.**	***E. coli***	***Salmonella* spp.**
All susceptible	26 (12.94)	46 (66.67)	26	45
1	33 (16.42)	6 (8.70)	TET (20)	SXT (3) TET (3)
2	40 (19.90)	2 (2.90)	BLA-TET (18)	AMG-BLA (1) CHL-SXT (1)
3	36 (17.91)	11 (15.94)	BLA-CHL-TET (13)	AMG-BLA-TET (9)
4	40 (19.90)	1 (1.45)	BLA-CHL-SXT-TET (11) AMG-CHL-SXT-TET (11)	AMG-BLA-SXT-TET (1)
5	20 (9.95)	2 (2.90)	AMG-BLA-CHL-SXT-TET (14)	AMG-BLA-BLI-SXT-TET (1) AMG-BLA-CHL-SXT-TET(1)
6	3 (1.49)	1 (1.45)	AMG-BLA-BLI-CHL-SXT-TET (2)	AMG-BLA-BLI-CHL-SXT-TET (1)
7	3 (1.49)	0 (0)	AMG-BLA-BLI-CHL-2GC-SXT-TET (2)	-
Non-MDR	99 (49.25)	54 (78.26)	99	54
MDR	102 (50.75)	15 (21.74)	102	15

Antimicrobial categories included: aminoglycosides, AMG (gentamicin, streptomycin); penicillin, BLA (ampicillin); β-lactam inhibitors, BLI (amoxicillin-clavulanate); phenicols, CHL (chloramphenicol); 2nd generation cephalosporins, 2GC (cefoxitin); folate pathway inhibitors, SXT (trimethoprim/sulfamethoxazole); and tetracycline, TET.

TABLE 4 | Comparison of AMR genes, virulence genes, plasmids and multilocus sequence type (MLST) in eight isolates with reduced susceptibility to ESCs and/or FQs.

Isolate	Species	MIC values	MLST	Resistance genotype	Plasmids	Virulence genes	QRDR mutations	
							QRDR	Amino acid substitution
1	*E. coli*	FOX 32	10	*bla*$_{TEM-1B}$, *tet(B)*	*IncR*, *IncY*	*gad*	Not determined	
2	*E. coli*	CIP 0.25	New (7573)	*aadA2*, *aadA1*, *bla*$_{TEM-1B}$, *qnrS1*, *floR*, *cmlA1*, *sul3*, *sul2*, *tet(A)*, *tet(M)*, *dfrA12*	*IncFIB(AP001918)*, *Col8282*, *p0111*, *IncFIA(HI1)*, *IncHI1B(R27)*, *IncHI1A*	*mchC*, *ipfA*, *mchB*, *iss*, *mcmA*, *mchF*, *astA*	Not Detected	
3	*E. coli*	CIP ≥4	10	*aadA1*, *aph(3′)-Ia*, *bla*$_{TEM-1B}$, *sul3*, *tet(A)*, *tet(M)*, *dfrA1*	*IncFII*, *IncX1*, *IncR*	*iss*, *gad*	DNA gyrase A subunit (GyrA)	Ser(83)—Leu Asp (87)—Asn Glu (678)—Asp Ser (828)—Ala
							DNA gyrase B subunit (GyrB)	Asp(185)—Glu
							Topoisomerase IV A subunit (ParC)	Ser(80)—Ile Glu(475)—Asp
							Topoisomerase IV B subunit (ParE)	Ile(136)—Val
4	*E. coli*	CIP ≥4	10	*aadA1*, *aph(3′)-Ia*, *bla*$_{TEM-1B}$, *sul3*, *tet(A)*, *tet(M)*, *dfrA1*	*IncX1*, *IncFII*, *IncR*	*iss*, *gad*	DNA gyrase A subunit (GyrA)	Ser(83)—Leu Asp (87)—Asn Glu (678)—Asp Ser (828)—Ala
							DNA gyrase B subunit (GyrB)	Asp(185)—Glu
							Topoisomerase IV A subunit (ParC)	Ser(80)—Ile Glu(475)—Asp
							Topoisomerase IV B subunit (ParE)	Ile(136)—Val
5	*E. coli*	CIP 0.5	10	*aadA2*, *aadA1*, *bla*$_{TEM-1A}$, *mef(B)*, *cmlA1*, *sul3*, *tet(A)*, *dfrA12*	*p0111*, *IncX4*, *IncFIA(HI1)*, *IncFIB(K)*	*celb*, *gad*	DNA gyrase A subunit (GyrA)	Ser(83)—Leu Glu (678)—Asp Ser (828)—Ala
							DNA gyrase B subunit (GyrB)	Asp(185)—Glu
							Topoisomerase IV A subunit (ParC)	Glu(475)—Asp
							Topoisomerase IV B subunit (ParE)	Ile(136)—Val
6	*E. coli*	FOX 32	4417	*aadA2*, *strA*, *strB*, *sul3*, *sul1*, *tet(A)*, *dfrA12*	*Col(MG828)*, *IncY*	*gad*, *celb*,*ipfA*	Not determined	

(Continued)

TABLE 4 | Continued

Isolate	Species	MIC values	MLST	Resistance genotype	Plasmids	Virulence genes	QRDR mutations	
							QRDR	Amino acid substitution
7	*Salmonella spp.*	CEF 2	469	*aadA2, strA, aph(3′)-Ic, strB, bla_{TEM-1B}, catA1, floR, sul1, sul2, tet(D), dfrA12*	*Col(MG828), IncA/C2*	*celb*		Not determined
8	*Salmonella spp.*	CEF 2	515	*dfrA5*	*IncFII(29), IncFIB(AP001918)*	-		Not determined

Virulence gene functions: gad: glutamate decarboxylase enzyme associated with acid tolerance; ipfA: adhesion that encodes long polar fimbriae that plays integral role in attachment of EPEC to the gut wall (Blum and Leitner, 2013) and can cause diarrhea in humans; iss: encodes increased serum survival protein which increases survival in animal serum (Miajlovic and Smith, 2014) and is recognized for its role in ExPEC virulence; mcmA: a microcin precursor gene encoding microcin M. Microcins are antimicrobial peptides secreted by members of the Enterobacteriaceae family and are involved in microbial competition within the intestinal tract (Vassiliadis et al., 2010); astA: encodes for heat stable enterotoxin EAST1 that is found mostly in enterohemorrhagic and enteroaggegative E. coli; celb encodes for the induction of cell lysis and the subsequent release of the endonuclease colicin E2 in E. coli (Mader et al., 2015); mchC, mchB and mchF are all involved in the production of microcin peptide antibiotics called in enterobacterial strains. mchB encodes for the synthesis of the microcin H47 (MccH47) peptide precursor. The product of mchC and other mch genes carry out the process of maturation of MccH47by adding an enterobactin derivative which is employed for MccH47 synthesis. After synthesis MccH47 is secreted through a type I apparatus that is formed by products of mchF and another mch gene (Poey et al., 2006).

to phylogenetic group A (Fischer et al., 2017). It was the most prevalent ST among the *E. coli* isolates submitted for whole genome sequencing (four of six isolates; 66.67%) and was overrepresented in a recent whole genome sequence analysis of porcine commensal *E. coli* isolated from two piggeries in Australia (Reid et al., 2017). ST10 is recognized as a potentially zooanthroponotic commensal clonal lineage that has also been identified as a cause of extraintestinal infections in humans in both hospital and community settings in the Netherlands and Canada. It has also been detected in poultry, wild birds, pigs and retail chicken and pork meat (Abraham et al., 2015). Previous studies have isolated an ESC-non-susceptible ST10 *E. coli* strain from a calf with diarrhea in Australia (Abraham et al., 2015), as well as dust and manure samples from piggeries in Germany (Fischer et al., 2017). Carriage by European farmers has also been previously reported (Fischer et al., 2017). However, its potential contribution to the spread of AMR between humans and animals is a very recent observation (Wang et al., 2016). Its frequent association with ESBL production, and widespread detection in humans, meat products and food animals, are important epidemiological traits (Manges and Johnson, 2012). Although the increased serum survival (*iss)* gene, recognized for its role in ExPEC infections (Miajlovic and Smith, 2014), was identified in half the porcine ST10 isolates obtained in the present study, the isolates do not conform to the molecular definition of an ExPEC strain as they do not contain $\geq$2 of the ExPEC associated virulence genes *papA* and/or *papC*, *sfa/focDE*, *afa/draBC*, *kpsM II*, and *iutA* (Guo et al., 2015).

In the absence of PMQR genes, mutational alterations in the FQ target enzymes DNA topoisomerase II and topoisomerase IV are the major mechanisms through which chromosomal resistance occurs in Gram-negative bacteria (Gruger et al., 2004). In isolates showing FQ non-susceptibility, such as the ST10 isolates identified in this study, DNA gyrase, the primary target in Gram-negative bacteria, normally possesses GyrA subunit substitutions at amino acid positions S83 and/or D87 and ParC subunit substitutions at S80 and E84 (Gruger et al., 2004). One of the most common mutations that results in high level resistance to FQs alters S83 to either L or W, which can result in an approximate 10-fold increase in MIC (Gruger et al., 2004). All three of the most common mutation sites (S83, D87 and S80) were present in the two FQ-resistant ST10 isolates (MICs $> 4\,\mu$g/ml) and a single isolate above the wild-type MIC (MIC 0.5 μg/ml) obtained in this study.

The isolate belonging to the new ST7573 contained the *ipfA* virulence gene, which encodes an adhesin that plays an integral role in attachment of enteropathogenic *E. coli* (EPEC) to the gut wall and has been found to be prevalent in both clinical and commensal *E. coli* isolated from human and bovine hosts (Blum and Leitner, 2013). This gene was identified in association with the *iss* serum survival gene and *astA* toxin genes, together with genes encoding microcins and microcin immunity. In a recent study by Blum and Leitner (2013), *iss* and *astA* were the most prevalent virulence factor genes identified in *E. coli* associated with bovine mastitis (Blum and Leitner, 2013). A recent whole genome sequence comparative analysis of 103 porcine commensal *E. coli* from two piggeries in Australia

identified a greater array of extraintestinal *E. coli* virulence genes in 14 ST10 isolates (Reid et al., 2017), but none would conform to the strict definition of an ExPEC strain (Guo et al., 2015). It is therefore difficult to infer if these genes are adaption genes commonly found in commensal *E. coli* or true virulence genes (Abraham et al., 2012).

Two *Salmonella* spp. isolates with MICs above the ceftiofur ECOFF were further characterized by whole genome sequencing. Interestingly, one of the isolates belonging to ST469, a sequence type commonly associated with the *Salmonella* spp. serotype Rissen. This serotype is commonly isolated from both humans and pork production systems in different parts of the world, notably Asia (Pornsukarom et al., 2015). The second ST515 (serotype Johannesburg) has been previously isolated from a mix of environmental, human and livestock isolates from Nigeria, the U.S.A and the U.K. and interestingly, from boneless camel meat in Australia (http://enterobase.warwick.ac.uk).

In conclusion, this study has identified *E. coli* isolates with MICs above the wild-type for ciprofloxacin that belong to diverse host range clonal lineages, such as ST10, in Australian piggeries, despite strict biosecurity and the absence of FQ selection pressure (Abraham et al., 2015). Such strains may have been introduced into the Australian piggery environment from an external source, possibly via humans, migratory birds or other vectors. Overall, however, the results of this study endorse the generally conservative approach to the use of CIAs in the Australian pig industry as only very low levels of non-susceptibility to these drugs were detected among both *E. coli* and *Salmonella* spp. isolates from healthy finisher pigs. This represents a baseline for benchmarking in future AMR surveillance programs.

AUTHOR CONTRIBUTIONS

AK performed experiments, data analysis, and drafted and prepared the manuscript. JB performed experiments and analyzed data. MO performed whole genome sequencing, sequencing analysis, and was involved in manuscript preparation. TL performed whole genome sequencing. SA, DJ, PM, CM, and DT were involved in experimental design development and manuscript preparation.

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Chapter 7

General discussion

7.1. General summary

E. coli is an important bacterial pathogen that is associated with extraintestinal disease in both humans and animals. It is the causative agent of many types of infection in cats and dogs; including urinary, respiratory, skin and soft tissue, gastrointestinal tract, joint, and wound infections (Guardabassi et al., 2004; Zogg et al., 2018a). *E. coli* also causes both intestinal and extraintestinal forms of disease in food-producing animal species, for example, newborn piglets can develop scours and septicaemia, weaners are prone to post-weaning diarrhoea and oedema disease, and sows can suffer from mastitis and cystitis (Zimmerman et al., 2012). Phylogenetic analyses have shown that commensal and intestinal pathogenic *E. coli* strains are typically associated with groups A, B1, and C, whereas virulent ExPEC strains are mainly associated with group B2 and, to a lesser extent, groups D, E, and F (Zogg et al., 2018b).

Antimicrobials are crucial for the treatment and control of a wide range of bacterial diseases in both companion and food-producing animals (Smith et al., 2016). AMR development in ExPEC isolates is an ongoing concern for both companion animal and human health. Increasingly, reports have identified MDR ExPEC causing infection in dogs and cats, in particular isolates resistant to CIAs including ESBL-producers (Marques et al., 2018). AMR in commensal and zoonotic bacteria isolated from food-producing animals also varies greatly throughout the world, especially in relation to use of and resistance to CIAs such as FQs and ESCs (Österberg et al., 2016). With increasing detection of multidrug resistance in animals, there is growing concern of the risk of animal-to-human transfer of AMR, especially in cases of close contact between humans and companion animals in household and hospital settings (Guo et al., 2013), and the risk of AMR transfer through the food chain with respect to commensal (e.g. *E. coli*) and zoonotic bacteria (e.g. *Salmonella*) isolated from food-producing animals

(Shaban et al., 2014). Given these increasing risks, it is fundamental that activities such as the identification of the main clonal lineages of bacteria responsible for AMR in animals is undertaken. The present study sought to identify the clonal lineages of *E. coli* isolated from extraintestinal infections in dogs and cats, and healthy pigs at slaughter in Australia, with particular focus on isolates resistant to FQs and ESCs. This type of study is crucial to evaluate the extent to which dogs and cats with UTIs are reservoirs for resistant clonal lineages such as ST131 and ST1193, as well as determine the risk posed by antimicrobial use in an important food-producing animal species, the pig (Shaban et al., 2014).

Bacteria are continuously evolving in response to many factors including antimicrobial exposure and selective pressures and AMR is known to change between geographical regions and over time (ECDC, 2015). These factors can result in the emergence and dissemination of new clonal lineages with unique AMR mechanisms (Hartmann et al., 2018). Due to the rapidly evolving nature of bacteria, it is critical that surveillance studies are regularly conducted to investigate the spread of AMR (Shaban et al., 2014).

AMR monitoring and surveillance programs play an important role in human medicine and have been undertaken in Australia over many years (https://agargroup.org.au). These programs aid human clinicians in both hospitals and the community to make appropriate empirical antimicrobial choices (Moyaert et al., 2017). Without similar AMR surveillance programmes focused on animal pathogens and commensals, veterinarians are directly impacted and may possibly prescribe inappropriate treatments.

Numerous studies have drawn links between ExPEC present in both humans and in-contact companion animals, indicating that certain clones are often shared between household members (Johnson et al., 2009). Until relatively recently, however,

national AMR veterinary surveillance programmes have only focused on commensal and zoonotic bacteria present in production animals at slaughter due to the concern of antimicrobial-resistant bacteria entering the food chain. Nevertheless, the monitoring of AMR in companion animals is now attracting similar attention (McMeekin et al., 2017), despite the fact that many national programs focused on AMR surveillance in animals neglect to include cats and dogs. Additionally, though many previous studies have described ExPEC resistant to CIAs (in particular AMR mediated by ESBLs) in companion animals, comparatively fewer studies have focused on the susceptible isolates likely to be obtained in a national surveillance program aimed at determining the prevalence of resistance to CIAs, in particular, FQs and ESCs.

Given the advent of relatively inexpensive, high-throughput WGS methodologies and the availability of large, national collections of isolates from companion animals (Saputra et al., 2017) and pigs (Shaban et al., 2014), this study sought to further investigate the following: First, the prevalence and significance of ExPEC-associated STs from phylogenetic group B2 were determined among Australia-wide collections of FQ-susceptible clinical isolates from cats (Chapter 2) and dogs (Chapter 3). Second, STs associated with FQ resistance, in particular human-associated clonal lineages ST131 and ST1193, were screened among FQR isolates in the same Australia-wide collection (Chapter 4). Third, the clinical features and WGS of isolates derived from two unusual cases of canine malakoplakia, a type of granulomatous inflammation of the bladder normally associated with *E. coli* infection in humans but rarely in dogs, were described (Chapter 5). Fourth, for comparative purposes, an Australia-wide collection of *E. coli* and *Salmonella* isolates obtained from healthy pigs at slaughter was subjected to AST with isolates resistant to CIAs further interrogated by WGS.

7.2. Major findings

7.2.1. ST73 is a dominant sequence type among FQ-susceptible phylogroup B2 cat and dog clinical isolates in Australia

ST73, a broad host range clonal group belonging to phylogroup B2 and associated with UTI, urosepsis and septicaemia in humans (Mora et al., 2018), and UTI in companion animals (Zogg et al., 2018b), was the dominant sequence type identified among group B2 FQ-susceptible cat clinical isolates (68/274, 25%; Chapter 2) according to both WGS (23/53, 43%) and PCR screening (45/221, 20%). This result is in line with another study that identified ST73 (by MLST) as the predominant ST among group B2 cat UPEC from the United States (12/55, 22%) (Liu et al., 2015). To the best of my knowledge, this is the only cat-focused study that has identified ST73 as most studies include just dog or both dog and cat isolates. In addition, most studies have a higher proportion of dog-source isolates compared to cats. This is an important feature given the key differences in predisposing factors to infection between each host species (Litster et al., 2011; Thompson et al., 2011). However, one additional study that studied both dog- and cat-source ESBL- and non-ESBL-producing *E. coli* in Switzerland and differentiated the isolates' source, identified ST73 in 39% (5/13) of cat-source isolates (Zogg et al., 2018b). Of note, both of these studies identified ST73 in susceptible rather than resistant strains. Historically ST73 has been largely associated with antimicrobial susceptibility especially to CIAs such as ESCs and FQs (Dale and Woodford, 2015; Doumith et al., 2015). However, resistant strains are now being identified in humans (Zogg et al., 2018b) as well as animals. A number of recently described ST73 strains are resistant to antimicrobials used in veterinary medicine and are positive for ESBL resistance genes (Bogema et al., 2019). In the United Kingdom and Australia, the number of MDR ST73 human isolates containing ESBLs has been on the rise (Alhashash et al., 2016; Bogema et al., 2019). Despite this, the prevalence

of ST73 in susceptible strains is still higher, at least in companion animals. In two studies in the United States investigating ESBLs and UPEC strains, ST73 was the most prevalent ST among UPEC strains (12/74, 16%) (Liu et al., 2015) but only the fifth most prevalent ST among the ESBL-positive strains (3/68, 4%) (Liu et al., 2016b). This is consistent with the present study where ST73 was identified in FQ-susceptible but not FQR strains in cats and only 9 out of 23 isolates were classified as MDR, with no resistance to CIAs reported. A single ST73 FQ-susceptible isolate contained SHV resistance genes but no other ESBL resistance genes were identified. These results suggest that the presence of ST73 in companion animals is likely to be under reported due to previous focus on MDR strains.

While not as prevalent as among cat-source isolates, ST73 has been sporadically isolated from dogs. In a study of ESBL MDR *E. coli* in both cats and dogs, ST73 was identified in two (2/44, 4.5%) dog-source isolates (Chen et al., 2019). ST73 has also been identified in a study of group B2 *E. coli* isolates from dogs in France (16/89, 17.9%) (Valat et al., 2020). In the present study (Chapter 3) ST73 was identified, but in lower numbers compared to cat-source isolates (25% and 13% of cat- and dog-source isolates, respectively). Similarly, to the cat-source isolates, ST73 was only identified among FQ-susceptible rather than FQR strains and no ESBL genes were identified suggesting that the association of ST73 with susceptible isolates holds true for both cats and dogs. In a study, which focused on 18 MDR and 15 susceptible isolates from UTIs in dogs, ST73 was only identified in the susceptible group (4/15, 27%) (Wagner et al., 2014), further backing up this hypothesis. Though usually considered a human-specific lineage, these studies suggest ST73 is most probably an ubiquitous urinary pathogen normally residing in the gut of companion animals, as it has been described in both healthy and diseased groups (Wagner et al., 2014; Liu et al., 2015; Bourne et al., 2019)

7.2.2 Cat, dog and human ST73 isolates are highly diverse but share some cross-host species clonal commonality

It has been suggested that unlike ST131 (Johnson et al., 2013), ST73 epidemiology in humans is not characterised by the emergence and expansion of a single dominant clone, but rather is associated with widespread circulation of multiple, phylogenetically diverse clusters (Alhashash et al., 2016). Increased incidence of MDR ST73 among humans in the UK and Australia was neither due to the emergence of a dominant clone, nor the widespread transmission of a promiscuous plasmid encoding resistance to multiple antimicrobials including ESCs (Bogema et al., 2019). Further analysis of these MDR ST73 combined datasets, and an overlay of geographical and temporal data, indicate that ST73 is globally disseminated and phylogenetically distributed into serotype-specific clusters (Bogema et al., 2019).

In the present study, comparison of WGS data obtained for the cat, dog and above mentioned human-source ST73 isolates enabled a highly discriminatory molecular epidemiological analysis to be undertaken. ST73 isolates from the three host species were again highly diverse and split into serotype-specific clusters in a SNP-based core genome phylogeny. Although most cat, dog and human ST73 isolates were located within host-specific sub-clusters, some intermingling of isolates from different hosts within sub-clusters was apparent. This suggests there is some potential for bi-directional transmission of ST73 between humans and companion animals. Additionally, cat and dog-source ST73 isolates appear just as diverse as human ST73 isolates, although they were more likely to be closely related within serotype-specific clusters than contemporaneous human isolates and none of the companion animal isolates were resistant to ESCs. Interestingly, the cat and dog isolates from Australia were more closely related to human isolates from the UK than to those from Australia.

This is most likely due to the existence of a transmission network or reservoir of clonally diverse, circulating ST73 strains that has yet to be fully defined.

In support of the clonal commonality of ST73 between humans and animals, frequent transmission and long term (more than three years) persistence of an ST73 strain was demonstrated between members of the same family, including the pet dog (Reeves et al., 2011). An earlier study found ST73 strains from humans, dogs, and cats shared closely related PFGE types, which although not as discriminatory as WGS SNP analysis, is certainly suggestive of the potential for cross-host species transmission (Johnson et al., 2008).

7.2.3 ST372 is the dominant sequence type identified among FQ-susceptible phylogroup B2 dog clinical isolates in Australia

ST372, a ST previously associated with UTIs in both humans and dogs (Blyton and Gordon, 2017; LeCuyer et al., 2018; Valat et al., 2020), was the predominant ST identified among FQ-susceptible phylogenetic group B2 clinical *E. coli* isolates from dogs in Australia (Chapter 3). In a study of dog-source ExPEC isolates from the United States and France, ST372 was identified in 22% (64/295) and 20.7% (102/492) of isolates, respectively (LeCuyer et al., 2018; Valat et al., 2020). The relatively high prevalence in the US and French studies is in line with the current study which identified 31% (24/77) of isolates that underwent WGS and 22% (53/240) of isolates that underwent ST-specific PCR as ST372. ST372 has now been identified as the most common antimicrobial-susceptible ST causing extraintestinal infection in dogs in three independent international studies, each involving large collections of isolates. Other studies on resistant canine ExPEC have identified ST372 (Liu et al., 2016b; Karkaba et al., 2017; Maeyama et al., 2018; Zogg et al., 2018b; Chen et al., 2019) but at much lower frequency, confirming the hypothesis that this ST is most likely associated with susceptible rather than resistant dog-source ExPEC strains. For instance, in a study

of CTX-M positive isolates, ST372 was the fifth most prevalent ST identified among only 4.5% (2/44) of isolates (Chen et al., 2019). These results confirm the importance of focusing on both susceptible as well as resistant dog-source ExPEC isolates as the significance of ST372 is likely to be under reported in studies focused solely on resistant collections.

It has been previously hypothesised that whilst there is possible clonal commonality among ExPEC isolated from cats, dogs and humans, certain clonal lineages appear to be dominant among each host species. Indeed, the prevalence of ST372 among dog-source clinical ExPEC has been suggested to be similar to that of ST73 among cat-source UTI isolates (LeCuyer et al., 2018). These results suggest that each host species may have a particular ST that comprises most of the *E. coli* uropathogens isolated despite evidence of broad overlap of several STs across different host species.

ST372 prevalence among human and cat-source ExPEC appears to be much lower and sporadic (Adler et al., 2012; Wagner et al., 2014), particularly in comparison to the much more prevalent ST73 clonal lineage. For instance, among FQ-susceptible group B2 cat-source isolates in Australia, ST372 frequency was only 8% (4/53) (Chapter 2). To the best of my knowledge, no other study has identified this ST in isolates from cats, however, this is not surprising as most studies only focus on ESC-resistant rather than susceptible *E. coli*. It has been suggested that the high frequency of ST372 in dogs is likely to be more reliant on specific host adaptations rather than AMR in general (ANSES, 2018). Despite its association with antimicrobial susceptibility and general lack of AMR genes, ST372 isolates have recently been found to contain ESBL genes. A study into the prevalence of faecal carriage of ESC- and carbapenem-resistant isolates from healthy dogs and cats in France identified an OXA-48-producing ST372 isolate (Melo et al., 2017). Furthermore, in a study of CTX-M-positive isolates

from companion animals in China, the two identified ST372 isolates both contained CTX-M genes (Chen et al., 2019). Likewise, in another study of ESC- and carbapenem-resistant isolates in France, one of the three ST372 isolates identified was CTX-M-positive (Valat et al., 2020). In addition, in a study of ESBL-producing dog isolates in the United States, three ST372 isolates carried the SHV-12 ESBL gene (Liu et al., 2016b). In the present study (Chapter 3) 25% (6/24) of ST372 dog isolates were classified as MDR, however, none contained ESBL genes. Similarly, of the 59 FQR isolates characterised in Chapter 4, none belonged to ST372 or ST73. This further confirms that while ESBL genes have been identified in some ST372 dog-source isolates, this ST is still predominately associated with antimicrobial susceptibility in Australia.

7.2.4 Prevalence of ST131 as a cause of FQR *E. coli* infections in Australian companion animals has remained low

ST131 is a MDR extraintestinal group B2 clonal lineage of high apparent virulence that has been identified throughout the world, though much less frequently in companion animals compared to humans. In Australia, two different studies have identified ST131 in companion animals; Platell et al. (2010), which investigated FQR clinical isolates from companion animals between 2007 and 2009 (Platell et al., 2010), and the present study (Chapter 4). These studies have identified ST131 in 7.2% (9/125) and 6.9% (6/59) of isolates, respectively. Other studies that have identified ST131 in companion animals include a study of canine urine samples in the United States, where ST131 was identified in 4% (12/295) of isolates (LeCuyer et al., 2018), and a study in Switzerland that identified ST131 in 7.8% (5/64) of urine samples from cats and dogs (Zogg et al., 2018b). Interestingly, in the two Australian-based studies, the prevalence of ST131 as a cause of FQR infections in companion animals has remained relatively constant over the five-year break between studies.

Of the multiple studies that have identified ST131 in companion animals, only one study from Japan estimated prevalence at two distinct time points similar to Platell et al. (2010) and the present study. The number of isolates analysed, however, was very low and therefore subject to much wider confidence intervals. In the Japanese study, ST131 was identified in two sets of ESBL-positive isolates, one obtained between 2003 and 2010 (4/11, 36.4%), and the other in 2015 (15/42, 35.7%) (Harada et al., 2012; Kawamura et al., 2017). These values were much higher than the present study and the study of Platell et al. (2010) and were more in line with the prevalence of ST131 among human FQR isolates. Further genomic analysis of these isolates may identify different selection foci in Japan compared to Australia. Despite the prevalence of ST131 in companion animals being higher in the Japanese study, this prevalence is in line with a similar study conducted in ESBL-positive isolates from a Japanese hospital between 2001 and 2012 where 43% (461/1079) of isolates were identified as ST131 (Matsumura et al., 2015).

A number of previous studies have identified similarities between clinical *E. coli* isolates from humans and companion animals in terms of their genomic background which suggests the possibility of zoonotic transmission (Liu et al., 2015). In the present study, sequenced whole genomes of the Australian cat- and dog-source ST131 isolates were compared to a set of internationally distributed ST131 isolates from humans and a small number of animals. This showed close relationships between the animal-source and human-source isolates and the absence of a separate companion animal-source clade. Some of the animal ST131 isolates even shared very high identity with human isolates. While this high degree of clonal commonality amongst human and companion animal ST131 isolates suggests the possibility of bi-directional between-species transmission, the lower prevalence of ST131 in

companion animals compared to humans suggests that companion animals are spillover hosts for this pandemic clonal lineage rather than the primary reservoir.

7.2.5 The newly emergent FQR clonal group ST1193, first identified in Australia was not identified among the cat- and dog-source FQR *E. coli* collection

ST1193 has recently emerged as an important, globally disseminated MDR human pathogen (Johnson et al., 2019). Despite being first identified in approximately 10% of phylogroup B2 clinical FQR *E. coli* isolates from humans and dogs in Australia (Platell et al., 2012) as well as several international studies, including isolates from companion animals (Maeyama et al., 2018; Zhang et al., 2018), ST1193 was not identified in the present study. Even though ST1193 is often regarded as the second most common ST among FQR isolates, its higher prevalence in human-source isolates and its absence in the present study suggests that it is predominately a human-source clone that has undergone anthroponotic transfer, suggesting that companion animals are also potential spillover hosts for this ST in addition to ST131.

Platell et al. (2012) discovered that the two Australian ST1193 isolates from dogs, separated from each other on the basis of possession of K1 or K5 capsular antigens, were indistinguishable from human ST1193 K1 and K5 isolates by PFGE analysis. This further suggests that ST1193 dog and human isolates were derived from a common ancestor and appear to have undergone anthroponotic-zoonotic transfer (Platell et al., 2012).

ST1193 has been identified in FQR isolates since its first isolation and multiple studies have identified CTX-M ESBLs in both human and companion animal ST131 isolates. One recent report, however, has identified carbapenemase-producing ST1193 strains in the United States (Gomes et al., 2020). This is the first report of KPC

in ST1193, however, carbapenemases have been identified in ST131 isolates since 2005 (Hong et al., 2005).

7.2.6 Apart from ST73, ST372 and ST131, multiple diverse STs each containing low numbers of isolates are associated with FQ-susceptible and FQR ExPEC from companion animals in Australia

Despite there being some evidence of predominant STs among both cat- and dog-source isolates, overall, a large degree of genomic diversity was identified in the cat- and dog-source Australian *E. coli* collection. This corroborates multiple international studies showing a high degree of ST diversity in both susceptible and resistant cat- and dog-source ExPEC. For instance, a study of 33 antimicrobial-resistant and -susceptible UTI *E. coli* isolates from dogs in Scotland did not identify a predominant ST (Wagner et al., 2014). In additional studies, one in Switzerland and two in the United States, 26, 31 and 40 different STs were respectively identified, with several STs newly identified (Liu et al., 2015; Liu et al., 2016b; Zogg et al., 2018b). In a total of 10 studies (Table 1) investigating a variety of ExPEC isolate types (though mostly resistant isolates) from both cats and dogs, between 30% and 64% (median 53%) of isolates were unique to each study. In addition, from these ten studies, six novel STs not present in Enterobase were identified among 1.7% to 13.5% (median 6.5%) of isolates. In most of these studies, the majority of STs were represented by only one or two isolates. In the present study investigating 53 FQ-susceptible group B2 cat clinical isolates subjected to WGS (Chapter 2), 18 different STs were identified with three of these being novel. Similarly in the collection of 77 dog FQ-susceptible isolates subjected to WGS (Chapter 3), 24 STs were identified with two being novel.

Table 1: Diversity of minor STs in *E. coli* isolates from cats and dogs

Sample type	No. of STs identified	% of isolates belonging to different STs	No. of novel STs	% of isolates belonging to a novel ST	Reference
37 ESBL/AmpC producing isolates	23	62.2	2	5.4	(Dierikx et al., 2012)
74 UPEC isolates	40	54.1	10	13.5	(Liu et al., 2015)
68 ESBL-producing isolates	31	45.6	6	8.8	(Liu et al., 2016b)
40 ESC resistant isolates	22	55	0	NA	(Liu et al., 2016a)
89 clinical isolates	38	42.7	5	5.6	(Karkaba et al., 2017)
42 ESBL producers	16	38.1	0	NA	(Kawamura et al., 2017)
24 CTX-M-positive isolates	13	54.2	0	NA	(Zhang et al., 2018)
44 CTX-M-producing isolates	28	63.6	0	NA	(Chen et al., 2019)
44 non-WT 3GC resistant isolates	23	52.3	0	NA	(Pepin-Puget et al., 2020)
89 B2 isolates	27	30.3	6	6.7	(Valat et al., 2020)

Despite this genetic heterogeneity, there are a number of minor STs that are common between studies in addition to ST73, ST372 and ST131. These include ST12 (Liu et al., 2015; Karkaba et al., 2017; Liu et al., 2017; LeCuyer et al., 2018; Valat et al., 2020), ST38 (Liu et al., 2017; Pepin-Puget et al., 2020), ST405 (Bogaerts et al., 2015; Liu et al., 2017; Pepin-Puget et al., 2020), and ST648 (Huber et al., 2013; Karkaba et al., 2017; Liu et al., 2017; LeCuyer et al., 2018; Bortolami et al., 2019; Valat et al., 2020) which are often associated with ESC-resistant infections and have been identified in both humans and animals (Schaufler et al., 2019). ST12 is the only one of

these four STs that belongs to phylogenetic group B2 and hence was the only ST to be identified in the FQ-susceptible population of companion animal ExPEC (19/591, 3%). Other minor STs represented among the FQ-susceptible isolates included ST80 in dogs (5/77, 7%) and ST127 in cats (4/53, 8%). While ST80 was also identified in the present study in cats and ST127 in dogs each represented few isolates. Among STs associated with FQR isolates (ST38, ST405 and ST648), ST38 was the most prevalent (13.6% of strains); ST405 was identified in 3% of strains (2/59), while only a single isolate was identified as ST648.

Although ST38 is considered a globally disseminated ESBL-producing ExPEC lineage in humans (Shaik et al., 2017) it has only sporadically been identified in companion animals. To the best of my knowledge, only one report in Japan (Kawamura et al., 2017) and two in Australia (Guo et al., 2015; Rusdi et al., 2018) have identified ST38 in dogs. ST38 has also been identified in 4% (11/284) of CIA-resistant *E. coli* from Australian gulls (Mukerji et al., 2019). In the two Australian studies, the prevalence of ST38 was higher (11/33, 33% and 9/21, 43%) compared to the present study; however, this is likely due to these samples being commensal isolates obtained from faecal samples rather than clinical isolates. In a study of ESBL-producing clinical isolates, ST38 was only identified in 12% of isolates, which is more in line with the present study. While all isolates in the aforementioned studies contained CTX-M resistance genes the present study was the only one to identify CTX-M-15. ST38 isolates from humans, however, have been identified with CTX-M-15 (Rodríguez et al., 2014).

7.2.7 Canine malakoplakia is associated with *E. coli* isolates including ST131 with a common array of ExPEC virulence factors

There are several instances of malakoplakia being recorded in animals in the literature – in a dog (Benzimra et al., 2019), three cats (Bayley et al., 2008; Rutland et

al., 2013; Cattin et al., 2016), three pigs (Gill et al., 1981; Taniyama et al., 1985; Gelmetti et al., 2014), and a monkey (Taketa et al., 2013). Of the few available studies that discuss malakoplakia in animals, none have characterised the bacterial isolates that were obtained; most just detail the clinical aspects of the disease.

Similarities can be found between malakoplakia and other disease conditions including granulomatous colitis (GC) of Boxer dogs and Crohn's disease in humans. Studies have shown that GC-associated *E. coli* lack genes associated with virulence which are present in diarrheagenic *E. coli*, additionally, they are able to invade epithelial cells and persist within macrophages (Simpson et al., 2006; Shaler et al., 2019). This behaviour is similar to that of adherent and invasive *E. coli* (AIEC) which is also associated with Crohn's disease in humans (Shaler et al., 2019). AIEC strains are considered highly associated with Crohn's disease and inflammatory bowel disease patients due to the suspicion that they induce chronic inflammation in their hosts by modifying the microbiota composition in the human intestine (Abdelhalim et al., 2020). Although AIEC strains lack most of the virulence factors found in intestinal pathogenic *E. coli* strains, they do share similar virulence factors with ExPEC strains (Abdelhalim et al., 2020). A study by Dogan et al. (2014) compared genome sequences between isolates of different origins including patients with Crohn's disease, dogs with GC, and mouse ileitis. This study did not identify a molecular property that is associated with the AIEC phenotype (Dogan et al., 2014). It did, however, reveal that there are certain factors associated with Crohn's disease-derived AIEC which are present in all B2 strains of *E. coli*. These factors included *pduC* (a putative glycerol dehydratase) and *chuA* (involved in haem acquisition). Additionally, Crohn's disease-associated AIEC which harboured *ipfA* (involved in cell attachment) presented a high level of invasion of epithelial cells and translocation through M cells (O'Brien et al., 2017).

Another study also found a significant association between the *chuA* gene and AIEC-like strains (Abdelhalim et al., 2020). The *chuA* gene is predominantly involved in iron uptake and increases the ability of AIEC strains to survive and persist within macrophages.

Due to the lack of studies, characterising *E. coli* isolated from cases of malakoplakia it was deemed significant to further investigate the six *E. coli* isolates identified from two cases of malakoplakia in dogs (Chapter 5). Of these six isolates, four different STs were identified, including the pandemic clonal lineage ST131.

Other studies have shown GC-associated strains in dogs share a variety of genes involved with iron acquisition and metabolism, notably *irp1*, *irp2*, *fyuA* (yersiniabactin), *chuA* (haemoglobin utilisation), *fepC* (ferric enterobactin transport ATP-binding protein), and *iroN* (siderophore receptor). However, the aerobactin-related genes, *iutA* and *iucD*, were not detected in these strains. Additionally, the *malX* gene, which acts as a marker for the pathogenicity islands of UPEC CFT073, and the *iss* gene, which encodes a protein responsible for serum resistance, were present in most strains. However, none of these strains were found to contain virulence genes of diarrheagenic *E. coli*, *LT*, *STa*, *STb*, *cnf1*, and the gamma variant of *eae*. These findings are in line with those of the present study as none of the six *E. coli* isolates in the current study were found to contain diarrheagenic *E. coli* virulence genes *LT*, *STa*, *STb*, *stx2*, or *cnf1*, though one isolate did contain *eae* (Isolate 2). All strains possessed *fepC* and *malX*, however, only one isolate (Isolate 3) contained *irp1*, *irp2*, *fyuA*, *chuA*, *fepC*, *iroN*, *malX*, *iss*, and *ibeA*. A number of these virulence genes were also found in other isolates, such as Isolate 2, which contained *iss* and Isolate 1 that harboured *irp1*, *irp2*, and *fyuA*. It should be noted that Isolates 1 and 3 were from different animals.

7.2.8 Low levels of non-susceptibility to CIAs were identified among *E. coli* and *Salmonella* spp. isolates from Australian pigs

In an Australia-wide population of *E. coli* and *Salmonella* spp. isolates from healthy finisher pigs low levels of non-susceptibility to CIAs (ESCs and FQs) were identified (Chapter 6). Non-susceptibly to both cefoxitin and ciprofloxacin was observed in only 1% (2/201) of *E. coli* isolates each and non-susceptibility to ceftiofur was not observed. In addition, the 69 *Salmonella* spp. isolates did not show any non-susceptibility to CIAs. These results are similar to several European surveillance studies. Among *E. coli* isolated from faecal samples of pigs in the Netherlands (MARAN, 2020), fattening pigs in Sweden (Swedres-Svarm, 2019) and caecal samples from healthy pigs in Denmark (DANMAP, 2018) all were susceptible to both cefotaxime and ceftazidime. Similarly, in the same studies, the frequency of resistance to ciprofloxacin was only 1%. However, in the previous year in the Netherlands, 9.9% of isolates were resistant to ESCs (MARAN, 2020). In France, 4% of isolates were resistant to cefoxitin, enrofloxacin, and 1% exhibited resistance to ceftiofur (RESAPATH, 2019). These levels are in contrast to China, where *E. coli* isolates from fresh faecal samples of healthy pigs in lairage yielded a high frequency (21.7%) of isolates resistant to ciprofloxacin (Fang et al., 2019). Interestingly, in the United States, studies focused on caecal contents from market swine and sows yielded *E. coli* cefoxitin-resistance frequencies of 21% and 2%, respectively. Similarly, the frequency of resistance to ciprofloxacin among the market swine *E. coli* was 4% while no resistance was observed among the sow isolates (The National Antimicrobial Resistance Monitoring System, 2019).

The difference in prevalence of resistance to CIAs worldwide is likely due to variations in legislation in regards to the use of CIAs in food-producing animals. The very low levels of resistance in *E. coli* and *Salmonella* spp. isolates from Australian

healthy finisher pigs at slaughter is likely due to the strict regulations regarding the use of antimicrobials in food-producing animals in this country. For instance, the use of ceftiofur was only allowed ‘off-label’ for individual cases of colibacillosis and has now been voluntarily withdrawn from use by specialist pig veterinarians in Australia and the use of FQs is banned entirely (Abraham et al., 2014a). Similarly, many European countries have banned or limited the use of CIAs in food-producing animals. For instance, in 2002, Danish regulations restricted the use of FQs in animal production and in pig production Danish farmers voluntarily stopped using cephalosporins following the emergence of resistance in commensal *E. coli* (DANMAP, 2018).

In the present study, a novel *E. coli* ST was identified. This isolate designated ST7573 was classified as MDR and was identified as possessing the FQ-resistance gene *qnrS1*. Out of the four isolates identified in the study showing MIC values above the wild-type for ciprofloxacin, this was the only isolate to contain a plasmid-mediated FQ-resistance gene. Plasmid-mediated quinolone-resistance genes are associated with low levels of quinolone non-susceptibility that is often below the clinical breakpoint threshold as outlined by CLSI. Despite this, PMQR genes can, in the presence of quinolones, enable the selection of higher level resistance resulting from chromosomal mutations and lead to treatment failure (Röderova et al., 2016). A previous study by Röderova et al. (2016) carried out in the Czech Republic during 2013 and 2014 isolated *E. coli* from turkey cloacal swabs sampled at slaughterhouses; 15.2% of which were susceptible to ciprofloxacin but carried the *qnrS1* gene (Röderova et al., 2016). The *qnrS1* gene was also discovered in FQ-susceptible *E. coli* from wastewater (n=3), environmental samples from chicken farms (n=2) and a hospital wound swab (n=1). A ciprofloxacin-susceptible isolate from a Chinese pig farm possessing *qnrS1* has also been identified (Huang et al., 2012).

7.3. Implications of findings and future work

MDR in companion animal ExPEC and commensal *E. coli* in pigs is of increasing concern especially as the prevalence of genes encoding resistance to CIAs is on the rise. Pandemic clonal MDR lineages are also becoming more prevalent worldwide. This study has shown that despite this worldwide increase in resistance among clinical *E. coli*, rates of resistance to CIAs among Australian companion animals isolates have remained low, as has the prevalence of pandemic clonal lineages such as ST131 and ST1193. The consistently lower prevalence of these pandemic clonal lineages among companion animal compared to human isolates suggests that companion animals are spill-over hosts rather than a predominant host, even though bi-directional transmission of ExPEC between pets and people does appear to occur.

Australia is in a unique position with regard to AMR issues in food, in part due to its strict biosecurity protocols, but also the strict measures in place on the use of CIAs in food-producing animals. The present study has confirmed that these are largely effective in terms of keeping CIA resistance frequency low, even though CIA-resistant isolates may still enter the food production cycle via other means (i.e. migratory birds).

Due to increasing reports of ExPEC and commensal isolates resistant to CIAs including ESBL-producers in both companion and food-producing animals worldwide and concerns over the risk of animal to human transfer of AMR it is crucially important that surveillance is maintained in an ongoing state, especially in regards to the main clonal lineages of *E. coli* responsible for AMR in animals. Thus, additional Australia-wide surveillance studies of *E. coli* carriage in healthy and sick companion animals need to be undertaken, as well as a comparison of strains from people and their pets to further study bi-directional transmission. In addition, ongoing surveillance of

indicator and zoonotic bacteria in food-producing animals needs to be carried out in a similar way to other countries worldwide that have established programmes.

7.4. Conclusions

This study provided crucial information on the prevalence of critically important AMR genes and the significance of certain phylogenetic lineages among ExPEC and other *E. coli* isolates from both companion and food-producing animals in Australia. It has shown that despite their increasing prevalence worldwide, populations of resistant isolates in Australian animals remain low. It has therefore provided a benchmark for future surveillance studies incorporating both AST and WGS.

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