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Carriage and population genetics of extended spectrum β-lactamase-producing *Escherichia coli* in cats and dogs in New Zealand

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ABSTRACT

The incidence of infections with extended spectrum β-lactamase producing *Escherichia coli* (ESBL-E) is increasing both in humans and animals. There is a paucity of data about the rate of faecal carriage of ESBL-E in pets.

In this study, faecal swabs collected from 586 pets (225 cats; 361 dogs) in Auckland, New Zealand, were analysed for the presence of ESBL-E by culture, and a questionnaire was delivered to the owners. The ESBL-E were characterised and data elicited by the questionnaires were used for a multivariable analysis, to investigate the factors associated with faecal ESBL-E carriage.

The prevalence of ESBL-E in faecal swabs was 6.4%. The β-lactamase genes detected in the ESBL-E were the blaCTX-M-14 (n = 2) and blaCMY-2 (n = 34). Several isolates displayed multilocus sequence types (ST) associated with human and animal infections. Multiple isolates sharing the same ST displayed different antibiograms and β-lactamase genes, reflecting horizontal gene transfer between and within ST. Variables independently associated with increased odds of ESBL-E carriage were: animal received systemic antimicrobial treatment in the six months before the sampling; presence of household members working in veterinary clinics; presence of household members travelling overseas in the six months before the sampling.

We conclude that pets are colonised by ESBL-E which are genotypically similar to the bacteria found to infect humans and animals. The statistical analysis suggested a number of eco-epidemiological factors associated with ESBL-E carriage. In particular, they suggest veterinary clinics may represent hot-spots of antimicrobial resistance.

1. Introduction

The emergence of antimicrobial resistance in bacteria of clinical relevance represents a serious global problem, and the factors that accelerate this process are still poorly understood. Pets are integral parts of modern households and share commensal bacteria with humans (Carvalho et al., 2016; Grinberg et al., 2017). Hence, the selective pressures promoting the colonisation with antimicrobial resistant bacteria are similar in humans and pets, and pets may be considered sentinels of resistance in the human eco-system.

The Gram-negative intestinal commensal *Escherichia coli* is commonly isolated from a broad range of infection sites in humans and animals (Rogers et al., 2011; Rzewuska et al., 2015; Yamamoto et al., 2014). Infections with extended-spectrum β-lactamase-producing and AmpC-producing *E. coli* strains (ESBL-E; AmpC-E) are increasingly associated with overt infections in humans and companion animals in New Zealand, and elsewhere (Drinkovic et al., 2015; Karkaba et al., 2017; Nebbia et al., 2014; Sauget et al., 2016). Infections with ESBL-E and AmpC-E are particularly problematic as these strains produce β-lactamases that confer resistance to a wide spectrum of β-lactams. Moreover, ESBL-E and AmpC-E are also more likely to be multi-drug resistant (MDR) than non-ESBL-E/AmpC-E (Jacoby, 2005), further complicating infection management.

In humans, ESBL-E/AmpC-E strains carried in the intestinal tract can cause ascending urinary tract auto-infections (Gopinath et al., 2014; Yamamoto et al., 1997). As *E. coli* strains are transmissible between people and pets in the household, and clones causing urinary tract infections in people have been identified also in the faeces of their pets (Johnson et al., 2008), there are concerns for potential zoonotic transmission of ESBL-E/AmpC-E strains from pets (Boerlin and Reid-

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Smith, 2008; Szmolka and Nagy, 2013).

ESBL-E/AmpC-E have been identified in the faeces of healthy cats and dogs in a number of studies, with subclinical carriage prevalences ranging between 1.3% and 5% (Ho et al., 2011). However, except one study (Gandolfi-Decristophoris et al., 2013), the factors associated with faecal colonisation with ESBL-E/AmpC-E in pets have not been adequately studied in robust epidemiological settings.

The aims of this study were to: 1) estimate the prevalence of faecal carriage of ESBL-E/AmpC-E in cats and dogs in Auckland, New Zealand; 2) determine factors statistically associated with the carriage of these bacteria by cats and dogs using multivariable analysis; and 3) describe aspects of population genetic structure of faecal ESBL-E/AmpC-E.

2. Materials and methods

2.1. Sampling strategy and collection of faecal samples and metadata

The target population was that of cats and dogs in greater Auckland, and the sample was obtained from a study population composed of cats and dogs visiting veterinary clinics in greater Auckland between June 2012 and June 2013. A census from 2006 indicated that around 68% of New Zealand households owned at least one pet. Hence, with 437,985 households in Auckland, the number of households with pets was estimated at 298,000 (Anonymous, 2011).

The veterinary clinics were recruited using a list of 131 clinics in greater Auckland provided by the Veterinary Council of New Zealand (http://www.vetcouncil.org.nz; accessed June 2012). Initially, emails were sent to all the clinics, and clinics that did not respond to this call were contacted again by phone or additional emails. Clinics expressing interest in participation were provided with a self-contained kit for sample collection and transport medium (Transystem®, Copan, Brescia, Italy). Cats and dogs were sampled by clinic personnel, by collecting rectal swabs during the consultation. In order to minimise selection bias, clinics were instructed to sample the first five animals presenting during the course of a normal working day, over a period of 2–3 weeks. Only one animal per owner was sampled, and the decision of which animal to sample was made by the sampling personnel. Owners must have been 18 years of age or older and signed a consent form. To enable the determination of factors associated with ESBL-E/AmpC-E carriage, owners were asked to complete a questionnaire during the visit. Swabs and completed questionnaires were sent to Massey University during the weekdays by overnight courier delivery, or held in the clinic fridge during weekends and sent the following Monday. Approval to collect samples and deliver questionnaire was granted by the Massey University human and animal ethics committees (protocols HEC 12/28 and MUAEC 12/46).

2.2. Laboratory isolation and characterisation of ESBL-E /AmpC-E

Culture for ESBL-E/AmpC-E from cat and dog faeces was performed using initial liquid enrichment, followed by a selection step on agarized media using antimicrobial-impregnated discs (modified from Bartoloni et al., 2006). In a preliminary trial performed in our laboratory, this method yielded more ESBL-E/AmpC-E-positive faecal samples than four other methods used in parallel (the results of the trial are available at the Massey University repository, http://hdl.handle.net/10179/12856).

Briefly, the swabs were immersed in buffered peptone water (BPW) and incubated overnight at 37 °C in aerobic conditions. In order to select for antimicrobial-resistant Enterobacteriaceae, the resulting bacterial suspension was diluted in normal saline to a turbidity equivalent to the 0.5 McFarland standard and a new swab was immersed and used to spread the bacteria onto the whole surface of two MacConkey agar plates (Fort Richards, Auckland, New Zealand). Within 15 min of inoculation, eight antimicrobial-impregnated discs were deposited on the plates' surfaces (four discs on each plate) and the plates were incubated as above. Discs used were cephalothin (30 µg); amoxicillin-clavulanic acid (30 µg); cefotaxime (30 µg); cefovecin (30 µg); and tetracycline (30 µg); gentamicin (10 µg); trimethoprim/sulphamethoxazole (25 µg); and enrofloxacin (5 µg) (Oxoid, Basingstoke, UK). Bacteria growing within the inhibitory zones for resistant Enterobacteriaceae in humans established by the most recent edition of Clinical Laboratory Standard Institute guidelines (CLSI, 2013) were assumed to be resistant Enterobacteriacea, while the diameter for cefovecin was provided by Zoetis, USA. A combined loopful growth from each sample showing bacterial growth around 2 or more β-lactam antimicrobial discs, which zone diameters suggested the presence of resistant organisms, was obtained from resistance zones, subcultured onto a selective-differential medium for *E. coli* (ECC CHROMagar®; Fort Richard Laboratories, Auckland) to obtain pure colonies, and incubated as above. A single presumptive *E. coli* colony was subcultured onto nutrient agar and these isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF, MYLA Version 3.2.0_5. Sys. Compliance: BioMérieux VITEK MS1.1.0; at Middlemore Hospital Laboratory, Auckland New Zealand). Subsequently, the susceptibility of each *E. coli* isolate to 12 antimicrobials was determined using the disc diffusion test (CLSI, 2013). Antimicrobials tested were: cephalothin (30 µg); amoxicillin-clavulanic acid (30 µg); cefoxitin (30 µg); cefotaxime (30 µg); ceftazidime (30 µg); imipenem (10 µg); tetracycline (30 µg); gentamicin (10 µg); trimethoprim/sulphamethoxazole (25 µg); enrofloxacin (5 µg) and aztreonam (30 µg) (Oxoid, Basingstoke, UK). The phenotypic detection of ESBL and AmpC production by the *E. coli* was tested according to the CLSI guidelines, and described in details (Karkaba et al., 2017). The ESBL genes, the plasmid-mediated AmpC β-lactamase genes, the chromosomal mutations in the *ampC* gene, and the multilocus sequence types (ST) of the ESBL-E/AmpC-E were determined by means of PCR-sequencing, as previously described (Karkaba et al., 2017).

2.3. Data analysis

Data elicited by the questionnaires were coded into dichotomous or categorical variables, except the age of the people living in the household which was fitted as a continuous variable (years and fractions of years). Statistical analyses were performed using the R statistical software (R version 3.0.3 2014©, The R Foundation for Statistical Computing Platform, Vienna, Austria).

Data from cats and dogs were initially analysed together after dropping host-specific variables such as breed. Subsequently, data on cats and dogs were analysed separately, mantaining the host-specific variables. Statistical modelling started with initial screening for associations between single variables and an outcome binary variable: presence/absence of ESBL-E and/or AmpC-E phenotypes in the sample. Variables resulting in p-values < 0.25 were included as explanatory variables (fixed effects) in multivariable logistic regression models, with clustering of animals within the clinics accounted for using a second level random intercept, as following:

$$Y_i = \beta_0 + \beta_1 x_{1j} + ... + \beta_n x_{nj} + u_{clinic(i)} + \varepsilon_i$$

where: Y = the outcome variable; β = *regression coefficient* (β_0 = intercept; $\beta_{1...n}$ = variable coefficients); x_{1i}, ..., x_{ki} = explanatory variables; u_i = random effect of clinic (which contains the j^{th} animal), with a normal distribution of a mean of zero and a variance of $\pi^2/3$; ε_i = residual, defined as ~ *Normal (0, σ^2)* and σ^2 = variance of residuals.

Models were built manually using backward stepwise elimination. The process progressed by elimination of the variable with the highest p-value, re-introduction of the previously eliminated variable and selection of the model between the two with the smallest Akaike Information Criterion score (Müller et al., 2013), until only variables with $p < 0.05$ remained. The Wald test (function "waldtest" in 'aod' package; Lesnoff and Lancelot, 2012) was used to assess model fitness in the last iterations. The random effects of clinics in the final model

Table 1
Univariate screening of the variables elicited by the questionnaire against the binary outcome of faecal carriage (yes/no) of extended spectrum β-lactamase (ESBL) and/or AmpC-β-lactamase-producing *E. coli*. In variables with Yes/No response, "No" was the reference category.

Variable	Categories	Odds Ratio (95% Confidence Interval), P-value		
		Dogs (n = 352)	Cats (n = 225)	Cats and dogs (n = 572)
Animal-level variables				
Species	Dogs	Reference Category		
	Cats			0.7 (0.3–1.4), 0.3
Cat Breeds	Domestic Short Hair		Reference Category	
	Pure Breed		0.7 (0.16-3.1), 0.6	
	Other + cross-breed		0.9 (0.16-5), 0.9	
Sex (1)	Male	Reference Category		
	Female	1.5 (0.6–3.4), 0.4	0.7 (0.2–2.3), 0.5	1.2 (0.6–2.3), 0.6
Sex (2)	Male neutered	Reference Category		
	Female neutered	1 (0.02–0.1), 0.9	0.5 (0.1–2.5), 0.4	0.8 (0.3–1.8), 0.6
	Male entire	0.9 (0.09-8.2), 0.8	1	0.8 (0.09-6.9), 0.6
	Female entire	5.4 (1.47–20.4), 0.02	4.9 (0.3–7.7), 0.2	6.2 (1.9–20.3), 0.002
Age of animal	0.08-1 year	Reference Category		
	> 1-10 years	0.3 (0.09–0.9), 0.03	0.5 (0.08–3.5), 0.52	0.3 (0.15–0.9), 0.04
	> 10 years	0.8 (0.2–2.7), 0.7	1.8 (0.3–10.9), 0.45	0.9 (0.3–2.5), 0.89
Other cat/dog in the same household in the last six months	Yes/No	0.49 (0.2–1.4), 0.1	0.6 (0.1–3.6), 0.6	0.5 (0.2–1.2), 0.1
Household member information				
Adults > 16yrs (not including owner) living in the household	Yes/No	1.4 (0.2–10.7), 0.8	0.46 (0.04–4.5), 0.5	1.3 (0.2–10.7), 0.8
Total number of adults living in the household	0	Reference Category		
	1	0.99	0.99	0.99
	2	0.99	0.99	0.99
	3	0.99	0.99	0.99
	> 3	0.99	0.99	0.99
Children living in the household < 16yrs of age?	Yes/No	1.7 (0.7–4.1), 0.2	2.1 (0.5–8.4), 0.3	1.6 (0.8–3.4), 0.1
Number of children living in the household	0	Reference Category		
	1	2.5 (0.9–6.6), 0.06	1.9 (0.4–8.9), 0.4	2.1 (0.9–4.6), 0.07
	> 1	0.92 (0.2–3.4), 0.9	2.7 (0.4–19.5), 0.3	1.2 (0.4–3.9), 0.7
Pets living in the same household				
Other cats in the household?	Yes/No	1.7 (0.7–4.5), 0.2	1.3 (0.26–6.1), 0.7	1.5 (0.7–3.2), 0.3
Total number of cats living in the household	0	Reference Category		
	1	0.4 (0.1–1.7), 0.3	1.2 (0.26–5.3), 0.8	0.7 (0.3–1.7), 0.4
	> 1	1.7 (0.6–4.3), 0.3	1.3 (0.29–5.9), 0.7	1.4 (0.7–3.2), 0.3
		Reference Category		
Other dogs in the household?	Yes/No	0.7 (0.2–2.7), 0.6	1.2 (0.3–6.4), 0.8	0.9 (0.3–2.6), 0.9
Number of dogs living in the household	0	Reference Category		
	1	0.9 (0.4–2.5), 0.9	0.9 (0.2–5), 0.9	1.3 (0.4–2.3), 0.9
	> 1	0.7 (0.2–2.7), 0.6	1.5 (0.3–8), 0.6	0.9 (0.3–2.7), 0.9
Other animal species in the household?	Yes/No	1	1	1
Antimicrobial Treatment/ Hospitalisation/ Surgery of the animal (in the last six months)				
Had antimicrobial treatment in the last 6 months	Yes/No	3.4 (1.4–8.4), 0.01	6.2 (1.5–22.6), 0.01	3.8 (1.8–7.7), 0.0001
Had topical antimicrobial treatment in the last 6 months	Yes/No	1.6 (0.5–5.2), 0.4	2.2 (0.2–22), 0.5	1.8 (0.6–5), 0.3
Had oral tablets/powder/liquid in the last 6 months	Yes/No	2.9 (1.2–6.8), 0.02	1.9 (0.4–9.9), 0.4	2.5 (1.3–5.3), 0.01
Had an injectable antimicrobial in the last 6 months	Yes/No	2.9 (1.1–7.7), 0.02	5.6 (1.5–21.5), 0.01	3.5 (1.6–7.5), 0.001
Animal had systemic antimicrobial treatment (oral/injection) in the last 6 months	None	Reference Category		
	Systemic treatment	3.3 (1.4–7.9), 0.005	4.5 (1.2–15.8), 0.02	3.5 (1.7–7), 0.0004
Hospitalised in the last 6 months	Yes/No	0.8 (0.09–6.8), 0.8	5 (0.7–34.4), 0.09	1.5 (0.4–5.4), 0.5
Had surgery in the last 6 months	Yes/No	1.7 (0.6–4.7), 0.3	1.7 (0.6–4.7), 0.9	1.5 (0.4–2.7), 0.9
Was hospitalised and/or had surgery in the last 6 months	Yes/No	1.5 (0.5–4.1), 0.4	1.3 (0.2–7.2), 0.7	1.3 (0.5–3.2), 0.5
Information on other animals living in the household				
Joined the household in the last 6 months?	Yes/No	2.5 (0.8–7.7), 0.1	1.5 (0.15–13.8), 0.7	2.3 (0.8–6.3), 0.08
Had oral antimicrobial treatment in the last 6 months	Yes/No	2.2 (0.8–6.3), 0.1	6.3 (1.4–30.8), 0.02	2.7 (1.2–6.3), 0.02
Was hospitalised for over 24 hours or/and had surgery in the last 6 months?	Yes/No	1.6 (0.4–5.3), 0.4	6 (1.2–29), 0.02	2.4 (0.9–6.1), 0.06
Information on people living in the household				
Household member works in healthcare industry	Yes/No	1.5 (0.6–4.1), 0.3	1.3 (0.3–5.7), 0.6	1.4 (0.7–3.3), 0.3
Household member works in a veterinary clinic	Yes/No	1.5 (0.4–5), 0.5	6.5 (1.2–30.4), 0.02	2.5 (0.9–6.5), 0.05
Household member works in human medical clinic	Yes/No	0.7 (0.15–3.3), 0.6	0.6 (0.07–5.7), 0.7	0.6 (0.2–2.4), 0.5
Household member had antimicrobial treatment in the last six months	Yes/No	0.8 (0.4–2.1), 0.7	1.3 (0.4–4.9), 0.6	1 (0.5–2), 0.9
Household member hospitalised > 24 hrs in the last six months	Yes/No	OR: 0.9999	OR: 0.9999	OR: 0.9999
Household member had surgery in the last six months	Yes/No	0.49 (0.1–2.3), 0.3	OR: 0.9999	0.43 (0.09–1.8), 0.2
Household member travelled overseas in the last six months	Yes/No	2.1 (0.9–5.1), 0.08	1.6 (0.4-5.5), 0.4	1.8 (0.9–3.8), 0.08
Number of countries visited	None	Reference Category		
	1	1.4 (0.02 – 4.5), 0.4	0.6 (0.6-9), 0.2	1.6 (0.7–3.8), 0.2
	> 1	2 (0.7–6), 0.1	OR: 0.9999	1.5 (0.5–3.9), 0.4

were compared using caterpillar plots, using the function "ranef" in the 'lattice' package of R (Sarkar, 2008). The biologically relevant variables of age, sex and species were included in all the models, regardless of their p-value. The variables elicited by the questionnaire and used for the statistical analysis are described in Table 1.

3. Results

A total of 586 animals attending 29 clinics were recruited (cats = 225, 39%; dogs = 361, 61%), corresponding to ~0.2% of the estimated population of cats and dogs in Auckland. There were 572/586 completed questionnaires from 28 clinics (dogs: 351; cats: 221), with a range of 1–31 and a median of 16 questionnaires per clinic.

3.1. Prevalence of ESBL-E/AmpC-E and occurrence co-resistance and multidrug resistance

A total of 38/586 (6.4%) swabs yielded ESBL-E and/ or AmpC-E (plasmid-mediated or chromosomal *ampC* mutations) by culture (cats: 12/225; dogs: 26/361; two-tailed Fisher's exact p = 0.12). Thirty-six out of 38 ESBL-E/AmpC-E were AmpC producers, one produced both AmpC and ESBL, and one was an ESBL-producer (Table 1). Twelve ESBL-E/AmpC-E (32%) were resistant only to β-lactam antimicrobials, 19 (50%) to one additional antimicrobial family, and 7 (18%) were MDR (i.e., displayed resistance to at least one compound from each of three or more antimicrobial families; Magiorakos et al., 2012). The highest co-resistance was observed to tetracycline (n = 25; 65%) and enrofloxacin (n = 10; 26%). None of the ESBL-E/AmpC-E isolates was resistant to imipenem or gentamicin (Supplementary Table 1).

3.2. ESBL and AmpC genes

The plasmid-mediated $bla_{CTX\text{-}M\text{-}14}$ gene was detected in two ESBL-E and the plasmid-mediated $bla_{CMY\text{-}2}$ gene was detected in 34/37 AmpC-E. The remaining three isolates displaying the AmpC phenotype were PCR-negative for the AmpC-β-lactamase genes tested. To assess a chromosomal origin of the AmpC-E phenotype, the three PCR-negative isolates were tested using a PCR for the detection of chromosomal mutations within the 191 base-pair region of the promoter and attenuator regions of the *ampC* gene (Caroff et al., 1999, 2000), and these mutations were detected in all three isolates.

3.3. Multilocus sequence typing results

The 38 ESBL-E/AmpC-E isolates belonged to 27 STs, of which nine belonged to clonal complexes (CCs) previously reported in the Warwick database (https://enterobase.warwick.ac.uk/species/ecoli/ allele_st_search). The remaining STs were assigned as singletons (clonal complex 'none') by the Warwick database, of which six were novel (i.e., not previously reported in the database). These novel STs were designated as ST4390, ST4391, ST4392, ST4393, ST4395, and ST4406 by the database curators. Details on the 38 ESBL-E/AmpC-E, including their antimicrobial susceptibility profiles, β-lactamase enzymes and STs are reported in Table 2 and Supplementary Table 1.

3.4. Statistical analysis results

Initial screening identified a number of variables associated at p < 0.25 with the presence of ESBL-E/AmpC-E in the sample (Table 1), and these were used for multivariable logistic regression modelling. To avoid correlated variables in the same model, a single variable on previous antimicrobial treatment: 'had systemic antimicrobial treatment (oral/injection) in the last 6 months' (binary variable - yes/no) was produced and included in the models.

The variables remaining significant (p < 0.05) in the final model combining cats and dogs were: 'had systemic antimicrobial treatment (oral/injection) in the last 6 months' (yes versus no: adjusted OR = 1.5, 95% CI 1.2–1.9, p = 0.001); presence of a household member working in a veterinary clinic (yes versus no: adjusted OR = 2.87, 95% CI: 1.1–7.6, p = 0.03); presence of a household member travelling overseas in the last 6 months (yes versus no, adjusted OR = 2.3, 95% CI: 1.1–4.8, p = 0.02) (Table 3). The random effects of the clinics ranged from -0.56 to 0.71, except for two clinics that were outliers. Further analysis indicated these clinics contributed more ESBL-E/AmpC-E isolates than any other clinics (7/38 and 6/38 ESBL-E/AmpC-E, respectively). The multivariable logistic regression modelling was repeated excluding these two clinics, and the statistical analysis remained unchanged.

The variables remaining significant (p < 0.05) in the final model in cats were: 'animal hospitalised and/or had surgery in the last 6 months' (yes versus no: adjusted OR = 8.7; 95% CI:1.3–55, p = 0.02); and presence of a household member working in a veterinary clinic (Table 3) (a number of biologically meaningful interaction terms were fitted iteratively in the final model, but only the interaction between age and antimicrobial treatment was significant in cats adjusted OR = 1.1; 95% CI: 1.02–1.2; p < 0.01).

The variables remaining significant (p < 0.05) in the final model in dogs were 'sex' (female entire versus male neutered, OR = 6.5, 95% CI:1.7–25.2, p = 0.009); 'had systemic antimicrobial treatment (oral/injection) in the last 6 months' (versus untreated: OR = 1.5, 95% CI: 1.1–2.2, p = 0.03), 'household member travelling overseas in the last 6 months' (yes versus no: OR = 2.2, 95% CI: 0.8–5.6, p = 0.08) (Table 3). Explored interactions in dogs were not significant.

Table 2
Multilocus sequence types (ST) and clonal complexes of extended spectrum β-lactamase (ESBL) and/or AmpC-β-lactamase-producing *E. coli* (as defined in the Warwick database) isolated from faeces of cats and dogs in this study.

	E. coli ST			
Clonal complex designation	PAmpC (n = 33)	*ampC* mutation (n = 3)	PAmpC + ESBL (n = 1)	ESBL (n = 1)
469	ST162[2]			
10	ST10[2]			
405		ST405[1]	ST405[1]	ST405[1]
57	ST57[1]			
206		ST206[1]		
155	ST58[1]			
38	ST38[1]			
590		ST590[1]		
46	ST46[1]			
Singletons	ST133[1], 295[1], 372[1], 457[3], 501[1], 540[3], 929[1], 963[2], 973[4], 2541[1], 2712[1], 4390[1*], 4391[1*], 4392[1*], 4393[1*], 4395[1*], 4406[1*]			

ESBL: Extended spectrum β-lactamase-producing *E. coli*; PAmpC: plasmid mediated AmpC-β-lactamase-producing *E. coli; ampC: E. coli* isolate with a chromosomal *ampC* mutation; ESBL + AmpC: isolates that have both ESBL and either PAmpC or *ampC*; Singletons: multilocus Sequence Types not associated with any known clonal complex.
For each ST, the number of isolates is denoted by superscripts.
* New ST (not previously reported in the Warwick database).

4. Discussion

We aimed to estimate the prevalence of intestinal carriage of ESBL-E/AmpC-E in cats and dogs in Auckland, New Zealand, and assess the presence of factors statistically associated with carriage. We isolated ESBL-E and/or AmpC-E from 6.4% of the faecal samples analysed. There were 2 (0.3%) ESBL-E and 36 (6.1%) AmpC-E (plasmid mediated

Table 3
Variables statistically associated ($p < 0.05$) with faecal carriage of ESBL and/or AmpC-producing *E. coli* in the final multivariable logistic regression model with random clinic effect.

Variable description	Variable's values	Odds Ratio (95%CI)	P-value
Cats and Dogs together			
Had systemic antimicrobial treatment (oral/injection) in the last 6 months	No (reference)		
	Yes	1.52 (1.2–1.94)	< 0.01
Household member works in a veterinary clinic	No (Reference)		
	Yes	2.87 (1.1–7.6)	0.03
Household member travelled overseas in the last six months	No (reference)		
	Yes	2.3 (1.1–4.8)	0.02
Cats only			
Hospitalised and/or had surgery in the last six months	No (Reference)		
	Yes	8.7 (1.3–55)	0.02
Household member works in a veterinary clinic	No (Reference)		
	Yes	5.2 (0.8–32)	0.06
Dogs only			
Had systemic antimicrobial treatment (oral/injection) in the last 6 months	No (reference)		
	Yes	1.5 (1.1–2.2)	0.003
Household member travelled overseas in the last six months	No (reference)		
	Yes	2.2 (0.8–5.59)	0.08

or chromosomal *ampC* mutations) isolates.

This ESBL-E carriage rate is similar to the rate reported in healthy dogs in the USA (0.8%) and the United Kingdom (0.5%) (Murphy et al., 2009; Wedley et al., 2011), but lower than the rate reported in other studies (Portugal: 1.3%, Costa et al., 2008; UK: 4.5% and 5.4%, Schmidt et al., 2015). The faecal carriage prevalence of ESBL-E in humans in New Zealand has been estimated at 5% (Upton et al., 2011). The AmpC-E prevalence was comparable to that reported in cats and dogs the Netherlands and the UK (Hordijk et al., 2013), but lower than the prevalence in Mexico (15%; Rocha-Gracia et al., 2015). However, meaningful comparisons between studies are problematic due to the different study designs.

The most common ST was ST973, represented by only four isolates. Interestingly, ST405 was represented by three isolates that carried different β-lactamase genes, probably reflecting horizontal gene transfer of genes (Borjesson et al., 2013). Several STs, such as ST405, ST10, ST88, ST162, ST372, ST38 and ST973 have been previously associated with human and animal infections abroad (Ewers et al., 2012; Hansen et al., 2014; Riley, 2014; Voets et al., 2013) and in New Zealand (Karkaba et al., 2017) (interestingly, ST131, which is the most common ST associated with human infections in New Zealand (Drinkovic et al., 2015; Heffernan et al., 2014) was not identified in this study). The ESBL/AmpC genes found in this study, such as the blaCTXM-14 and blaCMY-2 are also highly prevalent in clinical human and animal *E. coli* isolates in New Zealand and abroad (Bush, 2013; Ewers et al., 2012; Heffernan et al., 2007, 2009; Karkaba et al., 2017).

Statistical analysis identified several variables independently associated with increased odds of isolation of ESBL-E/AmpC-E (Table 3). Although only 25% of the animals had antimicrobial treatment in the six months before the sampling, about 55% (21/38) of the ESBL-E/AmpC-E were obtained from treated animals. This association remained significant in the multivariable analysis in dogs and cats, and in dogs only. This result is consistent with previous observations in humans and pets (Decristophoris et al., 2013; Espinosa-Gongora et al., 2015; Johard et al., 2015; Zhao et al., 2016), and provides additional evidence for the positive selection exerted by antimicrobial treatments on antimicrobial resistant bacteria colonising the gut. The effect of the single antimicrobial families could not be analysed due to the modest sample size within each group.

Studies in humans have found a higher carriage rate of ESBL-E/AmpC-E in healthcare workers compared with non-healthcare workers (Ben Sallem et al., 2012; Geser et al., 2012; Stromdahl et al., 2011). The higher carriage rate in healthcare workers might be due a colonisation with a peculiar bacterial flora occurring in healthcare centres, which is under selective pressure due to the extensive use of antimicrobials in these facilities. We found that cats from households with people working in veterinary clinics had increased odds of ESBL-E/AmpC-E carriage than their counterparts. To some extent, in terms of antimicrobial selective pressures, veterinary clinics may be comparable to human healthcare facilities. Interestingly, from the two clinics that contributed more isolates and skewed the random effects such that they were considered outliers (Supplementary Fig. 1), most of the ESBL-E/AmpC-E positive pets sampled belonged to the clinic staff. This sampling of animals belonging to staff was outside the study protocol and occurred without the authors' knowledge. However, the results of the two clinics were kept in the final model to determine the effect of other variables.

International travel has been associated with increased risk of intestinal colonisation and with infection with ESBL-producing Enterobacteriaceae in people in a number of studies (Kaspar et al., 2015; Tängdén et al., 2010; Valverde et al., 2015). In New Zealand, a study by Freeman et al. (2008) reviewed cases of community-onset urinary tract infections (UTI) caused by ESBL-E, particularly by CTX-M-15 *E. coli* and found that all the cases had a recent history of travel to the Indian subcontinent, without previous hospitalisation in New Zealand. In our study, the presence of a household member travelling overseas in the last six months was also associated with increased odds of ESBL-E/AmpC-E carriage in pets (dogs and cats together). Most of the travelling reported was to Australia (n = 105/182; 57%), followed by Asia (n = 28/182; 15%). However, studies have shown that animals staying at kennels may be at a greater risk for carriage of antimicrobial resistant *E. coli* (Harada et al., 2011; Procter et al., 2014), and we were unable to analyse the association with kennel boarding as we did not collect information about this variable.

Consistent with the literature (Jacoby, 2005), a high proportion (25/38; 65%) of the ESBL-E/AmpC-E were resistant to non-β-lactam antimicrobials, and 7/38 (18%) isolates were MDR.

Finally, our study has strengths and limitations. There is no agreed gold standard for the detection of faecal ESBL-E/AmpC-E by culture, but we used a culture method that performed better than four other methods used in parallel. To our knowledge, this is also one of the largest surveys of ESBL-E/AmpC-E carriage in household pets. Limitations include recall bias which may have occurred with some of the questions, and a potential for bias due to a skewed study population (pets arriving to veterinary clinics, rather than a random sample of all pets in the study area).

In summary, our study provides new knowledge on the factors governing the intestinal colonisation with ESBL-E/AmpC-E in

household pets. Many of the STs of ESBL-E/AmpC-E STs causing overt infections in humans and pets were found in this study. Statistical analysis identified positive associations between the odds of ESBL-E/AmpC-E carriage and previous antimicrobial treatment, frequent contact with veterinary clinics and international travel. These factors have been found to be associated with increased rate of carriage of antimicrobial resistant bacteria in humans.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.04.015.

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