Canine model for investigating the impact of oral enrofloxacin on commensal coliforms and colonization with multidrug-resistant *Escherichia coli*

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A model was developed in dogs to determine the impact of oral enrofloxacin administration on the indigenous coliform population in the gastrointestinal tract and subsequent disposition to colonization by a strain of multidrug-resistant *Escherichia coli* (MDREC). Dogs given a daily oral dose of 5 mg enrofloxacin kg$^{-1}$ for 21 consecutive days showed a significant decline in faecal coliforms to levels below detectable limits by 72 h of administration. Subsequently, faecal coliforms remained suppressed throughout the period of enrofloxacin dosing. Upon termination of antibiotic administration, the number of excreted faecal coliforms slowly returned over an 8-day period, to levels comparable to those seen prior to antibiotic treatment. Enrofloxacin-treated dogs were more effectively colonized by MDREC, evidenced by a significantly increased count of MDREC in the faeces ($7.1 \pm 1.5 \log_{10} \text{g}^{-1}$) compared with non-antibiotic-treated dogs ($5.2 \pm 1.2 \log_{10} \text{g}^{-1}$; $P = 0.003$). Furthermore, antibiotic treatment also sustained a significantly longer period of MDREC excretion in the faeces ($26.8 \pm 10.5$ days) compared with animals not treated with enrofloxacin ($8.5 \pm 5.4$ days; $P = 0.0215$). These results confirm the importance of sustained delivery of an antimicrobial agent to maintain and expand the colonization potential of drug-resistant bacteria *in vivo*, achieved in part by reducing the competing commensal coliforms in the gastrointestinal tract to below detectable levels in the faeces. Without *in vivo* antimicrobial selection pressure, commensal coliforms dominated the gastrointestinal tract at the expense of the MDREC population. Conceivably, the model developed could be used to test the efficacy of novel non-antibiotic strategies aimed at monitoring and controlling gastrointestinal colonization by multidrug-resistant members of the *Enterobacteriaceae* that cause nosocomial infections.

INTRODUCTION

Nosocomial infections have become increasingly prevalent in veterinary teaching hospitals (Boerlin et al., 2001; Prescott et al., 2002). Recent reports of extraintestinal infections in dogs due to multidrug-resistant *Escherichia coli* with resistance to third-generation cephalosporins and fluoroquinolones (MDREC) are a potential public health concern (Féria et al., 2002; Sanchez et al., 2002; Tesherger et al., 2000; Warren et al., 2001). Canine MDREC have been shown to possess β-lactamase and class-1 integron-associated resistance genes that have previously been identified in bacterial isolates from clinical infections in humans (Hanson et al., 2002; Townsend et al., 2002; Sanchez et al., 2002). This suggests the spread of common resistance mechanisms between canine and human isolates, possibly through the co-selection and transfer of multidrug-resistance plasmids. Furthermore, extraintestinal pathogenic *E. coli* isolates from dogs and humans are phylogenetically closely related and share common virulence genes, suggesting a potential for cross-species transmission (Johnson et al., 2001).

Dogs may therefore represent a previously overlooked reservoir of antimicrobial-resistance genes that may be transferred directly or indirectly to humans. Dogs that carry MDREC in their faeces may readily contaminate the veterinary hospital environment and are a potential source of transmission to other animals and humans (Warren et al., 2001). Risk factors for MDREC infections in human hosp-
tals include long hospitalization, lapses in infection control, immunosuppression, invasive procedures such as urinary or venous catheterization and treatment with broad-spectrum antimicrobials (Garau et al., 1999). Similar factors may also be present in veterinary hospitals, particularly increased use of the newer generation broad-spectrum antimicrobials such as fluoroquinolones (Cooke et al., 2002).

Each antimicrobial class causes ecological disturbances in different populations of the gastrointestinal microbiota (Edlund & Nord, 2000; Sakata et al., 1986). In humans, fluoroquinolones have little impact on gastrointestinal anaerobes and are regarded as 'gut-friendly' antimicrobials (Edlund & Nord, 1999). However, they have a profound effect on the aerobic population of the gut, totally suppressing or eliminating the Enterobacteriaceae (Reeves, 1986). This phenomenon is usually transitory, with populations returning to pre-antimicrobial administration numbers within 2 weeks of the cessation of therapy in the majority of studies (Edlund & Nord, 2000). Little is known about the effect of oral fluoroquinolones on the canine gastrointestinal microbiota and there have been no studies on the ecology of MDREC colonization.

**METHODS**

**Bacterial strain and media.** An enrofloxacin-resistant MDREC strain (C1) originally isolated from the urine of a 14-year-old castrated male corgi with pyelonephritis was used in the study (Warren et al., 2001). Strain C1 was grown in 100 ml LB broth at 37 °C to mid-exponential phase (OD600 = 0.7). The viable count was determined to be 7.3 × 10^8 cells ml⁻¹ on blood agar plates using the standard plate dilution method.

**Infection model.** Sixteen mixed-breed dogs were obtained from a local dog pound and housed in an air-conditioned isolation room set at 22–23 °C in individual dog runs. The dogs were fed commercial canned dog food and biscuits for the duration of the experiment. The dogs were walked each day of the trial for 30–60 min and the University of Queensland Animal Ethics and Experimentation Committee approved all experimental procedures. Prior to placement in the isolation room, fecal specimens from each dog were plated onto MacConkey agar containing 5 µg each of gentamicin and enoxacin ml⁻¹ to ensure the dogs were free from multidrug-resistant enteric bacteria. The dogs were then vaccinated against canine distemper, hepatitis, parovirus and parainfluenza virus infection (Canvac in 1; CSL), tested for heartworm and treated for fleas and intestinal worms with 50 mg praziquantel, 250 mg febantel, 49.8 mg pyrantel per 10 kg body weight (Drontal; Bayer Animal Health Australia). The dogs were randomly divided into four treatment groups of four dogs and received treatments according to the schedule in Table 1. After a 10-day acclimatization period, dogs in groups A and C received a daily oral dose of 5 mg enrofloxacin kg⁻¹ (Bayer Animal Health Australia) for 21 days. Dogs in groups A and B were given a single dose of 10 ml LB broth containing MDREC strain C1 (7.3 × 10⁸ bacteria) mixed into the food on day 7 of the antimicrobial treatment. Dogs in group D acted as untreated, uninoculated controls. Individual dogs were allowed to have contact with other dogs in the same treatment group but contact between treatment groups was prevented. Faecal specimens from each dog for viable total coliform and MDREC counts were obtained twice weekly until the conclusion of the trial on day 62 for groups A and B and day 48 for groups C and D.

**Total coliform and MDREC counts.** All counts were performed in duplicate with the final count expressed as the mean of the two counts. Faecal specimens from the dogs were processed within 2 h of collection. Serial 10-fold dilutions were made from 5 g faeces in 0.1 % peptone water (pH 7.3). Total coliform counts (TCC) were obtained by transferring 1 ml of the appropriate dilutions onto the surface of Petrifilm EC plates (3M Australia Ltd) according to the manufacturer's instructions as described previously (Bloch et al., 1996). The plastic Petrifilm EC plates were incubated in a humidified chamber at 35 °C for 24 h and the plates were examined for the presence of both E. coli and coliforms. Petrifilm EC plates were then reincubated for a further 24 h and the number of E. coli and coliforms was recorded using a colony counter. TCC and MDREC counts were expressed as the number of cfu (log₁₀) per gram wet weight of faeces. The level of detection was determined to be greater than 1 log₁₀ (g faeces)⁻¹. A dog was defined as being colonized with the MDREC challenge strain C1 if MDREC showing the same antimicrobial susceptibility profile was isolated from the faeces on two successive occasions. Antimicrobial disc diffusion susceptibilities were performed using 14 antimicrobials according to NCCLS standards (NCCLS, 1998).

**Statistical analysis.** Standard one-way analysis of variance with Dunnett’s multiple comparison test was used to compare the pre-

### Table 1. Treatment group schedule

<table>
<thead>
<tr>
<th>Group</th>
<th>Designation</th>
<th>Dogs</th>
<th>Treatment</th>
<th>Conclusion of trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>E+M+</td>
<td>1–4</td>
<td>Days 10–31</td>
<td>Day 17</td>
</tr>
<tr>
<td>B</td>
<td>E–M+</td>
<td>5–8</td>
<td>–</td>
<td>Day 17</td>
</tr>
<tr>
<td>C</td>
<td>E+M–</td>
<td>9–12</td>
<td>Days 10–31</td>
<td>–</td>
</tr>
<tr>
<td>D</td>
<td>E–M–</td>
<td>13–16</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Where indicated, dogs received enrofloxacin (5 mg kg⁻¹ per os) daily for 21 days. For MDREC treatment, dogs received a bolus dose of 7.3 × 10⁸ bacteria mixed into the food on day 7 of the antimicrobial treatment.
treatment TCC of groups A–C with the control group TCC obtained for the entire course of the trial (the pre-treatment TCC for groups A and C were obtained prior to administration of enrofloxacin and, for group B, prior to the administration of MDREC). TCC below the level of detection obtained for two dogs in group B, one dog in group C and one dog in group D were not included in the final analysis. The non-parametric Mann–Whitney test and the two-tailed unpaired t test (Graphpad Instat version 3.05) were respectively used to compare the duration of MDREC colonization and mean MDREC counts between groups A and B.

**RESULTS AND DISCUSSION**

In a study examining gastrointestinal carriage of fluoroquinolone-resistant Gram-negative organisms in humans, treatment with fluoroquinolones within the preceding 4 weeks of the trial was the only risk factor identified (Richard *et al.*, 2001). We hypothesized that similar patterns of antimicrobial use in veterinary hospitals may also be responsible for the increased isolation of MDREC with fluoroquinolone resistance from dogs (Cooke *et al.*, 2002). We therefore examined in a dog model the effects of enrofloxacin on faecal TCC and subsequent MDREC counts following oral administration of a strain of MDREC on day 7 of antimicrobial therapy.

TCC for each group during the course of the trial are shown in Fig. 1. One-way analysis of variance showed that variation between the four groups was just significant ($P = 0.046$). Using Dunnett’s multiple comparisons test, pre-treatment TCC for groups A and C were not significantly different from the control group (mean $= 7.3 \pm 1.3$), and only dogs in group B showed a significant difference (mean $= 6.4 \pm 1.1$; $P < 0.05$). However, this should not have influenced the outcome of the experiment, as it would be assumed that higher TCC would be more beneficial in competing with MDREC, unless the low counts are indicative of a higher proportion of adherent bacteria in the total coliform population. Occasionally, the TCC obtained from single animals in groups A, C and D were below the level of detection, despite the counts being repeated a number of times. In one dog in group D that developed otitis externa during the trial, these fluctuations in TCC coincided with the topical application...
tion of 0.5% framycetin prescribed for animal ethics reasons. However, for the other dogs, the TCC below the level of detection could not be linked to any change in the dogs’ environment.

Within 3 days of administration of enrofloxacin, faecal coliforms were eliminated or suppressed below the level of detection in group A (E+M+) and group C (E+M−) (Fig. 1). In group C, TCC remained suppressed for the entire duration of antimicrobial therapy and only reached detectable levels [TCC range: 3.7–7.8 log_{10} (g faeces^{-1})] by day 4 and pre-antimicrobial administration levels by day 8 (Fig. 1). Following dosing, the MDREC counts of dogs in group A (E+M+) were significantly higher (7.1 ± 1.5) than those of dogs in group B (E−M+) (5.2 ± 1.2; P = 0.003) (Fig. 2). In addition, group A dogs retained MDREC in their faeces for a significantly longer period (26.8 ± 10.5 days) than group B dogs (8.5 ± 5.4 days; P = 0.0286). In group A, dogs 1 and 4 were still colonized with MDREC at 38 and 31 days post-administration, respectively. MDREC were never isolated from any dogs in groups C (E+M−) or D (E−M−) during the trial, and all MDREC isolates recovered from the dogs had the same antimicrobial susceptibility profile as challenge strain C1.

These results suggest that canine MDREC do not compete well with resident coliforms in the absence of antimicrobial selection pressure. However, once established, MDREC may persist for long periods despite the return of the commensal coliforms to pre-antimicrobial treatment levels. In future studies, it will be interesting to treat dogs in group A (E+M+) with a second course of antimicrobials once they stop shedding MDREC in their faeces. This would determine whether MDREC have been eliminated completely by the other coliforms or whether they are still present in very small numbers in protected niches in the gastrointestinal tract and are able to proliferate and become detectable in the faeces again only in response to further antimicrobial selection pressure. However, in such studies, it would be important to confirm that exogenous sources of the MDREC challenge strain were completely eliminated from the dogs’ environment.

The results of the current study may, in part, provide an explanation for the occurrence of MDREC nosocomial infections in veterinary hospitals. Hospitalized dogs carrying MDREC would shed large numbers of the drug-resistant organisms in their faeces following treatment with enrofloxacin. This would contaminate the hospital environment, spreading MDREC to in-contact animals and, potentially, hospital employees by the faecal–oral route. A large number of MDREC carriers together with a heavily contaminated hospital environment would result in more opportunistic nosocomial MDREC infections in susceptible animals (Warren et al., 2001). In human hospitals, infection control measures and stringent antimicrobial prescribing guidelines are helpful in limiting colonization of patients with drug-resistant strains (Rupp & Fey, 2003). However, these measures may not achieve the same rate of success in veterinary hospitals due to the greater opportunity for faecal–oral transmission between animals. In addition, opportunistic wound or post-surgical infections are very common in companion animals because of reduced patient compliance, and veterinarians are more likely to prescribe prophylactic broad-spectrum antimicrobials to prevent their occurrence.

Novel methods of control are therefore required to limit multidrug-resistant organisms such as MDREC from becoming more prevalent in veterinary hospital settings (van der Waaij & Nord, 2000). The animal model developed in the present study will be useful for testing the efficacy of potential control agents, such as probiotic organisms or bacteriocins that are shown to inhibit the growth of multi-drug-resistant organisms specifically in vitro.

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**Fig. 2.** Changes in MDREC count in each dog in groups A (E+M+) and B (E−M+).
REFERENCES