Clonal group distribution of fluoroquinolone-resistant *Escherichia coli* among humans and companion animals in Australia

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**Objectives:** To determine the phylogenetic group distribution and prevalence of three major globally disseminated clonal groups (clonal group A (CGA) and O15:K52:H1, associated with phylogenetic group D, and sequence type ST131, associated with phylogenetic group B2) among fluoroquinolone-resistant extra-intestinal *Escherichia coli* isolates from humans and companion animals in Australia.

**Methods:** Clinical extra-intestinal fluoroquinolone-resistant *E. coli* isolates were obtained from humans (*n* = 582) and companion animals (*n* = 125), on Australia's east coast (October 2007–October 2009). Isolates were tested for susceptibility to seven antimicrobial agents, and for phylogenetic group, O type and clonal-group-specific single nucleotide polymorphisms by PCR.

**Results:** The fluoroquinolone-resistant isolates were typically resistant to multiple agents (median of four). Analysis revealed that clonal group ST131 accounted for a large subset of the human isolates (202/585, 35%), but for a much smaller proportion of the companion animal isolates (9/125, 7.2%; *P* ≤ 0.001). In contrast, CGA and O15:K52:H1 were uncommon among both human (7.2%) and companion animal (0.8%) isolates.

**Conclusions:** In Australia, a large proportion (42%) of recent fluoroquinolone-resistant extra-intestinal *E. coli* isolates from humans are represented by three major globally disseminated clonal groups, predominantly ST131, which by contrast is comparatively rare among fluoroquinolone-resistant *E. coli* from companion animals. In conjunction with Australia's ban on fluoroquinolone use in livestock, these results argue against a major domestic food animal or companion animal source for fluoroquinolone-resistant extra-intestinal *E. coli* among humans in Australia. However, both humans and companion animals are involved in the intercontinental emergence and dissemination of ST131.

**Keywords:** ST131, clonal group A, O15:K52:H1, multidrug resistance

**Introduction**

Fluoroquinolones are important therapeutic agents in both human and veterinary medicine; consequently, fluoroquinolone-resistant organisms are of concern in both sectors. The emergence and spread of fluoroquinolone-resistant *Escherichia coli* may be a result of the appearance and expansion of resistant mutants under the influence of antimicrobial selection pressure, and/or the dissemination of resistant clones. Recently, the emergence and dissemination of phylogenetic group B2 fluoroquinolone-resistant strains of sequence type ST131 (O25b:H4), predominantly causing urinary tract infections (UTIs) in humans, has been demonstrated globally, particularly in Europe,1 the USA2 and Canada.3 Other commonly identified, antimicrobial resistance-associated clonal groups of *E. coli* that cause UTI include clonal group A (CGA)4 and O15:K52:H1,1 both of which belong to phylogenetic group D.

ST131 has been recently reported from companion animals in Germany,5 Portugal6 and the USA,2 evidence suggesting that companion animals may play a role in disseminating this clonal group. Accordingly, we sought to determine the phylogenetic group distribution of recently isolated fluoroquinolone-resistant extra-intestinal *E. coli* from humans and companion animals in Australia, and to define in both host groups the prevalence of three major resistance-associated epidemic *E. coli* clonal groups (i.e. CGA, O15:K52:H1 and ST131), which to date have not been reported in either humans or companion animals in Australia.

**Materials and methods**

**Clinical** extra-intestinal fluoroquinolone-resistant *E. coli* isolates, predominately from urine, were obtained from humans (*n* = 582) over a 12 month period (October 2007–October 2008) from two private
Isolates were defined as MDR if they exhibited resistance to four or more antimicrobials including enrofloxacin. NA, not applicable.

Values in parentheses indicate the percentage of each trait with respect to clonal group.

Table 1. Distribution of fluoroquinolone-resistant E. coli isolated from humans and companion animals in Australia by phylogenetic group and MDR status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Prevalence of characteristic, number of isolates (column percentage)</th>
<th>Proportion of MDR isolates within each phylogenetic group or clonal group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>human isolates (n=582)</td>
<td>animal isolates (n=125)</td>
</tr>
<tr>
<td>Phylogenetic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>70 (12)</td>
<td>32 (26)</td>
</tr>
<tr>
<td>B1</td>
<td>52 (9)</td>
<td>21 (17)</td>
</tr>
<tr>
<td>B2</td>
<td>250 (43)</td>
<td>13 (10)</td>
</tr>
<tr>
<td>D</td>
<td>210 (36)</td>
<td>59 (47)</td>
</tr>
<tr>
<td>Total</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not applicable.
Isolates were defined as MDR if they exhibited resistance to four or more antimicrobials including enrofloxacin.
Values in parentheses indicate the percentage of each trait with respect to phylogenetic group.

Table 2. Distribution of fluoroquinolone-resistant E. coli isolated from humans and companion animals in Australia by clonal group and MDR status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Prevalence of characteristic, number of isolates (column percentage)</th>
<th>Proportion of MDR isolates within each phylogenetic group or clonal group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>human isolates (n=582)</td>
<td>animal isolates (n=125)</td>
</tr>
<tr>
<td>Clonal group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ST131</td>
<td>205 (35)</td>
<td>9 (7.2)</td>
</tr>
<tr>
<td>CGA</td>
<td>8 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>O15:K52:H1</td>
<td>33 (5.7)</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>

NA, not applicable; no clonal group A isolates among animal isolates.
Isolates were defined as MDR if they exhibited resistance to four or more antimicrobials including enrofloxacin.
Values in parentheses indicate the percentage of each trait with respect to clonal group.

Results and discussion

The 707 fluoroquinolone-resistant study isolates frequently exhibited co-resistance to other antimicrobial agents, including tetracycline (75%), cefotin (69%), trimethoprim/sulfamethoxazole (69%), amoxicillin/clavulanic acid (53%), gentamicin (39%) and cefoxitin (13%). Resistance scores ranged from 1 to 7 (median, 4). MDR status was highly prevalent overall (69%) and was significantly associated with companion animal isolates, of which 82% (102/125) were MDR, versus 66% (383/582) of human isolates (P≤0.001) (Table 1). This strong association with fluoroquinolone resistance and MDR phenotypes among companion animal isolates may reflect the use of fluoroquinolones as a last-line antimicrobial agent in companion animal medicine.

Phylogenetic group B2 accounted for nearly half (250/582, 43%) of the human isolates, compared with only 10% (13/125) of the veterinary isolates (P≤0.001). This suggests that companion animals are less important hosts of fluoroquinolone-resistant group B2 clinical strains compared with humans.

Analysis by SNP PCR of known antimicrobial resistance-associated clonal groups indicated that a large proportion of the fluoroquinolone-resistant human isolates (205/582, 35%; 82% of human group B2 isolates) corresponded to the recently recognized, globally disseminated group B2 lineage, ST131. In contrast, ST131 accounted for a much smaller proportion of companion animal isolates (9/125, 7.2%; P≤0.001) (Table 2). The marked ST131 prevalence disparity between human and companion animal isolates suggests that humans are the primary disseminators of ST131 in Australia.

Brisbane pathology laboratories servicing both hospitals (~80%–85% of submissions) and community clinics (~15%–20% of submissions) across a wide geographical area of eastern Australia with an estimated population size of 4–5 million (~20% of Australia’s total population). Isolates from companion animals (n=125; 120 canine and 5 feline) were obtained over a 24 month period (October 2007–October 2009) from one private and two university veterinary diagnostic pathology laboratories servicing Brisbane, Sydney and Melbourne. All isolates were screened for susceptibility to seven antimicrobial agents (amoxicillin/clavulanic acid, cefotin, cefotaxin, enrofloxacin, gentamicin, tetracycline and trimethoprim/sulfamethoxazole), as determined by disc diffusion according to CLSI guidelines.7,8 The resistance score was the number of antimicrobials to which an isolate exhibited resistance. Isolates with a resistance score ≥4 were defined as multidrug resistant (MDR). Phylogenetic groups (A, B1, B2 and D) were determined by multiplex PCR.9 Group B2 isolates were further screened by PCR for ST131-specific single nucleotide polymorphisms (SNPs) in mdh and gyrB3 and the ST131-associated O25b rfb variant.3 Similarly, group D isolates were examined for CGA-associated SNPs in fumC4 and gyrB3 and for an O15:K52:H1-associated SNP in fumC10 and the O15 rfb allele.11 Proportions were compared using χ² or Fisher’s exact test.
Despite the ample prevalence of phylogenetic group D (i.e. 36% and 47% of human and veterinary isolates, respectively), the group D-associated clonal groups CGA and O15:K52:H1 were uncommon, together accounting for only 7.1% and 0.8% of human and companion animal isolates, respectively (Table 2). Whilst this is the first report identifying these group D-associated lineages in Australia, they appear to be of limited significance amongst fluoroquinolone-resistant clinical isolates from humans and companion animals. Further investigation is required to determine whether other clonal groups are prominent among the remaining 81% of group D and 19% of B2 isolates, and if so the extent of the cross-species commonality.

The substantial prevalence of ST131 amongst phylogenetic group B2 fluoroquinolone-resistant E. coli isolates suggests that humans and, to a lesser extent, companion animals in eastern Australia are participants in the intercontinental emergence and dissemination of ST131. The prevalence of fluoroquinolone resistance among human E. coli isolates in Australia is currently considered to be low (<5%) compared with other parts of the world. This study confirms that a large proportion (35%) of human fluoroquinolone-resistant clinical isolates from Australia belong to the globally disseminated ST131 clonal group. This, plus the fact that fluoroquinolones cannot be administered legally to food production animals in Australia, argues against locally produced foods and associated livestock as an important source of Australian fluoroquinolone-resistant E. coli strains. Likewise, the low prevalence of ST131 among fluoroquinolone-resistant E. coli isolates from companion animals compared with humans presumptively exonerates companion animals as a source of ST131 among humans in Australia. In contrast, ST131 may represent an emerging problem in veterinary healthcare, perhaps due to transfer of such strains from humans to animals.

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Transparency declarations
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References