Phylogenetic diversity, antimicrobial susceptibility and virulence characteristics of phylogroup F Escherichia coli in Australia

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Unlike Escherichia coli strains belonging to phylogroup B2, the clinical significance of strains belonging to phylogroup F is not well understood. Here we report on a collection of phylogroup F strains recovered in Australia from faeces and extra-intestinal sites from humans, companion animals and native animals, as well as from poultry meat and water samples. The distribution of sequence types was clearly non-random with respect to isolate source. The antimicrobial resistance and virulence trait profiles also varied with the sequence type of the isolate. Phylogroup F strains tended to lack the virulence traits typically associated with phylogroup B2 strains responsible for extra-intestinal infection in humans. Resistance to fluoroquinolones and/or expanded-spectrum cephalosporins was common within ST648, ST354 and ST3711. Although ST354 and ST3711 are part of the same clonal complex, the ST3711 isolates were only recovered from native birds being cared for in a single wildlife rehabilitation centre, whereas the ST354 isolates were from faeces and extra-intestinal sites of dogs and humans, as well as from poultry meat. Although ST354 isolates from chicken

Abbreviations: FQ, fluoroquinolone; ST, sequence type.

The sequence data reported in this study have been deposited in GenBank and have the SRA Study Accession Number: SRP089829.

One supplementary table is available with the online Supplementary Material.
meat in Western Australia were distinct from all other ST354 isolates, those from poultry meat samples collected in eastern Australia shared many similarities with other ST354 isolates from humans and companion animals.

INTRODUCTION

It is well established that *Escherichia coli* exhibits extensive genetic structure and that strains of the species can be classified into four major and four minor phylogenetic groups (phylogroups). The great majority of *E. coli* isolates belong to the phylogroups known as A, B1, B2 and D. Strains of these phylogroups vary in their phenotypic and genotypic characteristics, ecological niche, lifestyle and propensity to cause disease (Tenaillon et al., 2010). The minor phylogroups are known as C, E and F and cryptic clade I (Clermont et al., 2013). Although these minor phylogroups have been recognized for several years, little is known about their geographic distribution, host preferences, phenotypic and genotypic characteristics or propensity to cause disease. Until recently, the only way of identifying strains belonging to the minor phylogroups was by MLST; this is the main reason why so little is known about the nature of these strains, as MLST characterization of large strain collections is often prohibitively expensive. However, Clermont et al. (2013) described an improvement to the classic triplex PCR method for phylogroup assignment (Clermont et al., 2000) that enables isolates belonging to the minor phylogroups to be identified.

Of the minor phylogroups, strains belonging to phylogroup F are of particular significance as they have been implicated as extra-intestinal pathogens of companion animals (Guo et al., 2015), horses (Ewers et al., 2014), cattle (Abraham et al., 2015) and humans (Lau et al., 2008). Phylogroup F strains have also been found at high frequency in the faeces of wild birds being treated in wildlife rehabilitation centres (Blyton et al., 2015). Further, many phylogroup F clinical isolates are resistant to fluoroquinolones (FQs) and/or expanded-spectrum cephalosporins.

The clinical significance of phylogroup F strains as causative agents of extra-intestinal infection and carriers of antimicrobial resistance determinants led us to examine phylogroup F strains isolated in Australia from extra-intestinal sites and faeces from a variety of host species, as well as from poultry meat and water samples. The strains were characterized for their antimicrobial resistance phenotype and, through whole-genome sequencing, their virulence gene profiles and phylogenetic relationships. A more detailed analysis of clonal complex CC354 was undertaken, as strains from this complex are frequently isolated from animals in clinical settings in Australia (Guo et al., 2015), are typically FQ resistant (Guo et al., 2015) and have been isolated from poultry meat products destined for human consumption (Ingram et al., 2013).

METHODS

Strains. The 87 strains examined here represent an ad hoc collection of published and newly obtained clinical, commensal, environmental and poultry meat isolates from diverse locales that were selected to represent the diversity of phylogroup F in Australia (Table 1). Also included were two National Center for Biotechnology Information reference strains (SMS-3-5 and 1A39) and several other isolates from non-Australian localities for which whole-genome sequence data were available (Table S1, available in the online Supplementary Material). The collection included 47 isolates recovered without antibiotic selection from the faeces of native Australian birds (Blyton et al., 2015) and mammals (Gordon & Cowling, 2003), from water samples (Power et al., 2005) and from humans living in Australia (Gordon et al., 2005, 2015; Blyton et al., 2014). In addition, the collection included 18 FQ-resistant isolates recovered from either dog faeces (Guo et al., 2015) or extra-intestinal isolates from humans (Turnidge et al., 2014) and dogs (Guo et al., 2015). As well, the collection included eight FQ-resistant isolates recovered from poultry meat samples collected in Western Australia (Ingram et al., 2013) and nine FQ-resistant isolates recovered from chicken meat products purchased from different retail outlets in Canberra, Australian Capital Territory (unpublished data). The basic metadata for all the phylogroup F strains examined here are presented in Table S1.

DNA extraction and whole-genome sequencing. Using ISOLATE II Genomic DNA kits (Bioline), genomic DNA was extracted from a 100 µl aliquot of a 5 ml lysogeny broth culture after overnight incubation at 35°C with shaking (150 r.p.m.). Genomic DNA was quantified using Qubit dsDNA BR assay kit (Invitrogen). Libraries were prepared using the Nextera XT DNA sample preparation kit (Illumina) and the Nextera XT index kit (Illumina), with 0.5 ng of input DNA, according to the manufacturer’s protocol.

Whole-genome DNA sequencing was performed on an Illumina MiSeq platform using a 600-cycle MiSeq Nextera XT version 3 reagent kit (2×300 paired-end reads). The raw genomic sequencing data files were assembled as *de novo* genome sequences and exported as FASTA files using CLC Genomics Workbench 8. The assemblies and annotations for all strains are available in Enterobase (http://enterobase.warwick.ac.uk/). The raw sequence read files have been deposited in GenBank and are associated with SRA study SRP089829.

In silico characterization. The strains were assigned to sequence types (STs) for the University of Warwick MLST scheme (http://enterobase.warwick.ac.uk/). The Centre for Genomic Epidemiology website (www.genomiccepidemiology.org) was used to characterize the strains using the VirulenceFinder (Joensen et al., 2014), ResFinder (Zankari et al., 2012), SeroTypeFinder (Joensen et al., 2015) and PlasmidFinder (Carattoli et al., 2014) tools. The presence of additional extra-intestinal virulence factors, beyond those detected by VirulenceFinder, was determined using CLC Genomics Workbench.

Comparative genomics. Phylogenetic relationships among the study isolates were inferred by aligning the strains to the reference phylogroup F strain SMS-3-5 using the Harvest suite of tools (Treangen et al., 2014). SNPs were extracted and were used to construct a phylogeny using PhylML (Guindon et al., 2010), together with a general time-reversible model of evolution. For CC354 strains, the alignment program Mauve (Darling et al., 2010) was used to determine the variable gene content.
Table 1. Source and site of isolation of the phylogroup F isolates characterized in the present study

<table>
<thead>
<tr>
<th>Isolate source</th>
<th>Site of isolation</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Intestinal biopsy</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Extra-intestinal</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Faeces</td>
<td>7</td>
</tr>
<tr>
<td>Domestic dog</td>
<td>Extra-intestinal</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Faeces</td>
<td>1</td>
</tr>
<tr>
<td>Wild mammal</td>
<td>Faeces</td>
<td>4</td>
</tr>
<tr>
<td>Chicken</td>
<td>Faeces</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Meat</td>
<td>17</td>
</tr>
<tr>
<td>Wild bird</td>
<td>Faeces</td>
<td>16</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Unknown</td>
<td>Unknown</td>
<td>3</td>
</tr>
</tbody>
</table>

which was extracted from the Mauve backbone file. DNA regions smaller than 100 bp were removed, as were regions present in all strains or only a single strain. The remaining regions were scored as being either present or absent.

For the CC354 isolates, the gene content of the plasmids harbouring resistance gene determinants was inferred using an iterative process. First, the assembly contigs containing antimicrobial resistance genes were identified, as were contigs containing other known plasmid-borne genes (examples are colicin B and ireN). The plasmid-associated contigs were then joined into a plasmid assembly for each isolate and the plasmid assemblies were aligned with the full assemblies of all CC354 isolates. Additional plasmid-associated contigs were identified, and the process was repeated until no new plasmid-associated contigs could be discovered. The resulting inferred plasmid assemblies were compared against the National Center for Biotechnology Information database by using BLAST to confirm that the resulting plasmid assemblies did not contain genes normally associated with the chromosome. The plasmid assemblies (contigs) for each strain were then aligned using Mauve and the variable gene content of each strain was extracted from the assemblies (contigs) for each strain were then aligned using Mauve and the plasmid assemblies. Additional plasmid-associated contigs were identified, and the process was repeated until no new plasmid-associated contigs could be discovered. The resulting inferred plasmid assemblies were compared against the National Center for Biotechnology Information database by using BLAST to confirm that the resulting plasmid assemblies did not contain genes normally associated with the chromosome. The plasmid assemblies (contigs) for each strain were then aligned using Mauve and the variable gene content of each strain was extracted from the backbone file.

Antimicrobial susceptibility testing. Susceptibility to clinically relevant antimicrobials was assessed by disk diffusion method on Mueller–Hinton agar plates (Acumedia, Neogen) as described by the European Committee on Antimicrobial Susceptibility Testing and was interpreted according to their published breakpoints (EUCAST, 2013). The antimicrobials represented different classes, namely, penicillins (ampicillin, 10 μg; amoxicillin/clavulanic acid, 20/10 μg), quinolones (nalidixic acid, 30 μg), FQs (ciprofloxacin, 5 μg), aminoglycosides (gentamicin, 10 μg), nitrofurans (nitrofurantoin, 100 μg), carbapenems (ertapenem, 10 μg), sulfonamides (trimethoprim/sulfamethoxazole, 23.75/1.25 μg), tetracyclines (tetracycline, 30 μg), first-generation cephalosporins (cefoxolin, 30 μg) and third-generation cephalosporins (cefotaxime, 30 μg; ceftriaxone, 30 μg; cefotiofur, 30 μg) (Becton, Dickinson). Inhibition zone diameters were measured using ProtoCOL 3 (Symbiosis).

RESULTS

Group F strain phylogenetic relationships

The assemblies of the 87 group F strains were aligned using SMS-3.5 as the genome reference strain. The analysis revealed 119 524 SNPs in the core genome of these strains; these were used to infer the phylogenetic relationships among the strains (Fig. 1). The 87 strains represented 21 STs, with ST354 represented by 27 isolates and ST59 and ST3711 represented by 12 isolates each.

The distribution of STs by source was clearly non-random. Only three ST354 isolates were from humans, while all ST59 and ST62 isolates (except strain TA326) were from humans. Similarly, all ST648 strains but one (30 1 R8) were extra-intestinal isolates from humans or companion animals, while all ST3711 isolates were recovered from native birds at one Western Australia locality.

Serotype diversity

By using an in silico approach, 49 % of the 87 isolates could be assigned an O type (Table S1). Ten O types were identified, with O1 being most common (20 %), followed by O8 (9 %) and O11 (6 %). In contrast, all isolates could be assigned an H type. Eleven H types were identified, with H34 being most common (57 %), followed by H7 (17 %).

Virulence determinants

In silico screening revealed that, overall, the phylogroup F isolates tended not to encode virulence factors implicated in extra-intestinal infection (Table S1). That is, no F isolates contained clyB, focG, sfaA, lpfA, tcpC or vat, and very few harboured afdD (4 %), etsC (3 %), hlyD (1 %), ireA (6 %), ireN (2 %), terC (1 %), cdB (2 %) or tsh (1 %). Virulence genes present in >10 % of isolates included fyuA (32 %), hra (23 %), ibeA (39 %), iutA (59 %), neuC (13 %), ompT (37 %), papC (17 %), sitA (79 %), trAT (53 %), usp (64 %), cah (70 %), iha (45 %), tia (23 %), upaG (11 %) and senB (13 %). It has been suggested that if an E. coli strain possesses two or more of the traits papAH, afd/draBC, sfa/focDE, kpsMT2 or iutA, it is capable of causing a urinary tract infection (Johnson et al., 2003). Only 13 (11 %) of the phylogroup F strains met this criterion, and all but two of these (DAEC9 and GNB211) are members of ST62 or ST59.

The presence of many virulence traits varied by ST. For example, five traits (fyuA, ibeA, iutA, usp and iha) tended to be uniformly present or absent in all representatives of a given ST. Likewise, the number of virulence factors detected per isolate varied by ST (P<0.001, Kruskal–Wallis test). That is, for the seven STs represented by more than three isolates each, the mean number of virulence factors per isolate was as follows: 7.0 (ST3637), 8.0 (ST3711), 11.0 (ST354 and ST648), 13.7 (ST62) and 16.2 (ST59).

Bacteriocins

Bacteriocin genes were detected in 39 of the isolates, with colicin genes seen in 35 isolates and microcin genes in eight (Table S1). Only five isolates carried multiple bacteriocin genes. The various bacteriocin genes differed significantly for overall prevalence and distribution by ST. That is, colicin E1, the most common bacteriocin (23 % of isolates overall), was restricted to ST354 and ST59. Colicin M, the
Fig. 1. Phylogenetic relationships among 87 phylogroup F strains based on core genome SNPs as inferred using PhyML (Guindon et al., 2010), together with a general time-reversible model of evolution. Bootstrap support values were 100% for all primary nodes. Green = native birds; red = Western Australia (strains with a WA prefix) or Australian Capital Territory poultry meat; black = non-human animals; blue = human; purple = water. Specimen type or source is listed for all non-faecal, non-water isolates.
next-most common bacteriocin (17% of isolates overall), was present in all ST3711 isolates. In contrast, microcin B17 was detected in only 6.9% of isolates (ST59), microcin H47 in 3.4% and colicins B and Ia/Ib in one isolate each. Microcin (colicin) V was not detected.

**Antimicrobial resistance phenotypes and determinants**

The susceptibility results must be interpreted in relation to the methods used to obtain the isolates, as summarized here. Most of the canine faecal and clinical isolates, as well as all human clinical isolates, were selected originally because they were FQ resistant. Antimicrobial selection was also used to recover resistant isolates from poultry meat. In contrast, no antimicrobial selection was used in isolating faecal or biopsy strains from humans in the Canberra region or from birds and native mammals.

Susceptibility patterns among isolates varied greatly by source and ST (Table S1). For example, by source, most human faecal isolates from the Canberra region were susceptible to all tested antimicrobials, whereas all avian faecal isolates from Western Australia were FQ resistant. Likewise, by ST, most ST354, ST648 and ST3711 isolates were FQ resistant, while ST59 and ST62 isolates were FQ susceptible. On average and irrespective of source, FQ-resistant isolates were resistant to four other antimicrobial classes, while FQ-susceptible isolates were resistant to \( \leq 1 \) antimicrobial class (P<0.001, Mann–Whitney test).

The number of antimicrobial resistance determinants detected by whole-genome sequencing also varied by ST (P<0.001, Kruskal–Wallis test) (Table S1). Resistance determinants were absent in ST3637 and were uncommon (<2 per isolate) in ST457, ST59 and ST62. In contrast, the mean number of resistance determinants per isolate was 5.5 for ST354 and 9.2 for ST648.

**Characteristics of CC354**

Almost half of the study isolates represented two closely related STs within phylogroup F, ST354 and ST3711. These two STs differed by a single MLST locus and hence were members of the same clonal complex, CC354. Because these isolates were all FQ resistant, this clonal complex was characterized more fully.

After alignment of the ST354 and ST3711 isolates’ genomes using Mauve and identification of the core genome, variable positions in the core genome were extracted and used to infer a phylogeny (Fig. 2). In this phylogeny, all ST3711 strains were closely related, while the ST354 strains exhibited some sub-clustering. That is, all of the ST354 Western Australia poultry isolates clustered and were distinct from the other ST354 strains. While many of the ST354 strains from poultry meat, humans and dogs were intermingled, there was a cluster of poultry meat isolates from eastern Australia that all encoded three copies of *iha*. The extent of among-strain similarity according to variable gene content was determined for the CC354 strains using a Principal Coordinates Analysis (Jaccard similarity metric). The resulting similarity patterns mimicked those observed when using the core genome data (data not shown).

All CC354 strains (i.e. ST354 and ST3711) contained *sitA* and *usp*. The ST354 strains additionally contained *ibeA*, *iha*, *iucC* and *iutA*, while the ST3711 strains did not. The Western Australia poultry meat isolates differed from other ST354 strains by containing *tia* and *hra* and lacking *cah*. Other differences between ST354 and ST3711 isolates or between the Western Australian and eastern Australian isolates related to proteins of unknown function or phage-related functions. Within ST354, no gene content feature categorically differentiated the eastern Australian poultry meat isolates from the human and companion animal isolates.

The plasmids hosted by CC354 isolates varied according to an isolate’s ST membership and source. The ST3711 isolates and GNB2829 (ST354) harboured an IncFIB plasmid encoding colicin M and containing a remnant of the colicin B activity gene. This plasmid also appeared to carry these strains’ plasmid-borne resistance determinants. In contrast, the ST354 Western Australia poultry meat isolates hosted an IncQ conjugative plasmid that carried these strains’ resistance determinants. Most of these isolates also harboured a colicin E1 plasmid. The balance of the ST354 strains appeared to harbour one of at least two different antimicrobial resistance plasmids: an IncQ plasmid or an IncFIA plasmid. Additionally, these eastern Australian isolates commonly also carried a colicin E1 plasmid in addition to the antimicrobial resistance plasmid.

The among-strain relationships based on the variable gene content of the inferred plasmid sequences (Fig. 3) broadly reflected the corresponding phylogenetic relationships, as inferred from core genome SNPs. The IncQ antibiotic resistance plasmids carried by the ST354 Western Australian poultry isolates were distinct from the IncQ plasmids hosted by the other ST354 isolates.

**DISCUSSION**

In this study, we defined the clonal structure of *E. coli* isolates of phylogroup F from diverse locales and ecologic sources in Australia and then compared clonal background with ecologic origin, locale, antimicrobial resistance, virulence gene content and plasmid repertoire. We found that isolate source and isolation method had a large impact on the STs identified. For example, over half of the phylogroup F isolates recovered from water samples belonged to a single lineage (ST3637), representatives of which were recovered only from water samples. In contrast, the great majority of isolates belonging to ST59 or ST62 were from humans, were recovered without antimicrobial selection and were FQ susceptible. Reference to the Warwick MLST database supports the observation that isolates representing ST62 and ST59 are most likely to be recovered from humans, as all 26 examples of ST59 and all 42...
examples of ST62 for which the database provides host data were from humans (strains from the present study excluded). Reference to the Warwick MLST database shows that ST354 strains have been isolated from humans and other animals, as was found in the present study.

Although the biases on how the phylogroup F study isolates were sampled in regard to antimicrobial resistance phenotype preclude firm conclusions, it is also important to note that these isolates were not selected on the basis of their ST. Therefore, the results do suggest that phylogroup F isolates isolated from human faeces without antimicrobial selection are likely to be FQ susceptible and to represent ST59 or ST62, while isolates belonging to CC354 are highly likely to be FQ resistant, as all of the present CC354 isolates were FQ resistant, including those recovered without antimicrobial selection.

The FQ-resistant ST3711 lineage of CC354 represented faecal isolates from native birds, chosen without respect to

Fig. 2. Phylogenetic relationships among CC354 strains based on core genome SNPs as inferred using PhyML (Guindon et al., 2010), together with a general time-reversible model of evolution. Bootstrap support values were 100% for all primary nodes. The presence of known colicin plasmids in a strain is also indicated. Green = native birds; red = Western Australia (strains with a WA prefix) or Australian Capital Territory poultry meat; black = non-human animals; blue = human.
their FQ resistance, and were observed only in one wildlife rehabilitation centre in Western Australia despite wild birds having been sampled from several other veterinary clinics and wildlife rehabilitation centres across Australia (Blyton et al., 2015). These were independent isolates, as all were collected from different individuals representing several avian species. The isolates are not identical but are highly similar, which suggests that a single ‘clone’ is circulating in this rehabilitation centre.

Guo et al. (2015), who reported on a collection of FQ-resistant \textit{E. coli} isolates recovered from the faeces or extra-intestinal sites of dogs, made a similar observation. Many of the phylogroup F dog isolates in their study came from the University Veterinary Teaching Hospital in Sydney, Australia. All of the F isolates \((n=16)\) from that facility were from dog faeces and were members of ST354. Such an outcome suggests that an ST354 ‘clone’ was circulating in this care facility.

To date, few studies have determined the relative abundance of phylogroup F strains in faecal samples from Australia in the absence of antimicrobial selection. Those that have been reported indicate that phylogroup F strains typically represent about 7\% of \textit{E. coli} isolates from human faeces (Clermont et al., 2013; Blyton et al., 2014) but are less common in birds and mammals [1\% in mammals (unpublished data) and 2\% in birds (Blyton et al., 2015)]. Therefore, it would be unlikely that every dog arriving at a veterinary hospital or every bird arriving at a rehabilitation centre would all harbour strains belonging to the same ST (354 or 3711). In turn, this suggests that strains belonging to CC354 have a propensity to persist and circulate in animal care facilities. Conditions favouring selection and maintenance of FQ-resistant strains do exist in Australian animal care facilities, as the FQ enrofloxacin is commonly used to treat injured wildlife, and both enrofloxacin and marbofloxacin are registered for the treatment of companion animals (Gillett, 2010).

On average, phylogroup F strains are unlikely to possess virulence traits associated with extra-intestinal infection; however, isolates belonging to ST59 and ST62 are an exception. Strains belonging to these STs are responsible for extra-intestinal infection (this study), harbour significantly more extra-intestinal virulence genes than most other phylogroup F isolates and, in particular, have a virulence gene profile linked to the ability to cause urinary tract infection. CC354 isolates likewise are also capable of causing extra-intestinal infection, but they are less likely than ST59 or ST62 isolates to harbour the genes typically associated with extra-intestinal infection, suggesting that they may carry as-yet-unrecognized traits that enhance a strain’s ability to cause extra-intestinal infection.

Some of the characteristics observed for the phylogroup F lineages ST59, ST62 and ST354 and ST3711 are mirrored by certain phylogroup B2 STs. Specifically, ST95, ST73 and ST131 are frequently encountered, human-associated STs known to cause extra-intestinal infection (Riley, 2014). However, ST95 and ST73 strains are unlikely to be FQ resistant and generally encode few other resistant determinants, while ST131 strains are typically FQ resistant and resistant to other antibiotics (Banerjee et al., 2013b; Tchesnokova et al., 2013). ST131 strains are also observed more frequently in environments such as hospitals and age-care facilities than are strains belonging to ST95 and ST73 (Banerjee et al., 2013a, b). ST59, ST62 and ST354 strains are frequently encountered phylogroup F strains also known to cause extra-intestinal infection. ST59 and ST62 are unlikely to be FQ resistant or resistant to other antimicrobials, while FQ resistance is common in ST354. The

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{upgma_tree.png}
\caption{UPGMA tree based on the variable gene content of the antimicrobial resistance plasmids inferred to be present in the CC354 isolates. When no incompatibility group is defined, the incompatibility group of the plasmid carrying the antimicrobial resistance genes could not be unambiguously determined. Green = native birds; red = Western Australia (strains with a WA prefix) or Australian Capital Territory poultry meat; black = non-human animals; blue = human.}
\end{figure}
evidence also suggests that ST354 is more likely to be encountered in care facilities.

It is not apparent why isolates from ST95, ST73, ST59 or ST62 should be less likely to be FQ resistant than those from ST131 or ST354. It seems unlikely that this dichotomy is due to differences in exposure to FQs, as rates of faecal carriage of all of these STs are broadly similar (within a phylogroup), and all these STs are capable of causing extra-intestinal infection. To argue otherwise would be to assume that hosts harbouring ST95, ST73, ST59 or ST62, intestinally or extra-intestinally, are less likely to be prescribed FQs compared to hosts that harbour ST131 or ST354. This may be true, if ST131 and ST354, for some non-antibiotic reason, associate preferentially with elderly, debilitated and hospitalized or institutionalized hosts, who in turn are more likely than others to receive antibiotics. However, if all these STs are exposed to FQs at broadly the same frequency, then this in turn would suggest that the acquisition of FQ resistance is dependent on the genomic background of the bacterial host and that some genomic backgrounds are either pre-adapted to or incompatible with the evolution of FQ resistance. Further experiments are required to determine if the ability of ST131 and CC354 isolates to establish and maintain themselves in health care environments relates directly to the evolution of FQ resistance or is due to traits that may predispose these strains to evolving FQ resistance.

The use of antimicrobials in animals destined for human consumption is of considerable concern (Collignon, 2015). In Australia, FQs cannot be administered to food-producing animals (Cheng et al., 2012) and Australia has extremely low prevalence of FQ-resistant Enterobacteriaceae among cattle, pigs and sheep (Abraham et al., 2014a, b). However, FQ-resistant E. coli ST354 strains have been recovered from poultry meat in both Western Australia and the Australian Capital Territory (Ingram et al., 2013; this study). In eastern Australia, ST354 isolates from humans, companion animals and poultry meat are closely related in terms of both their core genomes and their variable gene content and, at the population level, are indistinguishable. Indeed, poultry meat isolate W2-68 is virtually identical with several companion animal isolates. However, it cannot be concluded whether the presence of this strain in the poultry meat was due to its presence in the bird throughout the production cycle or contamination during processing and distribution of the poultry meat.

Although the other ST354 poultry meat isolates from eastern Australia were similar to isolates from humans and companion animals, they were not identical. This was most apparent for the cluster of poultry meat isolates that contained three copies of the adhesion-related gene iha, which were the only phylogroup F strains found to contain three copies of this gene. Further sampling will be required to determine if FQ-resistant ST354 strains containing three copies of iha can be recovered from humans or companion animals living in eastern Australia. Investigation and analysis of more ST354 isolates obtained directly from broilers is clearly required.

In conclusion, although phylogroup F is one of the least common E. coli phylogroups in Australia, it contains several lineages capable of causing extra-intestinal infection in humans, companion animals and wild birds. CC354 not only is a cause of extra-intestinal infection but also is highly likely to be FQ resistant, and it can be isolated from poultry meat products. Further studies are required to determine the prevalence and dissemination of FQ-resistant CC354 clones in Australia among community-dwelling humans, companion animals and poultry and the extent to which, within phylogroup F, FQ resistance is restricted to particular lineages.

ACKNOWLEDGEMENTS

 Portions of this work were funded by the Private Practice Fund of the Canberra Hospital and were in part based upon work supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (USA).

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Edited by: W. Achouak and R. Lan