Phylogenetic association of fluoroquinolone and cephalosporin resistance of D-O1-ST648

Escherichia coli carrying bla<sub>CMY-2</sub> from faecal samples of dogs in Japan

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This study aimed to investigate the genetic association between fluoroquinolone (FQ) and/or cephalosporin (CEP) resistance in Escherichia coli isolates from dogs, and the risk to human health. We characterized E. coli clinical isolates, derived from faecal samples of dogs attending veterinary hospitals, using phylogenetic grouping, determination of virulence factor (VF) prevalence, multilocus sequence typing (MLST) and O serotyping. The D group was the dominant phylogenetic group among strains resistant to FQ and/or CEP. In contrast, the dominant group among susceptible strains was group B2. Group D strains showed a significantly higher prevalence of VFs than strains belonging to groups A and B1, and were resistant to significantly more antimicrobials than group B2 strains. The phylogenetic distribution of FQ–CEP-resistant E. coli groups (FQ–CEPRECs) and FQ-resistant groups was significantly correlated (r=0.98), but FQ–CEPRECs and CEP-resistant E. coli groups were not correlated (r=0.58). Data from PFGE, O serotype and MLST analyses indicated that the majority of FQ-resistant strains derived from a particular lineage of phylogenetic group D: serotype O1 and sequence type (ST) 648. Some D-O1-ST648 strains carried bla<sub>CMY-2</sub>, showed multidrug resistance and possessed a higher prevalence of the VFs ksp<sub>MT</sub>, omp<sub>T</sub> and PAI compared with other group D strains. Our data indicate that the emergence of FQ-CEP-resistant E. coli is based primarily on FQ-resistant E. coli. Moreover, as strains of the D-O1-ST648 lineage have been found in clinical isolates derived from humans at a relatively high frequency, our findings indicate that the spreading of D-O1-ST648 strains may cause serious difficulties in both veterinary and human clinical fields in the future.

INTRODUCTION

Companion animals are an integral part of the daily life of many humans, and their medical treatment is similar to that of humans. For example, antimicrobials used for humans are also used in the treatment of companion animals. Inappropriate and excessive use of these antibiotics has led to increased resistance against these agents (Bergman et al., 2009). Recently, common clonal groups of fluoroquinolone (FQ)-resistant extra-intestinal Escherichia coli [serotype 25 (O25), sequence type (ST)131] have frequently been isolated from the human clinical field but has also been isolated from companion animals, although at an apparently lower rate (Platell et al., 2011). These results suggest that transmission of FQ-resistant extra-intestinal E. coli is caused mainly by transmission from humans to dogs. However, this evidence also suggests the opposite possibility: that some unknown clonal groups that are dominant in companion animals also harbour antimicrobial-resistant bacteria, and that transmission of these strains to humans poses a public health risk to humans.

Abbreviations: AMP, ampicillin; CEP, cephalosporin; CEPRECs, strains resistant to CEPs but not FQs; CFZ, cefazolin; CHL, chloramphenicol; CPD, cefpodoxime; DSM, dihydrostreptomycin; ENR, enrofloxacin; ESBL, extended-spectrum β-lactamase; FQ, fluoroquinolone; FQRECs, strains resistant to FQs but not CEPs; GEN, gentamicin; KAN, kanamycin; MLST, multilocus sequence typing; NAL, nalidixic acid; OTC, oxytetracycline; PMQR, plasmid-mediated quinolone resistance; QRDR, quinolone resistance-determining region; VF, virulence factor.
are frequently isolated from humans. For example, extended-spectrum β-lactamase (ESBL)-producing *E. coli* frequently show FQ resistance (Ruppé et al., 2009). Emergence of these FQ–CEPRECs has become a serious clinical problem.

FQ resistance is mainly caused by amino acid substitutions in the quinolone resistance-determining region (QRDR) of *gyrA* and *gyrB*, genes that encode DNA gyrase subunits, and in *parC* and *parE*, which encode topoisomerase IV subunits (Hopkins et al., 2005). On the other hand, third-generation CEP resistance is caused mainly by plasmid-mediated ESBLs and *ampC* genes, such as *blaCTX-M* and *blaCMY*, in *E. coli* and *Klebsiella pneumoniae* (Pérez-Pérez & Hanson, 2002). Therefore, the main mechanisms underlying acquisition of FQ and CEP resistance should be independent. However, previous studies have reported a significant correlation between the occurrence of FQ resistance and CEP consumption, and between CEP resistance and FQ consumption in *E. coli* (Bergman et al., 2009). Recent studies have indicated that a reason for this phenomenon may be the co-localization of ESBL genes and plasmid-mediated quinolone resistance (PMQR) determinants on the same genetic platforms (Kim et al., 2009a; Lavigne et al., 2006). However this cannot be the only reason, as for FQ–CEPRECs, which do not possess PMQR determinants, have also frequently been found (Ambrozic Avgustin et al., 2007; Kirchner et al., 2011).

Phylogenetic groups (A, B1, B2 and D) and virulence factor (VF) genes have been investigated as epidemiological markers in intestinal and extra-intestinal *E. coli* isolates in humans and animals (Clermont et al., 2000; Johnson et al., 2009; Takahashi et al., 2009; Yokota et al., 2012). In general, extra-intestinal virulent strains frequently belong to group B2, and to a lesser extent to group D. Less virulent indigenous intestinal strains are frequently from group A or B1 (Clermont et al., 2000). Several previous studies have shown that the phylogenetic group prevalent among FQ-resistant *E. coli* differs from that among FQ-susceptible *E. coli*. Takahashi et al. (2009) showed that, although phylogenetic group B2 was dominant, its prevalence was significantly less in FQ-resistant *E. coli* (49%) than in FQ-susceptible *E. coli* (78%) from human urinary isolates. Our own recent study indicated that FQ-resistant strains isolated from human clinical samples were mainly of the B2 group (80.4%) and in Japan, most (94.4%) of these B2 strains belonged to the O25:H4-ST131 lineage (Yokota et al., 2012). The other FQ-resistant strains were predominantly from group D. In isolates derived from dogs, the dominant groups among FQ-resistant *E. coli* were B1 and D (both 34%), and among FQ-susceptible *E. coli* was B2 (53%) (Johnson et al., 2009). Therefore phylogenetic grouping, which is a simple and inexpensive method, could be a useful marker indicating the association between genetic background and pathogenicity or antimicrobial resistance, and so could support epidemiological analysis in clinical *E. coli* isolates.

In this study, we characterized *E. coli* clinical isolates derived from dogs attending veterinary hospitals to elucidate the association of FQ and CEP resistance by using genetic analysis, including the determination of phylogenetic groups and VF prevalence. We also investigated the hazard to human health via canine FQ- and CEP-resistant extra-intestinal pathogenic *E. coli* by using multi-locus sequence typing (MLST) and O serotyping.

**METHODS**

**Bacterial strains.** One hundred and forty *E. coli* strains were isolated from 173 canine rectal samples using cotton swabs. These samples were recovered from dogs as follows: 93 dogs treated at Rakuno Gakuen University Animal Hospital (Ebetsu, Japan; strains named RE) and 80 dogs treated at eight community animal clinics (Ebetsu, Japan; strains named CE) in 2005. The case histories of the dogs were varied and included urethritis, cystitis, otitis externa, chronic diarrhoea, dermatitis, tumour, herniated intervertebral discs, keratitis, Cushings syndrome and septicemia. Canine rectal samples were collected immediately before administration of receiving any clinical treatment when the dogs visited the clinics or the university hospital. The percentages of dogs treated with FQ, CEP and any antimicrobials (for 6 months prior to sampling) were 22.2, 33.3 and 60.5%, respectively. The samples were incubated for 24 h at 37 °C in deoxycholate hydrogen sulfide lactose agar (Nissui) and subcultured in nutrient agar (Nissui). After incubation, the biochemical properties of these cultures were studied using triple-sugar iron medium (Nissui), lysine indole motility medium (Nissui) and an oxidase test. Final identification of *E. coli* was performed using API20E (bioMérieux). We classified the 140 isolated strains into groups according to FQ and/or CEP resistance as follows: 22 FQ–CEPRECs, 14 strains resistant to FQs but not CEPs (FQRECs), 10 strains resistant to CEPs but not FQs (CEPRECs) and 94 strains susceptible to both antimicrobials. Among the 94 susceptible strains, we randomly selected 35 and performed the following analyses. FQ- and CEP-resistant strains were defined as strains having MICs higher than 4 μg ml⁻¹ for enrofloxacin (ENR) and higher than 8 μg ml⁻¹ for cefpodoxime (CPD) following antimicrobial susceptibility testing.

**Susceptibility testing.** Susceptibilities to a panel of 10 antimicrobials were studied by the agar dilution method, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008), using an inoculum of 10⁶ c.f.u. Mueller–Hinton agar was obtained from Oxoid, and ENR from Bayer. Ampicillin (AMP), cefazolin (CFZ), CEP, gentamicin (GEN), kanamycin (KAN), dihydrostreptomycin (DSM), oxytetracycline (OTC) and chloramphenicol (CHL) were obtained from Sigma-Aldrich, and nalidixic acid (NAL) from Wako Junyaku. Resistance to DSM and OTC was defined by the Japanese Veterinary Antimicrobial Resistance Monitoring System (National Veterinary Assay Laboratory, 2009).

**Detection of QRDR mutations, PMQRs and β-lactamase genes.** Mutations in the QRDR of *gyrA*, *parC*, *parE* and *gyrB* were detected by direct DNA sequencing of PCR-amplified products, as described in previous studies (Sorlozano et al., 2007, Yang et al., 2004). *β*-Lactamase genes were detected by PCR and direct DNA sequencing. *blaTEM*, *blaSHV* and the chromosomal *ampC* promoter region were detected as described by Ruppé et al. (2009). Plasmid-mediated *ampC* was detected according to Pérez-Pérez & Hanson (2002), and *blaCTX-M* was detected as defined by Xu et al. (2005). The subtypes of detected *β*-lactamase genes were determined by nucleotide sequences using a BigDye Terminator v3.1 Cycle Sequencing kit with a 3130 Genetic Analyzer (Applied Biosystems). PMQR genes
ompT multiplex PCR, as reported previously (Takahashi et al., 2006). E. coli CE13). Among the 32 CEP-resistant strains (22 FQ–CEPRECs and 10 CEPRECs), FQREC strain possessed a PMQR, had three or four mutations in QRDRs and only one. All FQ-resistant strains (22 FQ–CEPRECs and 14 FQRECs)Association of antimicrobial susceptibility and phylogenetic groups

Association of VFs and phylogenetic groups

Group B2 strains harboured VF genes significantly more frequently, in particular sfa/foc, iroN, cvaC, hly, ompT, PAI and usp, than strains from other groups (P<0.01; Table 2). However, ETTT was markedly less frequent in group B2 than in other groups. Group D demonstrated the second highest prevalence of VFs. Groups A and B1 both demonstrated a low prevalence of VFs. Groups A and B2 demonstrated a higher prevalence of AMP and CHL resistance in group D was also higher than in strains of group B2. These findings indicate that group D strains tended to be multidrug-resistant compared with strains from other phylogenetic groups.

Among the 35 susceptible strains, those from group B2 were most common among the susceptible strains (14 isolates) compared with other phylogenetic groups (next most common were group B1, 12; group D, 5; and group A, 4). On the other hand, strains from group D were significantly more common among the 14 FQRECs (seven were from group D, three were from group B1 and two each were from groups A and B2) and the 22 FQ–CEPRECs (13 were from group D and three each were from groups A, B1 and B2). There was a significant correlation between FQRECs and FQ–CEPRECs in the distribution of phylogenetic groups (r=0.98, P=0.019), whereas no correlation was found between FQRECs and FQ–CEPRECs (10 strains; four were from group D, three each were from groups A and B1) and FQ–CEPRECs (r=0.58).

Of the β-lactamase genes, blaCTX-M2 was detected in 18 strains across all phylogenetic groups; however, nine of these strains belonged to group D. Four strains possessed blaCTX-M14: one strain each from groups A and B1 and two strains from group D. One strain possessing blaCTX-M27 was found in group B2.

PFGE analysis, O serotype and MLST in phylogenetic group D

To investigate further the association between FQ and CEP resistance, we focused on strains from group D, which were
more frequently isolated in FQ–CEPRECs and FQRECs. In
the dendrogram produced using the UPGMA algorithm,
group D strains were classified into four clusters (I–IV) of
50 % similarity and cluster I was divided into three
subclusters (Ia–Ic) of 60 % similarity according to the
Dice similarity index (Fig. 1). Clusters I and II were
composed mainly of FQRECs and FQ–CEPRECs; however,
clusters III and IV were composed of CEPRECs and
susceptible strains.

In O serotyping, 48.3 % of phylogenetic group D strains
(14/29 strains) were O1, and most of these belonged to
subclusters Ia and Ib. In MLST analysis, 10 of the 12 strains
belonging to subcluster Ia were identified as belonging to
the ST648 lineage. D-O1-ST648 strains were composed of
five FQRECs and five FQ–CEPRECs (eight strains from the
university animal hospital and two from different com-
munity animal clinics); all of these had two amino acid
substitutions in GyrA (S83L, D87Y or D87N) and one in
ParC (S80I) (Fig. 1). Eight and one strains additionally had
an amino acid substitution in ParC (E84G) and ParE
(S458A), respectively. All five FQ–CEPRECs belonging to
the D-O1-ST648 strains possessed bla\textsubscript{CMY-2} and four of
these were resistant to all antimicrobials tested, except for
KAN and/or GEN (Fig. 1). All D-O1-ST648 strains were
positive for ksp\textsubscript{MT}, omp\textsubscript{T} and ETTT and 90 % of these
possessed fim\textsubscript{H} and PAI. The prevalence of ksp\textsubscript{MT}, omp\textsubscript{T}
and PAI in D-O1-ST648 strains was significantly higher
than in other group D strains (P values were 0.0088, 0.0011
and 0.0062, respectively; Table 2). Clinical histories of dogs
from which D-O1-ST648 strains were isolated included
rhabdomyosarcoma (RE28), abdominal tumour (RE21),
mastocytoma (RE33), Cushing’s syndrome (RE17), her-
iniated intervertebral discs (RE39) and diarrhoea (CE13),
whilst the histories of others were unknown. The serotype
O25 or ST131 was not detected in any group (data not
shown). Other STs in the group D strains were identified as
ST10, ST68, ST69, ST354, ST394, ST405, ST493 and
ST2946. We also detected three novel STs, named
ST3556, ST3557 and ST3558.

DISCUSSION
Companion animals harbour antimicrobial-resistant bac-
teria in common with those harboured by humans, and
pose a public health risk to humans (Platell et al., 2011). In
particular, transmission of bacteria concomitantly resistant
to FQ and CEP from companion animals to humans poses
a higher risk to human health, because these two agents
have frequently been used in the human clinical field
(Bergman et al., 2009). In this study, we showed a
relationship between FQ and CEP resistance, and demon-
strated that a common \textit{E. coli} clonal group was frequently
isolated from dogs.

Among FQ–CEPRECs and FQRECs isolated from dogs in
Japan, group D was the dominant phylogenetic group,
whereas among susceptible strains was group B2 (Fig.

<table>
<thead>
<tr>
<th>Phylogenetic group</th>
<th>No. of resistant strains (%)</th>
<th>Mean no. of resistance</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>10 (83.3)</td>
<td>1.0 (1.0)</td>
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<tr>
<td>B</td>
<td>18 (88.7)</td>
<td>2.0 (2.0)</td>
</tr>
<tr>
<td>C</td>
<td>7 (58.6)</td>
<td>4.0 (4.0)</td>
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<tr>
<td>D</td>
<td>24 (82.8)</td>
<td>3.0 (3.0)</td>
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</table>

*Mean number of antimicrobial-resistant strains was calculated as the total number of antimicrobials showing resistance divided by the total number of isolates in each phylogenetic group.

**Significant difference versus group B1 (P<0.05).
***Significant difference versus group B2 (P<0.05).
### Table 2. Prevalence of VFs in *Escherichia coli* phylogenetic groups

<table>
<thead>
<tr>
<th>Phylogenetic group (no. strains)</th>
<th>Afa</th>
<th>fimH</th>
<th>iha</th>
<th>pap</th>
<th>sfa/foc</th>
<th>aer</th>
<th>fyuA</th>
<th>iroN</th>
<th>cnf1</th>
<th>hly</th>
<th>cvaC</th>
<th>kpsMT</th>
<th>ompT</th>
<th>traT</th>
<th>ibeA</th>
<th>PAI</th>
<th>usp</th>
<th>ETTT</th>
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<tr>
<td>A (12)</td>
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<td></td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
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<td>B1 (21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
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<tr>
<td>B2 (19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12</td>
<td>1</td>
<td>3</td>
<td>0</td>
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<td>0</td>
<td>12</td>
<td>12</td>
<td>2</td>
<td>14</td>
<td>8</td>
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<tr>
<td>D (29)</td>
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<td></td>
<td></td>
<td></td>
<td>0</td>
<td>12</td>
<td>0</td>
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<td>1</td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>2</td>
<td>14</td>
<td>8</td>
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<tr>
<td>D-O1- ST648 (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>10</td>
<td>0</td>
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<tr>
<td>Other D (strains belonged phylogenetic group D except ST648) (19)</td>
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<td></td>
<td></td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>10</td>
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<td>11</td>
<td>4</td>
<td>0</td>
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*aer*, aerobactin; *afa*, afimbrial adhesion; *cnf1*, cytotoxict necrotizing factor 1; *cvaC*, colicin V; *ETTT*, type III secretion system; *fimH*, type 1 fimbriae adhesion; *fimH*, yersiniabactin receptor for ferric yersiniabactin uptake; *hly*, α-haemolysin; *ibeA*, invasion of brain endothelium; *adhe*, catecholate siderophore receptor; *iha*, iron-regulated gene A homologue adhesion; *kpsMT*, group 2 capsule synthesis; *ompT*, outer-membrane protease T; *PAI*, pathogenic island marker of CFT073; *pap*, P fimbriae; *sfa/foc*, S/F1C fimbriae; *traT*, serum resistance associates; *usp*, uropathogenic-specific protein.

*Mean number of VFs was calculated as the total number of VFs divided by the total number of isolates in each phylogenetic group.*

†Significant difference versus group A ($P<0.05$).

‡Significant difference versus group B1 ($P<0.05$).

§Significant difference versus group B2 ($P<0.05$).

||Significant difference versus group D ($P<0.05$).

¶Significant difference versus other D (strains belonged phylogenetic group D except ST648) ($P<0.05$).
but not to CEPRECs (Fig. 1). In addition, the presence of FQ–CEPRECs were genetically highly related to FQRECs, risk for antimicrobial resistance during medical treatment. The presence of a group D strain in a dog should indicate a high risk for clinical infections, and these FQRECs, CEPRECs and CEPRECs carry a risk in clinical antimicrobial treatment regimens. Among these, the FQ-resistant phylogenetic group of E. coli, B2-O25:H4-ST131, which frequently possesses blaCTX-M-15 andaac(6′)-Ib-cr, has spread worldwide in humans (Peirano & Pitout, 2010; Takahashi et al., 2006). We observed B2-O25:H4-ST131 E. coli in 75.9 % (85/112 strains) of the FQ-resistant E. coli clinical isolates derived from humans in Hokkaido prefecture in Japan (Yokota et al., 2012). However, these strains did not carry the blaCTX-M-15 and aac(6′)-Ib-cr genes. In this study, we detected only two and three strains belonging to group B2 from FQRECs and FQ–CEPRECs, respectively, whereas group B2 was the most dominant group among the susceptible strains. In addition, E. coli strains belonging to phylogenetic group B2 showed a high prevalence of VFs and were frequently isolated from human clinical specimens (Clermont et al., 2000; Naseer et al., 2012; Takahashi et al., 2009). Thus, it has been recognized that group B2 strains are important in human E. coli infections, and these FQRECs, CEPRECs and CEPRECs carry a risk in clinical antimicrobial treatment regimens. Among these, the FQ-resistant phylogenetic group of E. coli, B2-O25:H4-ST131, which frequently possesses blaCTX-M-15 andaac(6′)-Ib-cr, has spread worldwide in humans (Peirano & Pitout, 2010; Takahashi et al., 2006). We observed B2-O25:H4-ST131 E. coli in 75.9 % (85/112 strains) of the FQ-resistant E. coli clinical isolates derived from humans in Hokkaido prefecture in Japan (Yokota et al., 2012). However, these strains did not carry the blaCTX-M-15 and aac(6′)-Ib-cr genes. In this study, we detected only two and three strains belonging to group B2 from FQRECs and FQ–CEPRECs, respectively, whereas group B2 was the most dominant group among the susceptible strains. In addition,

![Fig. 1. Genetic analysis and antimicrobial susceptibility of E. coli phylogenetic group D strains. Black, resistant; grey, intermediate; white, susceptible; ND, not detected; NT, not tested; Chr. ampC, mutation in chromosomal ampC promoter region.](image-url)
B2-O25:H4-ST131 FQ-resistant strains were not observed in this study. Although a previous study isolated B2-O25:H4-ST131 in E. coli isolates derived from dogs, the frequency was lower than those in isolates derived from humans (Peirano & Pitout, 2010; Platell et al., 2011; Takahashi et al., 2006; Yokota et al., 2012). Therefore, these results suggest that the risk of transmission of FQRECs, CEPRECs, and FQ–CEPRECs belonging to group B2 (including B2-O25:H4-ST131) from dogs and humans is low, and that these are not a major source of human E. coli infections.

On the other hand, we found that the major strain (27.8%, 10 of 36 FQ–CEPRECs/FQRECs) in the canine population in the clinical environment of Hokkaido prefecture in Japan was D-O1-ST648 (Fig. 1). This clonal group has also been reported in dogs; 42.9% (9/21 strains) of bla<sub>CMY-2</sub>-bearing CEPRECs obtained from stray dogs in Korea were D-ST648 (Tamang et al., 2012). Hadada et al. (2012) detected a D-O1-ST648 strain, possessing bla<sub>CTX-M-14</sub> and bla<sub>CMY-2</sub>-lactamase, in CFZ-resistant extra-intestinal pathogenic E. coli strains from a cat from a different region in Japan. Therefore, these results suggest that ST648 has spread in companion animals possessing β-lactamase genes, at least in Asia.

Clinical ST648 isolates have been also reported worldwide in humans since 2011. Our recent study on human isolates derived from Hokkaido prefecture showed that D-O1-ST648 was the second-most prevalent genotype among FQ-resistant strains (5/112 strains; 4.5% of the FQ-resistant isolates, followed by ST131; unpublished results, Yokota et al., 2012). Little et al. (2012) reported that ST648 strains isolated from human blood cultures carried the S83L and D87N substitutions in GyrA, and possessed both bla<sub>CMY-2</sub> and bla<sub>IMP-4</sub> genes. Remarkably, ST648 strains have also been reported as possessing the New Delhi metallo-enzyme carbapenemase in the UK (Hornsey et al., 2011). The D-O1-ST648 strains in this study also carried these amino acid substitutions in GyrA, and some carried the bla<sub>CMY-2</sub> gene (Fig. 1). As described above, Naseer et al. (2012) reported that ST648 was detected in four European countries, Norway, the UK, Spain and Sweden, and this clonal group was the second major clonal group (followed by ST131) in ESBL-producing E. coli strains derived from human clinical specimens. These findings indicate the possibility that transmission of this clonal lineage occurs between humans and dogs. In addition, VF genes (kpsMT, ompT, and PAI) were significantly more prevalent in D-O1-ST648 FQRECs/FQ–CEPRECs than in other group D strains (Table 2). This finding indicates that the spread of D-O1-ST648 FQRECs/FQ–CEPRECs may cause serious difficulties in the clinical field, as will be the spread of B2-O25:H4-ST131 strains.

However, many previous reports lack clinically important information regarding FQ or CEP susceptibility, VF profiles, phylogenetic group and O serotype in ST648 strains. Therefore, comprehensive surveillance for and implementation of appropriate interventions against D-(O1)-ST648 strains are required.

In conclusion, the majority of canine FQRECs and FQ–CEPRECs in Hokkaido prefecture, Japan, had genetic background features in common, indicating that they belong to the D-O1-ST648 group. These D-O1-ST648 FQRECs/FQ–CEPRECs, which have spread among humans and their companion animals, showed high VF prevalence and multidrug resistance, and may pose a serious challenge to management of infections in the future.

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