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Routine antibiotic therapy in dogs increases the detection of antimicrobial-resistant faecal *Escherichia coli*

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Background: Antimicrobial resistance (AMR) is a critical health problem, with systemic antimicrobial therapy driving development of AMR across the host spectrum.

Objectives: This study compares longitudinal carriage, at multiple timepoints, of AMR faecal *Escherichia coli* in dogs undergoing routine antimicrobial treatment.

Methods: Faecal samples ($n = 457$) from dogs ($n = 127$) were examined pretreatment, immediately after treatment and 1 month and 3 months post-treatment with one of five antimicrobials. Isolates were tested for susceptibility to a range of antimicrobials using disc diffusion for each treatment group at different timepoints; the presence/absence of corresponding resistance genes was investigated using PCR assays. The impact of treatment group/timepoint and other risk factors on the presence of resistance [MDR, fluoroquinolone resistance, third-generation cephalosporin resistance (3GCR) and ESBL and AmpC production] was investigated using multilevel modelling. Samples with at least one AMR *E. coli* from selective/non-selective agar were classed as positive. Resistance was also assessed at the isolate level, determining the abundance of AMR from non-selective culture.

Results: Treatment with β-lactams or fluoroquinolones was significantly associated with the detection of 3GCR, AmpC-producing, MDR and/or fluoroquinolone-resistant *E. coli*, but not ESBL-producing *E. coli*, immediately after treatment. However, 1 month post-treatment, only amoxicillin/clavulanate was significantly associated with the detection of 3GCR; there was no significant difference at 3 months post-treatment for any antimicrobial compared with pretreatment samples.

Conclusions: Our findings demonstrated that β-lactam and fluoroquinolone antibiotic usage is associated with increased detection of important phenotypic and genotypic AMR faecal *E. coli* following routine therapy in vet-visiting dogs. This has important implications for veterinary and public health in terms of antimicrobial prescribing and biosecurity protocols, and dog waste disposal.

Introduction

The gastrointestinal tract is an important reservoir for antimicrobial-resistant (AMR) Gram-negative organisms.[1,2] MDR (resistance to three or more antimicrobial classes),[3] ESBL- and AmpC-producing faecal *Escherichia coli* carried by dogs are of particular concern. They may act as a reservoir for self-infection, which may lead to further transmission of resistance genes, as well as pathogens and resistance genes potentially being transferred into other hosts, including people, other pets and the environment.[4–6] Systemic antimicrobial therapy selects for AMR Gram-negative bacteria, so increased use compounds AMR issues. In humans, even short-term therapy with ciprofloxacin, a cephalosporin or clindamycin can lead to long-term disturbance of commensal bacterial populations and prolonged carriage of AMR Enterobacteriaceae or anaerobic bacteria.[7,8]

There are a limited number of (mostly broad-spectrum) antimicrobials authorized for use in companion animals in the UK. Amongst these, β-lactams and fluoroquinolones are commonly utilized and critically important for the treatment of bacterial infections.[9] β-Lactam antimicrobials include oral cefalexin, oral amoxicillin/clavulanate, the most commonly prescribed, for dogs, by first-opinion veterinarians,[10,11] and injectable cefovecin (administered subcutaneously every 14 days). Enrofloxacin and

marbofloxacin are oral second-generation fluoroquinolones and clindamycin is an oral lincosamide antimicrobial.[7,12]

The overall effect of antimicrobial treatment on human commensal bacterial populations has been shown to depend on the pharmacokinetics, spectrum of activity, dose and treatment duration, and the levels of AMR bacteria present before treatment.[7] In dogs, a number of studies have shown that treatment with either β-lactam or fluoroquinolone antimicrobials may positively select for intestinal/faecal AMR *E. coli* for variable periods of time.[13–20] This study aimed to compare the extent and characteristics of AMR *E. coli* carriage in the faeces of community dogs before and after treatment with five different antimicrobials.

Materials and methods

Study population

Dogs attending veterinary consultations at three centres, including first-opinion and referral practice, in the North-West of England between June 2011 and September 2012 were recruited. Inclusion criteria were dogs diagnosed with a bacterial infection (skin, soft tissue, urinary tract, dental, respiratory tract, orthopaedic, gastrointestinal, ocular) requiring systemic antimicrobial therapy with one of five antimicrobials authorized for use, including cefalexin, amoxicillin/clavulanate, cefovecin, clindamycin or a fluoroquinolone (enrofloxacin or marbofloxacin). Exclusion criteria included antimicrobial therapy or veterinary admission within the previous 3 months and dogs aged <12 months (due to fluoroquinolone contraindication). Dogs were excluded if they were prescribed systemic antimicrobials during the follow-up period. The veterinarian in charge of the case selected and implemented the treatment plan (antimicrobial, dose, frequency and duration) according to clinical need. Before enrolment, all dog owners read the study outline and gave written informed consent. The University's Veterinary Science Ethics Committee approved the study protocol in June 2011.

Detection and characterization of faecal *E. coli*

E. coli isolation

Owners were asked to provide a fresh faecal sample from their dog pre-treatment, immediately after treatment and at 1 month and 3 months post-treatment. Samples were delivered in person or by first-class pre-paid return post. Faecal samples were refrigerated and processed on delivery (within 24–72 h of collection).

All faecal samples were processed by selective and non-selective methods, as previously reported.[21–24] In brief, equal volumes of faeces (5 g) and brain heart infusion broth with 5% glycerol (BHI-G) (5 mL) were homogenized before streaking onto plain eosin methylene blue agar (EMBA), EMBA impregnated with third-generation cephalosporins (1 μg/mL ceftazidime and 1 μg/mL cefotaxime) and spread-plating onto plain EMBA with antimicrobial discs (10 μg ampicillin, 30 μg amoxicillin/clavulanate, 1 μg ciprofloxacin, 30 μg chloramphenicol, 30 μg nalidixic acid, 30 μg tetracycline and 2.5 μg trimethoprim).[22] Following overnight aerobic incubation at 37°C, when present, 10 random colonies, morphologically resembling *E. coli*, were selected from plain EMBA and one colony from each (ceftazidime/cefotaxime) impregnated EMBA plate and/or growing within the inhibition zone around each antimicrobial disc was selected for further investigation; this methodology was used to investigate whether there is a reduction in diversity and/or emergence of low-prevalence AMR clones following antimicrobial selective pressure.[4,22,25] It was therefore possible to select a maximum of 19 isolates from each faecal sample. Selected colonies were sub-cultured onto nutrient agar for pure growth and incubated aerobically overnight at 37°C before Gram staining, biochemical analysis (catalase production, lack of oxidase, lactose fermentation, indole production and inability to use citrate as a carbon source). PCR assays for the *uidA* gene[26] confirmed isolates as *E. coli*. All antimicrobial discs were obtained from MAST Group Ltd, Liverpool, UK, and media from LabM Ltd, Bury, UK; cephalosporin powder was from Sigma–Aldrich Company Ltd, Gillingham, UK.

Antimicrobial susceptibility testing

All confirmed *E. coli* isolates underwent antimicrobial susceptibility disc-diffusion testing and interpretation as previously reported[24] according to BSAC guidelines[27] with the same panel of seven antimicrobial discs as used above. *E. coli* ATCC® 25922 (LGC Standards, Teddington, UK) cultured overnight on nutrient agar at 37°C was the control.

ESBL- and AmpC-producing E. coli

Isolates selected from third-generation-cephalosporin-impregnated EMBA and isolates from other selective and non-selective agar with phenotypic resistance to ampicillin or amoxicillin/clavulanate were further screened for third-generation cephalosporin resistance (3GCR) (10 μg of cefpodoxime) and phenotypic ESBL and AmpC production, according to the manufacturer's instructions (Extended Spectrum β-Lactamase Set D52C, MAST Group Ltd, Liverpool, UK, and AmpC detection set D69C, MAST Group, Liverpool, UK).[28,29] *E. coli* ATCC® 25922 (LGC Standards, Teddington, UK) cultured overnight on nutrient agar at 37°C was the control. Isolates with phenotypic ESBL or AmpC production were further tested for the presence of $bla_{\text{CTX-M}}$,[30] bla_{SHV}, bla_{TEM} and bla_{OXA} genes,[31] and bla_{AmpC} genes,[32] including $bla_{\text{CIT-M}}$ as a screen for bla_{CMY} (the most common AmpC gene in the UK).[24,33] If positive for $bla_{\text{CTX-M}}$, isolates were tested for the presence of CTX-M group-1, -2 and -9 genes,[34,35] as these are reported to be the most common CTX-M-group genes amongst animals in the UK.[24,36]

Statistical analyses

Sample-level prevalence of AMR over time

All isolates (from selective and non-selective agar) were included in the analysis. To account for multiple isolates per sample, microbiological data were collapsed to sample level, such that a sample with at least one resistant isolate was classed as resistant. Five resistance outcomes were considered: fluoroquinolone resistance (FQR), 3GCR, phenotypic ESBL or AmpC producing and MDR. The percentages of samples with each of the five resistance outcomes were calculated (including 95% CIs) for each treatment group/timepoint.

Isolate-level prevalence of AMR over time

To quantify the abundance of AMR *E. coli* for each treatment group/timepoint, 10 random isolates (if available) from non-selective EMBA were tested from each sample ($n = 3897$ isolates). The percentage of isolates (including 95% CIs) with resistance to each tested antimicrobial or MDR was determined for each treatment group/timepoint.

Risk factors for AMR faecal E. coli

Questionnaire data. A questionnaire investigating potential risk factors for AMR bacteria was completed by owners at the start of the study and at each faecal collection. The attending veterinary surgeon completed a one-page questionnaire detailing diagnosis, treatment regimen and previous antimicrobial treatment within the last 12 months. All questionnaire-derived information was available as potential explanatory variables for inclusion in multivariable modelling of AMR outcomes. Except for age, all variables were categorical. Collinearity between explanatory variables was assessed using two-by-two tables and Pearson's χ^2 test for independence or Fisher's exact tests if $N < 5$. A one-way between-groups analysis of variance (ANOVA) was used to investigate differences between animals of

Table 1. Pretreatment variables considered for inclusion in the final multivariable model, with the number and percentage of dogs in each treatment group and variable category

Variable	Cefalexin ($n = 32$)	Amoxicillin/clavulanate ($n = 28$)	Cefovecin ($n = 24$)	Clindamycin ($n = 29$)	Fluoroquinolone ($n = 14$)	Total ($n = 127$)	*P*
Mean age[a] (months)	44	50	68	79	83	62	**0.002**
Weight							**0.002**
small (<11 kg)	1 (5)	2 (10)	3 (15)	10 (50)	4 (20)	20 (16)	
medium (11–20 kg)	4 (25)	3 (19)	0	7 (44)	2 (13)	16 (13)	
large (>20 kg) (ref.)	27 (30)	23 (25)	21 (23)	12 (13)	8 (9)	91 (72)	
Gender							0.8
male (ref.)	19 (25)	17 (23)	12 (16)	17 (23)	10 (13)	75 (59)	
female	13 (25)	11 (21)	12 (23)	12 (23)	4 (8)	52 (41)	
Treatment duration							**0.001**
1 week (ref.)	6 (18)	16 (47)	0	10 (29)	2 (6)	34 (27)	
>1 to <3 weeks	12 (26)	9 (19)	9 (19)	11 (23)	6 (13)	47 (37)	
>3 weeks	14 (30)	3 (7)	15 (33)	8 (17)	6 (13)	46 (36)	
Recruitment site							**0.001**
first-opinion practice (ref.)	24 (33)	27 (37)	4 (5)	17 (23)	1 (1)	73 (57)	
referral consultation	8 (15)	1 (2)	20 (37)	12 (22)	13 (24)	54 (43)	
Diagnosis of pyoderma at enrolment[b]	28 (35)	3 (4)	23 (28)	16 (20)	11 (14)	81 (64)	**0.001**
Previous systemic antimicrobial treatment[c]	16 (26)	10 (16)	17 (28)	10 (16)	8 (13)	61 (48)	**0.048**
Previous β-lactam antimicrobial treatment[c]	11 (28)	7 (18)	9 (23)	7 (18)	6 (15)	40 (31)	0.41
Previous hospital admission[c]	17 (42)	12 (29)	5 (12)	6 (15)	1 (2)	41 (32)	**0.007**
In-contact human or pet received antimicrobials[d]	7 (26)	5 (19)	4 (15)	7 (26)	4 (15)	27 (21)	0.9
In-contact human or pet admitted to hospital or veterinary premises[d]	4 (15)	3 (12)	6 (23)	8 (31)	5 (19)	26 (20)	0.2
Owner works in healthcare	5 (21)	2 (8)	4 (17)	10 (42)	3 (13)	24 (19)	0.08
Multi-dog household	18 (32)	15 (26)	12 (21)	8 (14)	4 (7)	57 (45)	0.1
Enrolled dog regularly eats animal stools	7 (18)	5 (13)	8 (21)	11 (29)	7 (18)	38 (30)	0.08

ref., reference category for non-dichotomous variables.
Significant if $P < 0.05$ (bold text) (Pearson's χ^2 or [a]ANOVA).
[a]Age was the only continuous value and is represented by the mean age of dogs in each treatment group.
[b]Other infections ($n = 46$) include urinary tract/prostate ($n = 11$), abscess/bite wound ($n = 11$), dental ($n = 10$) and post-operative ($n = 8$).
[c]Within 12 months, but >3 months, as per enrolment criteria.
[d]Within 12 months of enrolment.

different ages (normal distribution) within treatment groups pretreatment (Table 1). To investigate significant pretreatment differences between treatment groups, for each AMR outcome (FQR, 3GCR, MDR and ESBL- and AmpC-producing *E. coli*) and questionnaire-derived variables, simple univariable and multivariable logistic regression analysis with a binomial distribution and logit link function were used. All questionnaire data analyses were undertaken using the SPSS software package (SPSS 20.0 for Mac, SPSS Inc., Chicago, IL, USA).

Multilevel models. Multilevel multivariable logistic regression modelling was used to examine differences between treatment groups/timepoints, including dog as a random-effect term (level-2 unit, due to repeated measurements in dogs). Faecal samples were the level-1 unit of interest. At enrolment all dogs were classed as 'untreated'. The different combinations of time ($n = 3$) and treatment group ($n = 5$) provided 15 categories for analyses.

Univariable analyses were initially performed; all variables showing some association with the resistance outcome ($P < 0.25$)[37] were considered for incorporation in the final multivariable model. Models were constructed using backwards stepwise procedures where variables with a Wald *P* value <0.05 were retained; treatment group/timepoint were always retained. Once a final multivariable model was generated, all variables significantly ($P < 0.05$) different between treatment groups at baseline were forced into the multilevel model to ensure that there was no confounding effect on remaining variables.

Univariable and multivariable calculations utilizing penalized quasi-likelihood estimates [second-order predictive quasi-likelihood (PQL) for all outcomes other than phenotypic ESBL, which was first-order marginal quasi-likelihood (MQL) due to lack of model convergence][38] were performed. First-order interaction terms were tested for all variables remaining in final models. The residuals ± 1.96 SD $\times$ rank (caterpillar plots) were calculated and graphed for each dog to check for outliers. Multilevel models were analysed using the MLwiN statistical software package (MLwiN Version 2.28 Centre for Multilevel Modelling, University of Bristol).

Results

Study population

One hundred and twenty-seven dogs were enrolled from three centres (Table S1, available as Supplementary data at *JAC* Online). All dogs provided samples pretreatment and at treatment end,

Table 2. Number (percentage) of samples that harboured at least one faecal *E. coli* positive for ESBL or AmpC resistance genes for each timepoint/treatment group

Treatment group	Timepoint and total samples	bla_{CTX-M}	CTX-M group 1	CTX-M group 9	bla_{CIT-M}	bla_{CTX-M} and bla_{CIT-M}	Phenotypic ESBL with bla_{TEM} and/or bla_{OXA}[a]
Cefalexin	D0 ($n = 32$)	1 (3)	1 (3)	0	4 (13)	0	0
	E ($n = 32$)	4 (13)	3 (9)	0	19 (60)	4 (13)	1 (3)
	M1 ($n = 27$)	2 (7)	2 (7)	0	9 (33)	0	1 (4)
	M3 ($n = 24$)	0	0	0	4 (17)	0	0
Amoxicillin/clavulanate	D0 ($n = 28$)	0	0	0	5 (18)	0	0
	E ($n = 28$)	1 (4)	1 (4)	0	9 (32)	0	1 (4)
	M1 ($n = 26$)	1 (4)	1 (4)	0	7 (27)	0	1 (4)
	M3 ($n = 25$)	1 (4)	0	1 (4)	5 (20)	0	0
Cefovecin	D0 ($n = 24$)	5 (21)	5 (21)	0	9 (38)	3 (13)	1 (4)
	E ($n = 24$)	6 (25)	4 (17)	1 (4)	15 (63)	4 (17)	1 (4)
	M1 ($n = 19$)	5 (26)	2 (11)	2 (11)	5 (26)	3 (16)	1 (5)
	M3 ($n = 18$)	1 (6)	1 (6)	0	7 (39)	2 (11)	1 (6)
Clindamycin	D0 ($n = 29$)	0	0	0	5 (17)	0	1 (4)
	E ($n = 29$)	1 (3)	0	0	3 (10)	0	1 (4)
	M1 ($n = 25$)	1 (4)	1 (4)	0	2 (8)	0	0
	M3 ($n = 23$)	0	0	0	1 (4)	0	0
Fluoroquinolone	D0 ($n = 14$)	2 (14)	2 (14)	0	4 (29)	1 (7)	1 (7)
	E ($n = 14$)	3 (21)	2 (14)	0	3 (21)	2 (14)	1 (7)
	M1 ($n = 7$)	1 (14)	1 (14)	0	1 (14)	0	0
	M3 ($n = 8$)	1 (13)	1 (13)	0	2 (25)	1 (13)	0
Treatment overall	D0 ($n = 127$)	8 (6)	8 (6)	0	27 (21)	4 (3)	3 (2)
	E ($n = 127$)	14 (11)	10 (8)	1 (1)	49 (39)	10 (8)	5 (4)
	M1 ($n = 105$)	9 (9)	7 (7)	2 (2)	24 (23)	3 (3)	4 (4)
	M3 ($n = 98$)	3 (3)	2 (2)	1 (1)	19 (19)	3 (3)	1 (1)
Total dogs, $n = 127$		22 (17)	16 (13)	3 (2)	63 (50)	13 (10)	13 (10)

Treatment overall, all antibiotics; Total dogs, dogs with ESBL or AmpC genes during the full study period (a dog was classed as positive if at least one isolate in one sample was positive); D0, pretreatment; E, treatment end; M1, 1 month post-treatment; M3, 3 months post-treatment.
[a]Sequencing was not performed to confirm carriage of genes bla_{TEM} and bla_{OXA}.

105 dogs provided samples at 1 month post-treatment and 98 dogs provided samples at 3 months post-treatment. Information regarding sample timepoints and the reasons for missing samples are described in the Supplementary data available at *JAC* Online.

Detection and characterization of faecal E. coli

E. coli was detected in 95% (434/457) of faecal samples. 3GCR was detected in 158 samples from 60% of dogs, phenotypic ESBL-producing *E. coli* were detected in 59 samples from 31% of dogs and AmpC-producing *E. coli* were detected in 138 samples from 60% of dogs (Table S2). Table 2 shows the number and percentage of samples with at least one faecal *E. coli* with ESBL- and/or AmpC-producing genes for each timepoint/treatment group. Carriage of bla_{CIT-M} was detected in the faecal samples of 50% of dogs during the full study period; bla_{DHA-1}/bla_{DHA-2} and bla_{MOX} were detected from only one dog each in addition to bla_{CIT-M}. The most commonly detected bla_{CTX-M} genes belonged to group 1 (13% of dogs) followed by group 9 (2% dogs); bla_{CTX-M} group-2 genes were detected in *E. coli* from a single dog at 1 month post-fluoroquinolone treatment.

Sample-level prevalence of AMR

Generally there was an increased percentage of samples with MDR, ESBL- or AmpC-producing *E. coli* following treatment with cefalexin, amoxicillin/clavulanate and cefovecin and an increased percentage of samples with FQR *E. coli* following cefalexin, cefovecin and fluoroquinolone treatment. However, the percentage of samples with resistance had generally declined by 3 months post-treatment (Figure 1).

Prevalence of AMR at the isolate level

During the full study period, isolates ($n = 3897$: pretreatment $n = 1097$, immediately post-treatment $n = 1011$, 1 month post-treatment $n = 911$ and 3 months post-treatment $n = 878$) were randomly selected from non-selective agar. For all treatment groups, the percentage of isolates with resistance to each tested antimicrobial and MDR increased immediately post-treatment compared with pretreatment, but declined by 3 months post-treatment (Figure 2); of note was the increased detection of MDR and a lack of fully susceptible isolates immediately after fluoroquinolone treatment (Figure 2).

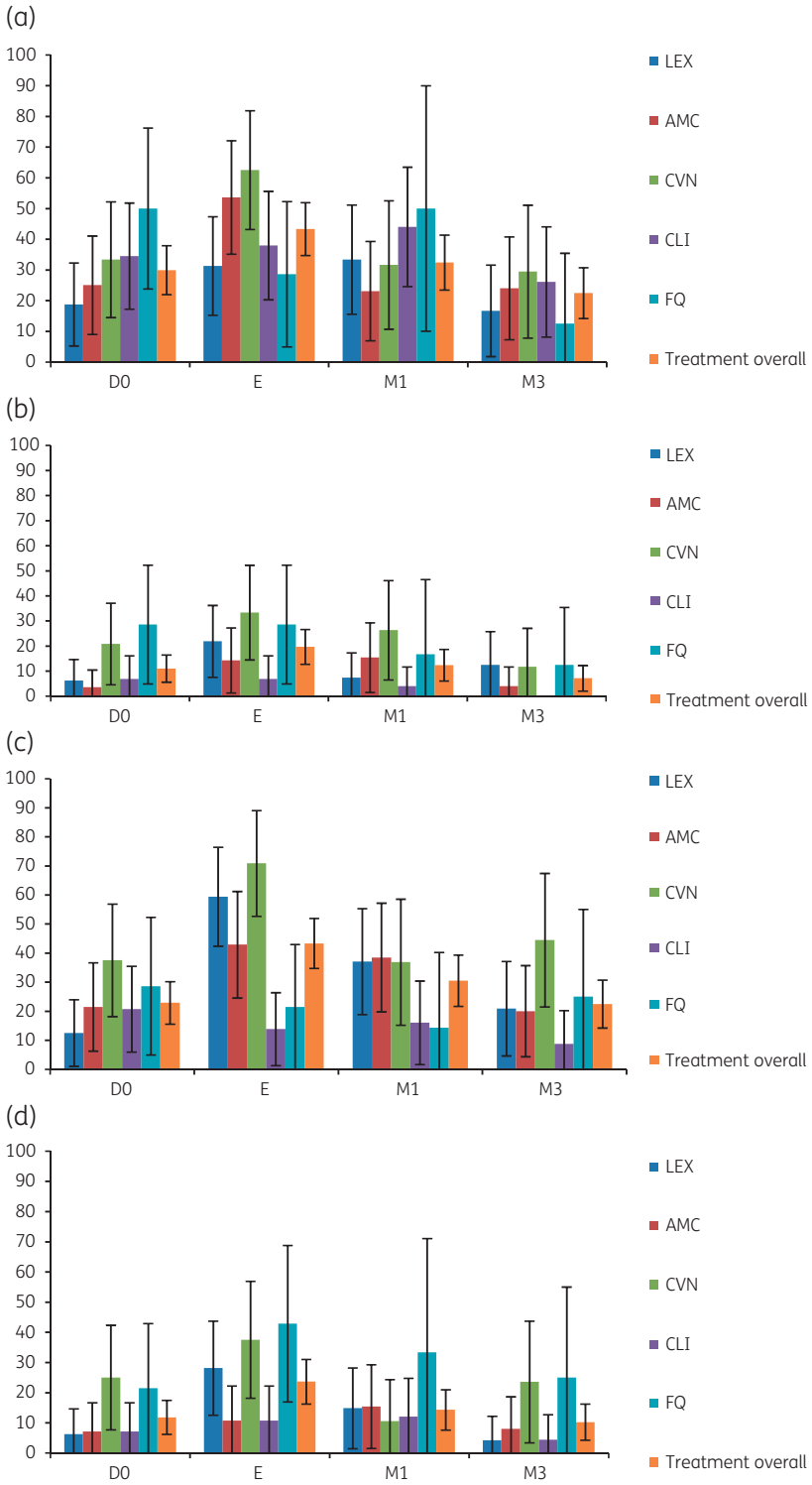

Figure 1. Percentage of samples with MDR (a), phenotypic ESBL-producing (b), fluoroquinolone-resistant (c) and Amp-C-producing (d) *E. coli* at each timepoint for each treatment group and treatment overall (95% CI). D0, pretreatment; E, treatment end; M1, 1 month post-treatment; M3, 3 months post-treatment; LEX, cefalexin; AMC, amoxicillin/clavulanate; CVN, cefovecin; CLI, clindamycin; FQ, fluoroquinolone.

Risk factors for AMR faecal E. coli

When compared with all pretreatment samples, MDR *E. coli* was significantly more likely to be detected following treatment with amoxicillin/clavulanate or cefovecin (Table 3). The risk of detecting 3GCR *E. coli* was higher following treatment with amoxicillin/clavulanate, cefalexin or cefovecin; for AmpC-producing *E. coli*,

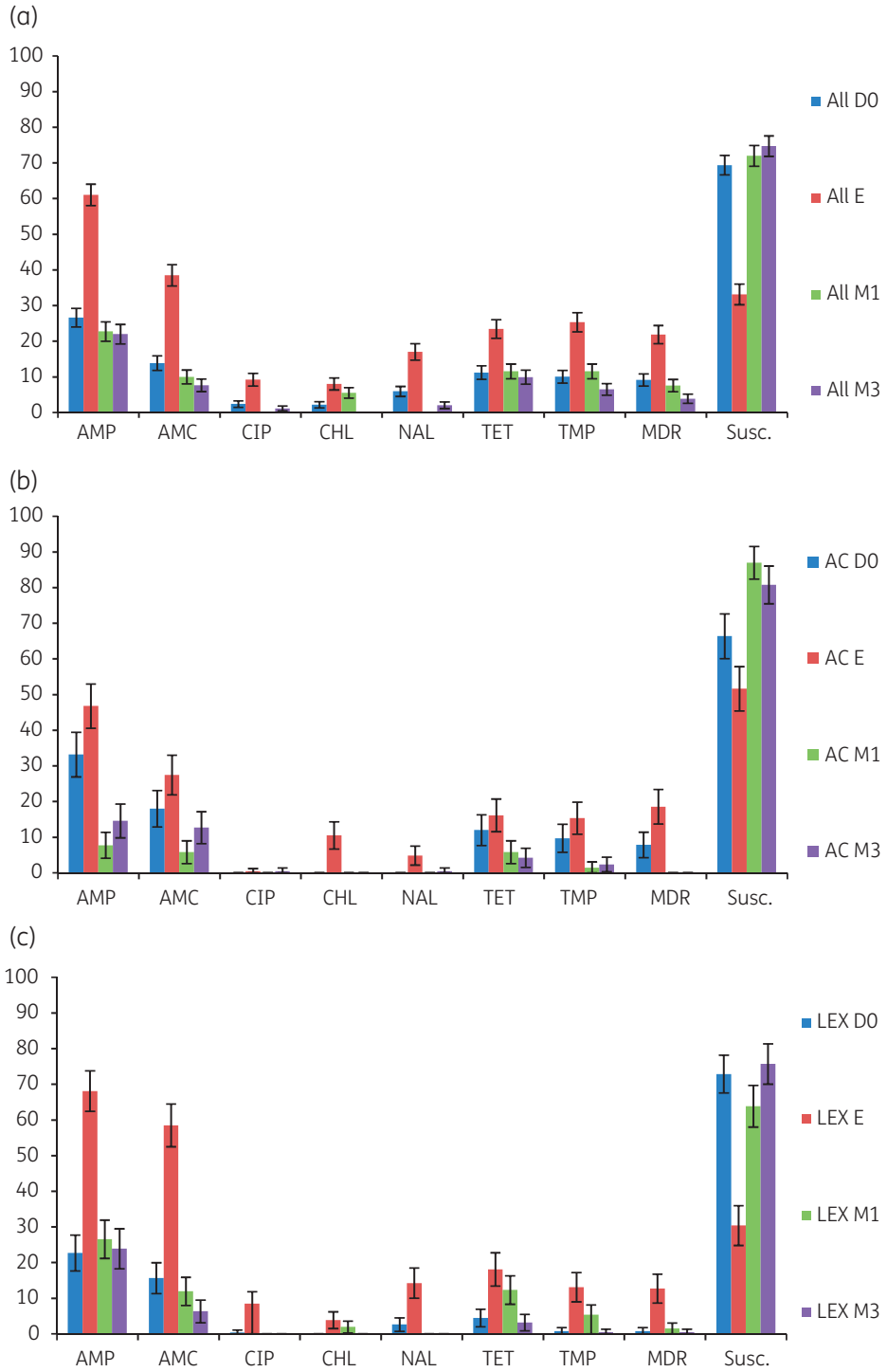

Figure 2. Percentage of isolates with resistance or susceptibility in each treatment group [overall treatment (a), amoxicillin/clavulanate treatment (b), cefalexin treatment (c), cefovecin treatment (d), clindamycin treatment (e) and fluoroquinolone treatment (f)] at each timepoint (95% CI). AMP, ampicillin; AMC, amoxicillin/clavulanate; CIP, ciprofloxacin; CHL, chloramphenicol; NAL, nalidixic acid; TET, tetracycline; TMP, trimethoprim; Susc., fully susceptible; All, all treatment groups; D0, pretreatment; E, treatment end; M1, 1 month post-treatment; M3, 3 months post-treatment; LEX, cefalexin; CVN, cefovecin; CLI, clindamycin; FQ, fluoroquinolone.

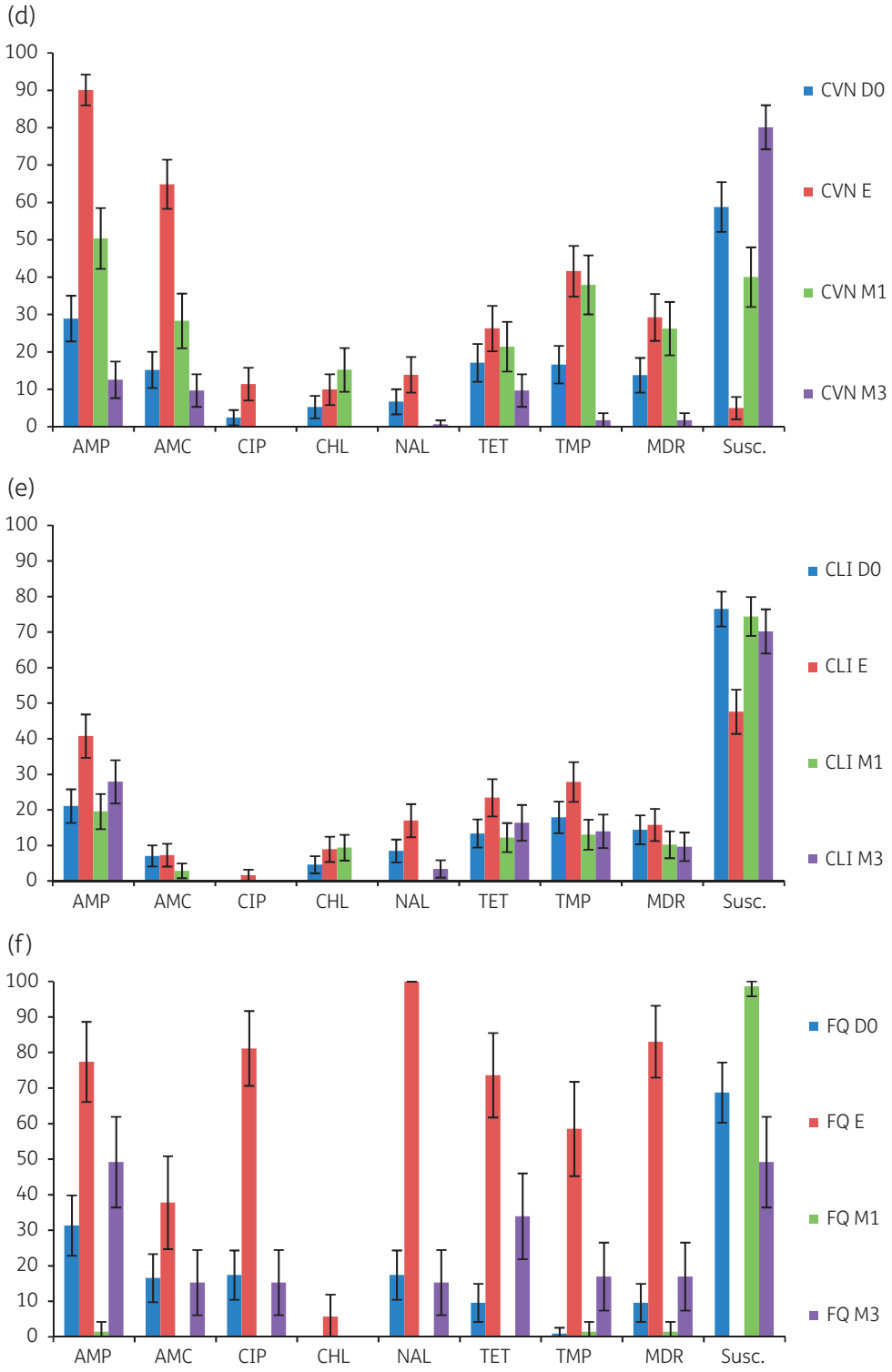

Figure 2. Continued

cefalexin or cefovecin therapies increased the risk of detection (Table 4). Finally, fluoroquinolone-resistant *E. coli* was more likely to be detected following treatment with cefalexin, a fluoroquinolone or cefovecin (Table 3). At 1 month post-amoxicillin/clavulanate, the risk of detecting 3GCR *E. coli* increased compared with pretreatment samples (Table 4); no other significant differences were detected at 1 month or 3 months post-treatment compared with pretreatment.

The final models also showed that there were positive associations between the following: living in a multi-dog household

Table 3. Multilevel multivariable results for the outcomes: FQR and MDR in 457 faecal samples from 127 dogs

	FQR			MDR		
Variable(s)	OR	95% CI	*P*	OR	95% CI	*P*
Time D0	ref.	–	–	ref.	–	–
Time E and cefalexin	**5.1**	**1.6–16.6**	**0.006**	1.7	0.6–5.0	0.4
Time E and amoxicillin/clavulanate	1.4	0.3–7.1	0.72	**5.0**	**1.5–16.0**	**0.007**
Time E and cefovecin	**7.0**	**1.9–25.5**	**0.003**	**8.0**	**2.1–30.6**	**0.002**
Time E and clindamycin	0.7	0.1–4.9	0.7	1.6	0.4–5.7	0.5
Time E and fluoroquinolone	5.6	1.2–25.7	0.03	0.8	0.2–4.0	0.8
Time M1 and cefalexin	2.02	0.5–8.8	0.35	2.1	0.7–7.0	0.2
Time M1 and amoxicillin/clavulanate	2.1	0.5–9.3	0.34	0.6	0.1–2.5	0.5
Time M1 and cefovecin	0.8	0.1–5.7	0.82	1.6	0.3–7.3	0.6
Time M1 and clindamycin	0.3	0.02–4.1	0.37	1.8	0.5–6.4	0.4
Time M1 and fluoroquinolone	2.7	0.3–22.9	0.34	1.8	0.2–14.1	0.6
Time M3 and cefalexin	0.4	0.03–5.6	0.53	0.7	0.2–2.8	0.6
Time M3 and amoxicillin/clavulanate	1.0	0.2–6.9	0.99	2.0	0.6–7.2	0.3
Time M3 and cefovecin	2.5	0.5–12.4	0.25	1.2	0.3–6.1	0.8
Time M3 and clindamycin	0.4	0.03–6.02	0.54	0.4	0.1–1.8	0.2
Time M3 and fluoroquinolone	2.9	0.4–23.0	0.32	0.3	0.02–4.5	0.4
Time treatment overall	–	–	**0.09**	–	–	**0.045**
Owner works in healthcare	–	–	–	**3.6**	**1.32–9.88**	**0.012**
Dog eats animal stools	**2.9**	**1.2–7.0**	**0.018**	–	–	–
Level 2 (dog) variance (standard error) VPC	1.6 (0.6) 32%	–	–	2.7 (0.7) 45%	–	–

D0, pretreatment; E, treatment end; M1, 1 month post-treatment; M3, 3 months post-treatment; VPC, variance partition coefficient.
P values are from the Wald χ^2 test; significant if $P < 0.05$ (bold text).

and 3GCR or ESBL-producing *E. coli*; recruitment from referral consultations and AmpC-producing *E. coli* (compared with first opinion); eating animal stools and FQR; owner working in healthcare and MDR; a 'diagnosis of pyoderma' and ESBL-producing *E. coli*; and body weight and AmpC-producing *E. coli* (dogs of small to medium weight were less likely than large dogs to have resistance).

Discussion

This study used a prospective, longitudinal design to examine the effect of different antimicrobials on the selection and carriage of AMR amongst faecal *E. coli* in a large cohort of vet-visiting dogs. Faecal samples were collected before treatment and at multiple timepoints, including 3 months, after completing therapy. Resistance to critically important antimicrobials was investigated (including third-generation cephalosporins and fluoroquinolones). Our findings suggest that single courses of systemic antimicrobials select for resistance immediately after treatment, but effects then wane. In particular, β-lactams selected for 3GCR and/or MDR, and cephalosporins and fluoroquinolones selected for FQR. This suggests that broad-spectrum antimicrobials authorized for the treatment of bacterial infections in dogs create a reservoir of AMR *E. coli* and potentially transmissible resistance genes within the canine gastrointestinal tract. Both can provide a source of environmental contamination, be transmitted to other hosts, including owners, or influence re-infection.

Selection of 3GCR including AmpC

Treatment with cefalexin and cefovecin significantly increased the risk of detecting 3GCR, in particular AmpC-producing *E. coli*. These results confirm those of Damborg *et al.*,[39] who examined cefalexin-only treatment in a small number of community dogs compared with untreated controls and also found an increase in AmpC-producing *E. coli*. Similarly, Lawrence *et al.*[20] reported increased AmpC-producing faecal *E. coli* 28 days after cefovecin-injection treatment in a small number of laboratory Beagles.

Impact of amoxicillin/clavulanate

It was surprising that amoxicillin/clavulanate (aminopenicillin plus β-lactamase inhibitor combination), the other β-lactam antimicrobial investigated in this study, did not significantly select for AmpC-producing *E. coli*, as did the cephalosporins. Treatment with amoxicillin/clavulanate is expected to select for 3GCR due to AmpC production, but not necessarily ESBL-mediated resistance. Clavulanate, a β-lactamase inhibitor, is less effective against AmpC-β-lactamases, so could select for these enzymes over other β-lactamases, including ESBL-variants.[40,41] Gibson *et al.*[16] also reported that, unexpectedly, they did not detect treatment with β-lactams or potentiated-β-lactams as risks for MDR AmpC-producing *E. coli* in hospitalized dogs; however, the findings of other studies support the selection of β-lactam resistance following treatment with amoxicillin without clavulanate.[15,18] Although the amoxicillin/clavulanate treatment group was of similar size to

Table 4. Multilevel multivariable results for 3GCR and phenotypic ESBL- or AmpC-producing *E. coli* in 457 faecal samples from 127 dogs

	3GCR			ESBL			AmpC		
Variable(s)	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Time D0	ref.	–	–	ref.	–	–	ref.	–	–
Time E and cefalexin	**8.7**	**2.9–25.9**	**<0.001**	1.6	0.5–4.8	0.42	**8.84**	**3.1–25.4**	**<0.001**
Time E and amoxicillin/clavulanate	**3.9**	**1.1–14.5**	**0.04**	1.3	0.2–7.6	0.77	**2.81**	**1.0–8.3**	**0.06**
Time E and cefovecin	**9.6**	**2.5–37.7**	**0.001**	2.5	0.8–8.3	0.12	**9.31**	**2.7–31.9**	**<0.001**
Time E and clindamycin	1.0	0.3–3.8	0.99	0.4	0.1–3.5	0.43	0.78	0.2–2.7	0.69
Time E and fluoroquinolone	0.6	0.1–3.2	0.51	2.4	0.5–11.5	0.29	0.47	0.1–2.6	0.39
Time M1 and cefalexin	2.2	0.7–6.9	0.182	0.5	0.1–2.5	0.41	2.76	1.0–8.0	0.06
Time M1 and amoxicillin/clavulanate	**5.3**	**1.5–19.7**	**0.013**	3.0	0.7–13.1	0.14	1.59	0.5–4.9	0.42
Time M1 and cefovecin	1.3	0.3–5.2	0.71	2.1	0.6–7.7	0.28	1.85	0.6–6.1	0.31
Time M1 and clindamycin	0.7	0.2–3.1	0.63	0.6	0.1–4.6	0.59	1.04	0.3–3.7	0.96
Time M1 and fluoroquinolone	0.7	0.1–5.3	0.69	0.9	0.1–10.4	0.91	0.42	0.03–5.8	0.52
Time M3 and cefalexin	0.8	0.2–2.6	0.57	0.9	0.2–3.8	0.91	0.62	0.2–2.3	0.47
Time M3 and amoxicillin/clavulanate	1.6	0.4–6.3	0.54	0.7	0.1–6.5	0.73	0.42	0.1–1.7	0.23
Time M3 and cefovecin	2.9	0.7–11.8	0.14	0.8	0.1–4.4	0.76	1.83	0.5–6.2	0.33
Time M3 and clindamycin	0.5	0.1–2.8	0.44	0.7	0.1–5.2	0.69	0.28	0.04–1.9	0.19
Time M3 and fluoroquinolone	0.8	0.1–6.6	0.87	1.1	0.1–11.2	0.92	0.96	0.1–7.2	0.97
Time treatment overall	–	–	**<0.001**	–	–	0.77	–	–	**<0.001**
Weight (large)	ref.	–	–	ref.	–	–	ref.	–	–
Weight (small)	–	–	–	–	–	–	0.5	0.2–1.4	0.17
Weight (medium)	–	–	–	–	–	–	**0.1**	**0.03–0.5**	**0.004**
Weight overall	–	–	–	–	–	–	–	–	**0.009**
Diagnosis of pyoderma	2.3	0.8–6.6	0.11	**3.63**	**1.18–11.13**	**0.024**	–	–	–
First opinion	RE	–	–	ref.	–	–	ref.	–	–
Referral consultation	2.1	0.9–5.2	0.11	–	–	–	**2.2**	**1.1–4.6**	**0.035**
Multi-dog household	**3.8**	**1.6–8.7**	**0.002**	**2.71**	**1.24–5.93**	**0.012**	–	–	–
Level 2 (dog) variance (standard error) VPC	2.4 (0.6) 43%	–	–	1.190 (0.5) 27%	–	–	1.8 (0.5) 35%	–	–

D0, pretreatment; E, treatment end; M1, 1 month post-treatment; M3, 3 months post-treatment; VPC, variance partition coefficient; ref., reference category.
P values are from the Wald χ^2 test; significant if $P < 0.05$ (bold text).

other treatment groups, apart from the fluoroquinolones, we cannot exclude a sample-size effect and larger studies are required to investigate these findings further; other factors, such as differing antimicrobial excretion and concentration within the intestinal tract, should be investigated.

Selection for ESBL, MDR and FQR

The detection of ESBL-producing *E. coli* was not associated with use of any antimicrobials administered in this study, as previously reported.[14,42,43] The percentage of samples positive for ESBL-producing *E. coli* was lower overall than other resistance outcomes. This may have reduced the power to detect significant associations, particularly as cefalexin and cefovecin were found to be a risk for 3GCR, an outcome including phenotypic ESBL- and AmpC-producing *E. coli*. Furthermore, previous studies in both healthy and hospitalized dogs[23,24,44] have reported a higher prevalence of canine faecal AmpC-producing compared with ESBL-producing *E. coli*.

In this study, the administration of both amoxicillin/clavulanate and cefovecin increased the risk of detecting MDR *E. coli* post-treatment. These results uphold previous work using a small number of dogs, where selection for MDR faecal *E. coli* followed treatment with ampicillin, amoxicillin, enrofloxacin or cefovecin;[15,18,20,45] retrospective risk analysis also identified cefalexin as a risk for MDR *E. coli* rectal carriage during hospitalization.[16] Cefovecin was also a risk for the detection of AmpC-producing *E. coli*, which are often MDR.[40]

The use of cephalosporins and fluoroquinolone antimicrobials increased the risk of detecting FQR post-treatment in this study. This upholds results from Boothe and Debavalya[18] (selection of MDR FQR *E. coli* following treatment with enrofloxacin in two laboratory dogs) and Lawrence *et al.*[20] (increased detection of enrofloxacin-resistant faecal *E. coli* after administration of cefovecin). As *E. coli* strains with high-level FQR are commonly resistant to cephalosporins,[46] this suggests co-selection of resistance.[2] However, we did not find an association between fluoroquinolone therapy and MDR or 3GCR at treatment end, possibly due to a small sample size in this treatment group.

Antimicrobial exposure recovery period

Chronic antimicrobial therapy likely maintains MDR amongst canine commensal *E. coli*.[47] Our study aimed to report AMR prevalence and risk factors at timepoints post-treatment, but in the absence of repeated antimicrobial prescriptions. We found that after amoxicillin/clavulanate treatment the risk of detecting 3GCR *E. coli* increased and remained higher at 1 month post-treatment, but at 3 months it had returned to pretreatment levels. The length of time for which significantly different levels of resistance were evident is longer than that suggested in previous work examining amoxicillin without clavulanate; Gronvold *et al.*[15] and Boothe and Debavalya[18] showed recovery at 2 weeks post-treatment. This may mean that whilst amoxicillin/clavulanate may have less overall impact on the selection for 3GCR than cephalosporins, the treatment effects are longer lasting. Further larger studies are required to corroborate and investigate the basis of these findings. For example, amoxicillin/clavulanate may have differing effects on other members of the microbiome (such as anaerobic bacteria) compared with cephalosporins, where higher-generation drugs have increasing activity against Gram-negative bacteria. For other treatment groups (cefalexin, cefovecin, clindamycin and fluoroquinolones) there was no association with resistance at 1 month post-treatment. Previous work, however, identified resistance at 21 days and between 17 and 37 days after enrofloxacin (Boothe and Debavalya[18] and Trott *et al.*,[13] respectively) and resistance at day 28 after cefovecin.[20]

Length of antimicrobial treatment

The length of antimicrobial treatment has been investigated particularly for human patients and shorter courses have been shown to reduce antimicrobial use, costs, adverse events and exposure to commensal organisms (and thereby AMR selection), without increasing morbidity or mortality.[48] The results of this study, however, did not suggest an association between treatment length and resistance, particularly given the shorter treatment length for amoxicillin/clavulanate; however, sample sizes were small in some groups, reducing the likelihood of detecting associations between variables. Overall, selection and persistence of resistance within the gastrointestinal tract is likely to be influenced by multiple factors, including antimicrobial class (broad or narrow spectrum), resistance type (MDR or not) and mechanism of resistance (transmissible or chromosomal),[18] pharmacokinetics/pharmacodynamics, the level of resistance present before therapy[7] and bacterial virulence/fitness.[49]

Magnitude of resistance

Quantifying resistance (assessing the number of isolates with an AMR trait) would be a better measure to detect changes over time than analysis at the sample level (sample classed as AMR if at least one isolate is AMR), particularly where there is high pretreatment AMR prevalence. High pretreatment prevalence can make it difficult to detect change following therapy, increase the risk of AMR in the following sample and influence recovery time. This study limitation was offset by selecting 10 random isolates from non-selective agar for each sample for analysis at the isolate level for each timepoint/treatment group. At the sample level, this study did not identify an association between cefalexin or fluoroquinolone therapy with MDR *E. coli*; however, there was an increase immediately at treatment end compared with pretreatment when examined at the isolate level; this concurs with our expectations and the findings of previous authors.[13,18,20,45] Concordantly, also at the isolate level, fluoroquinolone and cefovecin therapy appeared to have the most effect on fully susceptible *E. coli*; this was also noted at the sample level, where *E. coli* were not detected in over one-third of dogs directly after fluoroquinolone treatment. Both Lawrence *et al.*[20] and Trott *et al.*[13] reported significant inhibition of faecal *E. coli* and/or coliforms during and beyond treatment with cefovecin and enrofloxacin. Inhibition of susceptible isolates may create a vacant niche in the gastrointestinal tract for colonization with resistant or pathogenic bacteria.

Study implications

Antimicrobial therapy selects for MDR *E. coli* within the gastrointestinal tract of humans and dogs. These bacteria may be then shared between hosts (including between humans or between pets and between humans and pets) within households[4,5] and healthcare settings; carriage isolates may cause extra-intestinal infections.[16,50] Antimicrobial therapy is paramount to the successful treatment of many patients. Implementation of veterinary hospital prescribing guidelines can reduce overall use and misuse of important antimicrobials,[51] reducing selection pressure for AMR bacteria. This study provides important information on both the effect and the timescale of the effect following routine antimicrobial therapy in dogs. This information can be used to design biosecurity guidelines that limit transfer of such bacteria to in-contact individuals or to the environment, including barrier nursing,[52,53] appropriate disposal of dog waste[6] and strict hand hygiene.[54,55]

Conclusions

Antimicrobials impact not just the pathogens they are designed to target, but also the commensal microbiota. Our results suggest that treatment with many commonly used systemic antimicrobials (particularly β-lactams and fluoroquinolones) affects the commensal faecal flora of dogs, causing a shift towards a more resistant bacterial population of *E. coli*. There is a window of up to 1 month following the end of therapy when treated dogs are more likely to carry AMR faecal *E. coli*. Proactive strategies such as prudent antimicrobial prescribing and hospital biosecurity programmes are urgently needed to limit development and dissemination of AMR. In particular, policies for antimicrobial use during specific clinical conditions, alongside utilization of culture and susceptibility testing, could help reduce misuse and overuse of important antimicrobials. Full genome sequencing, e.g. deep sequencing of shotgun metagenomics of the microbiome, could help to elucidate the overall impact of therapy with different antimicrobials.

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Author contributions

Conceived and designed the experiments: V. M. S., G. P., T. N., N. M., S. D. and N. J. W. Collected samples: V. M. S., T. N. and N. M. Performed the experiments: V. M. S. Collated, analysed and interpreted the data. V. M. S., G. P. and N. J. W. Drafted and reviewed the manuscript: V. M. S., G. P., K. M. M. and N. J. W. All authors read and approved the final manuscript.

Supplementary data

Supplementary data (including Tables S1 and S2) are available as Supplementary data at *JAC* Online.

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