# Carriage of antimicrobial-resistant *Escherichia coli* and staphylococci in dogs in the community: molecular mechanisms

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by

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## Abstract

While previous studies have determined the prevalence of meticillin resistant *Staphylococcus aureus* (MRSA) and antimicrobial resistant (AMR) *Escherichia coli* in canine populations, few have included sufficiently large sample sizes and fewer still have characterised the isolates or investigated risk factors that might be associated with their carriage.

The main aims of the work presented in this thesis were; to determine the nasal prevalence of MRSA and other AMR staphylococci and the faecal prevalence of AMR *E. coli* in faeces in the canine population of mainland UK. The study also aimed to characterise the bacteria isolated using molecular techniques in order for comparisons to be made with isolates of human origin, and to determine the presence of potential risk factors associated with faecal carriage of AMR *E. coli.*

These objectives were achieved by carrying out two studies. The first study used frozen canine faecal samples collected during a cross sectional study of a semi-rural community in Cheshire to determine the prevalence of AMR *E. coli.* The second study collected faecal and nasal swabs from dogs visiting veterinary practices across mainland UK. Antimicrobial resistance of the isolates obtained from both studies were characterised using disc diffusion methods and PCR assays. In addition, isolates collected during the second study were subjected to multi-locus sequence typing and DNA micro array analysis of resistance and virulence genes. For antimicrobial resistant *E. coli*, risk factors associated with carriage were investigated.

The prevalence of MRSA in the canine population was found to be low at 1% and all isolates were identical to EMRSA-15, the main human endemic strain in many UK hospitals. The overall *S. aureus* prevalence was 7.5%, with a higher prevalence of 11.0% of *S. pseudintermedius*, in which no meticillin resistance was found. Meticillin resistant coagulase negative *Staphylococcus* spp. was found in 5.5% of dogs. AMR in the isolates varied between species; however resistance to fusidic acid was consistently high.

AMR *E. coli* was common in both studies (29.0% in community study and 44.8% in nationwide study). Resistance to ampicillin (24.0% and 37.2%), tetracycline (19.7% and 30.0%), trimethoprim (16.9% and 23.8%) and resistance to three or more antimicrobial classes (15.3% and 18.1%) was found to be high in both studies (community and nationwide respectively), while resistance to augmentin, chloramphenicol, ciprofloxacin and nalidixic acid was below 10% in both studies. A variety of genes responsible for resistance to expanded spectrum β-lactams was identified; including $bla_{\text{CTX-M-15}}$ and $bla_{\text{cmy2}}$, both of which have previously been identified in humans and dogs. A number of variables were found to be associated with resistance to antimicrobials, with previous prescription of antimicrobials and consumption of raw poultry meat remaining in the final model of more than one resistance outcome.

The carriage of MRSA and antimicrobial resistant *E. coli* could pose a potential problem both in terms of the welfare of the dogs carrying such bacteria as well as the zoonotic potential of the bacteria and resistance determinants.

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# Chapter One

## Introduction and Literature Review

## 1.1 Dog ownership

Pets, particularly dogs, have been a valued companion of humans for many years, and dog ownership has been suggested to have numerous positive effects on both physical and psychological health (Serpell 1991; Wells 2007). Conversely, there are also numerous hazards associated with dog ownership including potential development of allergies, bites and infectious diseases (Plaut, Zimmerman et al. 1996).

In the UK in 2010, it was estimated that 23% of households owned a dog with an approximate total population of 8 million (Pet Food manufacturers Association, "Pet ownership trends" http://www.pfma.org.uk/statistics). This suggests that the population of dogs in the UK has increased since 2004, when the estimation was 6.8 million. It is difficult to comment on whether the increase in population is related to an increase in the proportion of dog owning households since this data was not reported in the 2004 study. Studies in other countries have shown the percentage of dog owning households to be slightly higher, including in the USA (37.2%) (American Veterinary Medical Association, "US Pet Ownership – 2007" http://www.avma.org/reference/marketstats/ownership.asp) and Ireland (35.6%) (Downes, Canty et al. 2009). With such a high population and the close bond many people share with their pets, it is highly likely that bacteria can transfer between dogs and their owners, and this has sparked concerns that dogs may act as reservoirs for zoonotic bacteria, in particular antimicrobial resistant bacteria and resistance determinants (Guardabassi, Schwarz et al. 2004; Stenske, Bemis et al. 2009).

## 1.2 Use of antimicrobials in dogs

Given the close relationship shared between owners and their dogs, the issue of pet welfare has become very important and, with this, a predictable increase in the use of antimicrobials in companion animal medicine has been observed. Often, antimicrobials are prescribed in the absence of confirmatory laboratory testing (Hughes, Williams et al. 2012), which may result in the inappropriate use of antimicrobial agents in small animal medicine. While many countries publish data on

the use of antimicrobials in animals, the majority focus on their use is in food producing animals, with very little information relating to their use in companion animals being made available. In the UK, the Veterinary Medicines Directorate (VMD) publish such data annually, relating to sales of antimicrobials in both food producing and non-food producing animals. In 2009 (VMD 2009), sales of antimicrobials for use in non-food producing animals increased to 38 tonnes (of active ingredient) compared to 29 tonnes in 2004. It is not possible to break this down into sales for each individual animal species. However, the report does indicate that products for use only in the treatment of dogs to have increased from 4976 kg in 2004 to 12454 Kg in 2008. A report from Denmark also showed that sales of antimicrobials for use in pet animals has increased, with the highest sales being associated with the penicillins, especially in combination with a β-lactamase inhibitor (DANMAP 2009). In a retrospective case study of prescription records carried out in the USA (Wayne, McCarthy et al. 2011), amoxicillin-clavulanic acid was also the most frequently prescribed antimicrobial. It is also interesting to note that this study reported that 38% and 44.1% of prescriptions were given when there was no evidence of infection or any suspected infection, respectively. Conversely, data from Sweden shows a decreasing trend in sales of all antimicrobials used in dogs with the exception of macrolides and lincosamides (SVARM 2008). The report attributes this fall in sales to increased awareness after the first isolation of meticillin resistance *Staphylococcus aureus* and *S. pseudintermedius*, which led to increased media coverage, seminars and a revision of guidelines for use of antimicrobials in small animals.

Exposure of dogs to such high volumes of antimicrobials, as in humans can have important consequences. The commensal flora present in the animal may be disrupted, allowing for more pathogenic bacteria to occupy such niches and cause further disease. In addition, the pressures exerted upon commensal bacteria by antimicrobials may result in selection of resistance.

## 1.3 Antimicrobial resistance

Antimicrobial resistance is not a new phenomenon. In the same way that many of the antimicrobials are naturally occurring, so too are some of the mechanisms involved in resistance. It has been suggested that many antimicrobials are produced as a result of secondary metabolism (Vining 1990); with their toxic effect being more of a consequence than their specific function. They may however, provide producing organisms with an advantage over competitors occupying the same environmental niche. In order to avoid auto toxicity, many strategies are employed by the antimicrobial producing organism (Cundliffe 1989), including not producing the antimicrobial when undergoing rapid growth, modification of the antimicrobial targets, inactivation of intracellular antimicrobial and excretion of the antimicrobial into the extracellular environment. Bacteria living in the same niche as the antimicrobial producer will therefore be under a selection pressure to become resistant.

Even before the widespread use of penicillin, an enzyme capable of inactivating it was identified (Abraham and Chain 1940). It is also known that some resistance mechanisms may have had other functions, for example efflux pumps capable of transporting a wide range of substrates such as bile acids out of the cell, but are also able to actively pump out antimicrobials (Ma, Cook et al. 1995; Oethinger, Kern et al. 1998; Piddock 2006; Piddock 2006).

Resistance to antimicrobials can involve a number of different mechanisms and the specific details are reviewed later when discussing the individual antimicrobials and bacterial species. In broad terms, there are four main mechanisms by which bacteria can overcome the effect of antimicrobials:

1. Drug inactivation or modification e.g. β-lactamases responsible for penicillin resistance.
2. Alteration of the target of the antimicrobial e.g. mutations in the *gyrA* and p*arC* genes encoding topoisomerase/gyrase the target of quinolones.

3. Alteration of a metabolic pathway or e.g. the utilization of preformed folic acid rather than its precursor para-aminobenzoic acid (PABA) in sulphonamide resistance.
4. Reduced drug accumulation by either decreased uptake of the antimicrobial or by removal of the antimicrobial via efflux pumps.

Antimicrobial resistance can be either intrinsic (inherent) or acquired (Normark and Normark 2002). Intrinsic resistance represents a trait associated with all bacteria of a species or genus because of a lack of the bacterial target or the inability to accumulate the antimicrobial to sufficient levels. An example of this is the resistance of Gram negative bacteria to vancomycin due to the impermeability of the outer membrane to such large antimicrobial molecules (Hawkey 1998). Acquired resistance occurs in bacteria that are normally susceptible to the antimicrobial, either by mutations within the bacterial genome or by horizontal gene transfer via mobile genetic elements such as plasmids, integrons and transposons.

## 1.4 ***Escherichia coli***

*Escherichia coli* was first described by the German paediatrician Theobold Escherich in 1885 (Escherich 1989) as intestinal bacteria of infants as "short plump rods". *E. coli* is Gram negative, rod shaped, a facultative anaerobe and usually motile. The bacteria can possess many virulence factors enabling it to cause numerous diseases including urinary tract infections, sepsis, neonatal meningitis and gastrointestinal infections. *E. coli* can be classified in to six distinct groups, five of which are associated with gastrointestinal disease; enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC) and enteroinvasive *E. coli* (EIEC) (Nataro and Kaper 1998; Clarke 2001), and one that is responsible for infections at other sites, extra-intestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson 2000; Johnson and Russo 2002). ExPEC can be further sub-divided into *E. coli* responsible for urinary tract infections (UPEC), meningitis/ bacterial sepsis (MNEC) and avian infections (APEC).

As well as many strains being pathogenic, *E. coli* is also ubiquitously found in the gastrointestinal tract of many warm blooded animals, including dogs (Clapper and Meade 1963), and provides the host with some health benefits (Berg 1996). *E. coli* in the gut are responsible for the production of vitamin K; they prime the host immune system to facilitate a speedier response in the event of infection by pathogenic bacteria and may competitively exclude pathogenic bacteria competing for the same intestinal niche. However, the intestine is a prime location to be exposed to any antimicrobials that may be orally ingested or excreted into the intestinal lumen, and this exerts a tremendous selection pressure upon bacteria to develop or acquire resistance in order to survive. As such, commensal bacteria, specifically *E. coli* have been identified as providing a good indication of the prevalence and spread of antimicrobial resistance (van den Bogaard and Stobberingh 2000). In addition, due to the ever-present nature of *E. coli* in the canine intestine, comparisons of the strains of *E. coli* and the carriage of resistance can be made between different individuals. Development of antimicrobial resistance by commensal flora may have an impact not only for the individual dogs concerned, but also for the humans in close contact with them by either transfer of the resistant bacteria to humans, potentially leading to opportunistic infections that are difficult to treat, or transfer of the resistance determinants they have acquired to other, more pathogenic species (Guardabassi, Schwarz et al. 2004; Stenske, Bemis et al. 2009).

A number of antimicrobials, previously effective against infections caused by *E. coli* are no longer efficacious, with many mechanisms involved in resistance. The details of the antimicrobials commonly used to treat infections caused by *E. coli* and the mechanisms of resistance involved are discussed below.

### 1.4.1 β-lactam antibiotics

Penicillin and its related compounds (cephalosporins, monobactams and carbapenams) all possess a β-lactam ring and act by inhibiting cell wall synthesis. These antimicrobials bind to proteins, so-called penicillin binding proteins (PBPs), within the cell wall and prevent cross linkage of peptidoglycan strands thus compromising cell wall integrity.

## β-lactam resistance in *E. coli*

In *E. coli*, resistance to β-lactam antibiotics is mediated by the production of a diverse group of hydrolytic enzymes capable of inactivating the β-lactam ring. These β-lactamases were first described in 1940 (Abraham and Chain 1940), notably a few years prior to the first clinical use of penicillin.

## Classification of β-lactamases

A number of different methods for β-lactamase classification have been proposed, however two have been used predominantly. Ambler (Ambler 1980) proposed a scheme that is based on the amino acid sequence of the enzymes and the most recent description of the system comprises four classes. Class A enzymes (which includes the TEM and SHV β-lactamases) have a serine active site, as do the class C (AmpC β-lactamases) and D (including OXA β-lactamases) enzymes, while class B (metallo β-lactamases) enzymes utilize a metal cofactor, most often zinc, in substrate hydrolysis (Payne 1993). The second scheme, proposed by Bush and others is based on functional classification of β-lactamases (Bush 1989; Bush, Jacoby et al. 1995; Bush and Jacoby 2010). Group 1 (cephalosporinases) comprises the molecular class C enzymes and has two subgroups (1 and 1e). Group 2 (serine β-lactamases) enzymes are a large collection incorporating molecular classes A and D and is divided into six subgroups depending on substrate profiles and their resistance to inhibitors (2a to 2f), some of which are further divided. Finally, group 3 (metallo β-lactamases) comprises the molecular class B enzymes.

In most genera of bacteria, including *E. coli*, genes encoding β-lactamases are located on transferrable plasmids, allowing these genes to be disseminated amongst many bacterial species.

## TEM and SHV β-lactamases

The first plasmid mediated β-lactamase to be described was identified in a clinical isolate of *E. coli* from a patient in the 1960s, and was called TEM-1 after the name of

the affected patient, Temoneira (Anderson and Datta 1965). It was not long before the gene encoding TEM-1, $bla_{TEM}$, had spread and this gene is now common in many bacteria including *E. coli*, *Pseudomonas aeruginosa* and other members of the *Enterobacteriaceae* (Wiedemann, Kliebe et al. 1989). A second β-lactamase is TEM-2, which is the result of a single amino acid substitution at position 39 (glutamate for lysine) (Barthelemy, Peduzzi et al. 1985). Both TEM-1 and TEM-2 were shown to be resistant to penicillins and some early cephalosporins, such as cephalothin and β-lactamase inhibitors are effective against these two enzymes. At least 12 other TEM enzymes with this narrow spectrum of resistance have been described (http://www.lahey.org/Studies/) and are classified as molecular class A, functional group 2b.

Following extensive spread of the $bla_{TEM-1}$ and$bla_{TEM-2}$ genes, newer drugs that were not susceptible to these β-lactamases were developed. Such antimicrobials included the oxyimino-cephalosporins or third generation cephalosporins (for example ceftazidime, ceftriaxone and cefotaxime), which have an expanded spectrum of activity against bacteria compared to penicillin and its derivatives. It was not long, however, before β-lactamases capable of inactivating the newer drug were reported.

These enzymes were found to be mutated derivatives of the group 2b enzymes (TEM-1 and TEM-2) and, while retaining the substrate profile of the original enzymes and being susceptible to β-lactamase inhibitors, are also capable of hydrolysing the oxyimino-cephalosporins. The first to be described was named TEM-3 (Sougakoff, Goussard et al. 1988) and was the result of two amino acid substitutions of TEM-2. These mutations resulted in opening up of the catalytic site, thus extending the substrate profile of the enzyme. In subsequent years, the number of derivatives of the original enzymes has increased, each with substitutions at a relatively limited number of positions, resulting in subtle differences in substrate profiles. To date over 150 TEM-1 and TEM-2 derivatives have been described (http://www.lahey.org/Studies/). Due to their hydrolytic activity against the expanded spectrum β-lactam antibiotics, the collective name for these types of enzymes is extended spectrum β-lactamases (ESBL). ESBLs are molecular class A, functional group 2be enzymes (Ambler 1980; Bush and Jacoby 2010). In addition, some of the

derivatives from the original enzymes are also resistant to β-lactamase inhibitors and have been classified in a separate functional group to the ESBL enzymes, group 2br.

In *Klebsiella pneumoniae*, a different closely related β-lactamase is more common, but it has also been identified, to a lesser extent than TEM-1 and TEM-2, in *E. coli*. The enzyme is called SHV-1 (sulphydryl variable) and shares some degree of sequence homology with TEM-1 (68%) (Barthelemy, Peduzzi et al. 1986). SHV-1 and 30 other SHV variants (http://www.lahey.org/Studies/) are classified as molecular class A, functional group 2b enzymes (Ambler 1980; Bush and Jacoby 2010). The first SHV variant to display the ESBL phenotype, SHV-2 was found in a clinical isolate of *K. ozaenae* (Knothe, Shah et al. 1983; Kliebe, Nies et al. 1985), and was found to be the result of mutation of SHV-1 at amino acid position 238 (glycine for serine) (http://www.lahey.org/Studies/)(Barthelemy, Peduzzi et al. 1988), which is located around the active site of the enzyme. Like the TEM type ESBLs, many variants of the original narrow spectrum enzymes now exist (http://www.lahey.org/Studies/), including at least six that are resistant to inactivation by β-lactamase inhibitors.

CTX-M β-lactamases

CTX-M enzymes are so called for their higher levels of activity against cefotaxime rather than ceftazidime (Bauernfeind, Grimm et al. 1990; Bonnet 2004), the latter is the preferred substrate hydrolysed by the ESBL TEM and SHV variants. They are plasmid mediated β-lactamases, which are believed to have escaped from the chromosomal DNA of members of the genus *Kluyvera* (Bonnet 2004; Rodriguez, Power et al. 2004; Olson, Silverman et al. 2005). Currently, five clusters of CTX-M β-lactamases, grouped by similarity of amino acid sequence, have been described. These clusters are CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25. Within each cluster there is >94% identity compared with ≤90% identity between clusters (Bonnet 2004). To date over 110 enzymes of unique amino acid sequence have been described (http://www.lahey.org/Studies/) and they have been found in a number of different bacterial genera including *E. coli*, *Salmonella* spp, *Klebsiella* spp and *Enterobacter* spp. These enzymes are classified as molecular class A, functional

group 2be (Ambler 1980; Bush and Jacoby 2010) and all are susceptible to β-lactamase inhibitors.

Plasmid mediated AmpC β-lactamases

Most Gram negative bacteria, including *E. coli* carry a chromosomally mediated cephalosporinase (Sanders 1987; Jacoby 2009). In wild type bacteria, these AmpC β-lactamases can be either constitutively expressed at levels too low to be clinically relevant due to loss a regulatory gene (as in *E. coli*) (Honore, Nicolas et al. 1986), or are induced by the presence of the β-lactam antibiotic (as in *Citrobacter freundii* and *Enterobacter cloacae*) (Minami, Yotsuji et al. 1980; Lindberg, Westman et al. 1985; Lindberg and Normark 1987). β-lactam antibiotics vary in their ability to induce such genes, thus some can still be effective in these bacteria. However, mutational events in the chromosome can occur, which causes de-repression of the AmpC β-lactamase gene, and therefore expression of the enzyme at sufficiently high levels to inactivate β-lactam antibiotics that may have been effective in the wild type bacteria (Lindberg, Lindquist et al. 1987).

In the 1980's plasmids encoding AmpC β-lactamases were reported (Bauernfeind, Chong et al. 1989; Philippon, Arlet et al. 2002) and sequence analysis showed these enzymes to be closely related to the chromosomally encoded enzymes of *C. freundii*, *P. aeruginosa* and *E. cloacae* (Bauernfeind, Stemplinger et al. 1996). Bacteria harbouring plasmid mediated AmpC β-lactamases are typically more broadly resistant than classical ESBLs, with substrate profiles that may include the oxyimino-cephalosporins, cephamycins and β-lactamase inhibitors such as clavulanic acid, but not the carbapenams (Philippon, Arlet et al. 2002). AmpC β-lactamases belong to molecular class C, functional group 1 enzymes (Ambler 1980; Bush and Jacoby 2010).

Epidemiology of ESBLs

In human medicine, the prevalence and specific type of ESBL or plasmid mediated AmpC β-lactamase varies from country to country and can vary between individual

hospitals. Before 2003, the predominant types of ESBL were almost exclusively variants of the TEM and SHV enzymes from clinical isolates of hospital origin (Bradford 2001). However, after 2003, an increasing number of ESBL producing isolates were submitted from within the community, and CTX-M type ESBLs became more dominant both in the hospital and wider community settings (Munday, Whitehead et al. 2004; Woodford, Ward et al. 2004; Livermore and Hawkey 2005; Pitout, Gregson et al. 2005; Potz, Hope et al. 2006). While there is some degree of variation of the types of ESBLs that predominate, a certain few specific variants have spread worldwide. A very good example of this is the clonal spread of CTX-M-15, which can now be found in many countries worldwide (Baraniak, Fiett et al. 2002; Moubareck, Daoud et al. 2005; Nicolas-Chanoine, Blanco et al. 2008; Pitout, Gregson et al. 2009) including the UK (Lau, Kaufmann et al. 2008). Successful spread of specific gene variants have been shown to be linked to specific insertion sequences and transposons, (for example, CTX-M-9 and IS*CR1*(Novais, Canton et al. 2006), however, this is not the case with CTX-M-15, which has been shown to be mainly due to spread of epidemic clones carrying the $bla_{\text{CTX-M-15}}$ gene, specifically *E. coli* sequence type ST-131 by multi-locus sequence typing (Coque, Novais et al. 2008; Nicolas-Chanoine, Blanco et al. 2008; Blanco, Alonso et al. 2009; Woodford, Carattoli et al. 2009).

ESBL and β-lactamase producing *E. coli* in dogs

In dogs, β-lactam antimicrobials are indicated for treatment of a wide range of bacterial infections, such as pyoderma and respiratory and gastrointestinal tract infections, due to their broad spectrum of activity. Thus, they are widely used in veterinary practice.

There is a limited amount of data regarding the prevalence of ESBL and β-lactamase producing *E. coli* in dogs, with most studies relating to isolates obtained from clinical samples and very few investigating the prevalence in healthy dogs. Even fewer data are available relating to the genetic background of the ESBL/ AmpC phenotype. Table 1.1 below summarises the reports of ESBL producing *E. coli* isolated from dogs in different countries. The data show that often the specific gene identified is

that circulating within isolates of human origin, including *bla*$_{\text{CTX-M-15}}$ (Pomba, da Fonseca et al. 2009; Ewers, Grobbel et al. 2010).

**Table 1.1 Reports of ESBL producing *E. coli* isolated from dogs**

| Year | Country | Type of samples | β-lactamase genes detected | Reference |
|---|---|---|---|---|
| **2000** | Spain | Recurrent UTI | $bla_{\text{SHV-12}}$ | (Teshager, Dominguez et al. 2000) |
| **2002** | Portugal | Uropathogenic *E. coli* | $bla_{\text{SHV}}$, $bla_{\text{AmpC}}$ | (Feria, Ferreira et al. 2002) |
| **2004** | Portugal | Healthy animal faecal samples | $bla_{\text{CTX-M-1}}$, $bla_{\text{TEM-52}}$ | (Costa, Poeta et al. 2004) |
| **2005** | Italy | Healthy and sick animals | $bla_{\text{CMY-2}}$, $bla_{\text{SHV-12}}$, $bla_{\text{CTX-M-1}}$ | (Carattoli, Lovari et al. 2005) |
| **2006** | Australia | Clinical isolates | $bla_{\text{TEM}}$, $bla_{\text{CMY-7}}$ | (Sidjabat, Townsend et al. 2006) |
| **2008** | Portugal | Healthy animal faecal samples | $bla_{\text{CTX-M-1}}$, $bla_{\text{OXA-30}}$ | (Costa, Poeta et al. 2008) |
| **2008** | Chile | dogs and cats treated/ untreated with enrofloxacin | $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-14}}$ | (Moreno, Bello et al. 2008) |
| **2008** | Hong Kong | Stray dogs | $bla_{\textit{CTX-M-3}}$, $bla_{\textit{CTX-M-13}}$, $bla_{\textit{CTX-M-14}}$, $bla_{\text{CTX-M-}\textit{15}}$, $bla_{\text{CTX-M-}\textit{27}}$, $bla_{\text{CTX-M-}\textit{28}}$, $bla_{\text{CTX-M-}\textit{55}}$. | (Ho, Chow et al. 2011) |
| **2009** | Canada | Healthy animal faecal samples | $bla_{\text{CMY-2}}$ | (Murphy, Reid-Smith et al. 2009) |
| **2010** | China | Healthy and sick animals | $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-24}}$, $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-64}}$, $bla_{\text{CTX-M-65}}$ | (Sun, Zeng et al. 2010) |
| **2010** | USA | Urinary Tract Infections | $bla_{\text{SHV-12}}$, $bla_{\text{CTX-M-14}}$, $bla_{\text{CTX-M-15}}$ | (O'Keefe, Hutton et al. 2010) |
| **2010** | Europe | Clinical isolates | $bla_{\text{SHV-12}}$, $bla_{\text{CTX-M-15}}$ | (Ewers, Grobbel et al. 2010) |

### 1.4.2 Quinolone antimicrobials

Quinolones and fluoroquinolones inhibit DNA replication by binding to DNA gyrase and topoisomerase IV. Nalidixic acid was the first quinolone to be described and was approved for clinical use in 1967 (Lesher, Gruett et al. 1962; Emmerson and Jones 2003). However, its use was limited due to its inability to be efficiently absorbed, and newer, fluorinated compounds were developed and introduced, which were shown to have a wider spectrum of activity and better oral absorption. Examples of such fluoroquinolones include ciprofloxacin (2$^{nd}$ generation), levofloxacin (3$^{rd}$ generation) and moxifloxacin (4$^{th}$ generation).

#### Quinolone and fluoroquinolones resistance

Spontaneous chromosomal mutations can result in resistance by one of three mechanisms (Piddock 1998). Firstly, mutations in the genes encoding the subunits of DNA gyrase (*gyrA* and *gyrB*) or topoisomerase IV (p*arC* and p*arE*) (Sato, Inoue et al. 1986; Yamagishi, Yoshida et al. 1986; Heisig 1996) means that the antimicrobial can no longer bind to these target proteins with the same affinity. Studies have shown that mutations of these genes largely occur in a highly conserved region known as the quinolone resistance determining region (QRDR) (Hopkins, Davies et al. 2005), and that accumulation of mutations results in a higher degree of resistance (Bagel, Hullen et al. 1999). Mutations resulting in the reduced expression of porins on the bacterial cell membrane can also cause resistance to quinolones and fluoroquinolones since the drug cannot readily enter the cell and build up to sufficient concentrations. Finally, over expression of efflux pumps prevents the accumulation of the drug within the cell. Resistance mediated by the mechanisms discussed above, can only be spread by vertical or clonal transmission to the progeny of the resistant bacteria, and is not transferrable to other bacteria. However, plasmid mediated quinolone resistance has also been described, and this can spread between bacteria of different species and genera.

Plasmid mediated quinolone resistance (PMQR) has been shown to confer only low level transferable resistance to quinolones and fluoroquinolones to a broad host range, but it provides sufficient resistance to facilitate the selection of high level

resistance (Martinez-Martinez, Pascual et al. 1998). Tran and Jacoby demonstrated that the plasmid quinolone resistance gene, *qnr*, encoded a protein which protects DNA gyrase from inhibition by these antimicrobials (Tran and Jacoby 2002) and subsequent studies have shown that bacteria carrying PMQR generate chromosomal quinolone resistant mutants at a higher rate then those lacking in PMQR (Martinez-Martinez, Pascual et al. 1998). Three distinct families, each differing in nucleotide sequence by 40% or more, have been identified; *qnrA, qnrB* and *qnrS.* Within each family are numerous alleles differing by no more than 10% in nucleotide sequence (Jacoby, Cattoir et al.). To date there are 7, 31 and 4 *qnrA, qnrB* and *qnrS* alleles, respectively (http://www.lahey.org/qnrstudies/), which have been identified in many different genera of bacteria including *E. coli, Klebsiella* spp and *Enterobacter* spp. Recently, two further plasmid mediated quinolone resistance genes have been identified (*qnrC* and *qnrD*) in *Proteus mirabilis* and *Salmonella enterica* respectively (Cavaco, Hasman et al. 2009; Wang, Guo et al. 2009), but are currently rare and have not yet been reported in *E. coli.*

A variant of the aminoglycoside acetyltransferase (responsible for resistance to kanamycin, tobramycin and amikacin) also able to confer low level resistance to certain fluoroquinolones, AAC(6')-Ib-cr, was first described in 2006 (Robicsek, Strahilevitz et al. 2006) and reduces the activity of the antimicrobial by *N*-acetylation of the piperazinyl substituent of ciprofloxacin and norfloxacin. It was found to be acting in synergy with *qnrA*, which was also carried by the bacterial strains, but the *aac(6')-Ib-cr* gene has also since been found to confer resistance in the absence of *qnrA* (Park, Robicsek et al. 2006). Carriage of the *aac(6')-Ib-cr* gene has been found to be associated with carriage of $bla_{\text{CTX-M}}$ genes, in particular $bla_{\text{CTX-M-15}}$ (Karisik, Ellington et al. 2006; Machado, Coque et al. 2006).

A final mechanism of resistance to fluoroquinolones is the plasmid mediated efflux pump QepA, which was first described in a clinical *E. coli* isolate from Japan (Yamane, Wachino et al. 2007) and confers resistance by actively pumping the antimicrobial from the cytoplasm of the bacteria.

*Quinolone resistance in E. coli isolated from dogs*

Quinolones are generally prescribed to dogs in cases where the first line antimicrobial drugs are not appropriate (Guardabassi, Schwarz et al. 2004). Baytril® (Bayer Healthcare, Leverkusen, Germany) (enrofloxacin) is a fluoroquinolone antimicrobial licensed for use in various animal species including dogs and is indicated for the treatment of bacterial infections of numerous sites in particular urinary tract infections. Resistance to quinolones and fluoroquinolones has been reported in *E. coli* isolated from both healthy and sick dogs with prevalences ranging from 0-20% (Cohn, Gary et al. 2003; Carattoli, Lovari et al. 2005) including in the UK (Normand, Gibson et al. 2000). Mechanisms of fluoroquinolone resistance in bacteria isolated from dogs include mutations in the gyrase and topoisomerase IV genes (Saenz, Zarazaga et al. 2003), and plasmid mediated quinolone resistance determinants (Ma, Zeng et al. 2008; Pomba, da Fonseca et al. 2009).

### 1.4.3 Tetracyclines

Tetracyclines bind to the acceptor (A) site of the bacterial 30S ribosome preventing aminoacyl tRNA molecules from binding to this region, thus inhibiting protein synthesis. They are a broad spectrum bacteriostatic antimicrobial. The first generation tetracyclines, oxytetracycline and chlortetracycline, were discovered in the 1940's as products of *Streptomyces* spp. Increasing resistance to these antimicrobials led to the development of the second generation tetracyclines, minocycline and doxycycline and later the third generation tetracyclines, glycycline and tigecycline, which were derived from the original tetracyclines by chemical alteration (Chopra and Roberts 2001).

#### Tetracycline resistance

Genes found on mobile transferable elements such as plasmids confer resistance to tetracycline by one of three described mechanisms (Chopra and Roberts 2001; Roberts 2005; Thaker, Spanogiannopoulos et al. 2010). The genes can encode efflux pumps (for example, *Tet*M), which actively remove the drug from the cell, ribosomal protection proteins (RPP) (for example, *Tet*A) that protect the bacterial ribosome

from binding to tetracyclines or, less commonly, enzymes which inactive the drug (for example, *Tet*X). In *E. coli*, the most common mechanism of resistance described is the expression of the RPPs.

*Tetracycline resistance in E. coli isolated from dogs*

Oxytetracycline is prescribed for the treatment of infections in dogs including those caused by *Staphylococcus aureus* and *Streptococcus* spp. Tetracycline resistance has been reported in dogs with prevalences as high as 45% in some studies (Carattoli, Lovari et al. 2005; Authier, Paquette et al. 2006). The *tetA* and *tetB* gene have been reported in E. coli isolated from healthy dogs (Bryan, Shapir et al. 2004; Costa, Poeta et al. 2008).

### 1.4.4 Trimethoprim

Trimethoprim is a broad spectrum bacteriostatic antimicrobial agent that inhibits folic acid synthesis. It is a competitive inhibitor of the enzyme dihydrofolate reductase (DHFR), which is involved in the synthesis of tetrahydrofolate, a precursor in synthesis of DNA nucleotide thymidine.

Trimethoprim resistance

Numerous mechanisms of resistance are described (Huovinen 2001) including chromosomal mutations in the bacterial DHFR gene resulting in a reduced affinity for the antimicrobial, chromosomal mutations in the promoter region of the gene causing an increased concentration of the DHFR enzyme (Flensburg and Skold 1987) and reduction in accumulation of the antimicrobial by increased expression of efflux pumps or reduced expression of porins. Acquisition of a DHFR enzyme with reduced sensitivity or affinity for trimethoprim, encoded by *dfr* genes, typically associated with gene cassettes, has also been described (Amyes and Smith 1974; Heikkila, Skurnik et al. 1993; Gibreel and Skold 1998; Cocchi, Grasselli et al. 2007).

*Trimethoprim resistance in E. coli isolated from dogs*

Trimethoprim, used in combination with sulfamethoxazole (co-trimoxazole), another antimicrobial, which, like trimethoprim, acts by inhibiting part of the folic acid cycle, is prescribed to dogs for the treatment of skin, eye and ear infections and urinary and gastrointestinal tract infections caused by both Gram positive and negative infections.

Resistance to trimethoprim has been reported, ranging from 3% in faecal bacteria from healthy canines to 34% in canine clinical isolates (Normand, Gibson et al. 2000; Carattoli, Lovari et al. 2005; Murphy, Reid-Smith et al. 2009) and *dfr* genes have also been reported, including *dfrA12* (Costa, Poeta et al. 2008) and *dfrA17* (Sanchez, Stevenson et al. 2002).

## 1.5 *Staphylococcus* spp

Bacteria of the genus *Staphylococcus* are Gram positive, facultative anaerobes that form irregular clusters of cocci when viewed under the microscope. They are mostly harmless, commensal residents of the skin and mucosal membranes of humans and other animals (Clapper and Meade 1963; Williams 1963; Biberstein, Jang et al. 1984). However, a small number of species are regarded as important pathogens and it can often be the host's own commensal strains that, once past the protective skin barrier, are the cause of opportunistic infections (Casewell 1998). Staphylococci can be split into two distinct groups, based on their ability to produce the enzyme coagulase. Most attention is given to the coagulase positive staphylococci, which are typically regarded as being the more virulent and clinically important of the two.

### 1.5.1 Coagulase positive staphylococci

In humans, *S. aureus* is the most commonly isolated species, and is found consistently in the nares of around 25% of healthy humans, but varies depending on the study and population demographics (Kluytmans, vanBelkum et al. 1997; Abudu, Blair et al. 2001; Grundmann, Tami et al. 2002; Mainous, Hueston et al. 2006; Gorwitz, Kruszon-Moran et al. 2008). It is spread from one person to another by

contact with pus from infected wounds, skin to skin contact with an infected person, from the nares of healthy non symptomatic individuals and has been shown to persist on inanimate objects provided the right conditions to prevent desiccation are achieved (Neely and Maley 2000; Tolba, Loughrey et al. 2007). *S. aureus* can cause a wide range of infections including localised skin infections such as boils and abscesses, impetigo, endocarditis, septicaemia, toxic shock syndrome, gastroenteritis and scalded skin syndrome in infants

While *S. aureus* infections are of clinical importance in canine medicine, *S. pseudintermedius* is the most common coagulase positive species isolated from dogs (Clapper and Meade 1963; Devriese and Depelsmaecker 1987; Cox, Hoskins et al. 1988) and is an important causative agent of infections, in particular pyoderma and otitis (Hoekstra and Paulton 2002; Penna, Varges et al. 2010). Until 2007, such isolates from dogs were identified as the closely related *S. intermedius* (Devriese, Vancanneyt et al. 2005). However, after the first description of *S. pseudintermedius*, and subsequent reclassification of a group of clinical isolates from dogs (Sasaki, Kikuchi et al. 2007), it became widely accepted that all historical isolates of canine origin were in fact the new species *S. pseudintermedius* and that *S. intermedius* was found only in pigeons. *S. pseudintermedius* has also rarely been implicated in human infections (Van Hoovels, Vankeerberghen et al. 2006) in particular dog bite wounds (Talan, Staatz et al. 1989; Lee 1994).

### 1.5.2 Coagulase negative staphylococci

Coagulase negative staphylococci are found in abundance on the skin and mucosal surfaces of many animals. Due to their ubiquitous nature, coagulase negative staphylococci isolated from clinical samples were often dismissed as commensal contaminants, but with the increasing use of indwelling medical devices and the ability of coagulase negative staphylococci to form biofilms on such devices, their clinical significance has increased (Huebner and Goldmann 1999). Coagulase negative staphylococci have also been implicated as serving as a reservoir of resistance determinants for the coagulase positive species, in particular meticillin resistance (Silva, Mattos et al. 2001; Hanssen, Kjeldsen et al. 2004; Ziebuhr, Hennig et al. 2006).

In humans, the most commonly isolated species are *S. epidermidis* and *S. hominis* (Kleeman, Bannerman et al. 1993; Jarlov, Hojbjerg et al. 1996; Nagase, Sasaki et al. 2002), while in dogs very little investigation has been carried out. Mostly, reports go no further than to describe them as coagulase negative staphylococci. However, some of the species that have been isolated from dogs include *S. epidermidis, S. simulans, S. haemolyticus, S. saprophyticus, S. sciuri* and *S. xylosus* (Cox, Hoskins et al. 1988; Lilenbaum, Veras et al. 2000; Stepanovic, Dimitrijevic et al. 2001).

### 1.5.3 Meticillin resistance in *S. aureus*

Resistance to penicillin was first identified in *S. aureus* in the 1940s and was due to the production of an enzyme called penicillinase, which breaks down the β-lactam ring of penicillin and its related compounds (Kirby 1944; Kirby 1945). Meticillin (previously methicillin) was shown to be less susceptible to inactivation by the penicillinases of *S. aureus* (Knox and Smith 1963). This drug was first used in clinical medicine in the 1950s to treat penicillin resistant staphylococcal infections, but very shortly after its introduction resistance to meticillin was described (Lane 1962; Jevons, Coe et al. 1963). Meticillin resistant *S. aureus* (MRSA) was shown to produce a alternative penicillin binding protein (PBP) compared to its meticillin sensitive counterparts (Hartman and Tomasz 1981) and later studies attributed its production to expression of a gene called *mecA* (Archer and Niemeyer 1994). The *mecA* gene is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) (Katayama, Ito et al. 2000). This PBP (PBP2') has a lower affinity for the binding of β-lactam antibiotics and therefore, when the antimicrobial has inactivated the primary PBP, cross linkage of peptidoglycan can still take place and the organism can survive.

*S. aureus* is thought to have acquired meticillin resistance via horizontal transfer of the *mecA* gene and initial studies suggested only one such event in a single isolate of *S. aureus* (Kreiswirth, Kornblum et al. 1993). However, later studies support the theory that *S. aureus* acquired the *mecA* gene on a number of occasions (Enright, Robinson et al. 2002; Robinson and Enright 2003) and even now, new variants are being reported. For example, most recently, the new *mecA* gene variant isolated from

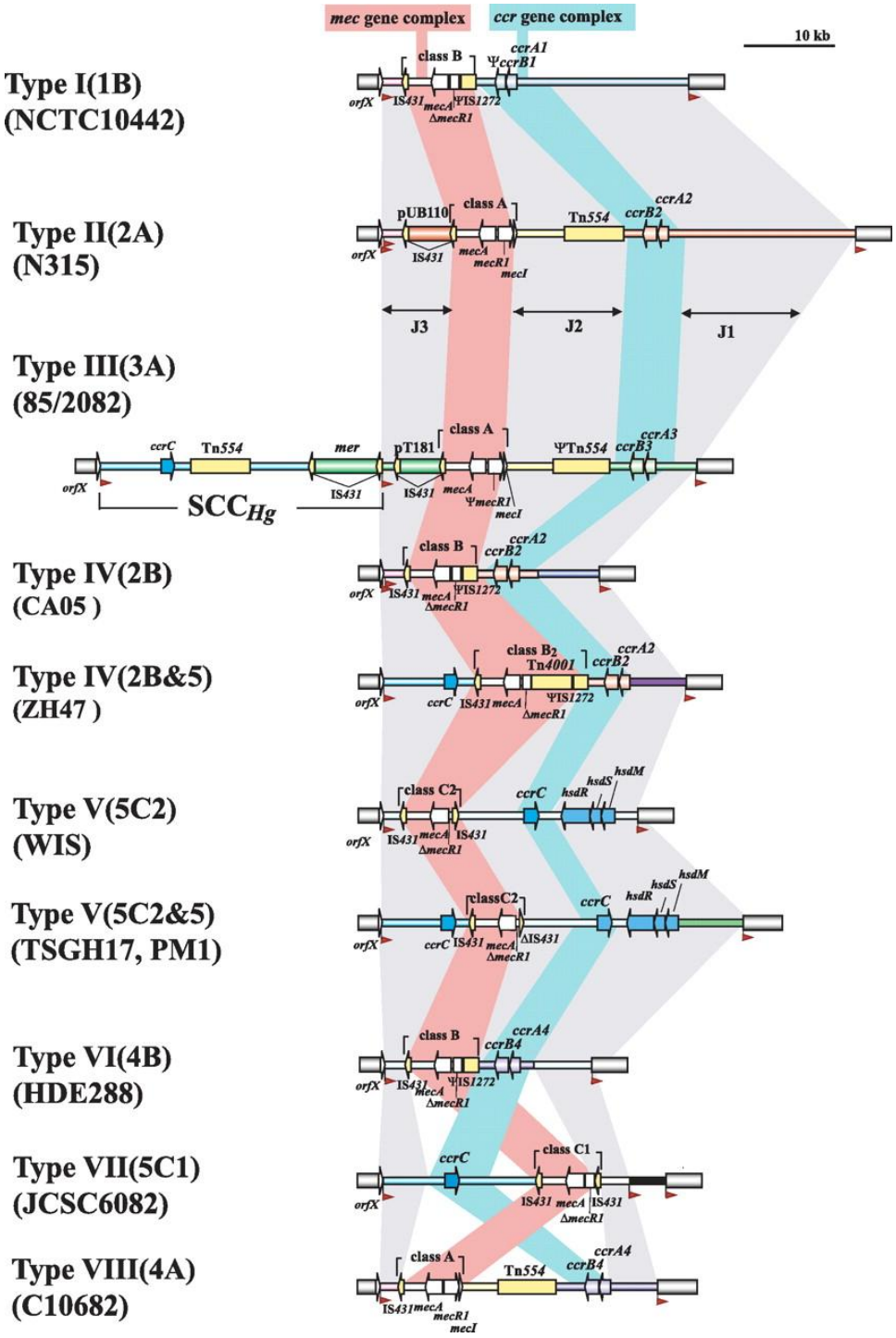
human and bovine populations in the UK and Denmark (Garcia-Alvarez, Holden et al. 2011) and in humans in Germany(Cuny, Layer et al. 2011).

SCC*mec*

In *S. aureus*, there are currently eight different types of SCC*mec* elements described (Ito, Katayama et al. 2001; Ma, Ito et al. 2002; Ito, Ma et al. 2004; Berglund, Ito et al. 2008; Ito, Hiramatsu et al. 2009; Zhang, McClure et al. 2009). SCC*mec* elements are characterised based on the composition of two main components (Ito, Hiramatsu et al. 2009). The *mec* gene complex comprises the *mecA* gene, its regulatory genes (which may be truncated) and insertion sequences. The *ccr* gene complex comprises a combination of different allotypes of *ccrA* and *ccrB* or an unaccompanied *ccrC* gene. SCC*mec* types can be further sub-divided based on the major characteristics of the joining (J) regions.

Figure 1.1 below illustrates the basic structures of the eight SCC*mec* types currently described (Ito, Hiramatsu et al. 2009).

**Figure 1.1 Basic structure of representative SCCmec cassettes. (Ito, Hiramatsu et al. 2009)**



SCC*mec* typing is commonly used in combination with multi-locus sequence typing of MRSA and one study suggests an association between the SCC*mec* type and virulence; for example the toxic shock toxin gene was significantly associated with SCC*mec* type II while the toxin gene *sea* was most often associated with SCC*mec type* III (Kim, Song et al. 2006). In addition, certain SCC*mec* types have been associated with hospital or community-acquired strains of MRSA as discussed below.

Healthcare associated MRSA

MRSA first became a problem in healthcare settings in the 1980s, when the first epidemic strains were described (Witte, Kresken et al. 1997), and is now endemic in many hospitals in many central European countries, Australia and parts of USA , often the result of acquisition of the *mecA* gene by already successful MSSA clones circulating within the hospital (Enright, Robinson et al. 2002; Robinson and Enright 2003). In Europe, there is a high degree of variation in the proportion of MRSA causing bacteraemia based on surveillance data submitted to the European Antimicrobial Surveillance System (EARSS) (Tiemersma, Bronzwaer et al. 2004), and this may be associated with "search and destroy" tactics employed in some northern European countries to screen patients on admission to hospital and subsequent isolation and decolonisation if MRSA is identified (VandenbrouckeGrauls 1996; Wertheim, Vos et al. 2004).

Many risk factors are recognised to be associated with acquisition of HA-MRSA including recent or prolonged hospitalisation, previous use of antimicrobials and surgery (Millar, Loughrey et al. 2007; McCarthy, Sullivan et al. 2010). Table 1.2 below summarises the main characteristics of HA-MRSA and its comparison to CA-MRSA. As well as the successful spread of specific clones within a hospital, some clones have also emerged to pandemic proportions, often being associated with many countries across more than one continent. Evidence for this is provided by large collections of HA-MRSA isolates from many different countries of origin being of a limited number of sequence types (Enright, Robinson et al. 2002). SCC*mec* types most commonly associated with HA-MRSA are I, II, III and IV. Some multi locus

sequence types have only been found to be associated with one SCC*mec* type, for example, ST22 has only been found to harbour SCC*mec* type IV. Conversely, other sequence types have been found to be associated with more than one SCC*mec* type, for example ST8, which can carry SCC*mec* type I, II, III and IV.

Community associated MRSA

In recent years, the number of cases of MRSA infections affecting individuals with no apparent exposure to health care settings and none of the risk factors associated with HA-MRSA has increased (Collignon, Gosbell et al. 1998; Okuma, Iwakawa et al. 2002; Saiman, O'Keefe et al. 2003; Maltezou and Giamarellou 2006) and has included populations such as athletes, prison inmates, children in day care facilities and military personnel (Adcock, Pastor et al. 1998; Stacey, Endersby et al. 1998; Zinderman, Conner et al. 2004; Cohen 2005). The Centres for Disease control and Prevention (CDC) defines these so called community-associated MRSA (CA-MRSA) as being isolated from a person within 48 hours of hospital admission or as an outpatient, who has no prior history of MRSA infection or colonization and no medical history in the last year of hospitalisation, dialysis, surgery or admission to a nursing home or hospice and has no in-dwelling catheters or other medical devices that penetrate the skin barrier (http://www.cdc.gov/MRSA/diagnosis/index.html#vs,). CA-MRSA are generally more susceptible to antimicrobials other than penicillins, and this may be linked to the fact that the most common SCC*mec* cassettes associated with CA-MRSA are the smaller SCC*mec* type IV and type V, which do not carry any other resistance genes (Baba, Takeuchi et al. 2002) (unlike some of those associated with SCC*mec* cassette found in HA-MRSA isolates (Hiramatsu, Katayama et al. 2002). CA-MRSA in the UK is typically susceptible to ciprofloxacin (Otter and French 2008). However, some isolates can be more virulent than HA-MRSA (Baba, Takeuchi et al. 2002). One such virulence factor that is often associated with CA-MRSA is Panton-Valentine leukocidin (PVL) (Vandenesch, Naimi et al. 2003), a cytotoxin responsible for tissue necrosis and destruction of leucocytes, which results in severe disease such as necrotising fascitis and necrotising pneumonia. The definitions that differentiate CA-MRSA from HA-MRSA have, however, begun to blur, since reports of CA-MRSA being responsible

for outbreaks in hospitals (O'Brien, Pearman et al. 1999; Saiman, O'Keefe et al. 2003; Maree, Daum et al. 2007; Popovich, Weinstein et al. 2008).

**Table 1.2 Comparison of HA and CA-MRSA infections**

| | **HA-MRSA** | **CA-MRSA** |
|---|---|---|
| **Population most at risk** | Hospital patients<br>Nursing homes<br>Immunocompromised | People in the community with no known contact or risk factors associated with healthcare settings<br>Prisons<br>Military<br>Sports/athletes |
| **Risk factors** | Recent hospitalisation<br>Antimicrobial use<br>In dwelling catheters | Close contact<br>Poor hygiene |
| **Types of infections** | Surgical wound infections<br>Bacteraemia and septicaemia<br>Infections of implants/ prostheses | Mostly skin and soft tissue infections<br>Rarely severe bacteraemia and septicaemia |
| **Commonly associated sequence types** | ST8, ST22, ST30, ST45, ST250 | ST1, ST30, ST59, ST80, ST398 |
| **SCC*mec* types** | Mainly I, II, III and IV | Mainly IV, V and VII |
| **Association with *pvl* gene** | Rarely | Often |
| **Resistance to other antimicrobials** | Multi-drug resistant | Rarely multi-drug resistant |

Meticillin resistance in other staphylococci

Meticillin resistance in other staphylococci is also mediated by the presence of the same SCC*mec* elements found in MRSA, along with many that largely cannot be typed using the scheme for MRSA SCC*mec* typing methods (Wisplinghoff, Rosato et al. 2003; Ruppe, Barbier et al. 2009; Garza-Gonzalez, Lopez et al. 2010). Coagulase negative staphylococci have been proposed as possible donors of the *mecA* determinant (Wielders 2001; Wu, de Lancastre et al. 2001; Wisplinghoff, Rosato et al. 2003), and this may account for the apparent diversity of SCC*mec* types since comparably fewer SCC*mec* elements have been identified in *S. aureus*.

### 1.5.4 Typing methods of staphylococci

In addition to typing the SCC*mec* of meticillin resistant isolates, molecular typing of both meticillin resistant and meticillin sensitive staphylococci is also important, particularly for the purposes of epidemiology and tracing of hospital outbreaks and subsequent infection control. There are a number of techniques available to type staphylococci, which are briefly discussed below with some of the major advantages and disadvantages discussed.

#### Pulsed field gel electrophoresis

Pulsed field gel electrophoresis is a techniques used to separate large DNA fragments following digestion of the bacterial genome using restriction enzymes (typically *SmaI* in *S. aureus*) (Schwartz and Cantor 1984; Tenover, Arbeit et al. 1995). Different strains of *S. aureus* differ slightly in their genome sequence, and therefore the number of restriction sites varies between strains. As a result, each strain will differ in the number and sizes of fragments produced by restriction. Separation of these bands by electrophoresis results in patterns of banding specific to strains allowing differentiation of different bacterial isolates. This technique is of particular use for epidemiological studies and investigation of outbreaks (Blanc, Struelens et al. 2001), but is timely, expensive and often difficult to compare results between different labs that might use slightly different methodology. Also, the livestock associated strain ST398 cannot be typed by this method due to lack of *SmaI* restriction sites (Bens, Voss et al. 2006).

#### Multi-locus sequence typing

Multi locus sequence typing (MLST) is a highly discriminatory technique first described in the 1990s (Maiden, Bygraves et al. 1998; Spratt 1999; Enright, Day et al. 2000), which involves sequencing of internal fragments of seven housekeeping genes. Each gene sequence is assigned an allele number and the combination of the seven different allele numbers relates to a specific allelic profile or sequence type (ST). Unlike PFGE, MLST allows for higher reproducibility and easier comparison between laboratories.

*Spa* typing

This method sequences the polymorphic X region of the *Staphylococcus* surface protein A (Harmsen, Claus et al. 2003; Strommenger, Braulke et al. 2008). Protein A is a virulence factor associated with binding of immunoglobulin molecules in particular IgG (Forsgren and Sjoquist 1966; Patel, Nowlan et al. 1987) and helps the bacteria evade phagocytic engulfment. *Spa* typing is a fast, reproducible method, used worldwide. Standardisation of nomenclature and the online database of *spa* types (http://www.spaserver.ridom.de), allows comparison of isolates from different countries, but, while inference of the ST of isolates from the spa type is possible, the information in the database may not be sufficient and can therefore result in further testing being necessary to allow sufficient discrimination between isolates.

### 1.5.5 Meticillin resistance in staphylococci isolated from dogs

MRSA

Isolation of MRSA from clinical canine samples was reported as early as 1972 (Olaojo 1972), but it is only in the past 10 to 15 years, since its clinical significance in veterinary medicine has increased, that a greater level of interest has been paid (Pak, Han et al. 1999; Tomlin, Pead et al. 1999). In the past few years, a number of studies have reported the incidence of MRSA isolated from mainly clinical isolates submitted for diagnostic purposes, dogs that were admitted to hospital, or healthy dogs visiting the veterinary practice. Table 1.4 below summarises the findings of these studies. Of those that have investigated healthy dogs, prevalences ranging from 0 – 3% (Rich and Roberts 2006; Vengust, Anderson et al. 2006; Bagcigil, Moodley et al. 2007; Boost, O'Donoghue et al. 2008; Griffeth, Morris et al. 2008; Kottler, Middleton et al. 2008; Abbott, Leggett et al. 2010; Morris, Boston et al. 2010; Gingrich, Kurt et al. 2011) and even as high as 7.8% in a rescue kennel (Loeffler, Pfeiffer et al. 2011) have been reported, but many have involved a limited number of samples from dogs. In clinical canine samples, reported prevalences have ranged from 0.2-7% (Loeffler, Boag et al. 2005; O'Mahony, Abbott et al. 2005; Ruscher, Lubke-Becker et al. 2009; Abbott, Leggett et al. 2010). A limited number of studies

have further characterised the MRSA isolated from both healthy and clinical canine samples, but all have found the strains circulating in the canine population to be the same as or similar to those endemic in the human hospitals in the country (Malik, Coombs et al. 2006; Moodley, Stegger et al. 2006). For example, in the United Kingdom, the dominant strain endemic in human hospitals is EMRSA-15, which has been identified among dogs and staff in a veterinary teaching hospital (Loeffler, Boag et al. 2005). In Ireland, MRSA has been isolated from dogs that were found to be indistinguishable by PFGE to those recovered from both veterinary personnel and human hospitals (O'Mahony, Abbott et al. 2005). A later study identified the predominant MRSA strain found in Irish hospitals, a strain similar to EMRSA-15, to also be isolated from both healthy and sick dogs (Abbott, Leonard et al. 2010).

Meticillin resistant *Staphylococcus pseudintermedius*

Isolation rates of meticillin resistant *S. pseudintermedius* (MRSP), and until its reclassification meticillin resistant *S. intermedius* (MRSI) (Sasaki, Kikuchi et al. 2007), has been reported be higher than that of MRSA (where studies have investigated both the prevalence of MRSA and MRSP) and ranges from 0.8-16.7% in healthy dogs (Vengust, Anderson et al. 2006; Epstein, Yam et al. 2009) and as high as 29.8% from nasal samples of sick dogs (Sasaki, Kikuchi et al. 2007) (Table 1.3). However, despite many reports suggesting a higher prevalence of MRSP in dogs compared to MRSA, studies in the United Kingdom and Canada isolated no MRSP (Loeffler, Pfeiffer et al. 2011) (Rubin and Chirino-Trejo 2011).

**Table 1.3 Summary of studies investigating meticillin resistant staphylococci in dogs**

| Animal | Healthy/ clinical samples | Percentage of samples * (Total tested) | Year | Country | Reference |
|---|---|---|---|---|---|
| **Dog** | Referral hospital Nose and mouth | Total staphylococci – 91%(45)<br>MRSA– 4% (45) | | UK | (Loeffler, Boag et al. 2005) |
| **Dog/ horse/ cat/ rabbit/ seal** | Clinical | MRSA – 0.7% (3400) | January 2003 – October 2004 | Ireland | (O'Mahony, Abbott et al. 2005) |
| **Dog** | Healthy | MRSA – 0% (200)<br>MRSI – 1.5% (200)<br>MR-CNS – 11.5% (200) | March – June 2005 | Slovenia | (Vengust, Anderson et al. 2006) |
| **Dog** | Healthy (vet visiting) | MRSA – 0.4% (255) | 2005 | UK | (Rich and Roberts 2006) |
| **Dog** | Healthy (vet visiting) | MRSA – 0% (100)<br>MR-CNS – 13% (100) | April – November 2005 | Denmark | (Bagcigil, Moodley et al. 2007) |
| **Dog** | Nasal swabs of inpatients and outpatients | SA – 8.8% (57)<br>MRSA – 1.8% (57)<br>MRSP – 29.8% (57) | January –March 2006 | Japan | (Sasaki, Kikuchi et al. 2007) |
| **Cat/ dog** | Healthy | SA – 13.1% (601)<br>MRSA – 3% (601) | | United States | (Kottler, Middleton et al. 2008) |
| **Dog** | Healthy | SA – 8.8% (830)<br>MRSA – 0.7% (830) | | Hong Kong | (Boost, O'Donoghue et al. 2008) |

| Animal | Healthy/ clinical samples | Percentage of samples * (Total tested) | Year | Country | Reference |
|---|---|---|---|---|---|
| **Dog** | Healthy (vet visiting) | CPS – 74% (50)<br>MSSA – 12% (50)<br>No MRSA<br>SI – 68% (50)<br>MRSI – 2% (50) | July 2005 – August 2006 | Pennsylvania , USA | (Griffeth, Morris et al. 2008) |
| | Dogs w/ inflammatory skin disease | MSSA – 8.5% (59)<br>MRSA – 1.7% (59)<br>MSSI – 81% (59)<br>MRSI – 6.8% (59) | | | |
| | Stray | SA – 0% (30) | | | |
| **Dog** | Clinical | MRSA – 0.2%(7490)<br>MRSP – 0.8% (7490) | 2007 | Germany | (Ruscher, Lubke-Becker et al. 2009) |
| **Dog** | Rescue dogs (Apparently healthy | SA – 0 (36)<br>MRSI – 16.7% (36) | | Hong Kong | (Epstein, Yam et al. 2009) |
| **Dog** | Pet therapy | No MRSA isolated<br>One report of MRSA after investigator petted a pug | June – August 2007 | Canada | (Lefebvre and Weese 2009) |
| **Dog** | Rescue Kennel (apparently healthy) | MRSA – 7.8 (129) | December 2008 – January2009 | UK | (Loeffler, Pfeiffer et al. 2010) |
| **Dog** | Rescue kennel (apparently healthy) | MRSA – 0.7% (302)<br>MRSP – 0% (302) | January 2007 – October 2008 | UK | (Loeffler, Pfeiffer et al. 2011) |

| Animal | Healthy/ clinical samples | Percentage of samples * (Total tested) | Year | Country | Reference |
|---|---|---|---|---|---|
| | Vet visiting | MRSA – 3.2% (402)<br>MRSP – 0% (402) | | | |
| **Dog** | Healthy | MRSA – 0.8% (258)<br>MRSP – 6.2%<br>MRSS – 0.8% | | USA | (Morris, Boston et al. 2010) |
| **Dog** | Clinical canine samples<br>Healthy (vet visiting)<br>Clinical samples | MRSA – 1.1% (2864)<br>MRSA – 0.8% (133)<br>MRSA – 7% (143) | 2003-2006<br>October 2005 – May 2006 | Ireland | (Abbott, Leggett et al. 2010) |
| **Dog** | Animal shelter (apparently healthy stray and adoptions) | MRSA – 0.5% (200)<br>MRSP – 3% (200) | May – August 2009 | USA | (Gingrich, Kurt et al. 2011) |
| **Dog** | Small animal hospital prior to admittance | MRSP – 7.4% (814) | September 2007 – January 2009 | Germany | (Nienhoff, Kadlec et al. 2011) |
| **Dog** | Healthy | SP – 87.4% (175)<br>MRSP – 0% (175) | May – November 2008 | Canada | (Rubin and Chirino-Trejo 2011) |

* MRSA meticillin resistant *S. aureus*, MRSI meticillin resistant *S. intermedius*, MR-CNS meticillin resistant coagulase negative *Staphylococcus* spp, SA *S. aureus*, CPS coagulase positive *Staphylococcus* spp, MSSA meticillin sensitive *S. aureus*, MSSI meticillin sensitive *S. intermedius*, MRSP meticillin resistant *S. pseudintermedius*, SP *Staphylococcus pseudintermedius*..

### 1.5.6 Significance of carriage of MRSA and meticillin resistant staphylococci in dogs to public health

Suggestions that dogs may act as a reservoir for infections and re-infections of MRSA and other meticillin resistant staphylococci for humans and other animals have been made as a result of case reports and studies (Duquette and Nuttall 2004; Epstein, Yam et al. 2009; Loeffler and Lloyd 2010). Therefore, while dogs may not be a primary reservoir of MRSA for humans, they do present an important secondary reservoir for re-infection or re-colonisation of humans.

Table 1.4 below summarises some case reports and studies investigating the role of the dog as a reservoir of MRSA. While some of these studies suggest a possible direction of transmission, this relates only directly to the specific infection/colonisation episode and it is impossible to identify, with any degree of certainty, the primary carrier. As discussed above, the strains of MRSA found in dogs are characteristic of the strains isolated in human hospitals (Baptiste, Williams et al. 2005; Loeffler, Boag et al. 2005; O'Mahony, Abbott et al. 2005; Malik, Coombs et al. 2006; Moodley, Stegger et al. 2006), and this makes the most likely scenario to be transfer of MRSA strains from humans to their pets or other animal contacts and subsequent colonisation or infection of the dog. Therefore, while dogs may not be a primary reservoir of MRSA for humans, they do present an important secondary reservoir for re-infection or re-colonisation of humans.

**Table 1.4 MRSA in humans associated with carriage/infections in dogs**

| Year | Case details | Reference |
|---|---|---|
| 1994 | Re-infection of two nurses following initial decolonisation | (Cefai, Ashurst et al. 1994) |
| 2000 | A nurse repeatedly identified as a MRSA carrier shortly after decolonisation and suffering from psoriasis. Pet dog nasally colonised with an identical strain | (van Duijkeren, Wolfhagen et al. 2004) |
| 2000-2001 | Recurrent infection in a patient with diabetes and his wife of an MRSA strain indistinguishable by PFGE to that isolated from their pet dog | (Manian 2003) |
| 2000-2004 | Infection or colonisation of identical MRSA strains in household pets and human contact (household members and veterinary personnel) | (Weese, Dick et al. 2006) |
| 2002 | Recurrent infection of a human patient with a PVL positive MRSA strain identical to that isolated from other household members including their pet dog | (van Duijkeren, Wolfhagen et al. 2005) |
| 2005 | A pet therapy dog found to carry MRSA after visiting a nursing home in which MRSA was known to be circulating | (Enoch, Karas et al. 2005) |
| 2007 | Pet dog euthanized following infection of MRSA found to be identical to a strain isolated from skin biopsies of owner | (Rutland, Weese et al. 2009) |

## 1.6 Importance of antimicrobial resistance in dogs

As discussed above, dogs play an important role in the lives of many people. Understanding the role that dogs may play in the spread and carriage of antimicrobial resistant *E. coli* and staphylococci is very important. Most studies have focused on clinical isolates; however, of equal if not higher importance is the situation in the healthy dog population where there is more opportunity for contact with people. Small studies have been conducted on healthy dog populations, but none in the UK and very few are on a large national scale. It would also be of use to gain an understanding of what factors might be associated with the carriage of antimicrobial resistant *E. coli*.

## 1.7 Aims and approaches

The overall aim of this work was to determine the prevalence of and risk factors for the carriage of antimicrobial resistant *E. coli* and staphylococci in healthy dogs in the UK. Genes responsible for such resistance were investigated and the strains types of some isolates, in order to allow comparison with those genes/strains prevalent in human isolates. To achieve these aims, two studies were carried out:

- A community based study of the prevalence of antimicrobial resistant *E. coli* in the faeces of dogs. (Chapter 3). This study made use of archived samples collected from a previous study investigating the prevalence of *Campylobacter* spp (Westgarth, Porter et al. 2009).
- A nationwide, cross sectional study of the faecal prevalence of antimicrobial resistant *E. coli* and nasal prevalence of staphylococci. (Chapters 4, 5 and 6). This study recruited dogs visiting vet practices during the study period and nasal and faecal swabs were collected from the dogs, as well as a questionnaire administered to allow statistical analysis of risks associated with carriage of antimicrobial resistant *E. coli.*

# Chapter Two

## General Materials and Methods

## 2.1 Sample collection

### 2.1.1 Collection of faecal samples for investigation of the prevalence of AMR *E. coli* in a semi-rural community in Cheshire

Details of the sample population and faecal sample collection from dogs in a semi-rural community in Cheshire have been described previously (Westgarth, Pinchbeck et al. 2007; Westgarth, Porter et al. 2009). Briefly, in a census based study of 1278 households, 260 were identified as dog owning. Owners were asked to provide a fresh faecal sample from their dog and complete a short questionnaire relating to medical history (for example recent history of vomiting, diarrhoea and antimicrobial use). In total, faecal samples were obtained from 183 healthy dogs over a period from August to November 2005. A faecal homogenate was prepared as described in section 2.2 below, and the samples stored at -70°C.

### 2.1.2 Collection of faecal and nasal samples from dogs visiting veterinary practice in the UK

#### Selection of veterinary practices

Dogs attending veterinary surgeries were recruited to the study. Practices were randomly selected from the Royal College of Veterinary Surgeons (RCVS) register and were contacted by telephone to identify the most appropriate member of staff to discuss the project with. An information sheet was then faxed to each practice. A follow up telephone call was made to the identified member of staff to find out if the practice would be willing to take part. If the person could not be contacted after four attempts, or the practices declined to take part, another practice was randomly selected from the register. Initially 50 practices agreed to take part, but due to a low return of samples, a further 37 were recruited.

#### Owner recruitment

A recruitment protocol was sent to each practice. Owners were recruited by veterinary staff during consultation; an owner information sheet was given owners

who agreed to take part, and an informed consent form was completed by both owner and veterinary staff.

Sample collection

For isolation of staphylococci, nasal swabs were taken by the veterinary staff and posted, along with the signed consent form, for processing. The owner was given a faecal sampling pot with a scoop and gloves to collect a fresh faecal sample from their dog at a convenient time. Owners were also given a questionnaire with questions relating to signalment, previous medical treatments, and previous antimicrobial use for both the dog and all other members of the household. The completed questionnaire and faecal sample were posted for processing.

Copies of all forms relating to veterinary practice and owner recruitment and data collection are included in Appendix One.

Ethical approval

Ethical approval for this study was sought and granted by the University of Liverpool Committee on Research Ethics in January 2008 (Reference Number: RETH000118) and by the Department for Environment, Food and Rural Affairs (Defra).

## 2.2 Processing of faecal samples for microbiological testing

On arrival, an equal volume of the faecal sample was added to 5 ml of brain heart infusion broth containing 5% glycerol (BHIG). After thorough mixing, the homogenate was processed for the isolation of antimicrobial resistant and extended spectrum β-lactamase (ESBL) producing *E. coli* as described below. The remainder of the homogenate was poured into a cryovial (Alpha Laboratories, Hampshire, UK) for long-term storage at -70°C, In addition, the neat faecal sample was also frozen.

## 2.3 Isolation of *E. coli* from the faecal homogenate

The faecal homogenate was directly plated, using a sterile swab, onto MacConkey agar and eosin methylene blue agar (all media LabM Ltd, Lancashire, UK) as

previously described (Bartoloni, Benedetti et al. 2006). The two plates were inoculated with the following antimicrobial discs (all discs Mast Group Ltd, Merseyside, UK), with potencies in parentheses: ampicillin (10 μg), augmentin (30 μg), chloramphenicol (30 μg), ciprofloxacin (1 μg), nalidixic acid (30 μg), tetracycline (30 μg) and trimethoprim (2.5 μg). After overnight incubation at 37°C, colonies growing around the discs (one per disc) and characteristic of *E. coli* were selected for antimicrobial disc susceptibility testing.

## 2.4 Isolation of ESBL producing *E. coli* from faecal homogenate

The faecal homogenate was streaked onto two EMBA plates, one supplemented with ceftazidime (1 μg/ml) and the other with cefotaxime (both antimicrobials supplied by Sigma-Aldrich Company Ltd, Dorset, UK) (1 μg/ml). The plates were incubated overnight at 37°C, after which one colony typical of *E. coli* was selected for antimicrobial disc susceptibility testing. In addition, for the nationwide study, to improve isolation of ESBL producing *E. coli*, 0.5 ml of the faecal homogenate was added to 3 ml of buffered peptone water (BPW) and incubated at 37°C overnight (Liebana, Batchelor et al. 2006). If no growth was observed on the initial ESBL screening plates, the BPW was plated onto the same media and incubated as above.

## 2.5 Antimicrobial disc susceptibility testing of *E.coli*

Agar disc diffusion testing was performed using the guidelines of the British Society for Antimicrobial Chemotherapy (Andrews 2007). Colonies less than 24 hours old were suspended in 3 ml of sterile distilled water ($dH_2O$), to a McFarland's standard of 0.5. After being vortexed, 0.5 ml of this suspension was added to 4.5 ml of sterile $dH_2O$. This suspension was used to inoculate the surface of an ISO Sensitest agar plate using a sterile swab. For all isolates, the susceptibility to seven antimicrobial agents was determined: ampicillin (10 μg), augmentin (30 μg), chloramphenicol (30 μg), ciprofloxacin (1 μg), nalidixic acid (30 μg), tetracycline (30 μg) and trimethoprim (2.5 μg). For those isolates suspected of ESBL production, a further panel of antimicrobial agents were tested: aztreonam (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefoxitin (30 μg), cefuroxime (30 μg), cephalexin (30 μg), trimethoprim/sulfamethoxazole (25 μg) and tazobactam (10 μg)/piperacillin (75 μg).

*E. coli* ATCC 25922 was used as a fully susceptible control in testing. Following overnight incubation at 37°C, the zone of inhibition around each disc was measured in mm and recorded. An isolate was considered resistant if a zone less than 10mm was recorded.

## 2.6 Double disc diffusion test for production of ESBL enzymes

Those isolates that were selected from the cephalosporin containing EMBA plates were tested for the production of classical ESBLs using MAST extended β-lactam ID discs (M'Zali, Chanawong et al. 2000). Fresh colonies of no more than 24 hours were suspended in 3 ml of sterile $dH_2O$ to an inoculum density of 0.5 McFarland. An ISO Sensitest agar plate was inoculated, using a sterile swab, and three pairs of cephalosporin discs (with and without clavulanic acid) were placed on the surface. The discs used were ceftazidime (30 µg) and ceftazidime (30 µg) plus clavulanic acid (10 µg), cefotaxime (30 µg) and cefotaxime (30 µg) plus clavulanic acid (10 µg) and cefpodoxime (30 µg) and cefpodoxime (30 µg) plus clavulanic acid (10 µg). All plates were incubated overnight at 37°C. The zones of inhibition around the discs were measured in mm and recorded. ESBL production was confirmed when the zone around the cephalosporin disc is expanded by a minimum of 5 mm in the presence of clavulanic acid. No expansion in zone size by clavulanic acid suggests the presence of either an AmpC β-lactamase, an inhibitor resistant ESBL variant or both an ESBL and an AmpC β-lactamase.

## 2.7 Isolation of staphylococci from nasal swabs

Prior to isolation of staphylococci, an enrichment step was carried out. Swabs were inoculated into nutrient broth supplemented with 6% sodium chloride (NaCl) and incubated overnight at 37°C. Samples were then streaked out onto mannitol salt agar (MSA) supplemented with aztreonam (20 µg/ml) (LabM Ltd, Lancashire, UK) for isolation of total staphylococci and on oxacillin-resistance screening agar (ORSA) supplemented with 2 µg/ml oxacillin (LabM Ltd, Lancashire, UK) and 25 units/ml polymyxin B (LabM Ltd, Lancashire, UK) for isolation of meticillin resistant staphylococci. Following incubation for 24 hours for MSA and up to 48 hours for

ORSA at 37°C, yellow colonies on MSA, blue colonies on ORSA and a selection of other isolates typical of staphylococci were selected for further investigation.

## 2.8 Antimicrobial disc susceptibility testing of staphylococci

Antimicrobial disc susceptibility testing was carried out using the same technique as described above for *E. coli* (Section 2.5). Columbia blood agar containing 2% NaCl was used and the following antimicrobial discs applied to the surface of the agar: co-trimoxazole (25 μg), ciprofloxacin (1 μg), fusidic acid (10 μg), gentamicin (10 μg), meticillin (5 μg), mupirocin (5μg), rifampicin (2 μg), teicoplanin (30 μg), tetracycline (10 μg) and vancomycin (5 μg). Agar plates were incubated at 30°C overnight for coagulase positive isolates and for up to 48 hours for coagulase negative isolates.

## 2.9 Biochemical identification of *E .coli* and staphylococci

### 2.9.1 Gram staining

A thin smear of one or two bacterial colonies was prepared on a glass slide by mixing with a loop full of $dH_20$. After drying in air and heat fixing using a Bunsen flame, the smear was stained. The slide was first flooded with crystal violet for one minute and washed in water. Grams Iodine was then added and left for 30 seconds. After washing in water, the slide was flooded with acetone (Fisher Scientific, Loughborough, UK) for a few seconds, and then washed a third time with water. Finally, the slide was flooded with safranin (all stains supplied by ProLab Diagnostics Inc, Cheshire, UK) for one minute before a final wash with water. After being allowed to dry, the smear was overlaid with immersion oil and viewed under X100 magnification.

### 2.9.2 Oxidase Test

A rapid test to determine the presence of the bacterial enzyme cytochrome oxidase was carried out. One drop of Test Oxidase™ reagent (ProLab Diagnostics Inc, Cheshire, UK) was placed onto a piece of Whatman filter paper. A thin smear was produced using one or two fresh bacterial colonies. The presence of cytochrome

oxidase results in oxidation of the oxidase reagent and a colour change from colourless to blue. *E. coli* is oxidase negative.

### 2.9.3 Catalase test

Isolates were examined for the presence of the catalase enzyme, which breaks down hydrogen peroxide ($H_2O_2$) to prevent intracellular accumulation. A drop of $H_2O_2$ (Sigma-Aldrich Company Ltd, Dorset, UK) was placed on a sterile plate or glass slide and one or two fresh bacterial colonies was added. The production of oxygen bubbles is indicative of the presence of catalase. Both *E. coli* and *Staphylococcus* spp. are catalase positive.

### 2.9.4 Test for lactose fermentation

A fresh colony of the test isolate was streaked onto a quarter of a MacConkey agar plate and incubated overnight at 37°C. Bacteria able to ferment the sugar lactose in the medium produce lactic acid, lowering the pH of the medium resulting in red/pink colonies. *E. coli* can ferment lactose and therefore appear red/pink on MacConkey agar.

### 2.9.5 Indole production

A tryptone soya agar (TSA) plate was streaked with fresh bacterial colonies and incubated overnight at 37°C. To test for the production indole, a piece of Whatman filter paper impregnated with Kovac's reagent (Sigma-Aldrich Company Ltd, Dorset, UK) was placed onto the agar surface. Presence of indole results in a colour change of the reagent to pink/red. *E. coli* is indole positive.

### 2.9.6 Citrate utilization

A Simmon's citrate agar plate was streaked with a fresh bacterial colony. After incubation at 37°C for 24-48 hours, the plates were examined. Bacteria with the enzyme citrase are able to utilize citrate, the sole carbon source in this medium. The pH of the medium is raised which results in a colour change of the medium from green to blue. Citrate negative bacteria, including *E. coli,* are unable to grow on this medium.

### 2.9.7 Tube coagulase test

Isolates were tested for the presence of the enzyme coagulase using rabbit coagulase plasma (Pro-Lab diagnostics, UK). One loop full of between two and four fresh colonies was emulsified into 0.5 ml of the reconstituted rabbit coagulase plasma and mixed gently. Isolates were incubated at 37°C and for between 4 and 24 hours. Every hour for the first four hours, samples were examined for coagulation by gently tipping the tube. If after four hours, there was no coagulation, the samples were re-incubated and examined again after 24 hours. *S. aureus* NTCC 25923 was included as a positive control for this test.

### 2.9.8 Staphylase test

Staphylococci were subjected to the Prolex Staph Latex kit (ProLab Diagnostics Inc, Cheshire, UK), which detects the presence of clumping factor A produced by *S. aureus*. One drop of staph test latex reagent was placed on a white test card and inoculated with one or two fresh colonies. The suspension was mixed and observed for no more than 20 seconds for signs of agglutination of the human fibrinogen. If a positive result is observed, the process is repeated using the negative control latex reagent. An isolate which possesses clumping factor will display rapid agglutination with the test reagent but not when the negative reagent is used. *S. aureus* NTCC 25923 was included as a positive control for this test.

## 2.10 Long term storage of isolates

Isolates were stored at -70°C on Microbank™ beads (ProLab Diagnostics, Cheshire, UK). A large loop full of the freshly grown isolate was added to the Microbank vial. After shaking to mix thoroughly, the excess liquid was removed using a sterile pastette.

## 2.11 Preparation of bacterial lysates for genotypic testing by PCR

### 2.11.1 *E. coli*

One or two fresh bacterial colonies were added to 500 µl of sterile $dH_2O$ in an eppendorf. After thorough mixing, the sample was placed in a heat block at 100°C for 20 minutes before being allowed to cool. Cell lysates were stored at 4°C.

### 2.11.2 Staphylococci

One or two fresh colonies were added to an eppendorf containing 10 µl of lysostaphin (1 mg/ml)(Sigma-Aldrich Company Ltd, Dorset, UK) and 90 µl of sterile $dH_2O$ and then incubated for 10 minutes at 37°C to allow the cell wall to be broken down, followed by 10 minutes at 100 °C. After cooling, 400 µl of sterile $dH_2O$ was added. Cell lysates were stored at 4°C.

## 2.12 Polymerase chain reaction (PCR)

All PCR reactions were carried out using a thermocycler (Applied Biosystems, California, USA) in either 96 well plates, eight strip tubes or individual 0.2 µl PCR tubes depending on the size of the batch. Thermo Scientific (UK) supplied all reagents required for PCR. For PCRs used for identification purposes, a Reddy PCR master mix containing 1.5mM $MgCl_2$, 0.625 U ThermoPrime *Taq* DNA polymerase, 75mM Tris-HCl, 20mM $(NH_4)_2SO_4$, 0.2mM of each dNTP and red dye for electrophoresis. If the PCR products were to be used for other procedures, for example sequencing or digestion with restriction enzymes, a PCR master mix with the same composition as stated above, excluding the red dye, was used. Unless otherwise stated, primers were used at a concentration of 1.25 µM and the reaction was carried out in a total volume of 25 µl containing 24 µl of Reddy PCR master mix and 1 µl of bacterial cell lysate prepared as described above (section 2.11). A negative control (molecular grade $H_2O$ only) was included in every PCR batch. All primers for PCR were supplied by IDT DNA technologies, UK. The sequences of each of the primers, the expected amplicon size of each and the reaction conditions for each PCR are described in Appendix Two.

### 2.12.1 B-D-glucuronidase *uidA* gene PCR for the identification of *E. coli*

Isolates identified as *E. coli* by biochemical testing (section 2.9) were further confirmed by the presence of the *uidA* gene. The primers used were uidAf and uidAr as previously described (McDaniels, Rice et al. 1996). *E. coli* ATCC 25922 was used as a positive control for the above PCR.

### 2.12.2 *bla*$_{TEM}$

PCR to detect the presence of the *bla*$_{TEM}$ gene was carried out using the following primers, TEMbF and TEMbR (Essack, Hall et al. 2001). A bacterial isolate known to carry the *bla*$_{TEM-1}$ variant of the gene, obtained from an in house culture collection, was used as a positive control for this PCR reaction.

#### Sequencing of *bla*$_{TEM}$ genes

For the purposes of sequencing, the same primers were used to produce a template using the master mix without the red dye in a total volume of 50 µl containing 2 µl of bacterial cell lysate. The PCR product was cleaned-up and sent for sequencing as described in section 2.13 below. In addition to the primers used for PCR reaction, the PCR product was also sequenced using three internal primers (described in Appendix Two) to ensure adequate sequencing of the middle section of the gene.

### 2.12.3 *bla*$_{SHV}$

PCR to detect the presence of the *bla*$_{SHV}$ gene was carried out using the following primers, SHVbF and SHVbR (Essack, Hall et al. 2001). A bacterial isolate known to carry the *bla*$_{SHV-12}$ variant of the gene, obtained from an in house culture collection, was used as a positive control for this PCR reaction.

### 2.12.4 *bla*$_{CTX-M}$

PCR to detect the presence of the *bla*$_{CTX-M}$ gene was carried out using the following primers, CTX-MU1 and CTX-MU2 (Boyd, Tyler et al. 2004). A bacterial isolate known to carry the *bla*$_{CTX-M-12}$ variant of the gene, obtained from an in house culture collection, was used as a positive control for this PCR reaction.

#### Sub-typing of *bla*$_{CTX-M}$ genes

Isolates found to carry a *bla*$_{CTX-M}$ gene were subjected to further PCR assays to assign the gene to a group. Simplex PCR reactions were carried out using the following primers: CTX-M-1f and CTX-M-1r for group 1, CTX-M-2f and CTX-M-

2r for group 2, and CTX-M-9f and CTX-M-9r for group 9 (Batchelor, Hopkins et al. 2005; Hopkins, Batchelor et al. 2006).

Sequencing of *bla*$_{CTX-M}$ genes

For the purposes of sequencing, the appropriate CTX-M grouping PCR was repeated with the PCR master mix without the red dye in a total volume of 50 µl containing 2 µl of the bacterial cell lysate. The PCR template was clean-up and sent for sequencing as described in section 2.13 below.

### 2.12.5 Multiplex PCR to detect the presence of *bla*$_{ampC}$ genes

The presence of *bla*$_{ampC}$ genes was investigated using a multiplex PCR containing six sets of *ampC* specific primers, which produce different sized amplicons, allowing easy differentiation of the specific families of the plasmid mediated *ampC* genes. The primers used were MOXMf, MOXMr, CITMf, CITMr, DHAMf, DHAMr, ACCMf, ACCMr, EBCMf, EBCMr, FOXMf, and FOXMr (Perez-Perez and Hanson 2002).

The reaction was carried out in a total volume of 25 µl containing 1.5 mM of $MgCl_2$, 20 mM of Tris-HCl, 0.2 mM of each of the deoxynucleoside triphosphates and 1.25 U of ThermoPrime *Taq* DNA polymerase. The primers were included at the following concentrations, 0.6 µM of primers MOXMf, MOXMr, CITMf, CITMr, DHAMf and DHAMr, 0.5 µM of primers , ACCMf, ACCMr, EBCMf and EBCMr, and 0.4 µM of primers FOXMf, and FOXMr. Positive controls for each of the primer pairs (excluding MOXM) were included in each reaction.

PCR for amplification of the full *cmy* gene for sequencing

For samples where a product of band size 462 bp was observed, which corresponds to product amplified by the CTIM primer pair, the full *bla*$_{CMY}$ gene was amplified using primers cmy25f and cmy2Dr (Liebana, Gibbs et al. 2004) and sent for sequencing (described in Section 2.13). The reaction was carried out in a total volume of 50 µl containing 48 µl of the master mix and 2 µl of bacterial cell lysate.

For sequencing of *bla*$_{CMY}$ genes, internal primers (detailed in Appendix Two) were also used to obtain the middle portion of the gene sequence.

### 2.12.6 Plasmid mediated quinolone resistance (*qnr*) genes

All *E. coli* isolates resistant to ciprofloxacin and/or nalidixic acid were subjected to a multiplex PCR to detect *qnrA, qnrB and qnrS* genes. The primers used were qnrAf, qnrAr, qnrBf, qnrBr, qnrSf and qnrSr (Robicsek, Strahilevitz et al. 2006). Bacterial isolates known to carry each of the three gene variants, obtained from an in house culture collection, were used as positive controls for this PCR reaction.

### 2.12.7 *dfr* genes

Isolates resistant to trimethoprim were subjected to a number of PCR assays to detect the presence of certain variants of dihydrofolate reductase (*dfr*) genes (Gibreel and Skold 1998; Lee, Oh et al. 2001). Multiplex PCRs were carried out for the detection of *dfrA1* and *dfrA9* (multiplex 1) and *dfrA7, dfrA12, dfrA13* and *dfrA17* (multiplex). *dfrA8* detection was carried out using a simplex PCR, while one set of primers was used for the detection of both *dfrA5* and *dfrA14.* All primers were used at a concentration of 2 µM. Control strains known to carry *dfrA1, dfrA12* or *dfrA14* were included in the relevant assay. The presence of restriction sites in *dfrA13* (*Eco*RV), *dfrA14* (*Eco*RI) and *dfrA17* (*Pst*I) allowed differentiation between these and *dfrA12, dfrA5* and *dfrA17* respectively.

The restriction enzyme reaction was carried out in a total volume of 25 µl which comprised 12.5 µl of the PCR product, 2.5 µl of the appropriate SuRE/Cut buffer (Roche Diagnostics, Sussex, UK) and 1 U of restriction enzyme (Roche Diagnostics, Sussex, UK). Following incubation at 37°C for an hour, DNA fragments were visualized by agarose gel electrophoresis (Section 2.12.14). The presence of two different sized bands demonstrated restriction of the PCR product by the enzyme.

### 2.12.8 Multiplex PCR for the identification of *tet* genes

Multiplex PCR reactions were carried out to detect the presence of tetracycline resistance genes in those isolates with phenotypic resistance to tetracycline (Ng, Martin et al. 2001). Multiplex assay A detected the presence of *tet(A), tet(E)* and

*tet(G),* and multiplex assay B detected the presence of *tet(B), tet(C)* and *tet(D).* Primers were used at the following concentrations, 0.5 µM or tetAf, tetAr, tetEf, tetEr, tetGf and tetGr, 0.2 µM of tetBf, tetBr, tetCf and tetCr, and 0.2 µM of tetDf and tetDr.

2.12.9 PCR for the detection of the *mecA* gene responsible for meticillin resistance

Isolates suspected of meticillin resistance were subjected to PCR to detect the presence of the *mecA* gene. The primers used were mecAf and mecAr (Vannuffel, Gigi et al. 1995). A MRSA isolate, EMRSA-15, obtained from an in house culture collection, was used as a positive control for this PCR reaction.

2.12.10PCRs for the identification of *S. aureus*

Isolates identified as *S. aureus* by phenotypic tests, as described above (Section 2.9), were subject to PCRs to detect the presence of the *S. aureus* specific *femA* (Francois, Pittet et al. 2003) and *nuc* (Brakstad, Aasbakk et al. 1992) genes. A bacterial isolate confirmed as *S. aureus* was included as a positive control for each of the PCR reactions below.

2.12.11PCR for identification of Coagulase positive staphylococci (CoPS)

PCR-restriction fragment length polymorphism approach for identification of *S. pseudintermedius*

The primers for this PCR assay detects the gene encoding phosphoacetyltransferase (*pta*), ptaF1 and ptaR1 (Bannoehr, Franco et al. 2009). This PCR reaction was carried out using the PCR master mix, without the red dye, in a total volume of 50 µl containing 2 µl of the bacterial cell lysate.

Following completion of the PCR reaction, 25 µl was incubated with 5U of the restriction enzyme *MboI* (New England Biolabs, Massachusetts, USA) at 37°C for 2 hours before being run on agarose gel alongside the undigested PCR product. When incubated with *MboI,* all *S. pseudintermedius* isolates produced two different sized restriction fragments of 213 and 107 base pairs (bp). Two bands were also present in *S.aureus* isolates, but these were of 156 and 164 bp, and therefore appeared as one

single band. The *pta* genes of other members of the *Staphylococcus intermedius* group (SIG) contained no restriction site, and therefore only one band of 310 bp was present in both digested and undigested samples.

Multiplex PCR for identification of CoPS

Any CPS that was not identified as either *S. aureus* or *S. pseudintermedius* by previous methods were subjected to a multiplex PCR in order to assign them to one of the seven CPS. The primers used were au-F3 and au-nucR, for identification of *S.aureus;* in-F and in-R3, for the identification of *S. intermedius;* sch-F and sch-R, for the identification of *S. schleiferi;* dea-F and dea-R, for the identification of *S. delphini* group A; deb-F and deb-R, for the identification of *S. delphini* group B; hy-F1 and hy-R1, for the identification of *S. hyicus;* and pse-F2 and pse-R5, for the identification of *S. pseudintermedius* (Sasaki, Tsubakishita et al. 2010).

Staphylococcal 16s rRNA PCR and sequencing

For any isolates that were unable to be assigned to species using the methods described above, the 16S rRNA gene was amplified using universal primers PA and PH* (Edwards, Rogall et al. 1989) and sequenced to allow identification.

Following amplification of the 16S rRNA gene, the samples were sent directly to Source BioScience for clean-up and sequencing. The obtained sequences were checked and edited as necessary using Chromas Pro (Technelysium Pty Ltd) to produce a contiguous sequence, and alignment to other 16S rRNA sequences was investigated using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi). A 16S rRNA sequence similarity of >98% was used to identify isolates at the species level.

2.12.12*Spa* gene PCR and sequencing for typing of *S. aureus*

The X region of the *Staphylococcus* protein A (*spa*) gene was amplified using primers 1113f and 1514r (Harmsen, Claus et al. 2003). The obtained sequence chromatograms were uploaded into the Ridom StaphType program (Ridom GmbH,

Germany), which automatically checks the sequences for quality, detects any *spa* repeats and assigns a *spa* type.

2.12.13SCC*mec* cassette typing of MRSA

The specific SCC*mec* type carried by the meticillin resistant staphylococci was investigated using a modified version of the method reported by (Oliveira and de Lencastre 2002). The primers corresponding to loci G and H, which differentiate variant I from IA and II from IIA respectively, were excluded from the assay. Two separate multiplex PCRs were performed to allow better differentiation between PCR products of similar size. Table 2.1 below summarizes the primers used in each multiplex and which SCC*mec* type the presence of a particular product corresponds to. Primers were used at a concentration of 0.2 µM.

**Table 2.1 Multiplex PCRs for typing of SCC*mec* cassettes of meticillin resistant staphylococci**

| PCR | Primer | SCC*mec* type |
|---|---|---|
| **A** | KDP F1 | II |
| | KDP R1 | |
| | MECI P2 | II, III |
| | MECI P3 | |
| | RIF5 F10 | III |
| | RIF5 R13 | |
| **B** | CIF2 F2 | I |
| | CIF2 R2 | |
| | DCS F2 | I, II, IV |
| | DCS R1 | |
| | RIF4 F3 | III |
| | RIF4 R9 | |

2.12.14Visualization of PCR products by agarose gel electrophoresis

PCR products were visualized on 1.5-2% (w/v) agarose gels. The gel was made using high pure low EEO agarose (Biogene Ltd, UK) and 1X Tris acetate EDTA (TAE) buffer (Sigma-Aldrich, UK). Ethidium bromide (Sigma Aldrich, UK) at a concentration of 0.25-0.4µg/µl was added to the gel prior to begin poured into the mould. When the Reddy PCR master mix was used, 12.5 µl of each sample was

added to each well. If the PCR product was to be used for sequencing or for restriction enzyme digestion, 5 µl of the product was added to 1 µl of 6X loading dye (AbGene, Epsom, UK) before being added to the wells. The first well of each gel contained 6 µl of Gel pilot 100bp Plus Ladder (AbGene, Epsom, UK) to allow accurate sizing of the PCR products. The gels were run in an electrophoresis tank at 120V for 25 minutes for small gels, while large gels were run for 75 minutes. Visualization and photography of the PCR products was under ultra violet (UV) light using an UVItech transilluminator and UVI Pro MW (UVI Tech, UK).

## 2.13 Sequencing of *bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{cmy}$, genes

All PCR reactions for preparation of templates for sequencing were carried out as described above for each specific gene. Following confirmation that the gene was successfully amplified by agarose gel electrophoresis (section 2.12.14), the product was purified by either PEG precipitation (Appendix Two), or the Wizard™ sv gel and PCR clean-up system (Promega, UK) following the manufacturer's instructions.

Either Source BioScience or Eurofins MWG Operon sequenced the purified products on both strands, with the same primers used for template preparation and internal primers as described above. The obtained sequences were viewed and checked using ChromasPro (Technelysium Pty Ltd). A contiguous sequence was constructed and compared with those in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain the specific gene variants.

# Chapter Three

## The prevalence of antimicrobial resistant *Escherichia coli* among dogs in a cross sectional, community based study

## 3.1 Introduction

Antimicrobial resistance (AMR) is a commonly encountered problem in both human and animal medicine. It can lead to failures in treatment, increased morbidity and mortality, and a greater financial burden on healthcare services. Use of antimicrobials may exert a selection pressure upon, and therefore select for bacteria that have acquired resistance. Such resistance can be acquired either by mutation of chromosomal DNA; or by horizontal transfer of resistance determinants via transmissible elements, such as plasmids. One particular mechanism of AMR is the production of extended spectrum β-lactamases (ESBL), enzymes capable of hydrolysing third generation cephalosporins (Livermore 2008). A further important resistance mechanism, due to their broad spectrum of resistance to β-lactams and the ineffectiveness of β-lactamase inhibitors, are plasmid mediated AmpC enzymes (Philippon, Arlet et al. 2002).

*Escherichia coli* can be readily isolated from the gastrointestinal tract of many animal species, including humans and dogs, and are therefore a good indicator of reservoirs of AMR (van den Bogaard and Stobberingh 2000). The presence of *E. coli* in the intestinal tract of humans, dogs and most animal species, results in its exposure to any antimicrobial agents that are administered and which enter the gastrointestinal tract. This exposure can select for *E. coli* which have acquired resistance determinants or mutations encoding antimicrobial resistance. In addition, there is the potential for AMR *E. coli* to act as a reservoir of resistance determinants for pathogenic bacteria (Guardabassi, Schwarz et al. 2004).

A significant quantity of antimicrobials sold in the UK is for veterinary use (VMD 2000). Since 2002, total veterinary sales of therapeutic antimicrobials have decreased (440 tonnes in 2002 to 387 tonnes in 2008), however, the total sales of drugs indicated for use in non food producing animals only, has increased, with a notable rise in sales of therapeutic antimicrobials licensed for use in dogs only (4.5 tonnes in 2002 to 7.3 tonnes in 2008) (VMD 2009). Increased use of antimicrobials in dogs, coupled with selection pressures for resistance may result in higher carriage of AMR bacteria.

Humans and dogs are often in close contact and as a result, there is a risk of transfer of bacteria, resistant or otherwise, from one to the other, which may influence the success of antimicrobial treatment required by the individual if such bacteria cause disease. AMR *E. coli* have been isolated from clinical samples from dogs (Normand, Gibson et al. 2000; Normand, Gibson et al. 2000) and other animals (Lanz, Kuhnert et al. 2003). In addition, faeces from healthy dogs (De Graef, Decostere et al. 2004; Costa, Poeta et al. 2008) and various other animals (Moyaert, De Graef et al. 2006) have been shown to harbour AMR *E. coli*. ESBL producing *E. coli* have been isolated from both healthy dogs and those with clinical infections (Moreno, Bello et al. 2008). However, with one exception in Sweden (SVARM 2006), previous studies investigating AMR *E. coli* in healthy dogs have been limited in their sample size, and in some countries, including the United Kingdom no such studies have been published.

The aim of the current study was to determine the prevalence of AMR *E. coli* and ESBL producing *E. coli* in the faeces from healthy dogs in a census-based, cross sectional study of a community in Cheshire, UK.

## 3.2 Materials and Methods

### 3.2.1 Collection of faecal samples

Details of the sample population and faecal sample collection from dogs in a semi-rural community in Cheshire have been previously described (Westgarth, Pinchbeck et al. 2007; Westgarth, Porter et al. 2009). Briefly, in a census based study of 1278 households, 260 were identified as dog owning. Owners were asked to provide a fresh faecal sample from their dog and complete a short questionnaire relating to medical history (for example recent history of vomiting, diarrhoea and antimicrobial use). Faecal samples were obtained from 183 healthy dogs over a period from August to November 2005. The fresh faecal samples were mixed with an equal volume of Brain Heart Infusion broth with 5% glycerol (BHIG) and the homogenate stored below -70°C. These faecal homogenates were thawed and processed as described below.

### 3.2.2 Isolation and identification of E. coli

AMR *E. coli* were detected using the direct plating method previously described (Bartoloni, Cutts et al. 1998; Bartoloni, Benedetti et al. 2006). The thawed faecal homogenate was plated directly onto MacConkey Agar and Eosin Methylene Blue Agar (EMBA) using a plain cotton swab and the following antimicrobial discs (MAST Group LTD), were applied; ampicillin (10µg); augmentin (30µg); chloramphenicol (30µg); ciprofloxacin (1µg); nalidixic acid (30 µg); tetracycline (30µg); and trimethoprim (2.5µg). After overnight incubation at 37°C, those colonies growing within the zone of inhibition around each disc on both sets of plates and whose morphology resembled *E. coli,* were selected for subsequent investigation. To screen for ESBL producing E.coli, the faecal homogenates were directly streaked onto EMBA containing ceftazidime (1µg/ml) and EMBA containing cefotaxime (1µg/ml), supplied by Sigma-Aldrich, (Liebana, Batchelor et al. 2006). In addition, to allow for non-selective isolation of *E.coli* Faecal homogenates were also directly streaked onto EMBA containing no antimicrobials. Three isolates from this plate were selected for subsequent antimicrobial testing. Presumptive *E. coli* resistant to at least one antimicrobial were confirmed by biochemical testing (Gram stain and testing for catalase production, lack of oxidase, lactose fermentation, indole production and inability to utilise citrate as a carbon source) and *uidA* PCR (McDaniels, Rice et al. 1996). All media were supplied by Lab M (IDG).

### 3.2.3 Antimicrobial susceptibility testing

Antimicrobial disc diffusion testing was performed in accordance with BSAC guidelines (Andrews 2007). The following antimicrobial discs were used: ampicillin (10µg); augmentin (30µg); chloramphenicol (30µg); ciprofloxacin (1µg); nalidixic acid (30µg); tetracycline (30µg); and trimethoprim (2.5 µg). For those isolates suspected of ESBL production, a further panel of nine antimicrobial agents were also tested; aztreonam (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefoxitin (30µg), cefuroxime (30µg), cephalexin (30µg), trimethoprim-sulfamethoxazole (25µg), gentamicin (10µg) and tazobactam (10µg)/piperacillin (75µg). The reference strain *E. coli* ATCC 25922 was used for quality control during testing.

### 3.2.4 Phenotypic confirmation of ESBL production

Those isolates which were selected from the cephalosporin containing EMBA plates, and therefore suspected of ESBL production, were tested using the MAST double disc diffusion method previously described (M'Zali, Chanawong et al. 2000). Briefly, an Iso-Sensitest agar plate was inoculated with the isolate and three pairs of cephalosporin discs (with and without clavulanic acid), were placed on the surface of the agar plate; ceftazidime (30µg) and ceftazidime (30µg) plus clavulanic acid (10µg); cefotaxime (30µg) and cefotaxime (30µg) plus clavulanic acid (10µg); and cefpodoxime (30µg) and cefpodoxime (30µg) plus clavulanic acid (10µg). The plates were incubated aerobically at 37°C for 18-24 hours and zone diameters around each disc were measured. ESBL production was confirmed when the zone around the cephalosporin disc was expanded in the presence of the clavulanic acid by a minimum of 5mm according the manufacturer's instructions (MAST Group Ltd). The AmpC phenotype was suggested when the presence of clavulanic acid did not result in a decrease in the zone of inhibition.

### 3.2.5 Characterisation of antimicrobial resistance genes

PCR assays were carried out on all isolates suspected of ESBL production for the presence of $bla_{SHV}$, $bla_{TEM}$, $bla_{CTX-M}$, $bla_{AmpC}$ and *qnr* genes (Essack, Hall et al. 2001; Perez-Perez and Hanson 2002; Boyd, Tyler et al. 2004; Robicsek, Strahilevitz et al. 2006). For isolates showing resistance to ampicillin, the presence of $bla_{TEM}$ and $bla_{SHV}$ genes was tested. Those isolates resistant to trimethoprim were examined by PCR for the presence of *dfrA1, dfrA5, dfrA7, dfrA8, dfrA9, dfrA12, drfA13, dfrA14* and *dfrA17* genes (Gibreel and Skold 1998; Lee, Oh et al. 2001). In addition, the presence of *qnrA, qnrB and qnrS* genes were tested by PCR in all isolates. Finally, isolates resistant to tetracycline were screened by PCR for the following genes: *tet(A), tet(B), tet(C), tet(D), tet(E)* and *tet(G*) (Ng, Martin et al. 2001). Conditions and references for each PCR assay are detailed in Appendix Two. A positive control (an isolate known to carry the gene under investigation) was included in each PCR assay.

### 3.2.6 Sequencing of *bla*$_{CMY}$ and *bla*$_{TEM}$ genes

For sequencing of the *bla*$_{CMY}$ gene, oligonucleotide primers were used to amplify the entire gene.(Liebana, Gibbs et al. 2004) For *bla*$_{TEM}$ sequencing, the amplicon was obtained using the primers for initial detection. The amplicons were cleaned using the Wizard SV gel and PCR clean-up system (Promega), and sequenced on both strands with additional primers used for the internal sequence (two for *bla*$_{CMY}$ sequencing and three for *bla*$_{TEM}$ sequencing). The sequences were compared with those in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to obtain the specific *bla*$_{CMY}$ *and bla*$_{TEM}$ gene variants.

## 3.3 Results

A total of 69 unique AMR *E. coli* isolates were collected from the faeces of 53 (29%, 95%CI 22.4-35.5) of the 183 dogs tested in this study. Thirteen dogs were found to harbour more than one unique isolate of *E. coli* whose antimicrobial resistance profiles differed. The susceptibility of isolates to seven antimicrobial agents is shown in Table 3.1 below.

**Table 3.1 Frequency of antimicrobial resistance of *E. coli* from 183 dog faecal samples**

| Antimicrobial agent | Total isolates | Number* | Percentage* | 95% CI |
|---|---|---|---|---|
| **Ampicillin** | 54 | 44 | 24.0 | 17.9-30.2 |
| **Augmentin** | 10 (+4 intermediate) | 7 | 3.8 | 1.0-6.6 |
| **Chloramphenicol** | 5 | 5 | 2.7 | 0.4-5.1 |
| **Ciprofloxacin** | 4 (+2 intermediate) | 4 | 2.2 | 0.1-4.3 |
| **Nalidixic acid** | 6 | 6 | 3.3 | 0.7-5.9 |
| **Tetracycline** | 46 | 36 | 19.7 | 13.9-25.4 |
| **Trimethoprim** | 33 | 31 | 16.9 | 11.5-22.4 |
| **MDR** | 30 | 28 | 15.3 | 10.4-20.5 |

* Number and percent of dogs carrying an isolate with resistance to at least one antimicrobial

Twenty-four percent of dogs (95%CI 17.9-30.2) harboured at least one isolate with ampicillin resistance, 19.7% (95%CI 13.9-25.4) with tetracycline resistance, and 16.9% (95%CI 11.5-22.4) had resistance to trimethoprim. The percentage of dogs with *E. coli* with resistance to the other antimicrobials tested was less than 4%.

Twenty eight (15.3%, 95%CI 10.4-20.5) of the 183 dogs sampled harboured at least one multidrug resistant (MDR, resistant to three or more antimicrobial classes) isolate. Thirty (44%) of the 69 AMR *E. coli* isolates were shown to be MDR and 19 (28%) were resistant to two antimicrobial classes. The resistance profiles of the 69 isolates are shown in Table 3.2 below.

**Table 3.2 Profile of resistance among the 69 *E. coli* faecal isolates from dogs**

| Resistance profile | Number of isolates (%) |
|---|---|
| **AMP, TET, TMP** | 18 (26.1) |
| **TET** | 9 (12.9) |
| **AMP** | 9 (12.9) |
| **AMP, TMP** | 7 (10.1) |
| **AMP, TET** | 7 (10.1) |
| **AMP, AMC, TET** | 4 (5.8) |
| **TET, TMP** | 2 (2.9) |
| **AMP, TMP, TET, CHL** | 2 (2.9) |
| **AMP, AMC, CIP, NAL** | 2 (2.9) |
| **AMP, AMC** | 2 (2.9) |
| **TMP** | 1 (1.4) |
| **NAL, TMP** | 1 (1.4) |
| **NAL, TET** | 1 (1.4) |
| **CIP, NAL, TET** | 1 (1.4) |
| **AMP, TMP, CHL** | 1 (1.4) |
| **AMP, AMC, CHL, TET** | 1 (1.4) |
| **AMP, AMC, CHL, CIP, NAL, TET, TMP** | 1 (1.4) |

AMC Augmentin, AMP Ampicillin, CHL Chloramphenicol, CIP Ciprofloxacin, NA Nalidixic acid, TET Tetracycline, TMP Trimethoprim.

The most common resistance profile was ampicillin-tetracycline-trimethoprim resistance, which was found in 26% of the isolates; followed by ampicillin only and tetracycline only resistance; both of which were found in 13% of isolates each; and ampicillin-trimethoprim and ampicillin-tetracycline resistance, which were each found in 10% of isolates.

Only one dog, which was found not to carry AMR *E. coli*, was recorded as being on a course of antimicrobials when the faecal sample was collected. Of 15 dogs reported to have had a course of antimicrobials in the previous month, five (33%) carried

AMR *E. coli*. This was also true of nine of 37 (24%) dogs whose owners reported antimicrobial use in the previous year. A similar prevalence however was observed among the dogs with no reported antimicrobial use.

The presence of *dfr* genes were investigated in those isolates resistant to trimethoprim, and of 33 trimethoprim resistant isolates, 11 were found to harbour *dfrA1*, 8 had *dfrA5* and 3 possessed *dfrA14* genes. The remaining 11 trimethoprim resistant isolates were negative for these genes in addition to *dfrA7, A8, A9, A12, A13* and *A17*. Of the 45 isolates resistant to tetracycline, 12 were found to harbour *tet(B)*, and none of the *tet* genes tested (*tetA, B, C, D, E* and *G*) could be detected in the remaining 33 isolates. Of the 54 ampicillin resistant isolates, 39 were found to harbour a $bla_{\text{TEM}}$ gene. None were positive for the detection of a $bla_{\text{SHV}}$ gene. No *qnr* genes were detected in any of the isolates tested, including those resistant to nalidixic acid.

The results relating to the nine *E. coli* isolates selected from the plates screening for ESBL production are in Table 3.3 below. Only one isolate was confirmed using the MAST double disc diffusion method as an ESBL-producer. A variety of resistance phenotypes were observed in these isolates, with only two sharing the same resistance profile. The nine isolates were tested for the presence of β-lactamase enzymes. A $bla_{\text{TEM}}$ gene was detected in two isolates, which were identified as $bla_{\text{TEM -1}}$ by sequencing. In seven isolates, $bla_{\text{AmpC}}$ was detected and were all identified as the $bla_{\text{CMY-2}}$ gene. No $bla_{\text{CTX-M}}$ or $bla_{\text{SHV}}$ genes were amplified from any of the isolates. A $bla_{\text{CMY-2}}$ gene was found in the only isolate to test positive for ESBL production, but no other genes tested were found.

**Table 3.3 Characteristics of nine isolates recovered by ESBL screening methods**

(The shaded row indicates the isolate positive for ESBL production.)

| Isolate ID | (CAZ+CA*)-CAZ (mm) | (CPD+CA)-CPD (mm) | (CTX+CA)-CTX (mm) | Genes amplified by PCR | Resistance profile |
|---|---|---|---|---|---|
| **066B(CFX)** | 0 | 0 | -1 | *bla*$_{CMY-2}$, *bla*$_{TEM-1}$, *tet(B)* | AMP, LEX, CXM, FOX, CAZ, CRO, TET |
| **084B** | -5 | 0 | -6 | *bla*$_{CMY-2}$ | AMP, AMC, LEX, CXM, FOX, CAZ, CRO |
| **084F** | 0 | 1 | -2 | *bla*$_{CMY-2,}$ *bla*$_{TEM-1}$ | AMP, AMC, LEX, CXM, FOX, CAZ, CRO, CIP, NA, TMP, SXT, TET, CHL |
| **091E** | 0 | 0 | -1 | *bla*$_{CMY-2}$ | AMP, AMC, LEX, CXM, FOX, CRO, ATM, CIP, NA |
| **141D** | 1 | 0 | -4 | none | AMP, TET |
| **156A** | 0 | 1 | **5** | *bla*$_{CMY-2}$ | AMP, AMC, LEX, CXM, FOX, CAZ, CRO, TET |
| **157A** | 0 | 1 | -5 | *bla*$_{CMY-2}$ | AMP, AMC, LEX, CXM, FOX, CAZ, CRO, TET |
| **157C** | 0 | 0 | -4 | *bla*$_{CMY-2}$ | AMP, AMC, LEX, CXM, FOX, CAZ, CRO, ATM, TET |
| **172G** | 0 | 0 | 4 | none | GEN, AMP, AMC, TZP, LEX, CRO |

AMC Augmentin, AMP Ampicillin, ATM Aztreonam, CAZ ceftazidime, CHL Chloramphenicol, CIP Ciprofloxacin, CRO Ceftriaxone, CXM Cefuroxime, FOX Cefoxitin, GEN Gentamicin, LEX Cefalexin, NA Nalidixic acid, SXT Trimethoprim-sulfamethoxazole, TET Tetracycline, TMP Trimethoprim, TZP Tazobactam-piperacillin.

*Disc contained both the cephalosporin and the β-lactamase inhibitor clavulanic acid.

## 3.4 Discussion

This study aimed to estimate the prevalence of AMR *E. coli* carriage in a community of healthy dogs and found that AMR carriage was widespread in the dogs sampled (29%). MDR *E. coli* was also found in these dog samples (15.3%). The most common antimicrobials the isolates were resistant to were ampicillin, tetracycline and trimethoprim. This is also reflected in the most common resistance phenotype observed and might reflect the mobile nature of the genes responsible for these resistance phenotypes, and that there are numerous variants of these resistance genes.

The prevalence observed for ampicillin resistance was higher than previously reported in healthy dogs in Portugal (Costa, Poeta et al. 2008), but similar to a study investigating, among others, healthy cat populations (Moyaert, De Graef et al. 2006). Resistance to other antimicrobials (augmentin, chloramphenicol, quinolones, tetracycline, and trimethoprim) is comparable across all three studies. The resistances observed in the present study are much higher than reported in a study of healthy dogs in Sweden (SVARM 2006), which may reflect differences in methodology. It is important to note that the dogs sampled in the present study were considered healthy based on questionnaire responses made by the owner, which may differ from other studies' definitions of healthy. When compared to studies investigating clinical isolates, the prevalence of resistance to ampicillin (Normand, Gibson et al. 2000) and tetracycline (De Graef, Decostere et al. 2004) was lower in the current population. Resistance to other antimicrobials was similar. In clinical canine isolates of *E. coli* in Denmark, the prevalence of AMR was comparable for all antimicrobials with the exception of nalidixic acid which was higher in the Danish study (12.5% compared to 3.3%) (Pedersen, Jensen et al. 2007). Differences in the prevalence of resistance observed may be due differences in the interpretation of the zone sizes or MICs observed or differences in how intermediate measurements were classified. This highlights how difficult it can be to compare different studies when a variety of methodologies and guidelines of interpretation are used and emphasises the need for more standardised methods. This may prove difficult to achieve both on an international level and between the human and veterinary profession. However it is likely that, compared to isolates from clinical cases, that apparently healthy,

household dogs sampled in the current study will have had less exposure to antimicrobials and hospital environments both of which have been shown previously to increase the prevalence of AMR (Dunowska, Morley et al. 2006; Moreno, Bello et al. 2008).

The gene responsible for conferring trimethoprim resistance could be determined in only 22 of the 33 isolates tested, the genes encoding *dfrA1*, *dfrA5* and *dfrA14* were detected and have been previously described in *E. coli* isolates of animal origin (Saenz, Brinas et al. 2004; Cocchi, Grasselli et al. 2007) and also from human isolates (Lee, Oh et al. 2001). Only *tet(B)* was detected in the tetracycline resistant isolates, which has been found in other isolates from animals (Saenz, Brinas et al. 2004; Costa, Poeta et al. 2008; Enne, Cassar et al. 2008). Those isolates that were negative for the genes tested are likely to harbour other genes or chromosomal mutations responsible for trimethoprim, and tetracycline resistance that were beyond the scope of this study and not tested.

The *qnrA*, *qnrB* and *qnrS* genes were not detected in any isolates. These genes are responsible for low level resistance to quinolones (Martinez-Martinez, Pascual et al. 1998). In addition to the *qnr* genes, other mechanisms of quinolone resistance may be involved to give higher levels of resistance, for example, mutations in the genes encoding the subunits of DNA gyrase (*gyrA* and *gyrB*) or topoisomerase IV (*parC* and *parE*) (Piddock 1998).

In total, nine suspected ESBL producing isolates were recovered. Subsequent testing only confirmed one of these to be positive for ESBL production, but as shown in table 4, the only gene found by PCR was a $bla_{\text{CMY-2}}$ gene. It is possible that the isolate carried a type of ESBL not tested in this study, for example, an OXA type ESBL. In the eight other isolates, two carried $bla_{\text{TEM-1}}$ genes, and six carried the plasmid mediated AmpC $bla_{\text{CMY-2}}$ gene. $Bla_{\text{CMY}}$ genes have previously been reported in isolates from dogs (Sidjabat, Townsend et al. 2006; Sidjabat, Hanson et al. 2007).

In summary, this study demonstrates a common occurrence of AMR, in particular MDR, among the faecal *E. coli* of healthy dogs living in the community, with a variety of mechanisms responsible for resistance. This is of concern, given the close

and frequent contact dogs have with humans and could pose a risk for spread of resistant bacteria or resistance genes. Larger studies are required to more accurately estimate the prevalence of AMR *E. coli* in healthy dogs and thus fully understand both the risk factors for such resistance and any risk posed to humans. It would also be prudent to carry out longitudinal studies to investigate if such antimicrobial resistance is maintained over time and how this relates to antimicrobial prescribing practices.

# Chapter Four

## Prevalence of *Staphylococcus spp* carriage in dogs

## 4.1 Introduction

Staphylococci are widely recognised as being present on the mucosal surfaces of healthy humans and animal species, but are also important opportunistic pathogens. In dogs, the two most clinically important species are the coagulase positive *Staphylococcus aureus* and *S. pseudintermedius* (formerly *S. intermedius*) (Devriese, Vancanneyt et al. 2005; Sasaki, Kikuchi et al. 2007), which are commonly associated with pyoderma, wound sepsis, and otitis. Coagulase negative staphylococci (CNS) have also been isolated from clinical samples (Lilenbaum, Veras et al. 2000). Extensive use of antimicrobials in the past has, inevitably resulted in the development and spread of resistance among staphylococcal species, in particular, resistance to meticillin, which can, in human medicine, be associated with a higher rate of negative outcomes of treatment (Cosgrove, Sakoulas et al. 2003).

Resistance to meticillin is most often mediated by the *mecA* gene, which encodes an alternative penicillin binding protein (PBP2a) that has a lower binding affinity for the β-lactams and confers resistance to these antibiotics including penicillins and cephalosporins.(Archer and Niemeyer 1994) The *mecA* gene is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) (Katayama, Ito et al. 2000). Furthermore, resistance to other antimicrobial classes can often be associated with meticillin resistance (MR) (Kim, Song et al. 2006), which further complicates treatment, therefore increasing morbidity, mortality and financial burden on human healthcare (Cosgrove 2006).

Meticillin resistant *S. aureus* (MRSA) was reported as early as 1999 from clinical samples from dogs (Pak, Han et al. 1999; Tomlin, Pead et al. 1999), and reports of both clinical isolates of both MRSA and MR *S. pseudintermedius* (MRSP) have since increased in frequency (van Duijkeren, Box et al. 2004; Jones, Kania et al. 2007; Griffeth, Morris et al. 2008; Ruscher, Lubke-Becker et al. 2009; Schwartz, Boettcher et al. 2009).

While the reporting of meticillin resistant staphylococci in canine clinical samples is important in order to monitor trends, the study of non-symptomatic carriage in healthy dog populations is equally worthwhile. Given the close relationship dogs

have with their owners, there is the potential for dogs to act as a reservoir for both MRSA and other resistant staphylococci (van Duijkeren, Wolfhagen et al. 2004; Van Hoovels, Vankeerberghen et al. 2006; Jones, Kania et al. 2007). Genetic characterisation of MRSA isolated from dogs also suggests transmission between humans and pets, since they carry the same strains that are prevalent in humans (Baptiste, Williams et al. 2005; Loeffler, Boag et al. 2005; O'Mahony, Abbott et al. 2005; Weese, Dick et al. 2006), but the direction of transmission is not clear.

Sampling of healthy dog populations has shown the prevalence of MRSA to be very low, ranging from 0% in studies from Europe (Vengust, Anderson et al. 2006; Bagcigil, Moodley et al. 2007) and Canada (Lefebvre and Weese 2009), to 0.4-4% in the UK (Rich 2005; Loeffler, Pfeiffer et al. 2010) and 0.7-0.8% in Hong Kong (Boost, O'Donoghue et al. 2008) and the USA (Morris, Boston et al. 2010). Studies have shown the prevalence of MRSP to be higher than that of MRSA in dogs in the USA (2-6.2%) (Griffeth, Morris et al. 2008; Morris, Boston et al. 2010) and Europe (1.5%) (Vengust, Anderson et al. 2006), however a recent study in the UK identified no MRSP (Loeffler, Pfeiffer et al. 2010). Carriage of MR-CNS in healthy dogs is also higher (11.5-13%) (Vengust, Anderson et al. 2006; Bagcigil, Moodley et al. 2007).

Although previous studies have reported the prevalence of meticillin resistance of staphylococci in healthy dog populations, including a large one in the London area of the UK (Loeffler, Pfeiffer et al. 2010), there have been few comprehensive studies to investigate the prevalence of MRS, including MRSA in the vet visiting dog population.

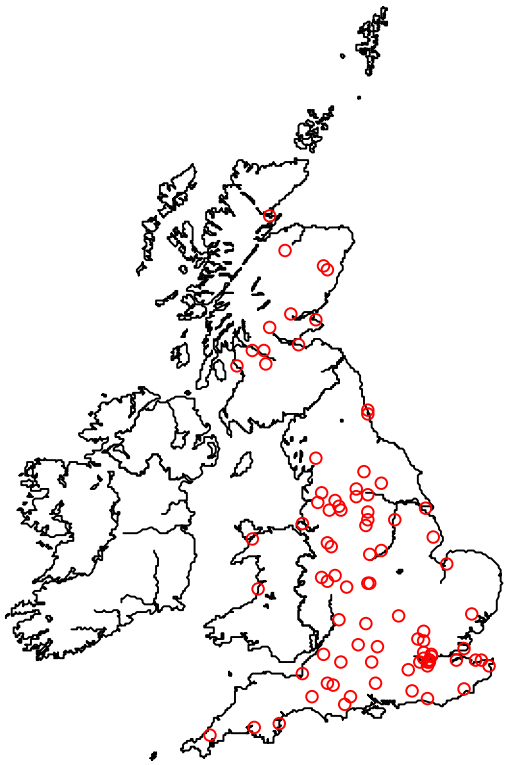
The aim of this study was to estimate the prevalence of nasal carriage of antimicrobial resistant, staphylococci, including meticillin resistance in vet visiting dogs in mainland UK. Antimicrobial resistance was investigated for all coagulase positive staphylococci (CPS) and MRCNS and the molecular characteristics of all MRSA and a subset of *S. aureus* isolates was investigated using DNA microarray analysis to determine carriage of virulence and antimicrobial resistance genes.

## 4.2 Methods

### 4.2.1 Study population

Dogs visiting veterinary practices in mainland UK were recruited for this study. Veterinary practices were randomly selected from the practices in the 2006 Royal College of Veterinary Surgeons (RCVS) register who indicated that they treated dogs. To estimate the prevalence of MRSA based on a prevalence of 2%, with a precision of 1% and 95% confidence intervals, it was calculated that a minimum of 753 dogs was required. Initially 50 practices were recruited and asked to sample 28 dogs (a total of 1400 samples) to take into account non-returns and a compliance rate of 70%. Figure 4.1 below shows the locations of the practices recruited into the study. Only dogs visiting the practice for consultations were included in this study, omitting any hospitalised dogs that may have become a carrier due to exposure in the hospital environment. Ethical approval for this study was obtained from the University of Liverpool Committee on Research Ethics in January 2008.

**Figure 4.1 Distribution of veterinary practices recruited to collect samples for nationwide study**



4.2.2 Nasal sample collection

Following informed consent from the owner, the attending veterinary personnel took nasal samples from the dog. A single swab (eSwab, Copan Italia SpA) was used to sample both nostrils, and the swab stored in AIMES transport medium. All samples were returned to the University of Liverpool by first class post.

4.2.3 Isolation of *Staphylococcus spp*

On arrival at the lab, swabs were enriched by overnight incubation in nutrient broth supplemented with 6.5% sodium chloride (NaCl) at 37°C. Using a 5µl loop, the broth

was streaked out onto mannitol salt agar (MSA) with aztreonam (20 μg/ml) for isolation of any staphylococci and on oxacillin-resistance screening agar (ORSA), with 2 μg/ml meticillin and 50 units/ml polymyxin B, for isolation of meticillin resistant staphylococci. Plates were incubated aerobically at 37°C for 24 hours and checked for growth, with ORSA plates incubated for a further 24 hours if no blue colonies were present. Colonies morphologically consistent with staphylococci were selected for further investigation, from MSA (up to two) and ORSA (one per sample). Staphylococci were presumptively identified on the basis of Gram stain, tube coagulase test using rabbit coagulase plasma (ProLab Diagnostics Inc), staphylase test (ProLab Diagnostics Inc), and a positive catalase test. Bacterial DNA was extracted from all isolates by digestion with lysostaphin (Sigma-Aldrich Company Ltd, Dorset, UK) for 10 minutes at 37$^{o}$C followed by incubation at 95°C for 10 minutes.

### 4.2.4 Assignment to species

Isolates were confirmed as *S. aureus* by PCR assays for the f*emA* and *nuc* genes. (Brakstad, Aasbakk et al. 1992; Francois, Pittet et al. 2003) Isolates of *S. pseudintermedius* were confirmed by a PCR assay for the *pta* gene, which encodes the enzyme phosphoacetyltransferase, and subsequent digestion of the amplicon by *MboI* (New England Biolabs) to give two different sized bands (Bannoehr, Franco et al. 2009). All other CPS isolates were assigned to species using a multiplex PCR, which distinguishes between all seven CPS species (Sasaki, Tsubakishita et al. 2010). For those CPS isolates that could not be assigned to species using the multiplex PCR, a 16S ribosomal DNA PCR was performed with subsequent analysis of the gene by sequencing (Edwards, Rogall et al. 1989).

### 4.2.5 Determination of antimicrobial resistance profile

Antimicrobial susceptibility testing was performed on all meticillin resistant staphylococci and all CPS by the Kirby Bauer method following the British Society for Antimicrobial Chemotherapy (BSAC) guidelines (Andrews 2007). Briefly, a Columbia blood agar (CAB) plate supplemented with 2% NaCl was inoculated for semi-confluent bacterial growth and the following antimicrobial discs were applied: co-trimoxazole (25 μg), ciprofloxacin (1 μg), fusidic acid (10 μg), gentamicin (10

μg), meticillin (5 μg), mupirocin (5 μg), rifampicin (2 μg), teicoplanin (30 μg), tetracycline (10 μg) and vancomycin (5 μg). The reference strain *S. aureus* ATCC 25923 was used as a fully susceptible control. All media was supplied by LabM-IDG (Bury, UK) and all antimicrobial discs by Mast Ltd (Liverpool, UK).

### 4.2.6 Determination of resistance to meticillin

Resistance to meticillin was investigated on those isolates selected from the ORSA plates. Isolates were streaked onto CAB supplemented with 2% NaCl with an oxacillin disc (1 μg) placed on the surface and MR was confirmed by PCR for the presence of the *mecA* gene (Vannuffel, Gigi et al. 1995).

### 4.2.7 Characterisation of SCC*mec* cassette type of MRSA isolates

SCC*mec* types were determined using a modified version of the method described by Oliveira et al (Oliveira and de Lencastre 2002).

### 4.2.8 *Spa* gene typing of *S. aureus* isolates

The variable region of the *staphylococcus* protein A (*spa*) gene, a virulence factor associated with interaction with IgG and evasion of phagocytosis, was amplified, as described previously (Harmsen, Claus et al. 2003) and sequenced by a commercial laboratory. Analysis of the sequence was carried out using Ridom StaphType software (Ridom GmbH, Germany), which assigned the isolate to a *spa* type. Associations of the assigned *spa* type with multi-locus sequence type (MLST) were determined using the Ridom *spa* server database (http://spaserver.ridom.de/).

### 4.2.9 Array analysis of MRSA and MSSA isolates

All MRSA isolates and a selection of MSSA isolates were subjected to DNA micro array based chip analysis using Identibac MRSA according to the manufacturer's instructions (Identibac, Surrey, UK). The array detects the presence of genes or gene groups associated with virulence (including staphylococcal enterotoxins, leukocidins and haemolysins), antimicrobial resistance (including aminoglycosides, β-lactams, chloramphenicol and vancomycin) and accessory gene regulators.

## 4.3 Results

### 4.3.1 Sample collection

Initially, 50 practices were recruited to the study and each asked to collect 28 samples. However, due to low compliance by veterinary practices, a further 37 practices were recruited to increase the number of samples collected to allow a more reliable estimate of the prevalence of carriage of antimicrobial resistant staphylococci. Of 87 veterinary practices, 14 failed to return any samples and only four returned all samples requested. Most practices returned between one and five samples each.

### 4.3.2 *Staphylococcus* carriage in dogs

A total of 724 nasal swabs were collected from dogs across mainland UK, of which 559 (77.2%) returned a completed questionnaire.

Table 4.1 shows a breakdown of the prevalence of staphylococci and total number of isolates collected. In total, 439 *Staphylococcus* isolates were obtained from 339 (55.1% (95% CI 51.5-58.7) dogs. Two hundred and seventy five (38.0% (95% CI 34.4-41.5) dogs were positive for carriage of at least one CNS and 140 (19.3% (95% CI 16.5-22.2) carried at least one CPS. More than one unique staphylococci isolate, with either different phenotypic characteristics or differing antimicrobial resistance profiles, was obtained from 39 (5%) samples, 16 (2%) of which carried a mixture of both CPS and CNS and one sample carried three different staphylococci.

**Table 4.1 Summary of staphylococci isolated from nasal swabs of 724 dogs in mainland UK**

| | Number of dogs (total unique isolates) | Prevalence (%) (95%CI) |
|---|---|---|
| **Staphylococci** | 399 (439) | 55.1 (51.5, 58.7) |
| **CoNS** | 275 (297) | 38.0 (34.4, 41.5) |
| **MR-CoNS** | 40 (40) | 5.5 (3.9, 7.2) |
| **CoPS** | 140 (142) | 19.3 (16.5, 22.2) |
| ***S. aureus*** | 54 (54) | 7.5 (5.5, 9.4) |
| **MRSA** | 7 (7) | 1.0 (0.3, 1.7) |
| ***S. pseudintermedius*** | 80 (81) | 11.0 (8.8, 13.3) |
| **MRSP** | 0 (0) | 0 |
| **Other CoPS** | 7 (7) | 1.0 (0.3, 1.7) |

CoNS = Coagulase negative staphylococci, MR-CoNS = Meticillin resistant CoNS, CoPS = Coagulase positive staphylococci, MRSA = Meticillin resistant *S. aureus*, MRSP = Meticillin resistant *S. pseudintermedius.*

### 4.3.3 Coagulase negative *Staphylococcus* species

In total 297 unique CNS isolates were archived. Twenty-two dogs carried two CNS isolates with differing resistance phenotypes, of which 17 dogs carried both MR-CNS and one MS-CNS. MR-CNS was detected in 58 samples; however, only 40 isolates (5.5% of dogs) were confirmed to be meticillin resistant by amplification of the *mecA* gene. All 40 *mecA* positive CNS isolates underwent antimicrobial disc susceptibility testing. Table 4.2 summarises the prevalence of resistance for these 40 isolates. Two isolates (5.0%) were resistant to meticillin only, and resistance to fusidic acid (n=38, 95% of MR-CNS) and ciprofloxacin (n=27, 67.2%) were most common among MR-CNS. Multidrug resistance (MDR, resistance to three or more antimicrobials was observed in 35 isolates (87.5%).

**Table 4.2 Percentage of antimicrobial resistance in the 182 staphylococci isolates tested**

| **Antimicrobial Agent** | **MR-CoNS (n=40)** | | **CoPS (n=142)** | | ***S. aureus* (n=54)** | | ***S. pseudintermedius* (n=81)** | |
|---|---|---|---|---|---|---|---|---|
| | Number resistant | % | Number resistant | % | Number resistant | % | Number resistant | % |
| **Ciprofloxacin** | 27 | 37.5 | 28 | 19.7 | 22 | 40.7 | 4 | 4.9 |
| **Co-trimoxazole** | 9 | 22.5 | 13 | 9.2 | 1 | 1.9 | 12 | 14.8 |
| **Fusidic Acid** | 38 | 95.0 | 62 | 43.7 | 29 | 53.7 | 29 | 35.8 |
| **Gentamicin** | 4 | 10.0 | 43 | 30.3 | 29 | 53.7 | 14 | 17.3 |
| **Meticillin** | 40 | 100.0 | 9 | 6.3 | 8 | 14.8 | 1 | 1.2 |
| **Mupirocin** | 8 | 20.0 | 1 | 0.7 | 1 | 1.9 | 0 | 0 |
| **Rifampicin** | 15 | 37.5 | 21 | 14.8 | 10 | 18.8 | 10 | 12.3 |
| **Teicoplanin** | 3 | 7.5 | 4 | 2.8 | 2 | 3.7 | 1 | 1.2 |
| **Tetracycline** | 11 | 27.5 | 27 | 19.0 | 1 | 1.9 | 24 | 29.6 |
| **Vancomycin** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **MDR** | 35 | 87.5 | 31 | 21.8 | 19 | 35.2 | 11 | 13.6 |

MR-CoNS = Meticillin resistant coagulase negative staphylococci, CoPS = Coagulase positive staphylococci, MDR = Multidrug resistance; resistance to three or more antimicrobials

### 4.3.4 Coagulase positive *Staphylococcus* species

One hundred and forty dogs (19.3%) carried a total of 142 CPS isolates (

Table 4.1). *S. aureus* was isolated from 54 (7.5%) dogs, with 80 (11.0%) dogs carrying at least one *S. pseudintermedius* isolate. One isolate was identified as *S. schleiferi* and six could not be assigned to species using the multiplex PCR. Sequencing of 16s ribosomal DNA of these six isolates identified two as *S. haemolyticus,* and one each as *S. schleiferi, S. saprophyticus* and *S. devriesei. S. haemolyticus, S. saprophyticus* and *S. devriesei* are regarded as coagulase negative species, but in this study some were found to be coagulase positive by the tube coagulase test. One remaining isolate could not be identified due to too many ambiguities in the obtained DNA sequence. One dog carried two *S. pseudintermedius* isolates, which differed by resistance phenotype, while another dog carried both a *S. aureus* and a *S. schleiferi* isolate.

*S. aureus*

Antimicrobial disc susceptibility testing was carried out for all 54 *S. aureus* (including MRSA) isolates and the susceptibility results are shown in Table 4.2. Resistance to at least one antimicrobial was observed in 30 isolates (55.6%). Resistance to both fusidic acid and gentamicin (both n=29, 53.7% of *S. aureus*) was most commonly observed, followed by ciprofloxacin resistance (n=22, 40.7%). MDR was observed in 19 isolates (35.2%). A subset of 20 MSSA isolates underwent *spa* typing and 18 previously described *spa* types were identified in single isolates. Two isolates were of unknown *spa* types.

Meticillin resistant *S. aureus*

Meticillin resistance was observed in eight *S. aureus* isolates and the presence of the *mecA* genes was confirmed in seven of these (1% of dogs). All seven confirmed MRSA isolates carried a SCC*mec* cassette type IV and were resistant to ciprofloxacin in addition to meticillin. In addition, resistance to gentamicin was observed in four isolates, fusidic acid in five isolates and rifampicin in four isolates. Five isolates were assigned to *spa* type t032, one to t022 and one to t3213, all of which are associated with ST22 and the human UK epidemic hospital associated strain EMRSA-15.

S. pseudintermedius

One *S. pseudintermedius* isolate was phenotypically resistant to meticillin, but was found to be *mecA* negative by PCR. The prevalence of antimicrobial resistance in the 81 *S. pseudintermedius* isolates is shown in Table 4.2. Twenty-five isolates (30.9%) were resistant to at least one antimicrobial and the most common resistance observed in these isolates was to fusidic acid (29 isolates, 35.8% of *S. pseudintermedius*) and tetracycline (24 isolates, 39.6%). Eleven isolates (13.6%) were resistant to three or more antimicrobials.

### 4.3.5 Array analysis of MRSA and MSSA isolates

All seven MRSA and a selection of MSSA isolates were subjected to DNA microarray analysis (26 in total) and the results are summarised in Figure 4.2. Regarding antimicrobial resistance, all MRSA isolates and 15 MSSA isolates harboured genes encoding resistance to β-lactams (*blaZ*) and two isolates (one MRSA and one MSSA) harboured genes encoding resistance to trimethoprim (*dfrA*). Two MRSA isolates harboured genes encoding resistance to macrolide-lincosamide-streptogrammin B antibiotics (*ermA* and *ermB*) and one MRSA isolate harboured genes encoding resistance to streptothricin (*sat*). The *mecA* gene was detected in all MRSA isolates and none of the MSSA isolates. None of the isolates tested harboured genes encoding resistance to either vancomycin (*vanA* or *vanB*) or mupirocin (*mupR*); however, one MSSA isolate did show resistance to mupirocin in antimicrobial disc susceptibility testing. Furthermore, no isolates harboured genes encoding resistance to tetracycline (*tetK* and *tetM*), but one MSSA strain displayed phenotypic resistance to tetracycline. Genes encoding staphylococcal enterotoxins were identified in all isolates, with *seX* and *seY* being most common (25 of 26 isolates); *seG, seI* and *seN* were also common, each being identified in 22 isolates. All isolates harboured genes encoding staphylococcal exotoxins. Most common were *set3, set6* and *set12* (all isolates), with only *set2* and *set21* being identified in less than 20 isolates (14 and 4 respectively). In addition, leukocidins were identified in all isolates, with *lukX* and *lukY* being most common (all isolates). *lukF* and *lukS* were also common (25 and 23 isolates respectively). In no strains were *lukF-PV* or *lukS-PV* genes detected, therefore all isolates were negative for PVL. Cluster analysis

(Figure 4.3) shows that all seven MRSA isolates formed a group with two of the MSSA isolates. The remaining MSSA isolates formed four separate groups.

**Figure 4.2 Presence of antimicrobial resistance and virulence factor genes among 28 MRSA and MSSA isolates by DNA microarray analysis**

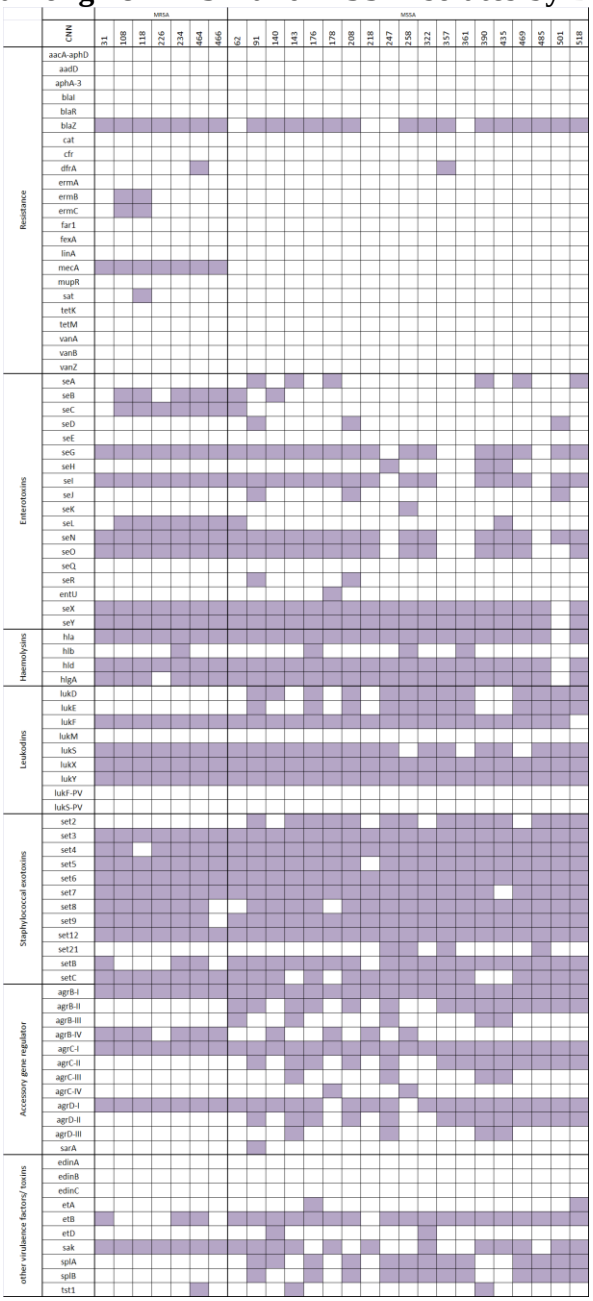
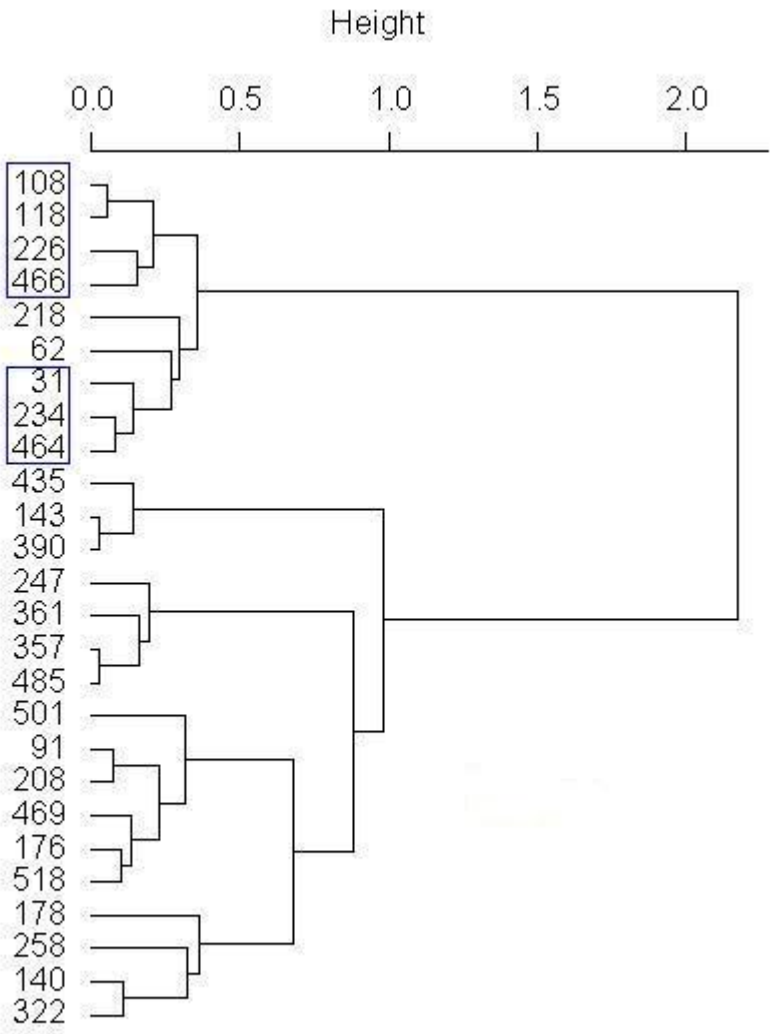



**Figure 4.3 Dendrogram showing relatedness of *S. aureus* isolates clustered by DNA microarray resistance and virulence genes**

Isolates enclosed in boxes are MRSA isolates. CCN – culture collection number assigned to each isolate prior to archiving of isolates


Height
0.0
0.5
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108
118
226
466
218
62
31
234
464
435
143
390
247
361
357
485
501
91
208
469
176
518
178
258
140
322

## 4.4 Discussion

The aim of this study was to estimate the prevalence of staphylococci carriage from nasal samples of dogs across mainland UK. The prevalence of total staphylococci observed in this study (55.1%) is less than the reported total staphylococci prevalence by Loeffler at al (91%) (Loeffler, Boag et al. 2005). However, the latter study took samples from a limited number of hospitalised dogs. In contrast, the present study investigated the prevalence in the larger, vet visiting dog community, omitting any hospitalised dogs, which may provide a closer representation of the UK population. Very few studies have reported the overall staphylococci prevalence, so it is difficult to make comparisons between different populations and countries. Carriage of CNS among dogs in this study (38.0%) is similar to the reported nasal carriage of another study of hospitalised dogs in the UK (Loeffler, Boag et al. 2005). The prevalence of MR-CNS (5.5%) is lower than that reported in Denmark (Bagcigil, Moodley et al. 2007) and Slovenia (Vengust, Anderson et al. 2006). In the latter study, both nasal and anal mucosal samples were collected, which may explain why the prevalence reported was higher than we report here.

It is reassuring that a low prevalence of MRSA was observed in this study, and this is the case with many other published studies in healthy dogs (Rich and Roberts 2006; Vengust, Anderson et al. 2006; Bagcigil, Moodley et al. 2007; Boost, O'Donoghue et al. 2008). The *spa* types of the MRSA isolates in this study have been found to be associated with MLST type ST22 (Shore, Rossney et al. 2010), from which the most prevalent MRSA clone circulating in UK hospitals comes (Johnson, Pearson et al. 2005; Ellington, Hope et al. 2010). This study therefore provides evidence that EMRSA-15 is present within the healthy dog population of the United Kingdom albeit at a very low level, which has been previously reported in hospitalised dogs in the UK (Baptiste, Williams et al. 2005; Loeffler, Boag et al. 2005).

The MSSA isolates were all identified as being of different *spa* types, and a number of these spa types have been shown to be associated with multi-locus sequence types (ST-5, ST-30 and ST-45) that are believed to be the MSSA precursors to many of the most successful MRSA clones circulating in both human hospitals and the

community (Robinson and Enright 2003). This again suggests sharing of the genotypes of *S. aureus* between dogs and humans.

Previous studies have shown that dogs are more likely to carry *S. pseudintermedius* than *S. aureus* (Biberstein, Jang et al. 1984; Griffeth, Morris et al. 2008; Epstein, Yam et al. 2009) and the current study found a higher prevalence of *S. pseudintermedius* in the canine population. However, no MRSP was identified in any of the samples, which was unexpected given that many other studies have found the MRSP prevalence to be higher than that of MRSA regardless of the type of population sampled (Vengust, Anderson et al. 2006; Griffeth, Morris et al. 2008; Epstein, Yam et al. 2009; Ruscher, Lubke-Becker et al. 2009). This lack of identification of MRSP in the present study is, however, in agreement with a recent study in the UK (Loeffler, Pfeiffer et al. 2010). In total, 20 isolates, which phenotypically displayed resistance to meticillin, were found not to carry the *mecA* gene. Non-*mecA* mediated meticillin resistance has been reported previously and a possible explanation for this could be due to hyper-production of β-lactamases (Mcdougal and Thornsberry 1986; Tomasz, Drugeon et al. 1989).

A high frequency of resistance was observed among the *Staphylococcus* isolates, with more than half (54.4%) of all isolates tested displaying resistance to at least one antimicrobial other than meticillin. High levels of resistance to fusidic acid (95.0% and 19.7%), ciprofloxacin (37.5% and 19.7%) and tetracycline (27.5% and 19.0%) was observed in both coagulase negative and positive isolates respectively. It is important to note, however, that only meticillin resistant coagulase negative staphylococci were subjected to antimicrobial susceptibility testing, which will cause the results observed to be disproportionately high in comparison to the observed prevalences in the coagulase positive staphylococci. It is possible, as suggested in other studies (Vengust, Anderson et al. 2006; Bagcigil, Moodley et al. 2007), that resistant coagulase negative staphylococci carriage in dogs may constitute an important reservoir for isolates capable of causing disease in both dogs and humans; or resistance determinants which may transfer to *S. aureus*. When comparing *S. aureus* and *S. pseudintermedius*, differences in antimicrobial resistance profiles were observed. For example, more *S. aureus* isolates were resistant to ciprofloxacin

and gentamicin, while more *S. pseudintermedius* isolates were resistant to co-trimoxazole. Resistance to fusidic acid was high in both species, but over 50% in *S. aureus*. These results are in agreement with studies of clinical isolates from dogs (Lilenbaum, Veras et al. 2000; Penna, Varges et al. 2010) and demonstrates the differences in antimicrobial resistance patterns in different staphylococcal species.

DNA microarray analysis showed that these isolates carry many virulence factors, which highlights their potential for pathogenicity. While all MRSA isolates clustered together, more variation was apparent between the MSSA isolates, which shows the higher level of diversity among the isolates compared to those with resistance to meticillin. The microarray results identified the presence or absence of resistance determinants which were not investigated by antimicrobial disc susceptibility testing, and this highlights the advantages of this approach and allows for higher discrimination between isolates.

Six coagulase positive isolates were unable to be assigned to species using the multiplex PCR (Sasaki, Tsubakishita et al. 2010) and 16s ribosomal DNA sequencing was carried out. Following this, four were identified as species more commonly found to be coagulase negative (*S. haemolyticus, S. saprophyticus* and *S. devriesei*), and one could not be identified at the species level. This highlights the difficulties associated with identification of staphylococci. Sequencing of 16s ribosomal DNA relies on variations in the sequences of bacterial species for identification, which can make it a difficult method when species of the same genus are so closely related and have very similar, conserved 16s sequences, as is the case with *Staphylococcus spp*.

It is possible that a degree of selection bias is present in this study. Anecdotal evidence, after discussions with participating veterinary practice, suggests that smaller breeds, whose nasal passages might be difficult to swab, were avoided. In addition, if the veterinary personnel felt the dog would not cooperate, they did not approach the owner for recruitment of their dog. It is not likely, however, that either of the issues would have substantially affected the results.

Since only a nasal swab was taken from each dog at the time of consultation, the carrier status of the dogs cannot be assessed. Swabs taken from a number of different sites, as in other studies (Loeffler, Boag et al. 2005; Vengust, Anderson et al. 2006; Griffeth, Morris et al. 2008) would provide more data relating to the dog as a whole. Additionally, this study does not take into account that a proportion of the population could be transient carriers as in the case of people (Kluytmans, vanBelkum et al. 1997), and may not continually carry staphylococci; thus were negative at the time of sampling. A longitudinal study investigating the population structure of staphylococci, in particular those isolates with antimicrobial resistance may be indicated to assess the importance of transient carriage.

This study provides further evidence that carriage of MRSA in healthy dogs is low and is in agreement with other studies that the same MRSA strains isolated from hospitalised dogs in the UK also circulate within the healthy population. The dogs in this study were found to carry MSSA strains commonly isolated from humans and the prevalence of resistance among the staphylococci was high. This therefore suggests that dogs in the community could be acting as a reservoir for both MSSA and resistant staphylococci.

# Chapter Five

# Prevalence and risk factors for carriage of antimicrobial resistant *Escherichia coli* in dogs

## 5.1 Introduction

Antimicrobial resistance is widely recognised as a common and increasing problem in the healthcare setting and within the community both in people and animals. Resistance to antimicrobials provides bacteria with an advantage over susceptible variants and could ultimately result in failure of treatment regimens. The result is higher rates of morbidity and mortality, but also an increased financial burden.

Antimicrobial resistant bacteria, for example *Escherichia coli*, isolated from veterinary samples are also commonly reported, in particular in dogs and other companion animals (Normand, Gibson et al. 2000). Pathogenic *E. coli*, are a common cause of gastro-intestinal infections, but the vast majority of humans, dogs and other warm blooded mammals carry commensal *E. coli* within the gut. However, such commensal bacteria may also cause opportunistic disease if outside their normal niche, for example in the urinary tract (Russo and Johnson 2000; Johnson and Russo 2002). Furthermore, the location of commensal *E. coli* means that exposure to antimicrobials prescribed orally to the individual is common, and, as a result, there is a selection pressure exerted upon the bacteria to develop resistance. This can be achieved either by chromosomal mutations in genes encoding the targets of the antimicrobials, or by acquisition of transferrable resistance genes from other members of the gut flora or transient bacteria passing through the gut. These bacteria may then themselves act as a reservoir for such resistance determinants (Guardabassi, Schwarz et al. 2004; Stenske, Bemis et al. 2009). As such, *E. coli* isolated from faecal samples can provide a good indication of the reservoir of resistance in the gut flora (van den Bogaard and Stobberingh 2000).

One resistance mechanism that is of particular concern is that mediated by extended spectrum β-lactamases (ESBL), which are capable of hydrolysing third generation cephalosporins, such as ceftazidime, cefotaxime, cefpodoxime and ceftiofur (Livermore 2008). *E. coli* harbouring such ESBL genes have become, in recent years, increasingly prevalent in hospitals and in the community in people (Munday, Whitehead et al. 2004; Pitout, Nordmann et al. 2005), as well as from clinical samples of canine origin (Ewers, Grobbel et al. 2010). Plasmid mediated AmpC enzymes, which also have a broad spectrum of resistance and are resistant to β-

lactamase inhibitors (Philippon, Arlet et al. 2002), have also been documented in dogs (Carattoli, Lovari et al. 2005; Sidjabat, Townsend et al. 2006).

Dogs have long been a companion of humans and a cross sectional study in 2007 indicated that 31% of households owned a dog with the estimate of total UK dog population to be 10.3 million (Murray, Browne et al. 2010). With such a large population and the close and frequent contact many people have with their pets, it is likely that bacteria are transferred between them, and this has sparked concerns that dogs may act as reservoirs for resistant bacteria and resistance determinants (Guardabassi, Schwarz et al. 2004; Damborg, Top et al. 2009; Johnson, Miller et al. 2009; Stenske, Bemis et al. 2009).

While antimicrobial resistant *E. coli* from clinical samples from dogs is often reported (Teshager, Dominguez et al. 2000; Pedersen, Jensen et al. 2007), it is the bacteria present in the faeces of dogs that the general human population are more likely to be exposed to. It is therefore important to gain an understanding of the prevalence of antimicrobial resistance among the *E. coli* resident in the healthy canine gut. Such studies have been carried out in both European countries (De Graef, Decostere et al. 2004; SVARM 2006; Costa, Poeta et al. 2008) and in Canada (Murphy, Reid-Smith et al. 2009). However, few have investigated the risk factors that might be associated with carriage of antimicrobial resistant *E. coli* by dogs in the community. Furthermore, the presence of antimicrobial resistant *E. coli* could also have a significant impact on the health and welfare of the dog. For example, transference of resistance determinants to bacteria that cause canine disease could result in difficult to treat infections the individual.

The aim of this study was to determine the faecal prevalence of antimicrobial resistant *E. coli,* including ESBL and AmpC β-lactamase producing *E. coli*, in the vet-visiting dog community of mainland UK. In addition, risk factors associated with carriage of antimicrobial resistance were investigated.

## 5.2 Methods

### 5.2.1 Study population

Dogs from mainland UK visiting veterinary practices were recruited for this study. Veterinary practices were randomly selected from the practices listed in the 2006 RCVS directory of veterinary practices who indicated that they treated dogs. Only dogs visiting the practice for consultations were included in this study, omitting any hospitalised animals. Sample size calculations were estimated based on an expected carriage rate of antimicrobial resistant *E. coli* of 50% (to give the largest sample size) with a precision of 5% and 95% confidence intervals. Assuming a conservative cluster variance of 0.01 between veterinary practices, then 555 faecal samples would be required from 50 veterinary practices. In addition to collection of faecal samples, nasal samples were also collected to determine the prevalence of MRSA, and is discussed in chapter four. Consequently, to allow for the higher number of samples required for investigation of prevalence of MRSA in nasal samples and a compliance rate of 70%, the 50 practices were asked to recruit 28 dogs each (a total of 1400). Ethical approval for this study was granted by the University of Liverpool Committee on Research Ethics.

### 5.2.2 Faecal sample collection and processing

Recruitment of the owners was carried out by the attending veterinary practice personnel during consultation. Following informed written consent, the owner was asked to provide a faecal sample from their dog at the next convenient opportunity. They were also requested to complete a six-page questionnaire comprising both tick box and free text questions. The questionnaire was designed using Cardiff TeleForm data capture software and piloted in-house and with the sample collection methods, at a veterinary practice, which did not participate in the main study. The questionnaire included questions relating to signalment, medical history of the dog over the previous three months (including antimicrobial use), use of antimicrobials by other household members (including other pets), and diet. The faecal sample and completed questionnaire were returned by first class post and processed immediately on receipt.

An equal volume of the faecal sample was added to 5ml of brain heart infusion broth with 5% glycerol (BHIG) and thoroughly mixed to create a faecal homogenate. A portion of this was stored below -70°C and the remainder used for the isolation of antimicrobial resistant *E. coli.* Isolation of both antimicrobial resistant *E. coli* and ESBL-producing *E. coli* have been described previously (Bartoloni, Benedetti et al. 2006; Liebana, Batchelor et al. 2006). Briefly, for antimicrobial resistant *E. coli*, the faecal homogenate was plated directly onto MacConkey and eosin methylene blue agar (EMBA) and antimicrobial discs (MAST group Ltd) applied to the surface: ampicillin (10 µg); augmentin (30 µg); chloramphenicol (30 µg); ciprofloxacin (1 µg); nalidixic acid (30 µg); tetracycline (30 µg); and trimethoprim (2.5 µg). Following overnight incubation at 37°C, colonies were selected if they were within the zone of inhibition around the antimicrobial discs and morphologically consistent with *E. coli.* For screening of samples for ESBL producing *E. coli*, two EMBA plates, one containing cefotaxime (1 µg/ml) and the other ceftazidime (1 µg/ml), were streaked with the faecal homogenate. If present, at least one isolate morphologically consistent with *E. coli* was selected from each plate. If no growth consistent with *E. coli* occurred, further EMBA plates were streaked with faecal homogenate following enrichment overnight in buffered peptone water. In addition, to allow for non-selective isolation of *E. coli*, an EMBA plate containing no antimicrobials was streaked with the faecal homogenate. Three isolates morphologically consistent with *E. coli* were selected for antimicrobial susceptibility testing.

Presumptive *E. coli* were confirmed by both biochemical testing (Gram stain, catalase production, lack of oxidase, fermentation of lactose, production of indole and inability to utilise citrate as a carbon source) and a PCR assay to detect the *uidA* gene (McDaniels, Rice et al. 1996).

### 5.2.3 Antimicrobial susceptibility testing

Antimicrobial disc susceptibility testing following British Society for Antimicrobial Chemotherapy (BSAC) guidelines (Andrews 2007) was performed on all isolates using the same antimicrobial discs as used for the isolation of *E. coli* above. The

reference strain *E. coli* ATCC 25922 was used as a fully sensitive control in all testing. Following overnight incubation at 37°C, the zone sizes in mm were recorded.

### 5.2.4 Phenotypic confirmation of ESBL production

The paired disc diffusion test (M'Zali, Chanawong et al. 2000) (MAST Group Ltd) was performed on isolates suspected of ESBL production, which were selected from the EMBA plates containing ceftazidime or cefotaxime. Three sets of antimicrobial discs were placed on an ISO-Sensitest agar plate inoculated for semi-confluent growth: ceftazidime (30µg) and ceftazidime/clavulanic acid (30µg/10µg); cefotaxime (30µg) and cefotaxime/clavulanic acid (30µg/10µg); and cefpodoxime (30µg) and cefpodoxime/clavulanic acid (30µg/10µg). Following overnight incubation, the zone sizes in mm were recorded. Production of an ESBL by an isolate was confirmed if the zone size was expanded by at least 5mm in the presence of clavulanic acid. A difference in zone size less than 5mm suggested the production of an AmpC β-lactamase or both an ESBL and an AmpC β-lactamase.

### 5.2.5 Confirmation of the presence of ESBL and AmpC β-lactamase genes by PCR

All isolates, suspected of harbouring either ESBL genes or $bla_{\text{AmpC}}$ genes were subjected to PCR testing for the presence of $bla_{\text{TEM}}$, $bla_{\text{SHV}}$, $bla_{\text{CTX-M}}$ and $bla_{\text{AmpC}}$ genes (Essack, Hall et al. 2001; Perez-Perez and Hanson 2002; Boyd, Tyler et al. 2004).

### 5.2.6 Statistical analysis

Questionnaire responses and antimicrobial resistance results were entered into an access database (Microsoft Office 2007) and exported to Stata (Version 9) for both univariable and multivariable analysis. In total, 11 different outcomes (any resistance, individual resistance to each of the seven tested antimicrobials, multidrug resistance (resistance to three or more antimicrobials), carriage of an ESBL producing *E. coli* and carriage of a $bla_{AmpC}$ gene) were tested for associations with any explanatory variables (questionnaire responses) using univariable binary logistic regression. Associations were considered statistically significant if $P<0.05$.

Multivariable logistic regression analyses were carried out. A multivariable model was built by step-wise elimination, initially including all variables with $P<0.3$ in

univariable analysis. Variables with P>0.05 in the model were sequentially removed (from highest P value to lowest) and a likelihood ratio test (LRT) carried out to compare the two models (with and without the variable). The variable remained in the model only if its removal resulted in a LRT P-value less than 0.05. Where the final model included more than two variables, tests for interactions between the variables were carried out. A LRT P-value of less than 0.05 suggested interaction between the two variables being tested and was retained in the final model.

## 5.3 Results

### 5.3.1 Study population and sample collection

Initially, 50 practices were recruited to the study and each asked to collect 28 samples. However, due to an unexpectedly low compliance by veterinary practices, a further 37 practices were recruited to increase the number of samples collected. These later practices were sent ten sample packs in the first instance with further packs sent out when all ten had been returned. Sixteen practices failed to return any faecal samples, with the median number of samples per practice returned being five (range 1.5-9).

In total, 581 faecal samples were returned, of which 574 also included the completed questionnaire. The median age of dogs recruited was 5 years (range 6 weeks to 17 years) and 64 different pure breeds were represented, with the most common type of dog being cross-breed (n=123, 21.4%), followed by Labrador (n=90, 15.7%).

### 5.3.2 Prevalence of antimicrobial resistance

At least one *E. coli* was isolated from 561 (96.6%) of the 581 faecal samples, with antimicrobial resistant *E. coli* being isolated from 260 (44.8%) faecal samples. A total of 436 unique *E. coli* isolates (up to nine per sample) were recovered based on their antimicrobial susceptibility profile. Table 5.1 below shows both the sample and isolate level prevalence of faecal carriage of antimicrobial resistant *E. coli*.

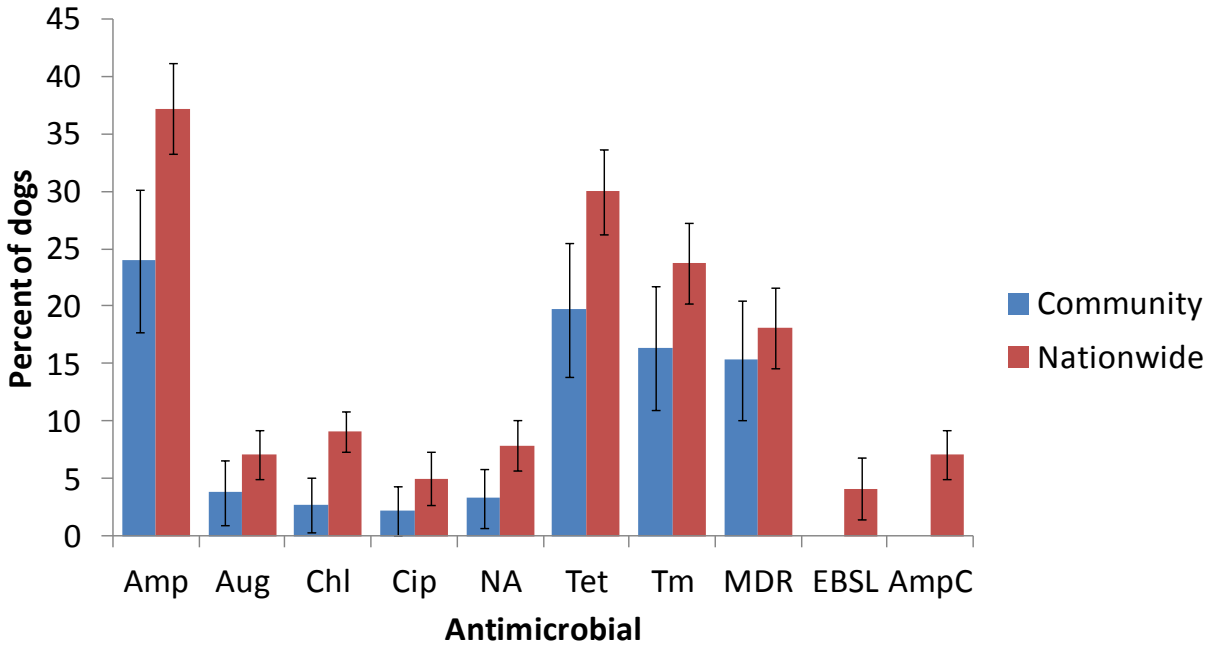
**Table 5.1 The prevalence of antimicrobial resistant *E. coli* isolated from faecal samples of dogs visiting veterinary practices in mainland United Kingdom**

| | Sample prevalence % (95% confidence interval) n=581 | Isolate prevalence % (95% confidence interval) n=436 |
|---|---|---|
| **Any resistance** | 44.8% (40.7-48.8) | |
| **Multidrug resistance** | 18.1% (14.9-21.2) | 31.9% (27.5-36.3) |
| **Ampicillin** | 37.2% (33.2-41.1) | 72.9% (68.8-77.1) |
| **Augmentin** | 7.1% (5.0-9.1) | 10.6% (7.7-13.4) |
| **Chloramphenicol** | 9.1% (5.7-10.1) | 13.5% (10.53-16.7) |
| **Ciprofloxacin** | 5.0% (3.2-6.8) | 8.0% (5.5-10.6) |
| **Nalidixic acid** | 7.9% (5.7-10.1) | 13.8% (10.5-17.0) |
| **Tetracycline** | 30.0% (26.2-33.7) | 62.8% (58.3-67.4) |
| **Trimethoprim** | 23.8% (20.3-27.2) | 41.3% (36.7-45.9) |
| **ESBL mediated resistance** | 4.1% (2.5-5.7) | 5.7% (3.6-7.9) |
| **AmpC mediated resistance** | 7.1% (5.0-9.1) | 9.6% (6.9-12.4) |

The most common resistances observed were to ampicillin (37.2% of dogs), tetracycline (30.0%) and trimethoprim (23.8%). Resistance to augmentin, chloramphenicol, ciprofloxacin and nalidixic acid was observed in less than 10% of dogs. Multidrug resistance (resistance to three of more different antimicrobial classes) was observed in 18.1% of dogs. ESBL producing *E. coli* were isolated from 4.1% of dogs and an AmpC β-lactamase producing *E. coli* from 7.1% of dogs.

Figure 5.1 below compares the prevalences of resistance in the two study populations. The prevalences of resistance to each of the antimicrobials is observed to be higher than those observed in the community population described in chapter three.

**Figure 5.1 Comparison of prevalence of resistance to antimicrobials observed in dogs in a semi-rural community and vet visiting dogs in mainland Great Britain.**




5.3.3 Univariable logistic regression analysis

Univariable analysis was carried out on the 574 samples with completed questionnaires for all 11 outcomes (Table 5.2 to Table 5.12 and Appendix Three). A total of 30 different variables were found be associated with one or more of the possible outcomes tested. Four variables were found to be associated with more than half of the eleven outcomes.

**Table 5.2 Univariable analysis of factors associated with carriage of any antimicrobial resistant *E. coli* in dogs for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P Value |
|---|---|---|---|---|---|---|---|
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.003** |
| No | 237 | 159 | 0 | | 1 | | 0.004* |
| Yes | 83 | 95 | 0.53 | 0.18 | 1.71 | 1.19-2.44 | |
| **Allowed off lead during walks** | | | | | | | **0.005** |
| No | 77 | 37 | 0 | | 1 | | 0.006* |
| Yes | 238 | 212 | 0.62 | 0.22 | 1.85 | 1.20-2.86 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.006** |
| No | 254 | 180 | 0 | | 1 | | 0.009* |
| Yes | 47 | 61 | 0.59 | 0.22 | 1.80 | 1.17-2.76 | |
| **Working Dog** | | | | | | | **0.02** |
| No | 292 | 218 | 0 | | 1 | | 0.02* |
| Yes | 19 | 29 | 0.72 | 0.31 | 2.04 | 1.12-3.74 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.04** |
| No | 249 | 177 | 0 | | 1 | | 0.04* |
| Yes | 65 | 70 | 0.42 | 0.20 | 1.51 | 1.03-2.23 | |
| **Fed raw poultry meat** | | | | | | | **0.04** |
| No | 309 | 239 | 0 | | 1 | | 0.05* |
| Yes | 8 | 15 | 0.89 | 0.45 | 2.42 | 1.01-5.81 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.04** |
| No | 210 | 149 | 0 | | 1 | | 0.05* |
| Yes | 103 | 104 | 0.35 | 0.18 | 1.42 | 1.01-2.01 | |

*Fishers exact test statistic

**Table 5.3 Univariable analysis of factors associated with carriage of multidrug resistant *E. coli* in dogs for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P Value |
|---|---|---|---|---|---|---|---|
| **Fed raw poultry meat** | | | | | | | **0.001** |
| No | 455 | 93 | 0 | | 1 | | 0.003* |
| Yes | 13 | 10 | 1.33 | 0.44 | 3.76 | 1.60-8.84 | |
| **Breed Size** | | | | | | | **0.002** |
| Small | 62 | 11 | 0 | | 1 | | |
| Medium | 116 | 17 | -0.19 | 0.42 | 0.83 | 0.36-1.87 | |
| Large | 153 | 54 | 0.69 | 0.36 | 1.99 | 0.98-4.06 | |
| Not specified | 140 | 21 | -0.17 | 0.33 | 0.85 | 0.38-1.86 | |
| **Given dog treats** | | | | | | | **0.02** |
| Never/ rarely | 94 | 32 | 0 | | 1 | | 0.03* |
| Sometimes/ often | 367 | 71 | -0.57 | 0.24 | 0.57 | 0.35-0.91 | |
| **Number of other dogs in household** | | | | | | | **0.04** |
| 0 | 264 | 49 | 0 | | 1 | | |
| 1 | 97 | 23 | 0.24 | 0.28 | 1.28 | 0.74-2.21 | |
| 2 | 42 | 9 | 0.14 | 0.40 | 1.15 | 0.53-2.52 | |
| 3 | 7 | 6 | 1.53 | 0.58 | 4.62 | 1.49-14.33 | |
| 4+ | 11 | 5 | 0.90 | 0.56 | 2.45 | 0.82-7.36 | |
| **Own a cat** | | | | | | | **0.04** |
| No | 349 | 67 | 0 | | 1 | | 0.05* |
| Yes | 114 | 35 | 0.47 | 0.23 | 1.60 | 1.01-2.53 | |
| **Breed Group** | | | | | | | **0.05** |
| Working | 13 | 7 | 0 | | 1 | | |
| Gundog | 151 | 46 | -0.57 | 0.50 | 0.57 | 0.21-1.50 | |
| Hound | 27 | 7 | -0.73 | 0.63 | 0.48 | 0.14-1.66 | |
| Terrier | 52 | 4 | -1.95 | 0.70 | 0.14 | 0.04-0.56 | |
| Utility | 26 | 4 | -1.25 | 0.71 | 0.29 | 0.07-1.16 | |
| Pastoral | 35 | 7 | -0.99 | 0.63 | 0.37 | 0.11-1.27 | |
| Toy | 28 | 7 | -0.77 | 0.63 | 0.46 | 0.13-1.60 | |
| Cross | 106 | 17 | -1.21 | 0.54 | 0.30 | 0.10-0.85 | |
| Not specified | 33 | 4 | -1.49 | 0.71 | 0.23 | 0.06-0.90 | |

*Fisher's exact test statistic

**Table 5.4 Univariable analysis of factors associated with carriage of ampicillin resistant *E. coli* in dogs for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P Value |
|---|---|---|---|---|---|---|---|
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.001** |
| No | 268 | 128 | 0 | | 1 | | 0.001* |
| Yes | 95 | 83 | 0.60 | 0.18 | 1.83 | 1.27-2.63 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.004** |
| No | 288 | 146 | 0 | | 1 | | 0.005* |
| Yes | 55 | 53 | 0.62 | 0.22 | 1.87 | 1.22-2.86 | |
| **Breed Size** | | | | | | | **0.004** |
| Small | 50 | 23 | 0 | | 1 | | |
| Medium | 98 | 35 | -0.25 | 0.32 | 0.78 | 0.41-1.45 | |
| Large | 114 | 95 | 0.57 | 0.29 | 1.77 | 1.01-3.12 | |
| Not specified | 101 | 60 | 0.26 | 0.30 | 1.29 | 0.72-2.33 | |
| **Neutered** | | | | | | | **0.02** |
| No | 85 | 69 | 0 | | 1 | | 0.02* |
| Yes | 276 | 142 | -0.46 | 0.19 | 0.63 | 0.43-0.92 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.02** |
| No | 239 | 120 | 0 | | 1 | | 0.02* |
| Yes | 117 | 90 | 0.43 | 0.18 | 1.53 | 1.08-2.18 | |
| **Given titbits** | | | | | | | **0.02** |
| Never | 29 | 31 | 0 | | 1 | | |
| Rarely | 124 | 52 | -0.94 | 0.31 | 0.39 | 0.22-0.72 | |
| Sometimes | 150 | 95 | 0.52 | 0.29 | 0.59 | 0.34-1.05 | |
| Often | 49 | 30 | -0.56 | 0.35 | 0.57 | 0.29-1.13 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.04** |
| No | 297 | 159 | 0 | | 1 | | 0.05* |
| Yes | 58 | 49 | 0.46 | 0.22 | 1.58 | 1.03-2.42 | |
| **Own a rodent** | | | | | | | **0.04** |
| No | 324 | 200 | 0 | | 1 | | 0.04* |
| Yes | 32 | 9 | -0.79 | 0.39 | 0.46 | 0.21-0.97 | |
| **Fed raw poultry meat** | | | | | | | **0.05** |
| No | 350 | 198 | 0 | | 1 | | 0.08* |
| Yes | 10 | 13 | 0.83 | 0.43 | 2.30 | 0.99-5.34 | |

*Fisher's exact test statistic

**Table 5.5 Univariable analysis of factors associated with carriage of augmentin resistant *E. coli* in dogs in the community for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.004** |
| No | 411 | 23 | 0 | | 1 | | 0.009* |
| Yes | 93 | 14 | 0.99 | 0.36 | 2.69 | 1.33-5.43 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.007** |
| No | 341 | 18 | 0 | | 1 | | 0.01* |
| Yes | 184 | 23 | 0.86 | 0.33 | 2.37 | 1.25-4.50 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.01** |
| No | 375 | 21 | 0 | | 1 | | 0.01* |
| Yes | 158 | 20 | 0.82 | 0.33 | 2.26 | 1.19-4.29 | |
| **Other animals in household** | | | | | | | **0.01** |
| No | 288 | 30 | 0 | | 1 | | 0.01* |
| Yes | 237 | 10 | -0.90 | 0.38 | 0.41 | 0.19-0.85 | |
| **Given titbits** | | | | | | | **0.02** |
| Never | 53 | 7 | 0 | | 1 | | |
| Rarely | 170 | 6 | -1.32 | 0.58 | 0.27 | 0.09-0.83 | |
| Sometimes | 221 | 24 | -0.20 | 0.46 | 0.82 | 0.34-2.01 | |
| Often | 76 | 3 | -1.21 | 0.71 | 0.30 | 0.07-1.21 | |
| **Own a cat** | | | | | | | **0.04** |
| No | 381 | 35 | 0 | | 1 | | 0.04* |
| Yes | 144 | 5 | -0.97 | 0.49 | 0.38 | 0.15-0.98 | |
| **Source of dog** | | | | | | | **0.04** |
| Breeder | 262 | 17 | 0 | | 1 | | |
| Rescue Kennel/ stray | 116 | 10 | 0.28 | 0.41 | 1.33 | 0.59-2.99 | |
| Newspaper/ word of mouth/ internet | 41 | 3 | 0.12 | 0.65 | 1.13 | 0.32-4.02 | |
| Family/friend | 79 | 4 | -0.25 | 0.57 | 0.78 | 0.26-2.39 | |
| Pet shop | 5 | 0 | | | | | |
| Other | 15 | 1 | -0.03 | 1.06 | 1.03 | 0.13-8.25 | |
| Self breed | 9 | 4 | 1.92 | 0.65 | 6.85 | 1.91-24.53 | |

*Fisher's exact test statistic

**Table 5.6 Univariable analysis of factors associated with carriage of chloramphenicol resistant *E. coli* in dogs in the community for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Own a cat** | | | | | | | **<0.001** |
| No | 390 | 26 | 0 | | 1 | | <0.001* |
| Yes | 124 | 25 | 1.11 | 0.30 | 3.02 | 1.68-5.43 | |
| **Number of other dogs in household** | | | | | | | **<0.001** |
| 0 | 292 | 21 | 0 | | 1 | | |
| 1 | 109 | 11 | 0.34 | 0.39 | 1.40 | 0.62-3.01 | |
| 2 | 46 | 5 | 0.41 | 0.52 | 1.51 | 0.54-4.21 | |
| 3 | 8 | 5 | 2.16 | 0.61 | 8.69 | 2.61-28.91 | |
| 4+ | 12 | 4 | 1.53 | 0.62 | 4.63 | 1.38-15.62 | |
| **Fed raw poultry meat** | | | | | | | **<0.001** |
| No | 505 | 43 | 0 | | 1 | | <0.001* |
| Yes | 14 | 9 | 2.02 | 0.46 | 7.55 | 3.09-18.45 | |
| **Working Dog** | | | | | | | **<0.001** |
| No | 471 | 39 | 0 | | 1 | | 0.001* |
| Yes | 36 | 12 | 1.39 | 0.37 | 4.03 | 1.94-8.36 | |
| **Other animals in household** | | | | | | | **0.001** |
| No | 301 | 17 | 0 | | 1 | | 0.001* |
| Yes | 213 | 34 | 1.04 | 0.31 | 2.83 | 1.51-5.19 | |
| **Given dog treats** | | | | | | | **0.003** |
| Never | 25 | 8 | 0 | | 1 | | |
| Rarely | 82 | 11 | -0.87 | 0.52 | 0.42 | 0.15-1.16 | |
| Sometimes | 250 | 15 | -1.67 | 0.49 | 0.19 | 0.07-0.49 | |
| Often | 155 | 18 | -1.01 | 0.48 | 0.36 | 0.14-0.92 | |
| **Own any other animal or livestock** | | | | | | | **0.005** |
| No | 467 | 40 | 0 | | 1 | | 0.01* |
| Yes | 47 | 11 | 1.01 | 0.37 | 2.69 | 1.31-5.68 | |
| **Given dog treats** | | | | | | | **0.01** |
| Never/ rarely | 107 | 19 | 0 | | 1 | | 0.01* |
| Sometimes/ often | 405 | 33 | -0.78 | 0.31 | 0.46 | 0.25-0.84 | |
| **Fed dry mixer** | | | | | | | **0.01** |
| No | 414 | 49 | 0 | | 1 | | 0.009* |
| Yes | 105 | 3 | -1.42 | 0.60 | 0.24 | 0.07-0.79 | |
| **Source of dog** | | | | | | | **0.02** |
| Breeder | 244 | 35 | 0 | | 1 | | |
| Rescue Kennel/ stray | 121 | 5 | -1.24 | 0.49 | 0.29 | 0.11-0.75 | |
| Newspaper/ word of mouth/ internet | 43 | 1 | -1.82 | 1.03 | 0.16 | 0.02-1.21 | |
| Family/friend | 78 | 5 | -0.81 | 0.50 | 0.45 | 0.17-1.18 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Pet shop | 5 | 0 | | | | | |
| Other | 14 | 2 | -0.004 | 0.78 | 1.00 | 0.22-4.57 | |
| Self breed | 10 | 3 | 0.74 | 0.68 | 2.09 | 0.55-7.97 | |
| **Other dogs in household** | | | | | | | **0.02** |
| No | 292 | 21 | 0 | | | | 0.03* |
| Yes | 222 | 31 | 0.66 | 0.30 | 1.94 | 1.09-3.47 | |
| **Breed Size** | | | | | | | **0.036** |
| Small | 70 | 3 | 0 | | | | |
| Medium | 123 | 10 | 0.64 | 0.68 | 1.90 | 0.51-7.12 | |
| Large | 179 | 28 | 1.29 | 0.62 | 3.65 | 1.08-12.39 | |
| Not specified | 150 | 11 | 0.54 | 0.67 | 1.71 | 0.46-6.33 | |

*Fisher's exact test statistic

**Table 5.7 Univariable analysis of factors associated with carriage of ciprofloxacin resistant *E. coli* in dogs in the community for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Fed raw poultry meat** | | | | | | | **<0.001** |
| No | 525 | 23 | 0 | | 1 | | 0.004* |
| Yes | 18 | 5 | 1.85 | 0.55 | 6.34 | 2.16-18.58 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.002** |
| No | 339 | 10 | 0 | | 1 | | 0.004* |
| Yes | 190 | 18 | 1.17 | 0.40 | 3.21 | 1.45-7.10 | |
| **Number of other dogs in household** | | | | | | | **0.006** |
| 0 | 302 | 11 | 0 | | 1 | | |
| 1 | 116 | 4 | -0.05 | 0.59 | 0.95 | 0.30-3.03 | |
| 2 | 46 | 5 | 1.09 | 0.56 | 2.98 | 0.99-8.98 | |
| 3 | 11 | 2 | 1.91 | 0.83 | 4.99 | 0.99-25.28 | |
| 4+ | 13 | 3 | 1.85 | 0.71 | 6.34 | 1.57-25.49 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.008** |
| No | 417 | 17 | 0 | | 1 | | 0.01* |
| Yes | 96 | 11 | 1.03 | 0.40 | 2.81 | 1.28-6.19 | |
| **Own a cat** | | | | | | | **0.008** |
| No | 402 | 14 | 0 | | 1 | | 0.01* |
| Yes | 136 | 13 | 1.01 | 0.40 | 2.74 | 1.26-5.98 | |
| **Given titbits** | | | | | | | **0.02** |
| Never/ rarely | 230 | 6 | 0 | | 1 | | 0.03* |
| Sometimes/ often | 302 | 22 | 1.03 | 0.47 | 2.79 | 1.11-7.00 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.03** |
| No | 382 | 14 | 0 | | 1 | | 0.04* |
| Yes | 164 | 14 | 0.85 | 0.39 | 2.33 | 1.09-5.00 | |
| **Breed Size** | | | | | | | **0.03** |
| Small | 71 | 2 | 0 | | 1 | | |
| Medium | 127 | 6 | 0.52 | 0.83 | 1.68 | 0.33-8.53 | |
| Large | 190 | 17 | 1.16 | 0.76 | 3.18 | 0.72-14.10 | |
| Not specified | 158 | 3 | -0.39 | 0.92 | 0.67 | 0.11-4.12 | |
| **Sex** | | | | | | | **0.03** |
| Male | 271 | 8 | 0 | | 1 | | 0.03* |
| Female | 275 | 20 | 0.90 | 0.43 | 2.46 | 1.07-5.69 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Breed Group** | | | | | | | **0.03** |
| Working | 16 | 4 | 0 | | 1 | | |
| Gundog | 185 | 12 | -1.35 | 0.63 | 0.26 | 0.07-0.90 | |
| Hound | 32 | 2 | -1.39 | 0.92 | 0.25 | 0.04-1.51 | |
| Terrier | 55 | 1 | -2.62 | 1.15 | 0.07 | 0.01-0.70 | |
| Utility | 27 | 3 | -0.81 | 0.83 | 0.44 | 0.09-2.25 | |
| Pastoral | 40 | 2 | -1.61 | 0.92 | 0.20 | 0.03-1.20 | |
| Toy | 34 | 1 | -2.14 | 1.16 | 0.12 | 0.01-1.14 | |
| Cross | 120 | 3 | -2.30259 | 0.80 8803 | 0.10 | 0.02-0.49 | |
| Not specified | 37 | 0 | | | | | |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.03** |
| No | 304 | 10 | 0 | | 1 | | 0.03* |
| Yes | 230 | 18 | 0.87 | 0.40 | 2.38 | 1.08-5.25 | |
| **Given titbits** | | | | | | | **0.03** |
| Never | 57 | 3 | 0 | | 1 | | |
| Rarely | 173 | 3 | -1.11 | 0.83 | 0.33 | 0.06-1.68 | |
| Sometimes | 231 | 14 | 0.14 | 0.65 | 1.15 | 0.32-4.14 | |
| Often | 71 | 8 | 0.76 | 0.70 | 2.14 | 0.54-8.44 | |
| **Anyone in the household work with farm animals** | | | | | | | **0.05** |
| No | 496 | 23 | 0 | | 1 | | 0.06* |
| Yes | 40 | 5 | 0.99 | 0.52 | 2.70 | 0.97-7.47 | |

*Fisher's exact test statistic

**Table 5.8 Univariable analysis of factors associated with carriage of nalidixic acid resistant *E. coli* in dogs in the community for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Fed raw poultry meat** | | | | | | | **<0.001** |
| No | 511 | 37 | | | 1 | | <0.001* |
| Yes | 15 | 8 | 2.00 | 0.47 | 7.37 | 2.93-18.5 | |
| **Number of other dogs in household** | | | | | | | **0.001** |
| 0 | 294 | 19 | | | 1 | | |
| 1 | 114 | 6 | -0.21 | 0.48 | 0.81 | 0.32-2.09 | |
| 2 | 43 | 8 | 1.06 | 0.45 | 2.88 | 1.19-6.98 | |
| 3 | 10 | 3 | 1.54 | 0.70 | 4.64 | 1.18-18.29 | |
| 4+ | 12 | 4 | 1.64 | 0.62 | 5.16 | 1.52-17.52 | |
| **Working Dog** | | | | | | | **0.003** |
| No | 476 | 34 | | | 1 | | 0.007* |
| Yes | 39 | 9 | 1.17 | 0.41 | 3.23 | 1.45-7.22 | |
| **Sex** | | | | | | | **0.01** |
| Male | 265 | 14 | | | 1 | | 0.02* |
| Female | 264 | 31 | 0.80 | 0.33 | 2.22 | 1.16-4.27 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.02** |
| No | 338 | 21 | | | 1 | | 0.02* |
| Yes | 183 | 24 | 0.75 | 0.31 | 2.11 | 1.14-3.90 | |
| **Breed Size** | | | | | | | **0.035** |
| Small | 69 | 4 | | | 1 | | |
| Medium | 124 | 9 | 0.22 | 0.62 | 1.25 | 0.37-4.22 | |
| Large | 182 | 25 | 0.86 | 0.56 | 2.37 | 0.80-7.6 | |
| Not specified | 154 | 7 | -0.24 | 0.64 | 0.78 | 0.22-2.77 | |
| **Given titbits** | | | | | | | **0.04** |
| Never/ rarely | 224 | 12 | | | 1 | | 0.04* |
| Sometimes/ often | 292 | 32 | 0.72 | 0.35 | 2.05 | 1.03-4.06 | |
| **Breed Group** | | | | | | | **0.04** |
| Working | 15 | 5 | | | 1 | | |
| Gundog | 178 | 19 | -1.14 | 0.57 | 0.32 | 0.10-0.98 | |
| Hound | 31 | 3 | -1.24 | 0.80 | 0.29 | 0.06-1.38 | |
| Terrier | 55 | 1 | -2.91 | 1.13 | 0.05 | 0.01-0.50 | |
| Utility | 27 | 3 | -1.10 | 0.80 | 0.33 | 0.07-1.59 | |
| Pastoral | 38 | 4 | -1.15 | 0.74 | 0.32 | 0.07-1.34 | |
| Toy | 32 | 3 | -1.27 | 0.79 | 0.28 | 0.06-1.33 | |
| Cross | 116 | 7 | -1.71 | 0.65 | 0.18 | 0.05-0.64 | |
| Not specified | 37 | 0 | | | | | |

*Fisher's exact test statistic

**Table 5.9 Univariable analysis of factors associated with carriage of tetracycline resistant *E. coli* in dogs in the community for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.003** |
| No | 315 | 111 | 0 | | 1 | | 0.005* |
| Yes | 82 | 53 | 0.61 | 0.21 | 1.83 | 1.22-2.76 | |
| **Fed raw poultry meat** | | | | | | | **0.004** |
| No | 392 | 156 | 0 | | 1 | | 0.008* |
| Yes | 10 | 13 | 1.18 | 0.43 | 3.27 | 1.40-7.61 | |
| **Working Dog** | | | | | | | **0.004** |
| No | 366 | 144 | 0 | | 1 | | 0.008* |
| Yes | 25 | 23 | 0.85 | 0.31 | 2.34 | 1.29-4.25 | |
| **Number of other dogs in household** | | | | | | | **0.02** |
| 0 | 232 | 81 | 0 | | 1 | | |
| 1 | 82 | 38 | 0.28 | 0.23 | 1.33 | 0.84-2.10 | |
| 2 | 29 | 22 | 0.78 | 0.31 | 2.17 | 1.18-4.00 | |
| 3 | 5 | 8 | 1.52 | 0.58 | 4.58 | 1.46-14.41 | |
| 4+ | 11 | 5 | 0.26 | 0.55 | 1.30 | 0.44-3.86 | |
| Not specified | 38 | 15 | 0.12 | 0.33 | 1.13 | 0.59-2.16 | 0.7 |
| **Breed Size** | | | | | | | **0.01** |
| Small | 53 | 20 | 0 | | 1 | | |
| Medium | 100 | 33 | -0.13 | 0.33 | 0.87 | 0.46-1.67 | |
| Large | 129 | 78 | 0.47 | 0.30 | 1.60 | 0.89-2.88 | |
| Not specified | 123 | 38 | -0.20 | 0.32 | 0.82 | 0.44-1.54 | |
| **Other dogs in household** | | | | | | | **0.02** |
| No | 232 | 81 | 0 | | 1 | | 0.03* |
| Yes | 165 | 88 | 0.42 | 0.18 | 1.53 | 1.06-2.19 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.02** |
| No | 291 | 105 | 0 | | 1 | | 0.02* |
| Yes | 114 | 64 | 0.44 | 0.19 | 1.56 | 1.07-2.27 | |
| **Own a fish** | | | | | | | **0.04** |
| No | 382 | 167 | 0 | | 1 | | 0.05* |
| Yes | 15 | 1 | -1.88 | 1.04 | 0.15 | 0.02-1.16 | |
| **Own any other animal or livestock** | | | | | | | **0.04** |
| No | 363 | 144 | 0 | | 1 | | 0.05* |
| Yes | 34 | 24 | 0.58 | 0.28 | 1.78 | 1.02-3.11 | |

*Fisher's exact test statistic

**Table 5.10 Univariable analysis of factors associated with carriage of trimethoprim resistant *E. coli* in dogs in the community for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Fed raw poultry meat** | | | | | | | **0.005** |
| No | 425 | 123 | 0 | | 1 | | 0.01* |
| Yes | 12 | 11 | 1.15 | 0.43 | 3.17 | 1.36-7.35 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.05** |
| No | 335 | 91 | 0 | | 1 | | 0.06* |
| Yes | 95 | 40 | 0.44 | 0.22 | 1.55 | 1.00-2.40 | |

*Fisher's exact test statistic

**Table 5.11 Univariable analysis of factors associated with carriage of an ESBL (TEM or CTX-M) producing *E. coli* in dogs in the community for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Fed raw poultry meat** | | | | | | | **<0.001** |
| No | 530 | 18 | 0 | | 1 | | <0.001* |
| Yes | 17 | 6 | 2.34 | 0.53 | 10.39 | 3.66-29.48 | |
| **Number of other dogs in household** | | | | | | | **0.005** |
| 0 | 301 | 12 | 0 | | 1 | | |
| 1 | 117 | 3 | -0.44 | 0.65 | 0.60 | 0.18-2.32 | |
| 2 | 49 | 2 | 0.02 | 0.78 | 1.02 | 0.22-4.71 | |
| 3 | 10 | 3 | 2.02 | 0.72 | 7.52 | 1.83-30.93 | |
| 4+ | 14 | 2 | 1.28 | 0.81 | 3.58 | 0.73-17.57 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.01** |
| No | 385 | 11 | 0 | | 1 | | 0.02* |
| Yes | 165 | 13 | 1.01 | 0.42 | 2.76 | 1.21-6.28 | |
| **Own a cat** | | | | | | | **0.02** |
| No | 404 | 12 | 0 | | 1 | | 0.03* |
| Yes | 138 | 11 | 0.99 | 0.43 | 2.68 | 1.16-6.22 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.03** |
| No | 421 | 13 | 0 | | 1 | | 0.05* |
| Yes | 99 | 8 | 0.96 | 0.46 | 2.62 | 1.06-6.48 | |

*Fisher's exact test statistic

**Table 5.12 Univariable analysis of factors associated with carriage of an *E. coli* harbouring a $bla_{AmpC}$ gene in dogs in the community for variables with p<0.05**

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Antibiotic prescribed during most recent visit** | | | | | | | **<0.001** |
| No | 433 | 23 | 0 | | 1 | | <0.001* |
| Yes | 90 | 17 | 1.27 | 0.34 | 3.56 | 1.83-6.93 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **<0.001** |
| No | 383 | 13 | 0 | | 1 | | <0.001 |
| Yes | 151 | 27 | 1.66 | 0.35 | 5.27 | 2.65-10.48 | |
| **Medication prescribed during most recent visit** | | | | | | | **<0.001** |
| No | 346 | 13 | 0 | | 1 | | <0.001* |
| Yes | 180 | 27 | 1.38 | 0.35 | 3.99 | 2.01-7.93 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | <0.001 |
| One off prescription | 5 | 1 | 0 | | 1 | | |
| Up to 5 days | 28 | 5 | -0.11 | 1.20 | 0.89 | 0.09-9.35 | |
| Up to 10 days | 39 | 6 | -0.26 | 1.18 | 0.77 | 0.08-7.77 | |
| Up to 2 weeks | 11 | 2 | 0.10 | 1.34 | 0.91 | 0.07-12.52 | |
| Up to 3 weeks | 2 | 2 | 1.61 | 1.48 | 5.00 | 0.27-91.52 | |
| Over 3 weeks | 1 | 1 | 1.61 | 1.79 | 5.00 | 0.15-166.60 | |
| Don't know | 4 | 0 | | | 1 | | |
| None prescribed | 433 | 23 | -1.33 | 1.12 | 0.27 | 0.03-2.37 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **<0.001** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 27 | 5 | 0 | | 1 | | |
| Up to 10 days | 30 | 6 | 0.08 | 0.66 | 1.08 | 0.30-3.95 | |
| Up to 2 weeks | 11 | 6 | 1.08 | 0.70 | 2.95 | 0.74-11.69 | |
| Up to 3 weeks | 4 | 1 | 0.30 | 1.22 | 1.35 | 0.12-14.73 | |
| Over 3 weeks | 7 | 0 | | | | | |
| Don't know | 2 | 0 | | | 1 | | |
| None prescribed | 416 | 18 | -1.45 | 0.54 | 0.23 | 0.08-0.68 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **<0.001** |
| No | 416 | 18 | 0 | | 1 | | <0.001* |
| Yes | 88 | 19 | 1.61 | 0.35 | 4.99 | 2.52-9.89 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.007** |
| No | 333 | 16 | 0 | | 1 | | 0.009* |
| Yes | 186 | 22 | 0.90 | 0.34 | 2.46 | 1.26-4.80 | |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.007** |
| No | 333 | 16 | 0 | | 1 | | 0.009* |
| Yes | 186 | 22 | 0.95 | 0.35 | 2.60 | 1.30-5.19 | |
| **Neutered** | | | | | | | **0.02** |
| No | 137 | 17 | 0 | | 1 | | 0.03* |
| Yes | 395 | 23 | -0.76 | 0.005 | 0.47 | 0.24-0.90 | |
| **Other animals in household** | | | | | | | **0.03** |
| No | 289 | 29 | 0 | | 1 | | 0.03* |
| Yes | 236 | 11 | 0.77 | 0.36 | 0.46 | 0.23-0.95 | |

*Fisher's exact test statistic

The consumption of raw poultry meat was found to be associated with nine resistance outcomes, increasing the risk in all cases; resistance to nalidixic acid (P<0.001), resistance to ciprofloxacin (P<0.001), resistance to chloramphenicol (P<0.001), ESBL mediated resistance (P<0.001), multidrug resistance (P= 0.001), resistance to tetracycline (P=0.004), resistance to trimethoprim (P=0.005), resistance to ampicillin (P=0.05) and resistance to any antimicrobial (P=0.05).

Being prescribed any antimicrobial in the three months prior to the veterinary visit was found to be associated with six outcomes, with an increased risk for all outcomes; AmpC mediated resistance (P<0.001), resistance to ampicillin (P=0.001), resistance to any antimicrobial (P=0.003), resistance to tetracycline (P=0.02), resistance to ciprofloxacin (P=0.03) and ESBL mediated resistance (P=0.01).

Being prescribed any antimicrobial during any previous consultations (excluding the most recent one) was associated with six outcomes; AmpC mediated resistance (P<0.001), resistance to ampicillin (P=0.004), resistance to augmentin (P=0.004), resistance to any antimicrobial (P=0.006), resistance to ciprofloxacin (P=0.008) and ESBL mediated resistance (P=0.03).

Similarly, breed size was also associated with six outcomes; multidrug resistance (P=0.002), resistance to ampicillin (P=0.004), resistance to tetracycline (P=0.01), resistance to nalidixic acid (P=0.03), resistance to ciprofloxacin (P=0.03) and resistance to chloramphenicol (P=0.04). For the first three outcomes, compared to small dogs, the risk is lower for medium dogs and higher for large dogs. However, for the last three outcomes, the risks increase as the size of the dog increases.

### 5.3.4 Multivariable logistic regression analysis

Multivariable logistic regression analysis was carried out for all 11 outcomes and the final models are shown in Table 5.13 to Table 5.22. Only raw poultry remained in the model for resistance to trimethoprim.

**Table 5.13 Multivariable model of variables associated with resistance to any antimicrobial in 557 dogs (17 missing values). Hosmer-Lemeshow P=1.0**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Allowed off lead during walks** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.48 | 0.23 | 1.62 | 1.03-2.53 | 0.04 |
| **Fed raw poultry meat** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.92 | 0.46 | 2.51 | 1.02-6.16 | 0.05 |
| **Regular contact with wild or farm animals during walks** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.43 | 0.21 | 1.52 | 1.01-2.29 | 0.05 |
| **Prescribed any antibiotic in previous three months** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.64 | 0.19 | 1.89 | 1.31-2.75 | 0.001 |

**Table 5.14 Multivariable model of variables associated with multi-drug resistance in 571 dogs (3 missing values). Hosmer-Lemeshow P=1.0**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Fed raw poultry meat** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.37 | 0.45 | 3.93 | 1.63-9.44 | 0.002 |
| **Breed size** | | | | | |
| Small | 0 | | 1 | | |
| Medium | -0.27 | 0.42 | 0.76 | 0.33-1.74 | 0.5 |
| Large | 0.63 | 0.37 | 1.87 | 0.91-3.83 | 0.09 |
| Not specified | -0.23 | 0.41 | 0.79 | 0.36-1.75 | 0.6 |

**Table 5.15 Multivariable model of variables associated with resistance to ampicillin in 539 dogs (35 missing values). Hosmer-Lemeshow P=0.8**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Breed size** | | | | | |
| Small | 0 | | 1 | | |
| Medium | -0.28 | 0.34 | 0.76 | 0.39-1.47 | 0.4 |
| Large | 0.6 | 0.30 | 1.86 | 1.03-3.38 | 0.04 |
| Not specified | 0.41 | 0.32 | 1.50 | 0.80-2.80 | 0.2 |
| **Neutered** | | | | | |
| No | 0 | | 1 | | |
| Yes | -0.52 | 0.21 | 0.60 | 0.40-0.90 | 0.01 |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.59 | 0.22 | 1.80 | 1.16-2.79 | 0.009 |

**Table 5.16 Multivariable model of variables associated with resistance to augmentin in 522 dogs (22 missing values). Hosmer-Lemeshow P=0.9**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Prescribed any antibiotic in previous three months** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.03 | 0.35 | 2.80 | 1.42-5.50 | 0.003 |
| **Other animals in household** | | | | | |
| No | 0 | | 1 | | |
| Yes | -1.06 | 0.39 | 0.35 | 0.16-0.74 | 0.006 |
| **Regular contact with wild or farm animals during walks** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.85 | 0.38 | 2.34 | 1.12-4.91 | 0.002 |

**Table 5.17 Multivariable model of variables associated with chloramphenicol resistance in 550 dogs (24 missing values). Hosmer-Lemeshow P=0.4**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Working dog** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.21 | 0.50 | 3.47 | 1.29-9.29 | 0.01 |
| **Own a cat** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.10 | 0.34 | 3.00 | 1.52-5.89 | 0.001 |
| **Own a cat** | | | | | |
| Working dog no cat | 0 | | 1 | | |
| Working dog with cat | -0.24 | 0.89 | 0.79 | 0.14-4.51 | 0.8 |
| **Fed raw poultry meat** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.80 | 0.53 | 6.03 | 2.12-17.14 | 0.001 |
| **Fed dry mixer** | | | | | |
| No | 0 | | 1 | | |
| Yes | -1.20 | 0.62 | 0.30 | 0.09-1.01 | 0.05 |

Explanation of interaction term

Not working dog no cat Odds ratio =1

Not working dog with a cat Odds ratio = 3.00

Working dog no cat Odds ratio = 3.47

Working dog with a cat odds ratio = 3.00* 3.47*0.79 = 8.22

**Table 5.18 Multivariable model of variables associated with resistance to ciprofloxacin1 in 527 dogs (47 missing values). Hosmer-Lemeshow P=0.6**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Breed Size** | | | | | |
| Small | 0 | | 1 | | |
| Medium | 1.31 | 1.13 | 3.71 | 0.40-31.17 | 0.2 |
| Large | 2.38 | 1.08 | 10.80 | 1.31-89.38 | 0.03 |
| Not specified | 0.51 | 1.21 | 1.66 | 0.16-17.73 | 0.7 |
| **Given titbits** | | | | | |
| Never/ rarely | 0 | | 1 | | |
| Sometimes/ often | 1.58 | 0.56 | 4.88 | 1.61-14.75 | 0.005 |
| **Antibiotic prescribed during most recent visit** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.14 | 0.49 | 3.12 | 1.21-8.06 | 0.02 |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.55 | 0.47 | 4.70 | 1.86-11.85 | 0.001 |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.16 | 0.46 | 3.18 | 1.30-7.81 | 0.01 |
| **Anyone in the household work with farm animals** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.33 | 0.62 | 3.78 | 1.11-12.83 | 0.03 |
| **Fed dry complete** | | | | | |
| No | 0 | | 1 | | |
| Yes | -1.49 | 0.52 | 0.23 | 0.08-0.62 | 0.004 |
| **Fed tinned or packet wet food** | | | | | |
| No | 0 | | 1 | | |
| Yes | -1.06 | 0.56 | 0.35 | 0.12-1.04 | 0.06 |

**Table 5.19 Multivariable model of variables associated with resistance to nalidixic acid in 566 dogs (8 missing values). Hosmer-Lemeshow P=0.8**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Fed raw poultry meat** | | | | | |
| No | 0 | | 1 | | |
| Yes | 2.04 | 0.49 | 7.68 | 2.93-20.16 | <0.001 |
| **Sex** | | | | | |
| Male | 0 | | 1 | | |
| Female | 0.97 | 0.33 | 2.64 | 1.38-5.05 | 0.003 |
| **Medication prescribed during most recent visit** | | | | | |
| No | 0 | | 1 | | |
| Female | 0.85 | 0.35 | 2.33 | 1.18-4.59 | 0.01 |

**Table 5.20 Multivariable model of variables associated with resistance to tetracycline in 561 dogs (13 missing values). Hosmer-Lemeshow P=1.0**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Regular contact with wild or farm animals during walks** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.56 | 0.22 | 1.75 | 1.12-2.70 | 0.01 |
| **Fed raw poultry meat** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.37 | 0.62 | 1.45 | 0.43-4.86 | 0.5 |
| **Regular contact with wild or farm animals during walks X Fed raw poultry meat** | | | | | |
| Animal contact no raw poultry | 0 | | 1 | | |
| Animal contact with raw poultry | 2.40 | 1.24 | 11.03 | 0.97-124.86 | 0.05 |
| **Any antibiotic in last 3 months** | | | | | |
| No | 0 | | 1 | | |
| Yes | 0.59 | 0.20 | 1.80 | 1.21-2.68 | 0.004 |

Explanation of interaction term
No animal contact no raw poultry Odd ratio = 1
No animal contact with raw poultry Odds ratio = 1.45
Animal contact no raw poultry Odds ratio = 1.75
Animal contact with raw poultry Odds ratio= 1.45*1.74*11.03 = 27.99

**Table 5.21 Multivariable model of variables associated with carriage of an ESBL (TEM or CTX-M) producing *E. coli* in 569 dogs (5 missing values). Hosmer-Lemeshow P=1.0**

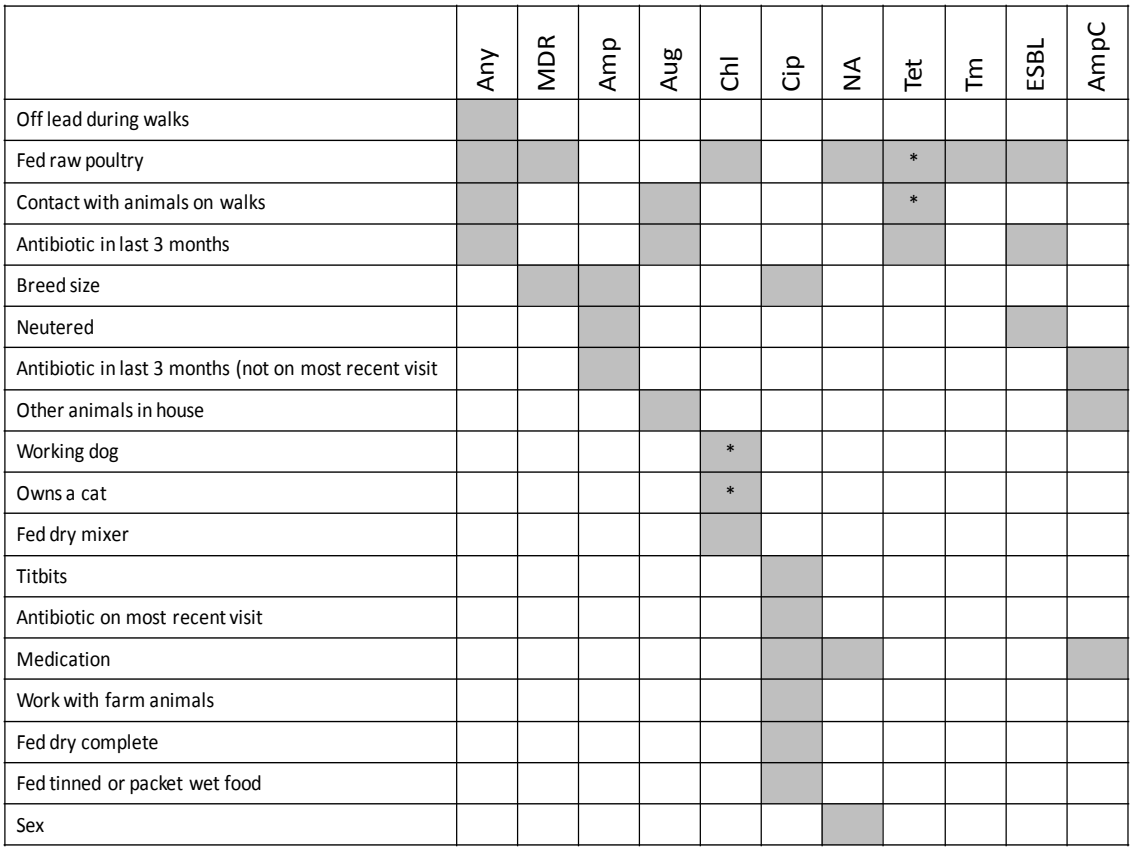
| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Prescribed any antibiotic in previous three months** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.36 | 0.46 | 1.88 | 1.59-9.48 | 0.003 |
| **Fed raw poultry meat** | | | | | |
| No | 0 | | 1 | | |
| Yes | 2.88 | 0.60 | 17.81 | 5.53-57.36 | <0.001 |
| **Neutered** | | | | | |
| No | 0 | | 1 | | |
| Yes | 3.38 | 2.06 | 1.22 | 1.02-11.13 | 0.05 |

**Table 5.22 Multivariable model of variables associated with AmpC β-lactamase mediated resistance in 527 dogs (47 missing values). Hosmer-Lemeshow P=0.6**

| Variable | Coefficient | SE | OR | 95% CI | P-Value |
|---|---|---|---|---|---|
| **Medication prescribed during most recent visit** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.12 | 0.37 | 3.06 | 1.49-6.31 | 0.002 |
| **Other animals in household** | | | | | |
| No | 0 | | 1 | | |
| Yes | -0.97 | 0.41 | 0.38 | 0.17-0.84 | 0.02 |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | |
| No | 0 | | 1 | | |
| Yes | 1.50 | 0.36 | 4.46 | 2.20-9.06 | <0.001 |

**Figure 5.2** below summarises the variables that remained in the final models of each outcome to allow comparison.

**Figure 5.2 Summary of variables in the final models of all outcomes**




Consumption of raw poultry was included in the final models of seven outcomes; resistance to any antimicrobial, multidrug resistance, resistance to tetracycline, resistance to nalidixic acid, resistance to chloramphenicol, resistance to trimethoprim and ESBL mediated resistance. Having received any antimicrobial in the previous three months was also commonly included in the final multivariable model; resistance to any antimicrobial, resistance to tetracycline, resistance to augmentin, and ESBL mediated resistance.

Following testing for interaction between variables, two of the final multivariable models included interaction terms. Interaction between consumption of raw poultry and contact with animals during walks was found to be significant in the final model of carriage of tetracycline resistant *E. coli*. Either eating raw poultry or having contact with animals during walks increases the risks only marginally (OR 1.45 and 1.75 respectively), however the risk is increased significantly if the dog both eats raw poultry and has contact with wild or farm animals during walks (OR 27.99).

In the final model for resistance to chloramphenicol, there was found to be interaction between having a cat in the household and dog being an actively working dog. Being both and working dog and owning a cat increased the risk significantly (OR 8.22) when compared to the effect of only either being a working dog or owning a cat (OR 3.00 and 3.47 respectively).

## 5.4 Discussion

This study found the prevalence of antimicrobial resistant *E. coli* to be common among the vet-visiting dog (44.7%) with the most common resistances being observed to ampicillin, tetracycline and trimethoprim. This supports previous studies, which also found these to be among the most common resistance phenotypes observed (De Graef, Decostere et al. 2004; Carattoli, Lovari et al. 2005; SVARM 2006; Costa, Poeta et al. 2008; Murphy, Reid-Smith et al. 2009), although at a higher prevalence in this study. This may be attributed to the ability of the numerous resistance determinants responsible being readily transmissible, or higher level of use of these and related drugs in animals due to their broad-spectrum action (VMD 2009). The observed levels of resistance to other individual antimicrobials in this study of vet visiting dogs appeared to be higher than in some previously reported studies (Carattoli, Lovari et al. 2005; Costa, Poeta et al. 2008; Murphy, Reid-Smith et al. 2009), including the findings discussed in chapter three, which investigated the prevalence in the community. This difference may be due to the type of population sampled (dogs in the community in chapter three compared to the vet visiting population). Furthermore, some of the dogs in this study reported recent use of antimicrobials and other studies excluded animals with any history of antimicrobial use. In addition, the sampling method in the current study used both selective and none selective methods to isolate antimicrobial resistant. Therefore, in general, a large number of isolates were screened (up to 17 per sample) and as many as six phenotypically resistant unique *E. coli* isolates were obtained from samples, with one per sample on average. Fewer resistant isolates may have been identified if only one or two isolates had been selected, which is the methodology adopted in some other studies. Multi-drug resistant isolates were found in 18.4% of dogs, however, it is

difficult to compare this with other studies since the definition of multi-drug resistance differs between studies and many studies do not report this.

Carriage of AmpC β-lactamase producing *E. coli* was higher (7.1%) than the prevalence of ESBL producing *E. coli* (4.1%), and these have both previously been reported in studies of healthy dogs (Costa, Poeta et al. 2004; Carattoli, Lovari et al. 2005; Sidjabat, Townsend et al. 2006; Sidjabat, Hanson et al. 2007). This may be attributed to the common use of amoxicillin and clavulanic acid (synulox™) in veterinary medicine (Escher, Vanni et al. 2011; Hughes, Williams et al. 2012), although this would need further investigation.

This study makes efforts to determine risk factors associated with carriage of antimicrobial resistant *E. coli* in the UK dog population. The finding that receiving antimicrobials in the last 3 months was associated with the carriage of antimicrobial resistant *E. coli,* remaining in four of the final models, is not surprising given the selection pressure this would exert upon commensal bacteria, however little is known in respect of how long after treatment such effects remain. A previous study found that administration of antimicrobials, specifically fluoroquinolones, to be associated with an increased risk of carriage of MDR *E. coli* in dogs (Gibson, Morton et al. 2011) and a longitudinal study of humans, investigating risk factors for selection quinolone resistance, also identified previous antimicrobial use as a risk factor for carriage of quinolone resistant *E. coli* on admission to hospital, with the prevalence in the study population increasing in the group prescribed quinolones (Yagci, Yoruk et al. 2009). In a study investigating the prevalence of antimicrobial resistant *E. coli* in faecal samples from horses, a similar association between carriage and use of antibiotics in the previous 10 days was evident (Maddox, Pinchbeck et al. 2011). Further work in this area may provide greater understanding of the lasting effects of short and long term antimicrobial use on the gut flora of dogs and carriage of resistant bacteria.

Eating raw poultry was also identified as a risk factor, remaining in the final model of many outcomes. Chickens have previously been identified as a potential reservoir for resistant bacteria and determinants (Costa, Vinue et al. 2009) and it is possible that ingestion of raw poultry could result in transfer of these to commensal bacteria. A study in Canada observed a high prevalence of antimicrobial resistant *Salmonella*

in raw food dog diets (Finley, Reid-Smith et al. 2008), further demonstrating an increased risk of carriage of antimicrobial resistant bacteria when dogs are exposed to raw meat. Based on these findings, it would be advisable for owners not to allow their dogs to eat raw poultry to reduce the risk associated with its consumption.

The presence of a household member who works with farm animals was found to be associated with an increased risk of carriage of ciprofloxacin resistant *E. coli*. Farm animals have been identified as a potential reservoir of antimicrobial resistance and determinants for the human population (Costa, Vinue et al. 2009; Checkley, Campbell et al. 2010; Smet, Martel et al. 2010; Ajayi, Oluyege et al. 2011), so it is not wholly surprising that exposure of household members to farm animals could result in an increased risk of carriage to the dogs by transfer from the household member to the dog. It is also possible and perhaps more likely, that dogs may have direct contact with the animals or they reside on the farm premises.

Being fed dry mixer, dry complete or tinned or packet wet food all had a protective effect in all cases where they remained in final model and this may further suggest that a cooked, pre prepared diet may be of more benefit, possibly by reducing their exposure to antimicrobial resistant *E. coli*, when compared to a raw diet.

Age of the dog was not included in the final model of any of the outcomes.

Two of the final models included interaction terms between two of the variables. In the final model for chloramphenicol resistance, the risk of carriage is only slightly increased by consumption of raw poultry or contact with animals during walk when considered alone, but the risk is markedly increased when the dog both consumes raw poultry and is allowed contact with animals during walks. It could be expected that these two variables may increase the risk of carriage of antimicrobial resistant *E. coli*, but any biological reasons why the two would interact to increase the risks further is not obvious.

The sampling method adopted in this study may have introduced some selection bias. For example, during busy periods owners may not have been approached for recruitment. However, the large numbers of animals recruited may have limited this. Other limitations of the study include collection of faecal samples on only one occasion. This study gives the prevalence of carriage of antimicrobial *E. coli* at a single time point, and it may be possible that this is transient in nature. Studies

involving sample collection on multiple occasions could provide a more complete assessment of the carriage rates of antimicrobial resistant *E. coli* in faecal samples of dogs, however such studies would require large resources or similar. Many of the dogs sampled were found to harbour antimicrobial resistant *E. coli*, which may have implication for public health specifically in relation to dogs being a potential reservoir for resistant bacteria and determinants. This also has important implications for the welfare of the individual dogs with respect to future treatments. In addition, several potential risk factors associated with the carriage of antimicrobial *E. coli* by dogs in the community and knowledge of such risk factors will allow better education for both owners and veterinary surgeons on how to minimise these risks.

# Chapter Six

## Molecular characterisation of antimicrobial resistant *Escherichia coli* isolated from the faeces of vet visiting dogs in mainland UK

## 6.1 Introduction

Antimicrobials are regularly used to treat infections in both human and animal medicine. Antimicrobial use, however, exerts a selection pressure that may drive the development of resistance and spread of resistance genes. Resistance to expanded spectrum β-lactams, particularly the third generation cephalosporins, is of increasing importance, with the limited treatment options make managing infections with these resistant bacteria very challenging. Resistance to third and fourth generation cephalosporins is mediated by either genes encoding extended spectrum β-lactamases (ESBLs) (Bradford 2001; Livermore and Hawkey 2005) or AmpC β-lactamases (Philippon, Arlet et al. 2002), and these genes are often found in *Escherichia coli* and other *Enterobacteriaceae*.

While most *E. coli* isolated from the gastrointestinal tract of warm blooded animals are members of the commensal gut flora, many are also capable of causing disease. *E. coli* can cause diarrhoea, but is also associated with urinary tract infections, septicaemia, meningitis, and skin and soft-tissue infections. Pathogenic *E. coli* have acquired specific virulence factors that enhance their disease causing potential by a variety of mechanisms (Mcdaniel, Jarvis et al. 1995; Russo, McFadden et al. 2002; Kaper, Nataro et al. 2004; Johnson, Wannemuehler et al. 2008), and the virulence factors associated with enteric disease are different to those associated with extra-intestinal disease.

Dogs have regular close contact with people, and, there is potential for transfer of bacteria in both directions. Dogs are potential reservoirs for *E. coli*, and have been shown to carry the same strains as humans (Stenske, Bemis et al. 2009). More importantly, they have been implicated as possible reservoirs for pathogenic *E. coli* (Johnson, Johnston et al. 2008), particularly those that may cause extra-intestinal disease (Johnson, Stell et al. 2001; Starcic, Johnson et al. 2002) and that are antimicrobial resistant (van den Bogaard and Stobberingh 2000; Guardabassi, Schwarz et al. 2004; Lloyd 2007). In addition, ESBL producing *E. coli* have recently become an increasing clinical problem in canine medicine (Steen and Webb 2007;

Ewers, Grobbel et al. 2010; Ewers, Grobbel et al. 2011; Timofte, Dandrieux et al. 2011).

In a previous study investigating the prevalence of antimicrobial resistant *E. coli* carriage in the vet visiting dog population of the mainland UK (chapter five), we screened 581 faecal samples for ESBL or AmpC β-lactamase producing *E. coli*. Fifty-eight isolates from 56 dogs (9.6%) were identified as either ESBL or AmpC producers by phenotypic testing and PCR. The aim of this study was to characterise the full antimicrobial susceptibility profile, strain types and carriage of virulence and resistance genes of these 58 isolates, and compare the results to isolates of human origin.

## 6.2 Methods

### 6.2.1 Isolation of ESBL and AmpC β-lactamase producing *E. coli*

Faecal samples were collected from dogs visiting veterinary practices in the mainland UK, as discussed in chapter five. Briefly, presumptive ESBL and AmpC β-lactamase producing *E. coli* were isolated using eosin methylene blue agar (LabM-IDG, Bury, UK) supplemented with either ceftazidime (1µg/ml) or cefotaxime (1µg/ml) (Liebana, Batchelor et al. 2006). If no growth was observed, the method was repeated using faecal samples enriched overnight in buffered peptone water. At least one isolate morphologically consistent with *E. coli* (if present) was selected per plate. *E. coli* were confirmed using both biochemical methods (Gram stain, catalase production, lack of oxidase, fermentation of lactose, production of indole and inability to utilise citrate as a carbon source) and PCR to detect the presence of the *uidA* gene (McDaniels, Rice et al. 1996).

### 6.2.2 Double disc diffusion testing for presence of ESBL and AmpC β-lactamases

The paired disc diffusion method was used as previously described (M'Zali, Chanawong et al. 2000) (Mast Group Ltd, Merseyside, UK) for phenotypic confirmation of the presence of β-lactamase enzymes. Three pairs of antimicrobial discs (ceftazidime, cefpodoxime and cefotaxime at 30 µg) with and without clavulanic acid (10 µg) were applied to an ISO-Sensitest agar plate (LabM-IDG,

Bury, UK) inoculated for semi-confluent growth. After incubation overnight at 37°C, the zones of inhibition around the plates were measured and recorded. Production of ESBL enzymes is inferred if the presence of the clavulanic acid increased the zone size by at least 5mm, while the presence of an AmpC β-lactamase is suggested when the clavulanic acid has no effect on the zone size.

### 6.2.3 Antimicrobial disc susceptibility testing

Antimicrobial disc susceptibility testing following British Society for Antimicrobial Chemotherapy guidelines(Andrews 2007) was performed. The following antimicrobial discs were used: ampicillin (10µg), augmentin (30µg), aztreonam (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefoxitin (30µg), cefuroxime (30µg), cefalexin (30µg), chloramphenicol (30µg), ciprofloxacin (1µg), nalidixic acid (30µg), tazobactam (10µg), piperacillin (75µg), tetracycline (30µg), trimethoprim (2.5 µg) and trimethoprim-sulfamethoxazole (25µg). Plates were incubated overnight at 37°C and the zones of inhibition around each disc were recorded. The reference strain *E. coli* ATCC 25922 was used as a fully susceptible control.

### 6.2.4 Characterisation of extended spectrum β-lactamase and *bla*$_{\text{AmpC}}$ genes

PCR was used to detect the presence of *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{AmpC}}$ genes using previously described methods (Essack, Hall et al. 2001; Perez-Perez and Hanson 2002; Boyd, Tyler et al. 2004; Batchelor, Hopkins et al. 2005), and the specific *bla*$_{\text{CTX-M}}$ or *bla*$_{\text{CMY}}$ gene variant was determined by sequence analysis.

Following assignment of the specific *bla*$_{\text{CTX-M}}$ gene group, PCR primers specific to each group were used (

Table 6.1) (Boyd, Tyler et al. 2004; Batchelor, Hopkins et al. 2005) to sequence the PCR product on both strands using an ABI 3730 DNA sequence analyser. The sequences of the PCR products were compared to sequences published on GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

**Table 6.1 Primers used for sequencing analysis of blaCTX-M genes**

| CTX-M group | Primer Name | Forward primer (5'-3') | Annealing Temperature (°C) |
|---|---|---|---|
| 1 | Ctxmgrp1F<br>Ctxmgrp1R | CCCATGGTTAAAAAATCACTGC<br>CAGCGCTTTTGCCGTCTAAG | 60 |
| 2 | Ctxmgrp2F<br>Ctxmgrp2R | ATGATGACTCAGAGCATTCGC<br>TCAGAAACCGTGGGTTACGAT | 55 |
| 3 | Ctxmgrp9F<br>Ctxmgrp9R | ATGGTGACAAAGAGAGTGCAAC<br>TTACAGCCCTTCGGCGATG | 60 |

For isolates identified as positive for *bla*$_{CMY}$ genes using multiplex PCR (Perez-Perez and Hanson 2002), the entire gene was amplified (Liebana, Gibbs et al. 2004) and sequenced to determine the specific *bla*$_{CMY}$ gene present. An additional set of primers (those used in the multiplex, CITMf and CITMr) were used to obtain the internal sequence of the gene. All sequences were compared to those submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

### 6.2.5 DNA array analysis of *E. coli*

*E. coli* isolates were subjected to DNA micro array based chip analysis using Identibac *E. coli* and Identibac AMR–ve according to the manufacturer's instructions (Identibac, Surrey, UK). Identibac *E. coli* detects the presence of virulence genes associated with *E. coli*, which include toxins, secretion systems and adherence factors. Identibac AMR-ve detects the presence of genes responsible for resistance to a selection of antimicrobials including the quinolones, sulphonamides, tetracyclines, aminoglycosides, chloramphenicol, trimethoprim, β-lactams and erythromycin in Gram negative bacteria. This work was carried out at the Animal Health and Veterinary Laboratories Agency (AHVLA).

### 6.2.6 Multi-Locus Sequence Typing analysis of *E. coli*

Typing of the isolates was performed at the VLA by multi-locus sequence typing (MLST)by sequencing of internal fragments of seven housekeeping genes; *adk* (adenylate kinase), *fumC* (fumarate hydratase), *gyrB* (DNA gyrase), *icd* (isocitrate/isopropylmalate dehydrogenase), *mdh* (malate dehydrogenase), *purA* (adenylosuccinate dehydrogenase) and *recA* (ATP/GTP binding motif) (Wirth,

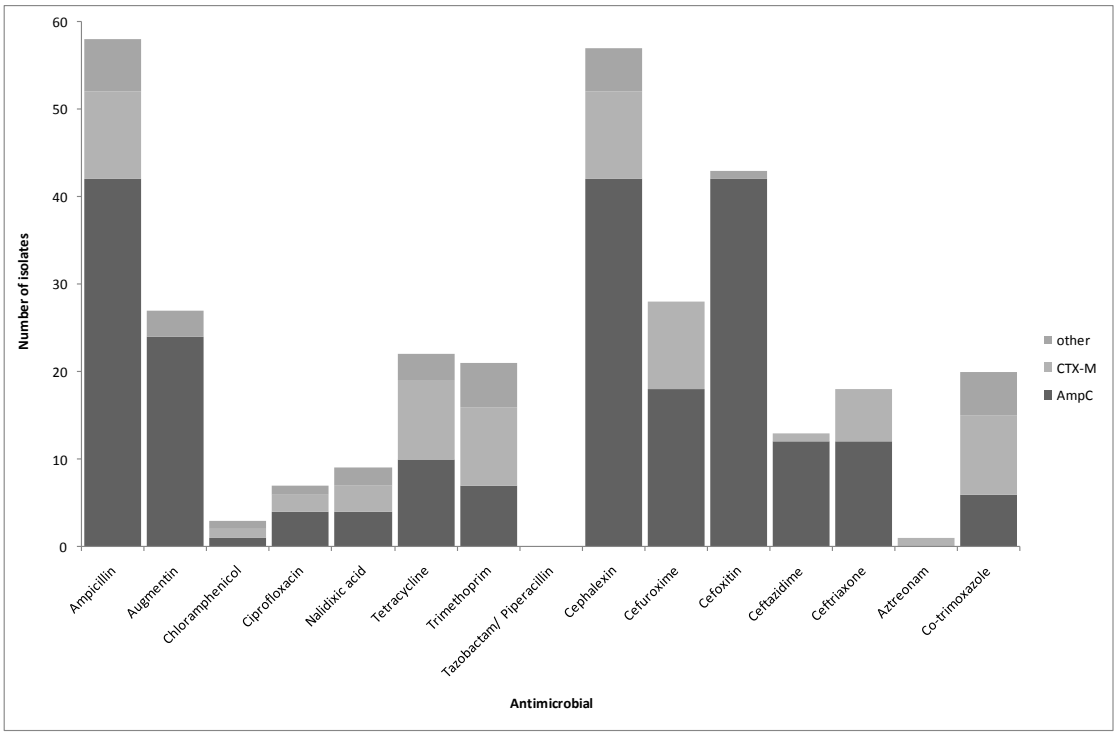
Falush et al. 2006). Sequences were compared to those in the *E. coli* MLST database (http://mlst.ucc.ie/mlst/dbs/Ecoli) and an allele number assigned for each loci, with novel sequences submitted to the database and a sequence type assigned.

## 6.3 Results

### 6.3.1 Antimicrobial disc susceptibility testing

Antimicrobial disc susceptibility testing of the 58 isolates showed that there was a high degree of variation (Figure 6.1 below). Thirty-nine different resistance profiles were observed with the most common being resistance to ampicillin, cephalexin and cefoxitin (10 isolates). As expected, all isolates were resistant to ampicillin (1). Fifty-four of 58 isolates were resistant to cefalexin and 43 of 58 to cefoxitin, which included all the AmpC β-lactamase producing isolates. All ten CTX-M producing isolates were resistant to cefalexin and cefuroxime. All isolates were susceptible to tazobactam and piperacillin, while all but one isolate was susceptible to aztreonam. Multi-drug resistance was observed in 56 of 58 isolates with resistance to three or four antimicrobial classes being most common (17 and 18 isolates respectively).

**Figure 6.1 Prevalence of resistance among the 58 ESBL and AmpC Prevalence of resistance among the 58 ESBL and AmpC producing E. coli**




## 6.3.2 Characterisation of ESBL and AmpC β-lactamase genes

Of the 58 isolates, 21 were found to carry a *bla*$_{TEM}$ gene. Ten carried a *bla*$_{CTX-M}$ gene, most of which were found to be of CTX-M group 1 (five *bla*$_{CTX-M-1}$, two *bla*$_{CTX-M-15}$ and one *bla*$_{CTX-M-3}$). One isolate carried *bla*$_{CTX-M-14/18}$ (CTX-M group 9) and one *bla*$_{CTX-M-20}$ (CTX-M group 2). A *bla*$_{AmpC}$ gene was found in 42 isolates, which were all identified as *bla*$_{CMY-2.}$ No isolates carried a *bla*$_{SHV}$ gene. Table 6.2 below summarises the genes identified in the isolates and the phenotype of resistance observed.

**Table 6.2 Prevalence of resistance among 58 *E. coli* isolates from canine faecal samples**

| Genes detected | Full antimicrobial resistance phenotype* | Number of isolates |
|---|---|---|
| $bla_{TEM,}$ $bla_{cmy2}$ | Pen, 1st Gen, 2nd Gen, 3rd Gen | 1 |
| $bla_{TEM,}$ $bla_{cmy2}$ | Pen, Chl, Q, Tet, Tm, 1st Gen, 2nd Gen | 1 |
| $bla_{TEM,}$ $bla_{cmy2}$ | Pen, Q, Tet, Tm, 1st Gen, 2nd Gen | 3 |
| $bla_{TEM,}$ $bla_{cmy2}$ | Pen, Tet, 1st Gen, 2nd Gen | 1 |
| $bla_{TEM,}$ $bla_{cmy2}$ | Pen, Tet, Tm, 1st Gen, 2nd Gen | 1 |
| $bla_{TEM,}$ $bla_{cmy2}$ | Pen, Tm, 1st Gen, 2nd Gen | 1 |
| $bla_{TEM,}$ $bla_{cmy2}$ | Pen, Tm, 1st Gen, 2nd Gen, 3rd Gen | 1 |
| $bla_{cmy2}$ | Pen, 1st Gen | 1 |
| $bla_{cmy2}$ | Pen, 1st Gen, 2nd Gen | 16 |
| $bla_{cmy2}$ | Pen, 1st Gen, 2nd Gen, 3rd Gen | 12 |
| $bla_{cmy2}$ | Pen, Tet, 1st Gen, 2nd Gen | 2 |
| $bla_{cmy2}$ | Pen, Tet, 1st Gen, 2nd Gen, 3rd Gen | 2 |
| $bla_{TEM,}$ $bla_{CTX\text{-}M\text{-}1}$ | Pen, Q, Tet, Tm, 1st Gen, 2nd Gen | 1 |
| $bla_{TEM,}$ $bla_{CTX\text{-}M\text{-}1}$ | Pen, Tet, Tm, 1st Gen, 2nd Gen | 1 |
| $bla_{TEM,}$ $bla_{CTX\text{-}M\text{-}3}$ | Pen, Tet, Tm, 1st Gen, 2nd Gen, 3rd Gen | 1 |
| $bla_{TEM,}$ $bla_{CTX\text{-}M\text{-}15}$ | Pen, Q, Tet, Tm, 1st Gen, 2nd Gen, 3rd Gen | 1 |
| $bla_{TEM,}$ $bla_{CTX\text{-}M\text{-}1}$ | Pen, Tet, Tm, 1st Gen, 2nd Gen | 1 |
| $bla_{TEM,}$ $bla_{CTXM14/18}$ | Pen, Chl, Tet, Tm, 1st Gen, 2nd Gen, 3rd Gen | 1 |
| $bla_{TEM}$ | Pen | 1 |
| $bla_{TEM}$ | Pen, Chl, Q, Tm, 1st Gen | 1 |
| $bla_{TEM}$ | Pen, Q, Tet, Tm, 1st Gen | 1 |
| $bla_{TEM}$ | Pen, Tet, Tm, 1st Gen | 1 |
| $bla_{TEM}$ | Pen, Tet, Tm, 1st Gen, 2nd Gen | 1 |

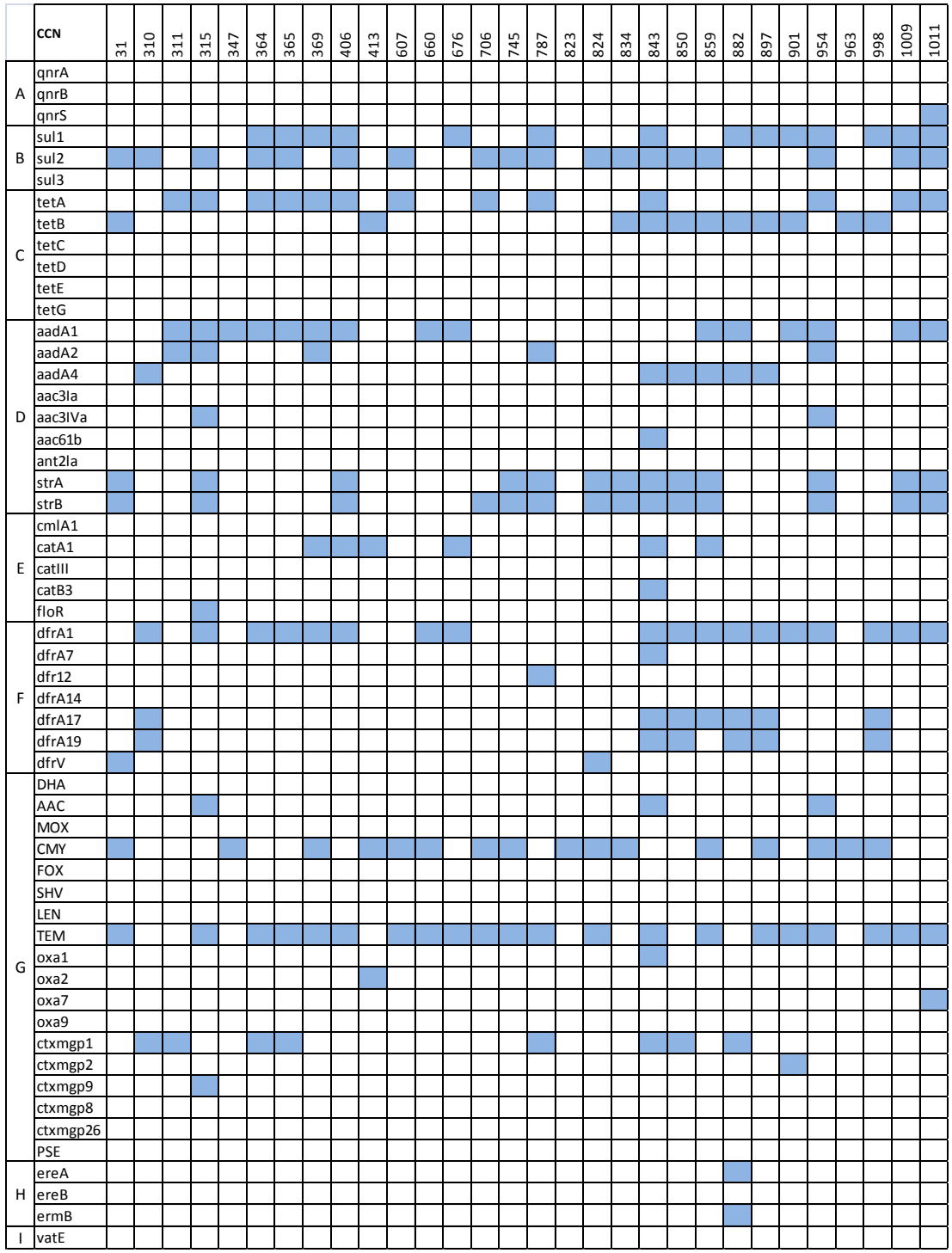
| Genes detected | Full antimicrobial resistance phenotype* | Number of isolates |
|---|---|---|
| *bla*$_{TEM}$ | Pen, Tm, 1st Gen | 1 |
| *bla*$_{CTX-M-1}$ | Pen, Tet, 1st Gen, 2nd Gen, 3rd Gen | 1 |
| *bla*$_{CTX-M-1}$ | Pen, Tet, Tm, 1st Gen, 2nd Gen | 1 |
| *bla*$_{CTX-M-1}$ | Pen, Tm, 1st Gen, 2nd Gen, 3rd Gen | 1 |
| *bla*$_{CTX-M-15}$ | Pen, Q, Tet, Tm, 1st Gen, 2nd Gen, 3rd Gen, Mon | 1 |

*1$^{st}$ Gen cephalexin, 2$^{nd}$ Gen cefuroxime/ cefoxitin, 3$^{rd}$ Gen ceftazidime/ ceftriaxone, Chl chloramphenicol, Mon aztreonam, Pen ampicillin/ augmentin, Q ciprofloxacin/ nalidixic acid, Tet tetracycline, Tm trimethoprim/ co-trimoxazole.

### 6.3.3 Microarray analysis of *E. coli*

A subset of 30 isolates also underwent microarray analysis. Isolates were chosen due to their resistance to multiple classes of antimicrobials including all isolates positive for carriage of a $bla_{\text{CTX-M}}$ gene and a selection of those found to carry either a $bla_{\text{TEM}}$ or $bla_{\text{AmpC}}$ genes. The results observed using the DNA microarray analysis show good agreement with *in vitro* testing of antimicrobial susceptibility (Figure 6.2). Of the 30 isolates tested, 23 harboured genes encoding resistance to tetracycline (*tetA* and *tetB*) and 20 harboured genes encoding resistance to trimethoprim (*dfrA1, dfrA7, dfr12, dfrA17* and *dfrV*). Genes encoding resistance to aminoglycosides were also identified in 25 isolates (*aadA1, aadA2, aadA4, aac3Iva, aac6lb, strA* and *strB*), whilst 24 isolates harboured genes encoding resistance to sulphonamides (*sul1* and *sul2*). Seven isolates were found to harbour genes encoding resistance to chloramphenicol (*catA1*, *catB3* and *floR*). Erythromycin resistance genes (*ereA* and *ermB*) were identified in a single isolate and no genes encoding resistance to streptogrammins were detected.

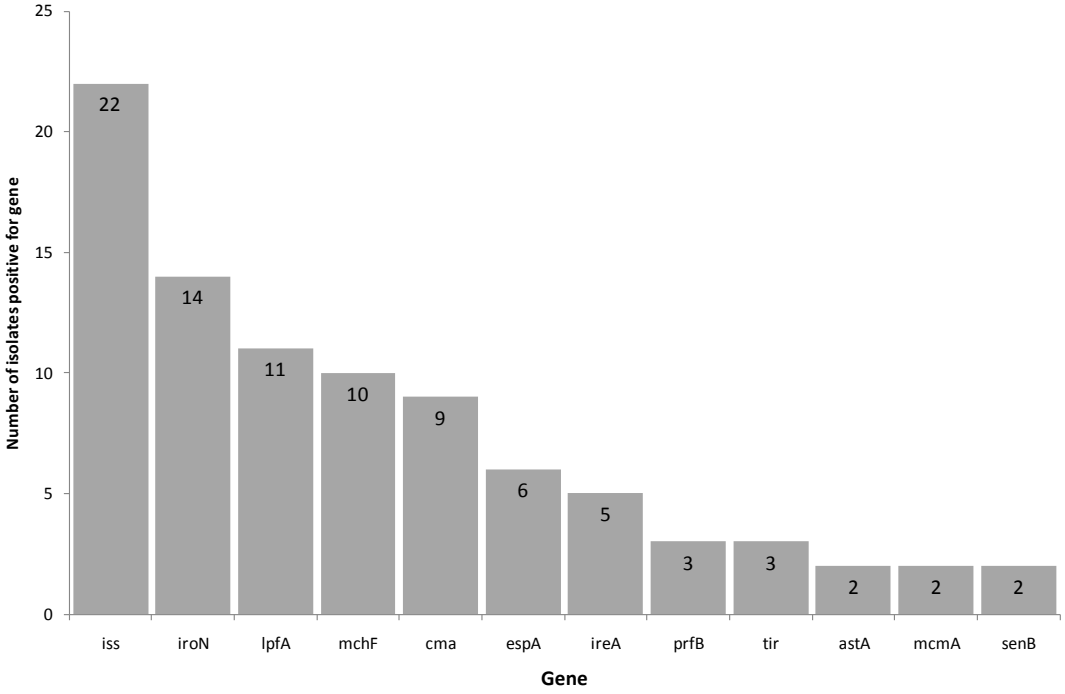
**Figure 6.2 Presence of antimicrobial resistance genes among the 30 ESBL and AmpC β-lactamase producing E. coli isolated from canine faecal samples**




A-quinolone resistance, B-sulphonamides, C-tetracyclines, D-aminoglycosides, E-chloramphenicol, F-trimethoprim, G-β-lactamases, H- erythromycin, I-streptogrammin.

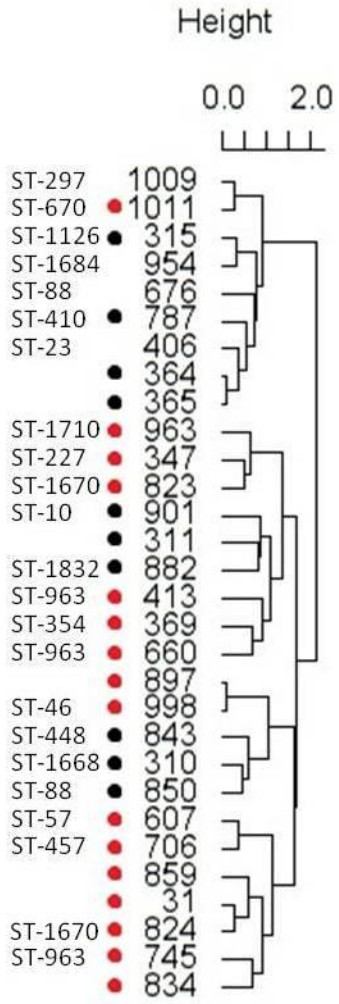
Twenty-three different genes associated with virulence were detected (Figure 6.3). The most common virulence factor identified was *iss* (22 isolates), which is involved in increased resistance to serum, followed by *iroN* (14 isolates), which is involved in iron uptake. A number of genes associated with the locus of enterocyte effacement (LEE) were detected in eight isolates.

**Figure 6.3 Virulence genes detected in 30 ESBL and AmpC β-lactamase producing E. coli isolated from canine faecal samples**




Cluster analysis (Figure 6.4), based on the presence or absence of virulence and antimicrobial resistance genes revealed seven groups. Four of these groups contained only isolates which harboured a $bla_{cmy2}$ gene, with one single $bla_{cmy2}$ carrying isolate clustering separately from the rest. Furthermore, when considering the specific CTX-M enzyme produced, isolates carrying $bla_{CTX\text{-}M\text{-}1}$ did not appear to cluster.

**Figure 6.4 Dendrogram of E. coli clustered by DNA microarray resistance and virulence genes. Red dots indicate presence of *bla*$_{\text{CTX-M}}$ gene. Black dots indicate presence of *bla*$_{\text{cmy2}}$ gene**




### 6.3.4 Multi-Locus Sequence Typing

The 30 isolates studied by microarray also underwent multi-locus sequence typing, although seven could not be typed due to poor sequence or because no amplicon could be obtained. In total, 19 different sequence types (STs) were identified. Sixteen occurred only once and three (ST-1684, ST-1710 and ST-1832) were novel sequence types. The most commonly identified STs were ST-963 (three isolates), ST-88 (two isolates) and ST-1670 (two isolates). With the exception of the three ST-963 isolates, which were all found to carry the *bla*$_{\text{CMY}}$ gene, no patterns relating to ST and

carriage of specific *bla*$_{CTX-M}$ or *bla*$_{CMY}$ genes were evident. Additionally, with the exception of two of the ST-963 isolates clustering together, no patterns were evident when clustering was based on the presence of virulence and resistance genes. The two isolates that carried *bla*$_{CTX-M-15}$ were found to be ST-410 and ST-448 and not the UK pandemic clone ST-131.

## 6.4 Discussion

This is the first study to report a detailed analysis and comparison of antimicrobial resistant *E. coli* isolates from dogs. The findings demonstrate that *E. coli* isolated from dog faeces show highly variable antimicrobial resistance, virulence and sequence type. Overall, however, the range of antimicrobial resistance and virulence factors was similar to that seen in human isolates.

The most common *bla*$_{CTX-M}$ genes were from group one. Half of the isolates positive for this gene carried *bla*$_{CTX-M-1}$, which has previously been identified in isolates from healthy canine faecal samples from Portugal and Chile (Costa, Poeta et al. 2004; Costa, Poeta et al. 2008; Moreno, Bello et al. 2008). However, MLST data revealed that the isolates carrying *bla*$_{CTX-M-15}$ were not ST-131, which is the human epidemic clone associated with CTX-M-15 in the UK (Lau, Kaufmann et al. 2008) and has been isolated from canine clinical isolates in Portugal (Pomba, da Fonseca et al. 2009) and across Europe (Ewers, Grobbel et al. 2010). Three novel sequence types were identified during this study; ST1684, ST1710 and ST1832, which were shown not be highly related with regards to presence of virulence factors and resistance genes. These isolates may suggest the emergence of new ST specifically capable of colonisation of dogs, or simply that isolates of these STs have not yet been identified in humans. With the increasing number of isolates submitted to the MLST database, it is possible that *E. coli* of human or other animal origin with these STs may be identified in future.

The only gene associated with the AmpC β-lactamase phenotype identified from our isolates was *bla*$_{CMY-2}$, which has previously been identified in *E. coli* from canine clinical samples in Italy and Canada (Sanchez, Stevenson et al. 2002; Carattoli,

Lovari et al. 2005), and healthy dogs from the USA (Murphy, Reid-Smith et al. 2009). This suggests that carriage of *bla*$_{\text{CMY-2}}$ may be widespread among canine *E. coli* isolates, and thus dogs may present an important reservoir for human infection, particularly in the USA, where this gene has been increasingly associated with *Salmonella* (Dunne, Fey et al. 2000; Winokur, Brueggemann et al. 2000; Winokur, Vonstein et al. 2001), but also in the UK (Woodford, Reddy et al. 2007). The reason for the high frequency of this one variant of the plasmid mediated AmpC β-lactamases in canine isolates is unknown, but it may be due to spread of a few specific plasmids(Hopkins, Liebana et al. 2006). One other possibility is the integration of the *bla*$_{\text{CMY-2}}$ gene into many diverse plasmids facilitating widespread dissemination (Carattoli, Tosini et al. 2002). It is also possible that the extensive global use of amoxicillin-clavulanic acid in veterinary medicine may have selected for this type of resistance.

Microarray analysis of antimicrobial resistance genes showed that many isolates harboured genes encoding resistance to a wide range of antimicrobials. These antimicrobial resistance genes are also commonly identified in isolates of human origin (Frye, Jesse et al. 2006; Walsh, Cooke et al. 2010; Leverstein-van Hall, Dierikx et al. 2011). The predominant genes and mechanisms of resistance for each of the antimicrobials tested concur with other studies of resistance genes in canine isolates (Lanz, Kuhnert et al. 2003; Costa, Poeta et al. 2008). This provides further evidence that antimicrobial resistance in bacteria isolated from dogs is often mediated by the same mechanisms as those of human origin.

The virulence array detected a wide variety of genes associated with virulence, but by far the most commonly identified were those associated with extra-intestinal pathogenic *E. coli* (ExPEC) in human isolates (Russo, McFadden et al. 2002; Johnson, Wannemuehler et al. 2008). It has been suggested that dogs may be a potential reservoir for this pathotype (Johnson, Stell et al. 2001; Johnson, Johnston et al. 2008). It is also interesting to note that a number of isolates harboured virulence genes associated with the locus of enterocyte effacement (LEE), which are found in enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) (Mcdaniel, Jarvis et al. 1995; Kaper, Nataro et al. 2004; Anjum, Mafura et al. 2007). These are

important pathogens in human enteric disease, and, again, dogs may be a potential reservoir for zoonotic colonisation and infection.

There were a number of discrepancies between the microarray results and antimicrobial disc diffusion susceptibility. For example, one isolate, which harboured *tetA* showed no resistance to tetracycline *in vitro*. Furthermore, one isolate showed resistance to chloramphenicol, but no resistance genes were identified, while five isolates harboured genes encoding resistance to chloramphenicol but showed no associated resistance *in vitro.* This could be explained by silencing of the resistance genes (Enne, Delsol et al. 2006), the absence of effective promoter sequences or insufficient expression to result in observable resistance. Finally, one isolate was found to harbour the plasmid mediated quinolone resistance gene *qnrS* during microarray testing, but no such genes were detected during PCR testing (see chapter five).

It is difficult to draw firm conclusions from the sequence typing data, as only 30 of the 58 isolates were typed, and this wasn't successful in seven of these. Typing all 58 isolates may have provided evidence of some common sequence types, but nevertheless there was a high degree of diversity among ESBL and AmpC β-lactamase producing isolates from these dogs. It is not possible to comment on the whole picture of antimicrobial resistance in healthy dogs as this study focused only on isolates with resistance to third generation cephalosporins. It would therefore be worthwhile to study other isolates, including antimicrobial susceptible isolates, to determine any relationship between virulence, sequence type and antimicrobial resistance on a larger scale.

In conclusion, this study shows that dogs can carry *E. coli* isolates have a similar range of virulence and antimicrobial resistance genes to those isolated from human clinical samples. In particular, virulence factors were associated with the ExPEC pathotype. Dogs could therefore act as reservoirs of pathogenic bacteria, and resistance and virulence genes. However, given the increasing significance of these isolates causing disease in dogs, these findings also highlight the importance of better understanding the role of these isolates.

# Chapter Seven

# Final Discussion

## 7.1 General discussion

Antimicrobial resistance in the canine population has, in recent years, become an issue of increasing importance. As pets are often integral family members, the welfare of dogs, including treatment of infections, is of importance. In addition, given this close bond shared between dogs and their owners and other contacts (family, friends, veterinary professionals), the issue of public health with regards to antimicrobial resistance and transmission of bacteria of clinical importance (for example MRSA) has gained greater recognition (Seguin, Walker et al. 1999; van den Bogaard and Stobberingh 2000; Winokur, Vonstein et al. 2001; Duquette and Nuttall 2004; Weese, Dick et al. 2006). Therefore, knowledge of the burden of antimicrobial resistance on the canine population is of great significance. The work presented in this thesis had three main aims which were to determine the prevalence of meticillin resistant *Staphylococcus aureus* (MRSA) and antimicrobial resistant *E. coli* and within the canine population in mainland UK, to assess their molecular characteristics and to identify any possible risk factors associated with carriage. These aims have been largely accomplished and in the case of carriage of antimicrobial resistant *E. coli* the prevalence of two different dog populations, namely those within the community and the vet visiting, has been estimated.

The prevalence of MRSA within the vet visiting dog population of mainland UK was found to be quite low (1%), which is in agreement with the prevalences reported in many other studies in the UK (0%-4%) and worldwide (Rich and Roberts 2006; Vengust, Anderson et al. 2006; Bagcigil, Moodley et al. 2007; Boost, O'Donoghue et al. 2008). This study found a higher, although not significantly, prevalence of *S. pseudintermedius* (11.0%) than *S. aureus* (7.5%), a finding mirrored in other studies (Griffeth, Morris et al. 2008; Gingrich, Kurt et al. 2011). *S. aureus* and MRSA are most suitably adapted to colonisation of the human or bovine mucosal surfaces, and the findings of this study provide further evidence that *S. aureus* is perhaps less well adapted than *S. pseudintermedius* to the colonisation of dogs. Given such a low prevalence in the population studied, the risks for humans posed by these dogs could be considered very low. However, the risks to humans posed by populations with higher prevalences (hospitalised dogs or dogs with recurrent

infections such a pyoderma) should not be taken lightly (Loeffler, Boag et al. 2005; Griffeth, Morris et al. 2008). Analysis of risk factors could not be undertaken as a result of the low prevalence in the population studied. This study also confirmed that the strains of MRSA carried by dogs (characterised by sequence type, *spa* type and SCC*mec* type) are identical to those endemic within the human hospital setting (Baptiste, Williams et al. 2005; O'Mahony, Abbott et al. 2005; Coelho, Torres et al. 2011). No meticillin resistant *S. pseudintermedius* were isolated during this study, a similar finding to another study in the UK (Loeffler, Pfeiffer et al. 2010), but different from other studies elsewhere that have found the prevalence of MRSP to be higher than MRSA (Sasaki, Kikuchi et al. 2007; Griffeth, Morris et al. 2008; Ruscher, Lubke-Becker et al. 2009; Morris, Boston et al. 2010). Some of these studies, however, investigated prevalences in clinical samples and dogs with pre-existing inflammatory skin disease, which may account in part for the differences observed. *Staphylococcus pseudintermedius* (formally *S. intermedius*) has been identified as a common commensal of dogs (Sasaki, Kikuchi et al. 2007) and MRSP is now emerging as an important pathogen in dogs associated with pyoderma and otitis (Weese and van Duijkeren 2010), often also harbouring genes conferring resistance to other antimicrobials (Perreten, Kadlec et al. 2010).

Antimicrobial resistance testing was carried out on all coagulase positive staphylococci and all meticillin resistant coagulase negative staphylococci (MR-CNS), which has been rarely undertaken in other studies. High levels of resistance were found to certain antimicrobials such as, fusidic acid (43.7%), gentamicin (30.3%), ciprofloxacin (19.7%) and tetracycline (19.0%). Furthermore, carriage of multidrug resistant staphylococci was found in (21.8%) of dogs. The prevalence of resistance did vary depending on the specific staphylococcal species. It is possible that these bacteria may provide an important source of resistance determinants for other, more pathogenic bacteria. Carriage of such resistance determinants might also provide the bacteria with an advantage as an opportunistic pathogen in the host dog.

In contrast to the low prevalence of MRSA, the prevalence of antimicrobial resistant *E. coli* in the UK vet visiting dog population was high (44.8%), with the most prevalent resistances being to antimicrobials commonly used in canine medicine,

such as ampicillin, tetracycline and trimethoprim (24.0%, 19.7% and 16.9% respectively in the community; and 37.2%, 30.0% and 23.8% respectively in the vet visiting population). Carriage of antimicrobial resistance at such a high level, in particular multidrug resistance (15.3% in the community and 18.1% in the vet visiting population), emphasises that dogs may be a potential reservoir of both resistant enteric bacteria and resistance determinants. It also raises an important issue with regards to potential treatment options for the individual dogs in the future, should such bacteria cause an infection.

A subset of the *E. coli*, selected for their carriage of $bla_{\text{CTX-M}}$, $bla_{\text{TEM}}$ or $bla_{\text{cmy2}}$ and their resistance to numerous antimicrobials, were subjected to MLST and micro array analysis of virulence and resistance genes. A great deal of variation in both sequence type (ST) and genotypic resistance and virulence factors was evident. This is the first such study to characterise in such detail *E. coli* isolated from healthy dogs and provides valuable information regarding the genetic background of antimicrobial resistance, particularly ESBL-producing *E. coli*. Two of the ten isolates that carried $bla_{\text{CTX-M}}$ genes were identified to carry $bla_{\text{CTX-M-15}}$, but none were found to be ST-131, the highly successful human pandemic strain (Lau, Kaufmann et al. 2008; Nicolas-Chanoine, Blanco et al. 2008), responsible for the worldwide clonal spread of $bla_{\text{CTX-M}}$. *E. coli* of this ST have previously been identified in companion animals in several European countries (Pomba, da Fonseca et al. 2009; Ewers, Grobbel et al. 2010), but not at the time of writing in the UK.

This study also provides important information relating to statistical analysis of the potential risk factors associated with carriage of antimicrobial resistant *E. coli* including recent prescription of antimicrobials and being fed raw poultry. As would be expected, previous use of antimicrobials regularly remained significant in the final models of resistance outcomes. This supports the careful and rational use of antimicrobials in dogs (Ungemach, Muller-Bahrdt et al. 2006; Escher, Vanni et al. 2011). In some models, the diet of the dog was significantly associated and this shows how the types of food eaten can have a great impact on the flora of an animal. Owning another animal, particularly a cat was found to be associated with antimicrobial resistance of *E. coli* found in the faecal samples, as well as having

contact with farm and wild animals during walks. This may provide an indication as to possible sources of either antimicrobial resistant *E. coli* or, perhaps more likely, specific resistance determinants from environmental sources.

This study does have some limitations, both in relation to the study design and methods. As with many cross sectional studies, there is a degree of unavoidable bias. Practices were recruited by random selection from the Royal College of Veterinary Surgeons register and contacted by phone. Each practice was asked to recruit 28 dogs to the study. However, veterinary personnel may not have had time or consciously chosen not to ask all owners depending on how cooperative the dog may have been. Also, using veterinary surgeries to recruit owners for the nationwide cross sectional study meant that only dogs visiting the vet were recruited. A different approach could have been to contact owners directly for recruitment by use of mailing information to vet patient lists. This would however prove very expensive and logistically difficult.

Isolation of antimicrobial resistant *E. coli* from the dogs in the community was carried out on previously frozen faecal samples. This may have had an impact on the recoverability of *E. coli* and may have lowered the prevalence observed in this population. In a study investigating recovery of faecal flora from frozen faecal samples (Bonten, Nathan et al. 1997), a decrease in recovery after freezing for up to four weeks was shown. However, the authors did find that storage in glycerol, in which the community faecal samples were stored, improved recovery rates compared to other methods tested.

An aim of this study was to carry out analysis of some of the molecular characteristics of the isolates obtained. DNA microarray analysis was carried out to investigate the presence of certain antimicrobial resistance and virulence determinants among the isolates, which showed that many of the isolates carried genes detected in isolates of human origin. For example virulence genes associated with ExPEC , EPEC and EHEC, all common pathogens of humans. This supports other studies which suggest a fair amount of overlap between isolates of human and animal origin with regards to virulence determinants (Johnson, Stell et al. 2001; Johnson, Stell et al. 2001; Johnson, Johnston et al. 2008).

This study only took samples from the dogs at a single point in time. However, with *Staphylococcus* in particular, some individuals may be only intermittent carriers. At the point of sampling, these individuals may have been negative, but this does not mean that they have never been carriers or that they will not be in the future. A more complete estimation of prevalence could be achieved by collecting samples at multiple, regular time points in a longitudinal study.

## 7.2 Further work

While this study has gone to great lengths to estimate the prevalence of antimicrobial resistant bacteria in the canine population of mainland UK and has taken steps to characterise many of the isolates collected, there is still a significant amount of information that the study could provide. Multi-locus sequence typing (MLST) was carried out on all $bla_{\text{CTX-M}}$ and a selection of $bla_{\text{cmy2}}$ or $bla_{\text{TEM}}$ carrying *E. coli*. In addition, all MRSA and a selection of MSSA were subjected to MLST. While, this has provided valuable data relating to the strains of bacteria carried by dogs, it is highly likely that some of the isolates that did not undergo MLST may be different to those already identified in this study and provide a more comprehensive representation of the bacteria carried by dogs and how these relate to those of human and other animal origin. In addition, three of the *E. coli* were found to be of a novel ST, it is therefore possible that further novel STs would be identified if MLST was carried out on more isolates.

As mentioned in chapter four, the prevalence of MRSA was too low for any meaningful statistical analysis to be carried out. A larger, possibly multi-laboratory study, would allow for a higher number of dogs to be sampled, thus allowing identification of risk factors associated with carriage of MRSA to be carried out. However, the information this would provide would not be worth the expense and time needed to execute such a study. Nonetheless, it is possible that the prevalence of *S. aureus* and *S. pseudintermedius* is sufficiently high enough for some statistical analysis, but how useful this would be remains to be seen.

Given the number of journal articles and case studies where animals have been identified as maintaining MRSA in the household (Cefai, Ashurst et al. 1994; Manian 2003; Weese, Dick et al. 2006; Nienhoff, Kadlec et al. 2009) and relatedness of isolates between family members (Boost, O'Donoghue et al. 2008), a study that follows a number of households in which all family members (both human and animal) are sampled this could provide important information about the persistence of MRSA within the family as a whole rather than just considering the individuals. From this kind of research, it may be possible to gain substantial evidence regarding source attribution, identify the index case within the household and what factors may contribute to acquisition and maintenance of MRSA. A small study has been carried out (Faires, Tater et al. 2009), however since this only involved 22 households at a single time point, only limited information could be obtained. Therefore a larger scale, longitudinal study could provide a more detailed picture of MRSA in the household.

## 7.3 Final conclusions

The findings of this study clearly indicate a high prevalence of carriage of antimicrobial resistant *E. coli, in dogs* with a number of factors associated with its carriage including previous use of antimicrobials (further supporting the prudent use of antimicrobials in dogs) and consumption of raw poultry meat. The molecular characteristics of the isolates were diverse, as well as the profiles of antimicrobial resistance. MRSA carriage is reassuringly low in the dog population studied and those that were isolated were all found to be identical to those commonly isolated from UK hospitals. This clearly suggests transmissions between species, although this study does not offer any indication of the direction. Carriage of antimicrobial resistant bacteria by dog is just one increasingly important aspect of a much wider issue both with respect to canine welfare and the zoonotic potential.

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# Appendices

## Appendix One

Vet information sheet

Vet sampling protocol

Owner information sheet

Informed consent form

Nationwide questionnaire

UNIVERSITY OF LIVERPOOL

***Detection of antibiotic resistant bacteria in animal faecal and nasal swab samples.***

Dear Colleague,

Thank you for your agreement to take part in this study being carried out to investigate the prevalence of antibiotic resistant bacteria in dogs in the community. This study principally aims to investigate staphylococcal species and *Escherichia coli* in the nose and gastrointestinal tract, respectively.

You are one of many veterinary practices across the country who is being asked to obtain samples from 28 dogs each. We are seeking samples from only one dog from each household and the dogs that you sample can be healthy, visiting for routine appointments or sick dogs. We do, however, request that you do not recruit dogs that have been admitted to the hospital. Please note that due to the high volume of samples we aim to obtain, we will be unable to provide you with individual results.

For this study, we will require you, with the signed consent of the owner, to obtain a nasal swab from their dog during the consultation and ask the owner to provide us with a faecal sample when the dog next defecates. We will provide you with a sampling pack, which contains everything you will need to swab the nose and give to the owner for collection of the faeces. Also included will be an informed consent form, a questionnaire for the owner to complete and two pre paid envelopes; one for you to return the nasal swab and signed consent form, and one to give to the owner to return the faecal sample pot and questionnaire. We will in addition provide information letters for owners to read telling them of the study and asking for their participation. These can be handed out to clients in the waiting room to read to avoid delay during the consultation.

**For the nasal swab**

Using the sterile swab provided, insert the cotton end about 1cm into the nasal passage and move around the inside of the nostril. Repeat this with the other nostril using the same swab. Place the swab back into its container. On the label, enter the date of the sample, dog and owner information and, using the prepaid return envelope, return it to us with the signed consent form.

**For the faecal swab**

The faecal sample will need to be collected by the owner when their dog next defecates, so please provide the owner with the faecal sample pack.

**Questionnaire**

We will also be asking the owners to fill out a short questionnaire, which contains questions relating to the dog's current and previous health, veterinary treatment, diet, environment and contact with other animals. We are also asking owners if they would be willing to participate in the second phase of the investigation involving a longitudinal study for which we would deal with the owner directly. This completed questionnaire should be returned with the faecal sample by the owner.

Samples returned to us will be subjected to a number of analytical tests to characterise the isolated bacteria. Such tests will include antibiotic resistance

profiling, biochemical testing, polymerase chain reaction (PCR) to identify genes responsible for resistance to antimicrobials. We will also be able to perform epidemiological analysis on the data when we relate this back to the information provided on the questionnaires and identify potential risk factors associated with carriage of antibiotic resistant bacteria in dogs.

**Of what benefit is this study to Small Animal Veterinary medicine?**

Antimicrobial resistant bacteria pose a huge problem to veterinary medicine as the number of antibiotics available to treat infections caused by them is severely reduced. Furthering our knowledge of how antibiotic resistance develops, is spread and understanding the epidemiology of antimicrobial resistance will help us to prevent further development and expansion of resistance to ensure the efficacy of the already limited antimicrobials available.

Thank you again for agreeing to take part in this study and in advance for collection of the samples. Should you have any further questions about this work, please feel free to contact us on 0151 794 6027 or at antibiotic.resistance@liverpool.ac.uk.

Yours sincerely

Amy Wedley BSc (Hons)
University of Liverpool
Veterinary Clinical Sciences Department
Leahurst
CH64 7TE
0151 7946027
antibiotic.resistance@liverpool.ac.uk

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***Detection of antibiotic resistant bacteria in animal faecal and nasal swab samples.- Protocol for Vets.***

During or before consultation, ask dog owner whether they might be interested in taking part in study.

⇩

If they are interested give the owner the information sheet to read. If they have any questions, answer them if you feel happy to or refer them to the contact details provided on the information sheet.

⇩

If owner agrees to participate, ask them to read and sign the consent form provided at the front of the sample pack. Please ask them to initial each box on the consent form.

⇩

After consent form has been signed, take sterile nasal swab insert the cotton end about 1cm into the nasal passage and move around the inside of the nostril. Repeat this with the other nostril using the same swab. Place the swab into its container, break off and seal the container. This should already be numbered with the same unique identification number as the paperwork and faecal sample pot.

⇩

Place nasal swab and completed consent form in the empty pre-paid envelope, seal and post back to the University of Liverpool for processing.

⇩

Give the owner the pre-paid envelope containing questionnaire, faecal sample pot and glove.

⇩

Explain to the owner that they need to fill in the questionnaire as accurately as possible and collect a fresh faecal sample in the pot provided when their dog next defecates. They then need to send the completed questionnaire and sample to the University of Liverpool in the envelope provided.

⇩

**Many thanks for your time and help.**

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***Detection of antibiotic resistant bacteria in animal faecal and nasal swab samples: Information Sheet***

Dear dog owner,

Your veterinary surgeon has kindly agreed to help the University of Liverpool Veterinary School with a new study looking at antibiotic resistant bacteria. As part of this, you and your dog are invited to take part. We would be very grateful if you would allow us to take some samples from your dog and ask you some questions.

Please read the following information carefully and please ask if you would like more information or if there is anything you do not understand. Your vet may be able to answer some questions; otherwise my contact details are at the end of this letter. We would like to stress that you do not have to accept this invitation and should only agree to take part if you want to. If you decide not to participate this will **not** affect the veterinary treatment of your animal

## Why are we getting these samples?

All animals carry bugs (such as bacteria) in their guts and nasal passages (and other places too), and most of them cause no problem. Bacteria which are not killed by antibiotics (antibiotic resistant bacteria) are now becoming more of a concern in animal and human medicine. We are trying to see how much antibiotic resistance there is in the normal bacteria which animals carry. This will allow us a greater understanding of how antibiotic resistance occurs and hopefully can lead to the development of new ways to combat the problem. It will also give us more information so that we can develop ways to combat the spread of resistance, so that in the future we will not run out options for treating infections.

## What samples are we collecting?

If you agree to take part, your vet will take a swab from your dog's nose (nasal swab). This is a routine procedure with very little to risk to your animal. It does not hurt and will not cause distress to your dog. Your vet will also provide you with a sample pot and a pair of gloves to collect a sample of your dog's poo (faecal sample) when he or she next produces fresh faeces. You will be provided with a pre-paid envelope to send the sample back to us.

## Anything more involved?

We would also like to know a bit about your dog and the household it lives in, so we have a short questionnaire for you also. Your vet will give you the questionnaire for you to fill in and send back with your dog's faecal sample. It is important that you answer all questions to the best of your knowledge to provide us with accurate information.

## Further information

Samples and information obtained from the questionnaire may be retained for up to six years and possibly used in future projects. All data will be stored in a secured database only accessible by people working on the project. If you decide you want

to withdraw from the study you may do so without explanation, and any information you have given can be destroyed.

All answers from the questionnaire will be kept strictly confidential. Results from the study will be printed in veterinary journals and also in the non-veterinary animal press, but no-one will be identifiable from any published work.

## What next?

If you are happy to allow your dog to become involved, then please read and sign the consent form, and the vet can start getting the samples. Please note, unfortunately due to the large number of samples, we will not be able to give you back any individual results from your dog.

Many thanks,

Amy Wedley BSc (hons)

University of Liverpool
Veterinary Clinical Sciences Department
Leahurst Campus
CH64 7TE
0151 794 6027
antibiotic.resistance@liverpool.ac.uk

**If there are any problems, please let us know by contacting Amy Wedley on 0151 794 6027, and we will try to help. If you remain unhappy or have a complaint which you feel you cannot come to us with then you should contact the Research Governance Officer on 0151 794 8290 (ethics@liv.ac.uk). When contacting the Research Governance Officer, please provide details of the name or description of the study (so that it can be identified), the researcher(s) involved, and the details of the complaint you wish to make.**

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***Detection of antibiotic resistant bacteria in animal faecal samples: Informed Consent Form***

***Please read the following information carefully. You may also request a copy for yourself.***

**Research Study:** *"Detection of antibiotic resistant bacteria in animal faecal and nasal swab samples."*

**Researcher:** Amy Wedley

**Please initial box**

1. I confirm that I have read and have understood the information sheet dated December 2009 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my rights being affected. If I do not participate this will **not** affect the veterinary treatment of my animal. ☐

3. I understand that, under the Data Protection Act, I can at any time ask for access to the information I provide and I can also request the destruction of that information if I wish. I understand that I may refuse to answer particular questions and individual privacy will be maintained in all published and written data from the study. ☐

4. I allow participation of myself and my animal in the above study. ☐

If you agree with the above-stated conditions please sign below:-

| | | |
|---|---|---|
| Participant Name | Date | Signature |
| Name of Person taking consent | Date | Signature |
| Researcher | Date | Signature |

**The contact details of lead Researcher are:**

Amy Wedley
National Centre for Zoonosis Research
Leahurst Campus
University of Liverpool
Neston
CH64 7TE
0151 795 6027
antibiotic.resistance@liverpool.ac.uk

Ref

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# Antibiotic Resistance in Dogs Questionnaire

*All information from this questionnaire is strictly confidential and will be available only to the investigators. Individual privacy will be maintained in any published /written data*

Please answer the questions by marking an "X" in the box, or writing your answer in the spaces provided (using block capital letters). Where boxes are given, only write one letter in each box. Please use blue or black ink only.

☒ Male ☐ Female

LIKE THIS OR THIS

*Please answer all questions to the best of your knowledge. If you are uncertain of any of the answers please indicate. You may decline to answer any question.*

## QUESTIONS ABOUT YOU

Date of veterinary visit / /

Your contact details:-

Forename

Surname

Address

Line 2

Line 3

Line 4

Postcode Phone ( )

## QUESTIONS ABOUT YOUR DOG

1. Name

2. Age Years Months Weeks

Is this: ☐ Exact ☐ Estimate ☐ Don't know age

3. Breed ☐ Pedigree *(please specify below)* ☐ Cross *(please specify below)* ☐ Don't know

4. Sex ☐ Male ☐ Female ☐ Don't know

5. Has s/he been neutered ☐ Yes ☐ No ☐ Don't know

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6. How long have you owned him/her? Years Month Weeks

7. Where did you get him/her from?

Breeder | Family/ Friend | Rescue Kennel | Pet Shop

Newspaper advert | Don't know | Other

8. Is your dog a working dog? Yes | No | Don't know

If, yes what type of work does s/he do?

## QUESTIONS ABOUT YOUR DOG'S DIET

9. What is s/he fed? *(Cross all that apply)*

Tinned meat | Raw chicken | Dry Mixer | Cooked chicken

Dry complete | Raw red meat | Cooked red meat | Don't know

Other

10. Is s/he fed commercial dog treats?

Never | Rarely | Sometimes | Often | Don't know

11. Is s/he fed human titbit/ scraps?

Never | Rarely | Sometimes | Often | Don't know

## QUESTIONS ABOUT YOUR DOG'S HEALTH

12. What was the reason for your visit today?

Vaccination *Go to Question 13* | Presenting Complaint *(please state below)*

Was any medication prescribed? Yes | No | Don't know

If yes, was it an antibiotic? Yes | No | Don't know

If antibiotic, what was prescribed? *(if known please provide details of drug name and dose)*

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For how long was this antibiotic prescribed?

☐ One-off injection ☐ Up to 5 days ☐ Up to 10 days ☐ Up to 2 weeks

☐ Up to 3 weeks ☐ Over 3 weeks ☐ Don't know

**13. Has your dog received any veterinary treatment (other than today)in the last 3 months?**

☐ Yes *(please provide details)* ☐ No *Go to Question 14* ☐ Don't know *Go to Question 14*

Was any medication prescribed? ☐ Yes ☐ No ☐ Don't know

If yes, was it an antibiotic? ☐ Yes ☐ No ☐ Don't know

If antibiotic, what was prescribed? *(if known please provide details of drug name and dose)*

For how long was this antibiotic prescribed?

☐ One-off injection ☐ Up to 5 days ☐ Up to 10 days ☐ Up to 2 weeks

☐ Up to 3 weeks ☐ Over 3 weeks ☐ Don't know

Was your dog left at the vet premises? ☐ Yes ☐ No ☐ Don't know

If yes, for how long was s/he there for?

## WALKING YOUR DOG

**14. Where is your dog most often walked?** *(Please cross only one)*

☐ Park ☐ Playing fields ☐ Public footpaths ☐ Roads

☐ Woods ☐ Canals ☐ Beach ☐ Farmland

☐ Other

**15. Is your dog allowed close contact with other dogs during walks?**

☐ Yes ☐ No ☐ Don't know

**16. Does your dog have regular contact with wild or farm animals during walks?**

☐ Yes ☐ No ☐ Don't know

**17. Do you regularly allow your dog off the lead during walks?**

☐ Yes ☐ No ☐ Don't know

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## ABOUT YOUR HOUSEHOLD

**18. Are there any other dogs in the household?**

☐ Yes ☐ No ☐ Don't know If yes, how many? ☐☐

**19. Do you own any other animals (other than dogs)?**

☐ Yes ☐ No ☐ Don't know

If yes, what animals? *(please cross all that apply)*

☐ Cat ☐ Bird ☐ Rabbit ☐ Rodent

☐ Reptile ☐ Don't know

☐ Other

**20. Where does your dog usually sleep?**

☐ Outside ☐ Garage ☐ Kitchen ☐ Living Room

☐ Bedroom floor ☐ Bedroom on the bed of a family member

☐ Don't know ☐ Other

**21. Does anyone in your household work with farm animals?**

☐ Yes ☐ No ☐ Don't know

If yes, please state which animals are worked with

**22. Has anyone in your family (including other pets) to your knowledge in the last month taken antibiotics?**

☐ Yes ☐ No ☐ Don't know

If yes, was this a ☐ Family member ☐ Pet

What antibiotic was prescribed? *(if known)*

**23. Does anyone in your household work in medical or veterinary healthcare?**

☐ Yes ☐ No ☐ Don't know

If yes, in what setting?

☐ Hospital ☐ Community Nursing ☐ GP Surgery ☐ Nursing Home

☐ Dentist ☐ Veterinary Practice ☐ Don't know

☐ Other

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**24. Has anyone in your household attended hospital in the last month?**

☐ Yes ☐ No ☐ Don't know

If yes, why?

☐ Admission to hospital ☐ Visit ☐ Outpatient appointment ☐ Don't know

☐ Other

**AND FINALLY**

**25. Would you be willing for your dog to take part in the second part of this studty which involves collecting faecal samples from your dog, in pots provided, weekly for 4 weeks and montly thereafter for a total period of 6 months?**

☐ Yes ☐ No ☐ Don't know

**Date of faecal sample collection** ☐☐ / ☐☐ / ☐☐☐☐

**Date of questionnaire completion** ☐☐ / ☐☐ / ☐☐☐☐

**Thank you for your time**

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## Appendix Two

PCR primers and expected size of amplicons

PCR reaction conditions

PEG precipitation protocol

PCR primers and expected size of amplicons

| Target Gene | Primer name | Primer (5'-3') | Expected amplicon size(bp) | Reference |
|---|---|---|---|---|
| *uidA* | uidAf | CCAAAAGCCAGACAGAGT | 623 | (McDaniels, Rice et al. 1996) |
| | uidAr | GCACAGCACATCAAAGAG | | |
| $bla_{TEM}$ | tembf | ATGAGTATTCAACATTTCCGTG | 861 | (Essack, Hall et al. 2001) |
| | tembr | TTACCAATGCTTAATCAGTGAG | | |
| | temiA (internal)$^{+}$ | TTCTGTGACTGGTGAGTACT | | |
| | temiB (internal)$^{+}$ | GAGTAAGTAGTTCGCCAGTT | | |
| | temiC (internal)$^{+}$ | CTGCAGCAATGGCAACAAC | | |
| $bla_{SHV}$ | shvbf | ATGCGTTATATTCGCCTGTG | 865 | (Essack, Hall et al. 2001) |
| | shvbr | GTTAGCGTTGCCAGTGCTCG | | |
| $bla_{CTX-M}$ | CTX-MU1 | ATGTGCAGYACCAGTAARGTKATGGC | 593 | (Boyd, Tyler et al. 2004) |
| | CTX-MU2 | TGGGTRAARTARGTSACCAGAAYCAGCGG | | |
| $bla_{CTX-M}$ group 1 | CTX-M-1f | ATGGTTAAAAAATCACTGCG | 876 | (Batchelor, Hopkins et al. 2005) |
| | CTX-M-1fr | TTACAAACCGTCGGTGAC | | |
| $bla_{CTX-M}$ group 2 | CTX-M-2f | ATGATGACTCAGAGCATTCGC | 893 | (Hopkins, Batchelor et al. 2006) |
| | CTX-M-2r | TCAGAAACCGTGGGTTACGAT | | |

| Target Gene | Primer name | Primer (5'-3') | Expected amplicon size(bp) | Reference |
|---|---|---|---|---|
| *bla*$_{\text{CTX-M}}$ group 9 | CTX-M-9f | ATGGTGACAAAGAGAGTGCAAC | 876 | (Batchelor, Hopkins et al. 2005) |
| | CTX-M-9fr | TTACAGCCCTTCGGCGATG | | |
| *bla*$_{\text{AmpC}}$ (Multiplex) | MOXMf | GCTGCTCAAGGAGCACAGGAT | 520 | (Perez-Perez and Hanson 2002) |
| | MOXMr | CACATTGACATAGGTGTGGTGC | | |
| | CITMf (internal)* | TGGCCAGAACTGACAGGCAAA | 462 | |
| | CITMr (internal)* | TTTCTCCTGAACGTGGCTGGC | | |
| | DHAMf | AACTTTCACAGGTGTGCTGGGT | 405 | |
| | DHAMr | CCGTACGCATACTGGCTTTGC | | |
| | ACCMf | AACAGCCTCAGCAGCCGGTTA | 645 | |
| | ACCMr | TTCGCCGCAATCATCCCTAGC | | |
| | EBCMf | TCGGTAAAGCCGATGTTGCGG | 302 | |
| | EBCMr | CTTCCACTGCGGCTGCCAGTT | | |
| | FOXMf | AACATGGGGTATCAGGGAGATG | 190 | |
| | FOXMr | CAAAGCGCGTAACCGGATTGG | | |
| *cmy* (sequencing) | cmy25f | CAATGTGTGAGAAGCAGTC | 1146 | (Liebana, Gibbs et al. 2004) |
| | cmy2dr | CGCATGGGATTTTCCTTGCTG | | |

| Target Gene | Primer name | Primer (5'-3') | Expected amplicon size(bp) | Reference |
|---|---|---|---|---|
| *qnrA* | qnrAf | ATTTCTCACGCCAGGATTTG | 516 | (Robicsek, Strahilevitz et al. 2006) |
| | qnrAr | GATCGGCAAAGGTTAGGTCA | | |
| *qnrB* | qnrBf | GATCGTGAAAGCCAGAAAGG | 469 | |
| | qnrBr | ACGATGCCTGGTAGTTGTCC | | |
| *qnrS* | qnrSf | ACGACATTCGTCAACTGCAA | 417 | |
| | qnrSr | TAAATTGGCACCCTGTAGGC | | |
| *dfrA1* | D1 | ACGGATCCTGGCCTGTTGGTTGGACGC | 254 | (Gibreel and Skold 1998; Lee, Oh et al. 2001) |
| | D2 | CGGAATTCACCTTCCGGCTCGATGTC | | |
| *dfrA9* | dfr9F | ATGAATTCCCGTGGCATGAACCAGAAGAT | 398 | |
| | dfr9R | ATGGATCCTTCAGTAATGGTCGGGACCTC | | |
| *dfrA5/14* | D3 | GTTGCGGTCCAGACATAC | 253 | |
| | D4 | CCGCCACCAGACACTA | | |
| *dfrA8* | D5 | TCGAGCTTCATGCCATTT | 453 | |
| | D6 | TCTTCCATGCCATTCTGC | | |
| *dfrA12/13* | D7 | CCGTGGGTCGATGTTTGATG | 485 | |
| | D8 | GCATTGGGAAGAAGGCGTCAC | | |
| *dfrA7/17* | D9 | GTCGCCCTAAAACAAAGTTA | 195 | |
| | D10 | CGCCCATAGAGTCAAATGT | | |

| Target Gene | Primer name | Primer (5'-3') | Expected amplicon size(bp) | Reference |
|---|---|---|---|---|
| *tetA* | TetAf | GCTACATCCTGCTTGCCTTC | 210 | (Ng, Martin et al. 2001) |
| | TetAr | CATAGATCGCCGTGAAGAGG | | |
| *tetB* | TetBf | TTGGTTAGGGGCAAGTTTTG | 659 | |
| | TetBr | GTAATGGGCCAATAACACCG | | |
| *tetC* | TetCf | CTTGAGAGCCTTCAACCCAG | 418 | |
| | TetCr | ATGGTCGTCATCTACCTGCC | | |
| *tetD* | TetDf | AAACCATTACGGCATTCTGC | 787 | |
| | TetDr | GACCGGATACACCATCCATC | | |
| *tetE* | TetEf | AAACCACATCCTCCATACGC | 278 | |
| | TetEr | AAATAGGCCACAACCGTCAG | | |
| *tetG* | TetGf | GCTCGGTGGTATCTCTGCTC | 468 | |
| | TetGr | AGCAACAGAATCGGGAACAC | | |
| *mecA* | mecAf | TGGCTATCGTGTCACAATCG | 310 | (Vannuffel, Gigi et al. 1995) |
| | mecAr | CTGGAACTTGTTGAGCAGAG | | |
| *femA* | femAf | TGCCTTTACAGATAGCATGCCA | 703 | (Francois, Pittet et al. 2003) |
| | femAr | AGTAAGTAAGCAAGCTGCAATGACC | | |
| *nuc* | nuc1 | GCGATTGATGGTGATACGGTT | 279 | (Brakstad, Aasbakk et al. 1992) |
| | nuc2 | AGCCAAGCCTTGACGAACTAAAGC | | |

| Target Gene | Primer name | Primer (5'-3') | Expected amplicon size(bp) | Reference |
|---|---|---|---|---|
| *pta* | ptaF1 | AAAGACAAACTTTCAGGTAA | 320 | (Bannoehr, Franco et al. 2009) |
| | ptaF2 | GCATAAACAAGCATTGTACCG | | |
| (*nuc*) | au-F3 | TCGCTTGCTATGATTGTGG | 359 | (Sasaki, Tsubakishita et al. 2010) |
| *S. aureus* | au-nucR | GCCAATGTTCTACCATAGC | | |
| (*nuc*) | in-F | CATGTCATATTATTGCGAATGA | 430 | |
| *S. intermedius* | in-R3 | AGGACCATCACCATTGACATATTGAAACC | | |
| (*nuc*) | sch-F | AATGGCTACAATGATAATCACTAA | 526 | |
| *S. schleiferi* | sch-R | CATATCTGTCTTTCGGCGCG | | |
| (*nuc*) | dea-F | TGAAGGCATATTGTAGAACAA | 661 | |
| *S. delphini* group A | dea-R | CGRTACTTTTCGTTAGGTCG | | |
| (*nuc*) | hy-F1 | CATTATATGATTTGAACGTG | 793 | |
| *S. hyicus* | hy-R1 | GAATCAATATCGTAAAGTTGC | | |
| (*nuc*) | pse-F2 | TRGGCAGTAGGATTCGTTAA | 926 | |
| *S.pseudintermedius* | pse-R5 | CTTTTGTGCTYCMTTTTGG | | |
| (*nuc*) | deb-F | GGAAGRTTCGTTTTTCCTAGAC | 1135 | |
| *S.delphini* Group B | deb-R4 | TATGCGATTCAAGAACTGA | | |
| 16s | PA | AGAGTTTGATCCTGGCTCAG | ~1530 | (Edwards, Rogall et al. 1989) |
| | PH* | AAGGAGGTGATCCAGCCGCA | | |

| Target Gene | Primer name | Primer (5'-3') | Expected amplicon size(bp) | Reference |
|---|---|---|---|---|
| *spa* | 1113f | TAAAGACGATCCTTCGGTGAGC | variable | (Harmsen, Claus et al. 2003) |
| | 1514r | CAGCAGTAGTGCCGTTTGCTT | | |
| SCCmec typing | CIF2 F2 | TTCGAGTTGCTGAGAAGAAGG | 495 | (Oliveira and de Lencastre 2002) |
| | CIF2 R2 | ATTTACCACAAGGACTACCAGC | | |
| | KDP F1 | AATCATCTGCCATTGGTGATGC | 284 | |
| | KDP R1 | CGAATGAAGTGAAAGAAAGTGG | | |
| | MECI P2 | ATCAAGACTTGCATTCAGGC | 209 | |
| | MECI P3 | GCGGTTTCAATTCACTTGTC | | |
| | DCS F2 | CATCCTATGATAGCTTGGTC | 342 | |
| | DCS R1 | CTAAATCATAGCCATGACCG | | |
| | RIF4 F3 | GTGATTGTTCGAGATATGTGG | 243 | |
| | RIF4 R9 | CGCTTTATCTGTATCTATCGC | | |
| | RIF5 F10 | TTCTTAAGTACTCGCTGAATCG | 414 | |
| | RIF5 R13 | GTCACAGTAATTCCATCAATGC | | |

*Primers used as internal primers for sequencing of full $bla_{cmy}$ gene

+Primers used for internal sequencing of whole $bla_{\mathrm{TEM}}$ gene

**PCR product clean-up (PEG precipitation)**

1. Aliquot 60 µl 20% (w/v) $PEG_{8000}$, 2.5M NaCl per well, using a multichannel pipette, seal wells with adhesive film, vortex and spin briefly spin the plate at 500 rcf to ensure mix is at the bottom of the wells. Incubate the plates for either 15 min at 37 °C, 30 min at 20 °C or overnight at 4 °C. (Longer incubations do not have a detrimental effect on the clean up procedure).

2. Spin at 2750 rcf at 4 °C for 60 min.

3. To remove PEG, place folded blue tissue into the bottom of the centrifuge plate holders and gently invert the plate onto the blue tissue. Spin at 500 rpm for 6 sec.

4. Wash pellet twice with 150 µl 70% ice-cold ethanol. i.e add 150 µl per well and spin at 2750 rcf for 10 min. Remove ethanol by inversion of plate onto blue tissues, and then spin the inverted plate on folded clean blue at 500 rpm for 60 sec. Repeat.

5. Air dry plate on bench for 10 min.

6. Re-suspend the pellet in STERILE milliQ water. Re-suspension volume is dependent on intensity of PCR product observed following PCR e.g. Barely visible products are re-suspended in 5 µl with more intense products re-suspended in volumes of up to 50 µl. Seal lid carefully, vortex and spin briefly.

7. Resuspended products can be stored long-term at -20 °C, or short term at 4 °C.

## Appendix Three

Full results of univariable analyses

Full univariable results for carriage of *E. coli* with resistance to any antimicrobials

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.8** |
| <1 | 31 | 25 | 0 | | | | |
| 1 | 44 | 34 | -0.04 | 0.35 | 0.96 | 0.48-1.91 | |
| 2 | 19 | 14 | -0.09 | 0.44 | 0.91 | 0.38-2.18 | |
| 3 | 26 | 15 | -0.33 | 0.42 | 0.72 | 0.31-1.63 | |
| 4 | 195 | 166 | 0.05 | 0.29 | 1.06 | 0.60-1.86 | |
| **Breed** | | | | | | | **0.8** |
| Pedigree | 241 | 194 | 0 | | | | 0.8* |
| Cross | 70 | 53 | -0.06 | 0.21 | 0.94 | 0.63-1.41 | |
| **Breed Group** | | | | | | | **0.4** |
| Working | 9 | 11 | 0 | | | | |
| Gundog | 103 | 94 | -0.29 | 0.47 | 0.75 | 0.30-1.88 | |
| Hound | 20 | 14 | -0.56 | 0.57 | 0.57 | 0.19-1.75 | |
| Terrier | 37 | 19 | -0.87 | 0.53 | 0.42 | 0.15-1.19 | |
| Utility | 21 | 9 | 1.05 | 0.60 | 0.35 | 0.11-1.14 | |
| Pastoral | 22 | 20 | 0.30 | 0.55 | 0.74 | 0.26-2.17 | |
| Toy | 20 | 15 | 0.49 | 0.56 | 0.61 | 0.20-1.86 | |
| Cross | 70 | 53 | 0.48 | 0.48 | 0.62 | 0.24-1.60 | |
| Not specified | 18 | 19 | -0.15 | 0.56 | 0.86 | 0.29-2.57 | |
| **Breed Size** | | | | | | | **08** |
| Small | 44 | 29 | 0 | | | | |
| Medium | 88 | 45 | -0.25 | 0.30 | 0.78 | 0.43-1.4 | |
| Large | 99 | 108 | 0.50 | 0.28 | 1.66 | 0.96-2.85 | |
| Not specified | 89 | 72 | 0.20 | 0.29 | 1.23 | 0.70-2.15 | |
| **Neutered** | | | | | | | **0.07** |
| No | 76 | 78 | 0 | | | | 0.07* |
| Yes | 242 | 176 | -0.34 | 0.19 | 0.71 | 0.49-1.03 | |
| **Working Dog** | | | | | | | **0.02** |
| No | 292 | 218 | 0 | | | | 0.02* |
| Yes | 19 | 29 | 0.72 | 0.31 | 2.04 | 1.12-3.74 | |
| **Given dog treats** | | | | | | | **0.6** |
| Never | 17 | 16 | 0 | | | | |
| Rarely | 47 | 46 | 0.04 | 0.41 | 1.04 | 0.47-2.30 | |
| Sometimes | 148 | 117 | -0.17 | 0.37 | 0.84 | 0.41-1.73 | |
| Often | 102 | 71 | 0.30 | 0.38 | 0.74 | 0.35-1.56 | |
| **Given titbits** | | | | | | | **0.1** |
| Never | 28 | 32 | 0 | | | | |
| Rarely | 110 | 66 | -0.64 | 0.30 | 0.53 | 0.29-0.95 | |
| Sometimes | 131 | 114 | -0.27 | 0.29 | 0.76 | 0.43-1.34 | |
| Often | 42 | 37 | -0.26 | 0.34 | 0.77 | 0.39-1.51 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Given dog treats** | | | | | | | **0.2** |
| Never/ rarely | 64 | 62 | 0 | | | | 0.2* |
| Sometimes/ often | 250 | 188 | -0.25 | 0.20 | 0.78 | 0.52-1.15 | |
| **Given titbits** | | | | | | | **0.2** |
| Never/ rarely | 138 | 98 | 0 | | | | 0.23* |
| Sometimes/ often | 173 | 151 | 0.21 | 0.17 | 1.23 | 0.88-1.72 | |
| **Reason for visit** | | | | | | | **0.1** |
| Vaccination/ worming | 132 | 81 | 0 | | | | |
| Presenting complaint | 140 | 125 | 0.38 | 0.19 | 1.46 | 1.01-2.10 | |
| Check up | 13 | 11 | 0.32 | 0.43 | 1.38 | 0.59-3.22 | |
| Work at clinic | 9 | 10 | 0.59 | 0.48 | 1.81 | 0.71-4.65 | |
| Vaccination/ presenting comp | 7 | 15 | 1.25 | 0.48 | 3.49 | 1.37-8.93 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 3 | 2 | 0.08 | 0.92 | 1.09 | 0.18-6.64 | |
| Other | 6 | 7 | 0.64 | 0.57 | 1.90 | 0.62-5.86 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.04** |
| No | 210 | 149 | 0 | | | | 0.05* |
| Yes | 103 | 104 | 0.35 | 0.18 | 1.42 | 1.01-2.01 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.2** |
| No | 259 | 197 | 0 | | | | 0.2* |
| Yes | 53 | 54 | 0.29 | 0.22 | 1.34 | 0.88-2.04 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.6** |
| One off prescription | 4 | 2 | 0 | | | | |
| Up to 5 days | 14 | 19 | 1.00 | 0.93 | 2.71 | 0.43-16.96 | |
| Up to 10 days | 23 | 22 | 0.65 | 0.92 | 1.91 | 0.32-11.52 | |
| Up to 2 weeks | 7 | 6 | 0.54 | 1.03 | 1.71 | 0.23-12.89 | |
| Up to 3 weeks | 1 | 3 | 1.79 | 1.44 | 6.00 | 0.35-12.89 | |
| Over 3 weeks | 1 | 1 | 0.69 | 1.66 | 2.00 | 0.08-51.60 | |
| Don’t know | 3 | 1 | -0.41 | 1.44 | 0.67 | 0.04-11.29 | |
| None prescribed | 259 | 197 | 0.42 | 0.87 | 1.52 | 0.28-8.39 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.3** |
| No | 237 | 159 | 0 | | | | 0.4* |
| Yes | 83 | 95 | 0.53 | 0.18 | 1.71 | 1.19-2.44 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.098** |
| No | 184 | 130 | 0 | | | | 0.1* |
| Yes | 128 | 120 | 0.28 | 0.17 | 1.33 | 0.95-1.86 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.1** |
| No | 203 | 146 | 0 | | | | 0.1* |
| Yes | 107 | 101 | -0.33 | 0.18 | 1.31 | 0.93-1.85 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **06** |
| No | 254 | 180 | 0 | | | | 09* |
| Yes | 47 | 61 | 0.59 | 0.22 | 1.80 | 1.17-2.76 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.07** |
| One off prescription | 4 | 3 | 0 | | | | |
| Up to 5 days | 11 | 21 | 0.93 | 0.85 | 2.55 | 0.48-13.46 | |
| Up to 10 days | 17 | 19 | 0.40 | 0.83 | 1.49 | 0.29-7.63 | |
| Up to 2 weeks | 6 | 11 | 0.89 | 0.92 | 2.44 | 0.40-14.75 | |
| Up to 3 weeks | 2 | 3 | 0.69 | 1.19 | 2 | 0.19-20.61 | |
| Over 3 weeks | 4 | 3 | -165.00 | 1.08 | 1 | 0.12-8.31 | |
| Don’t know | 2 | 0 | | | | | |
| None prescribed | 254 | 180 | -0.57 | 0.77 | 0.94 | 0.21-4.27 | |
| **Left at veterinary premises** | | | | | | | **0.7** |
| No | 239 | 187 | 0 | | | | 0.8* |
| Yes | 24 | 21 | 0.11 | 0.31 | 1.12 | 0.60-2.07 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.7** |
| No | 69 | 51 | 0 | | | | 0.8* |
| Yes | 245 | 197 | 0.08 | 0.21 | 1.09 | 0.72-1.64 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.04** |
| No | 249 | 177 | 0 | | | | 0.04* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Yes | 65 | 70 | 0.42 | 0.20 | 1.51 | 1.03-2.23 | |
| **Allowed off lead during walks** | | | | | | | **05** |
| No | 77 | 37 | 0 | | | | 06* |
| Yes | 238 | 212 | 0.62 | 0.22 | 1.85 | 1.20-2.86 | |
| **Other dogs in household** | | | | | | | **0.2** |
| No | 180 | 133 | 0 | | | | 0.3* |
| Yes | 133 | 120 | 0.20 | 0.17 | 1.22 | 0.87-1.70 | |
| **Number of other dogs in household** | | | | | | | **0.3** |
| 0 | 180 | 133 | 0 | | | | |
| 1 | 67 | 53 | 0.07 | 0.22 | 1.07 | 0.70-1.64 | |
| 2 | 24 | 27 | 0.42 | 0.30 | 1.52 | 0.84-2.76 | |
| 3 | 4 | 9 | 1.11 | 0.61 | 3.05 | 0.92-10.10 | |
| 4+ | 9 | 7 | 0.05 | 0.52 | 1.05 | 0.38-2.90 | |
| Not specified | | | | | | | |
| **Other animals in household** | | | | | | | **0.9** |
| No | 177 | 141 | 0 | | | | 0.9* |
| Yes | 136 | 111 | 0.02 | 0.17 | 1.02 | 0.73-1.43 | |
| **Own a cat** | | | | | | | **0.8** |
| No | 232 | 184 | | | | | 0.8* |
| Yes | 81 | 68 | 0.06 | 0.18 | 1.06 | 0.73-1.54 | |
| **Own a bird** | | | | | | | **0.9** |
| No | 290 | 233 | 0 | | | | 1.0* |
| Yes | 23 | 19 | 0.03 | 0.32 | 1.03 | 0.55-1.93 | |
| **Own a rabbit** | | | | | | | **1.0** |
| No | 293 | 236 | 0 | | | | 1.0* |
| Yes | 20 | 16 | -68027.00 | 0.35 | 0.99 | 0.50-1.96 | |
| **Own a rodent** | | | | | | | **0.2** |
| No | 286 | 238 | 0 | | | | 0.2* |
| Yes | 27 | 14 | -0.47 | 0.34 | 0.62 | 032-1.22 | |
| **Own a reptile or amphibian** | | | | | | | **0.2** |
| No | 309 | 245 | 0 | | | | 0.2* |
| Yes | 4 | 7 | 0.79 | 0.63 | 2.21 | 0.64-7.63 | |
| **Own a fish** | | | | | | | **0.3** |
| No | 302 | 247 | 0 | | | | 0.3* |
| Yes | 11 | 5 | -0.59 | 0.55 | 0.56 | 0.19-1.62 | |
| **Own any other animal or livestock** | | | | | | | **0.8** |
| No | 282 | 225 | 0 | | | | |
| Yes | 31 | 27 | 0.09 | 0.28 | 1.09 | 0.63-1.88 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Where the dog usually sleeps** | | | | | | | **0.7** |
| Outside | 10 | 13 | 0 | | | | |
| Downstairs | 162 | 132 | -0.47 | 0.44 | 0.63 | 0.27-1.48 | |
| Upstairs | 125 | 100 | -0.49 | 0.44 | 0.62 | 0.26-1.46 | |
| Whole house | 14 | 8 | -0.82 | 0.61 | 0.44 | 0.13-1.46 | |
| Outside and downstairs | 1 | 1 | -0.26 | 1.48 | 0.77 | 0.04-13.87 | |
| **Anyone in the household work with farm animals** | | | | | | | **0.2** |
| No | 289 | 230 | 0 | | | | 0.23* |
| Yes | 21 | 24 | 0.36 | 0.31 | 1.44 | 0.78-2.64 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.3** |
| No | 257 | 215 | 0 | | | | 0.4* |
| Yes | 56 | 37 | -0.24 | 0.23 | 0.79 | 0.50-1.24 | |
| **Who took antibiotics** | | | | | | | **0.8** |
| No | 30 | 20 | 0 | | | | |
| Yes | 19 | 16 | 0.23 | 0.45 | 1.26 | 0.53-3.02 | |
| No one/ not specified | 257 | 214 | 0.22 | 0.30 | 1.25 | 0.69-2.26 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.7** |
| No | 223 | 177 | 0 | | | | 0.8* |
| Yes | 91 | 77 | 0.06 | 0.18 | 1.07 | 0.74-1.53 | |
| **Healthcare setting** | | | | | | | **0.6** |
| Human | 27 | 26 | 0 | | | | |
| Animal | 61 | 51 | -0.14 | 0.34 | 0.87 | 0.45-1.67 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 223 | 177 | -0.14 | 0.33 | 0.82 | 0.46-1.46 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.2** |
| No | 223 | 192 | 0 | | | | 0.3* |
| Yes | 90 | 62 | -0.22 | 0.19 | 0.80 | 0.55-1.17 | |
| **Reason for hospital visit** | | | | | | | **0.6** |
| Admission | 12 | 12 | 0 | | | | |
| Visit | 10 | 8 | -0.23 | 0.63 | 0.80 | 0.23-2.73 | |
| Outpatient/ A&E | 67 | 41 | -0.49 | 0.45 | 0.61 | 0.25-1.49 | |
| Other/ not specified | 1 | 1 | 194.00 | 1.47 | 1.00 | 0.06-17.90 | |
| No attendance | 223 | 192 | -0.15 | 0.42 | 0.86 | 0.38-1.96 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Source of dog** | | | | | | | **0.5** |
| Breeder | 156 | 123 | 0 | | | | |
| Rescue Kennel/ stray | 75 | 51 | -0.15 | 0.22 | 0.86 | 0.56-1.32 | |
| Newspaper/ word of mouth/ internet | 27 | 17 | -0.22 | 0.33 | 0.80 | 0.42-1.53 | |
| Family/friend | 45 | 38 | 0.07 | 0.25 | 1.07 | 0.65-1.75 | |
| Pet shop | 3 | 2 | -0.17 | 0.92 | 0.85 | 0.14-5.15 | |
| Other | 7 | 9 | 0.49 | 0.52 | 1.63 | 0.59-4.50 | |
| Self breed | 4 | 9 | 1.05 | 0.61 | 2.85 | 0.86-9.49 | |
| **Fed tinned or packet wet food** | | | | | | | **0.4** |
| Yes | 210 | 159 | 0 | | | | 0.4* |
| No | 107 | 95 | 0.16 | 0.18 | 1.17 | 0.83-1.66 | |
| **Fed dry mixer** | | | | | | | **0.5** |
| No | 254 | 209 | 0 | | | | 0.5* |
| Yes | 63 | 45 | -0.14 | 0.22 | 0.87 | 0.57-1.33 | |
| **Fed dry complete** | | | | | | | **0.1** |
| No | 68 | 68 | 0 | | | | 0.1* |
| Yes | 249 | 186 | -0.29 | 0.20 | 0.75 | 0.51-1.10 | |
| **Fed raw poultry meat** | | | | | | | **0.04** |
| No | 309 | 239 | 0 | | | | 0.05* |
| Yes | 8 | 15 | 0.89 | 0.45 | 2.42 | 1.01-5.81 | |
| **Fed cooked poultry meat** | | | | | | | **0.2** |
| No | 247 | 209 | 0 | | | | 0.2* |
| Yes | 70 | 45 | -0.27 | 0.21 | 0.76 | 0.50-1.15 | |
| **Fed raw red meat** | | | | | | | **0.3** |
| No | 311 | 246 | 0 | | | | 0.4* |
| Yes | 6 | 8 | 0.52 | 0.55 | 1.69 | 0.58-4.92 | |
| **Fed cooked red meat** | | | | | | | **1.0** |
| No | 307 | 246 | 0 | | | | 1.0* |
| Yes | 10 | 8 | -16273.00 | 0.48 | 1.00 | 0.39-2.57 | |
| **Sex** | | | | | | | **0.9** |
| Male | 155 | 124 | | | | | 0.9* |
| Female | 165 | 130 | -0.02 | 0.17 | 0.98 | 0.71-1.37 | |

Full univariable results for carriage of multidrug resistant *E. coli*

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.5** |
| <1 | 49 | 7 | 0 | | | | |
| 1 | 66 | 12 | 0.24 | 0.51 | 1.27 | 0.47-3.47 | |
| 2 | 29 | 4 | -0.04 | 0.67 | 0.97 | 0.26-3.58 | |
| 3 | 32 | 9 | 0.68 | 0.55 | 1.97 | 0.67-5.82 | |
| 4 | 290 | 41 | 0.54 | 0.43 | 1.71 | 0.74-3.94 | |
| **Breed** | | | | | | | **0.2** |
| Pedigree | 352 | 83 | 0 | | | | 0.2* |
| Cross | 106 | 17 | -0.39 | 0.29 | 0.68 | 0.39-1.20 | |
| **Breed Group** | | | | | | | **0.05** |
| Working | 13 | 7 | 0 | | | | |
| Gundog | 151 | 46 | -0.57 | 0.50 | 0.57 | 0.21-1.50 | |
| Hound | 27 | 7 | -0.73 | 0.63 | 0.48 | 0.14-1.66 | |
| Terrier | 52 | 4 | -1.95 | 0.70 | 0.14 | 0.04-0.56 | |
| Utility | 26 | 4 | -1.25 | 0.71 | 0.29 | 0.07-1.16 | |
| Pastoral | 35 | 7 | -0.99 | 0.63 | 0.37 | 0.11-1.27 | |
| Toy | 28 | 7 | -0.77 | 0.63 | 0.46 | 0.13-1.60 | |
| Cross | 106 | 17 | -1.21 | 0.54 | 0.30 | 0.10-0.85 | |
| Not specified | 33 | 4 | -1.49 | 0.71 | 0.23 | 0.06-0.90 | |
| **Breed Size** | | | | | | | **02** |
| Small | 62 | 11 | 0 | | | | |
| Medium | 116 | 17 | -0.19 | 0.42 | 0.83 | 0.36-1.87 | |
| Large | 153 | 54 | 0.69 | 0.36 | 1.99 | 0.98-4.06 | |
| Not specified | 140 | 21 | -0.17 | 0.33 | 0.85 | 0.38-1.86 | |
| **Neutered** | | | | | | | **0.4** |
| No | 123 | 31 | 0 | | | | 0.5* |
| Yes | 346 | 72 | -0.19 | 0.24 | 0.83 | 0.52-1.32 | |
| **Working Dog** | | | | | | | **0.1** |
| No | 422 | 88 | 0 | | | | 0.1* |
| Yes | 35 | 13 | 0.58 | 0.35 | 1.78 | 0.91-3.50 | |
| **Given dog treats** | | | | | | | **0.06** |
| Never | 22 | 11 | 0 | | | | |
| Rarely | 72 | 21 | -0.54 | 0.44 | 0.58 | 0.24-1.39 | |
| Sometimes | 223 | 42 | -0.98 | 0.41 | 0.38 | 0.17-0.83 | |
| Often | 144 | 29 | -0.92 | 0.42 | 0.40 | 0.18-0.92 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Given titbits** | | | | | | | **1.0** |
| Never | 48 | 12 | 0 | | | | |
| Rarely | 145 | 31 | -0.16 | 0.38 | 0.86 | 0.41-1.80 | |
| Sometimes | 20 | 45 | -0.11 | 0.36 | 0.90 | 0.44-1.83 | |
| Often | 65 | 14 | -0.15 | 0.44 | 0.86 | 0.37-2.03 | |
| **Given dog treats** | | | | | | | **0.02** |
| Never/ rarely | 94 | 32 | 0 | | | | 0.03* |
| Sometimes/ often | 367 | 71 | -0.57 | 0.24 | 0.57 | 0.35-0.91 | |
| **Given titbits** | | | | | | | **1.0** |
| Never/ rarely | 193 | 43 | 0 | | | | 1.0* |
| Sometimes/ often | 265 | 59 | 0 | 0.22 | 1.00 | 0.65-1.54 | |
| **Reason for visit** | | | | | | | **0.3** |
| Vaccination/ worming | 186 | 27 | 0 | | | | |
| Presenting complaint | 209 | 56 | 0.61 | 0.26 | 1.85 | 1.12-3.04 | |
| Check up | 19 | 5 | 0.59 | 0.54 | 1.81 | 0.63-5.26 | |
| Work at clinic | 14 | 5 | 0.90 | 0.56 | 2.46 | 0.82-7.38 | |
| Vaccination/ presenting comp | 16 | 6 | 0.95 | 0.82 | 2.58 | 0.93-7.17 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 4 | 1 | 0.54 | 1.14 | 1.79 | 0.19-15.99 | |
| Other | 10 | 3 | 0.73 | 0.69 | 2.07 | 0.53-7.99 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.2** |
| No | 299 | 60 | 0 | | | | 0.3* |
| Yes | 164 | 43 | 0.27 | 0.22 | 1.31 | 0.85-2.02 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.2** |
| No | 378 | 78 | 0 | | | | 0.2* |
| Yes | 83 | 24 | 0.34 | 0.26 | 1.40 | 0.84-2.35 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.4** |
| One off prescription | 5 | 1 | 0 | | | | |
| Up to 5 days | 24 | 9 | 0.63 | 1.16 | 1.87 | 0.19-18.32 | |
| Up to 10 days | 37 | 8 | 0.08 | 1.16 | 1.08 | 0.11-10.56 | |
| Up to 2 weeks | 9 | 4 | 0.80 | 1.25 | 2.22 | 0.19-25.72 | |
| Up to 3 weeks | 2 | 2 | 1.61 | 1.48 | 5.00 | 0.27-91.52 | |
| Over 3 weeks | 2 | 0 | | | | | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 378 | 78 | 0.31 | 1.10 | 1.03 | 0.12-8.95 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.5** |
| No | 328 | 68 | 0 | | | | 0.5* |
| Yes | 143 | 35 | 0.17 | 0.23 | 1.18 | 0.75-1.86 | |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **1.0** |
| No | 258 | 56 | 0 | | | | 1.* |
| Yes | 204 | 44 | -0.01 | 0.22 | 0.99 | 0.64-1.54 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.8** |
| No | 286 | 63 | 0 | | | | 0.9* |
| Yes | 172 | 36 | -0.05 | 0.23 | 0.95 | 0.61-1.49 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **1.0** |
| No | 357 | 77 | 0 | | | | 1.0* |
| Yes | 88 | 19 | 0 | 0.28 | 1.00 | 0.58-1.74 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.3** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 29 | 3 | 0 | | | | |
| Up to 10 days | 26 | 10 | 1.31 | 0.71 | 3.72 | 0.92-15.00 | |
| Up to 2 weeks | 14 | 3 | 0.78 | 0.88 | 2.07 | 0.37-11.60 | |
| Up to 3 weeks | 4 | 1 | 0.88 | 1.27 | 2.42 | 0.20-29.23 | |
| Over 3 weeks | 4 | 3 | 1.98 | 0.98 | 7.25 | 1.07-49.03 | |
| Don’t know | 2 | 0 | | | | | |
| None prescribed | 357 | 77 | 0.73 | 0.62 | 2.08 | 0.62-7.02 | |
| **Left at veterinary premises** | | | | | | | **0.2** |
| No | 349 | 77 | 0 | | | | 0.3* |
| Yes | 40 | 5 | -0.57 | 0.49 | 0.57 | 0.22-1.48 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Allowed close contact with other dogs during walks** | | | | | | | **0.9** |
| No | 98 | 22 | 0 | | | | 0.9* |
| Yes | 363 | 79 | -0.03 | 0.27 | 0.97 | 0.57-1.63 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.09** |
| No | 356 | 70 | 0 | | | | 0.1* |
| Yes | 104 | 31 | 0.42 | 0.24 | 1.52 | 0.94 | |
| **Allowed off lead during walks** | | | | | | | **0.2** |
| No | 98 | 16 | 0 | | | | 0.2* |
| Yes | 364 | 86 | 0.37 | 0.30 | 1.45 | 0.81-2.58 | |
| **Other dogs in household** | | | | | | | **0.08** |
| No | 264 | 49 | 0 | | | | 0.1* |
| Yes | 199 | 54 | 0.38 | 0.22 | 1.46 | 0.95-2.24 | |
| **Number of other dogs in household** | | | | | | | **0.04** |
| 0 | 264 | 49 | 0 | | | | |
| 1 | 97 | 23 | 0.24 | 0.28 | 1.28 | 0.74-2.21 | |
| 2 | 42 | 9 | 0.14 | 0.40 | 1.15 | 0.53-2.52 | |
| 3 | 7 | 6 | 1.53 | 0.58 | 4.62 | 1.49-14.33 | |
| 4+ | 11 | 5 | 0.90 | 0.56 | 2.45 | 0.82-7.36 | |
| Not specified | | | | | | | |
| **Other animals in household** | | | | | | | **0.2** |
| No | 267 | 51 | 0 | | | | 0.12* |
| Yes | 196 | 51 | 0.31 | 0.22 | 1.36 | 0.89-2.09 | |
| **Own a cat** | | | | | | | **0.04** |
| No | 349 | 67 | 0 | | | | 0.05* |
| Yes | 114 | 35 | 0.47 | 0.23 | 1.60 | 1.01-2.53 | |
| **Own a bird** | | | | | | | **0.9** |
| No | 429 | 94 | 0 | | | | 0.8* |
| Yes | 34 | 8 | 0.07 | 0.41 | 1.07 | 0.48-2.39 | |
| **Own a rabbit** | | | | | | | **0.8** |
| No | 433 | 96 | 0 | | | | 1.0* |
| Yes | 30 | 6 | -0.10 | 0.46 | 0.90 | 0.37-2.23 | |
| **Own a rodent** | | | | | | | **0.2** |
| No | 426 | 98 | 0 | | | | 0.2* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Yes | 37 | 4 | -0.76 | 0.54 | 0.47 | 0.16-1.35 | |
| **Own a reptile or amphibian** | | | | | | | **1.0** |
| No | 454 | 100 | 0 | | | | 1.0* |
| Yes | 9 | 2 | 0.01 | 0.79 | 1.01 | 0.21-4.74 | |
| **Own a fish** | | | | | | | **0.06** |
| No | 447 | 102 | 0 | | | | 0.09* |
| Yes | 16 | 0 | | | | | |
| **Own any other animal or livestock** | | | | | | | **0.1** |
| No | 420 | 87 | 0 | | | | 0.1* |
| Yes | 43 | 15 | 0.52 | 0.32 | 1.68 | 0.90-3.17 | |
| **Where the dog usually sleeps** | | | | | | | **0.5** |
| Outside | 18 | 5 | 0 | | | | |
| Downstairs | 240 | 54 | -0.21 | 0.53 | 0.81 | 0.29-2.28 | |
| Upstairs | 188 | 37 | -0.34 | 0.54 | 0.71 | 0.25-2.03 | |
| Whole house | 16 | 6 | 0.30 | 0.70 | 1.35 | 0.34-5.28 | |
| Outside and downstairs | 1 | 1 | 1.28 | 1.50 | 3.60 | 0.19-68.34 | |
| **Anyone in the household work with farm animals** | | | | | | | **0.8** |
| No | 425 | 94 | 0 | | | | 0.7* |
| Yes | 36 | 9 | 0.12 | 0.39 | 1.13 | 0.53-2.43 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.3** |
| No | 390 | 82 | 0 | | | | 0.4* |
| Yes | 73 | 20 | 0.26 | 0.28 | 1.30 | 0.75-2.26 | |
| **Who took antibiotics** | | | | | | | **0.9** |
| Family member | 39 | 11 | 0 | | | | |
| Pet | 27 | 8 | 0.05 | 0.53 | 1.05 | 0.37-2.96 | |
| No one/ not specified | 389 | 82 | -0.29 | 0.36 | 0.75 | 0.37-1.52 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.7** |
| No | 326 | 74 | 0 | | | | 0.8* |
| Yes | 139 | 29 | -0.08 | 0.24 | 0.92 | 0.57-1.48 | |
| **Healthcare setting** | | | | | | | **0.9** |
| Human | 42 | 11 | 0 | | | | |
| Animal | 94 | 18 | -0.31 | 0.43 | 0.73 | 0.32-1.68 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Does not work in healthcare | 326 | 74 | -0.14 | 0.36 | 0.87 | 0.43-1.76 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.3** |
| No | 335 | 80 | 0 | | | | 0.3* |
| Yes | 129 | 23 | -0.29 | 0.26 | 0.75 | 0.45-1.24 | |
| **Reason for hospital visit** | | | | | | | **0.4** |
| Admission | 18 | 6 | 0 | | | | |
| Visit | 17 | 1 | -1.73 | 1.13 | 0.18 | 0.02-1.62 | |
| Outpatient/ A&E | 92 | 16 | -0.65 | 0.54 | 0.52 | 0.18-1.51 | |
| Other/ not specified | 2 | 0 | | | | | |
| No attendance | 335 | 80 | 0.33 | 0.49 | 0.72 | 0.28-1.86 | |
| **Source of dog** | | | | | | | **0.5** |
| Breeder | 221 | 58 | 0 | | | | |
| Rescue Kennel/ stray | 109 | 17 | -0.52 | 0.30 | 0.59 | 0.33-1.07 | |
| Newspaper/ word of mouth/ internet | 37 | 7 | -0.33 | 0.44 | 0.72 | 0.31-1.70 | |
| Family/friend | 72 | 11 | -0.54 | 0.36 | 0.58 | 0.29-1.17 | |
| Pet shop | 4 | 1 | -0.05 | 1.13 | 0.95 | 0.10-8.69 | |
| Other | 12 | 4 | 0.33 | 0.60 | 1.27 | 0.40-4.08 | |
| Self breed | 10 | 3 | 0.13 | 0.67 | 1.14 | 0.30-4.29 | |
| **Fed tinned or packet wet food** | | | | | | | **0.3** |
| Yes | 307 | 62 | 0 | | | | 0.3* |
| No | 161 | 41 | 0.23 | 0.22 | 1.26 | 0.81-1.95 | |
| **Fed dry mixer** | | | | | | | **0.3** |
| No | 376 | 87 | 0 | | | | 0.4* |
| Yes | 92 | 16 | -0.29 | 0.30 | 0.75 | 0.42-1.34 | |
| **Fed dry complete** | | | | | | | **0.3** |
| No | 107 | 29 | 0 | | | | 0.3* |
| Yes | 361 | 74 | -0.28 | 0.25 | 0.76 | 0.47-1.22 | |
| **Fed raw poultry meat** | | | | | | | **01** |
| No | 455 | 93 | 0 | | | | 03* |
| Yes | 13 | 10 | 1.33 | 0.44 | 3.76 | 1.60-8.84 | |
| **Fed cooked poultry meat** | | | | | | | **0.6** |
| No | 372 | 84 | 0 | | | | 0.7* |
| Yes | 96 | 19 | -0.13 | 0.28 | 0.88 | 0.51-1.51 | |
| **Fed raw red meat** | | | | | | | **0.3** |
| No | 455 | 102 | 0 | | | | 0.5* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Yes | 13 | 1 | -0.07 | 1.04 | 0.34 | 0.04-2.65 | |
| **Fed cooked red meat** | | | | | | | **0.4** |
| No | 452 | 101 | 0 | | | | 0.8* |
| Yes | 16 | 2 | -0.58 | 0.76 | 0.56 | 0.13-2.47 | |
| **Sex** | | | | | | | **0.08** |
| Male | 237 | 42 | 0 | | | | 0.08* |
| Female | 234 | 61 | 0.39 | 0.22 | 1.47 | 0.95-2.27 | |

Full univariable results for carriage of *E. coli* with resistance to ampicillin

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.4** |
| <1 | 37 | 19 | | | | | |
| 1 | 52 | 26 | -0.03 | 0.37 | 0.97 | 0.47-2.01 | |
| 2 | 22 | 11 | -0.27 | 0.46 | 0.97 | 0.39-2.42 | |
| 3 | 30 | 11 | -0.34 | 0.45 | 0.71 | 0.29-1.73 | |
| 4 | 217 | 144 | 0.26 | 0.30 | 1.29 | 0.71-2.34 | |
| **Breed** | | | | | | | **0.7** |
| Pedigree | 274 | 161 | | | | | 0.8* |
| Cross | 80 | 43 | -0.09 | 0.21 | 0.91 | 0.60-1.39 | |
| **Breed Group** | | | | | | | **0.2** |
| Working | 12 | 8 | | | | | |
| Gundog | 116 | 81 | 0.05 | 0.48 | 1.08 | 0.51.2.68 | |
| Hound | 22 | 12 | -0.20 | 0.58 | 0.82 | 0.26-2.55 | |
| Terrier | 42 | 14 | -0.69 | 0.55 | 0.50 | 0.17-1.47 | |
| Utility | 24 | 6 | -0.98 | 0.65 | 0.38 | 0.11-1.33 | |
| Pastoral | 25 | 17 | 0.02 | 0.55 | 1.02 | 0.34-3.02 | |
| Toy | 22 | 13 | -0.12 | 0.58 | 0.89 | 0.29-2.74 | |
| Cross | 80 | 43 | -0.22 | 0.49 | 0.51 | 0.31-2.12 | |
| Not specified | 20 | 17 | 0.24 | 0.56 | 1.27 | 0.42-3.84 | |
| **Breed Size** | | | | | | | **0.004** |
| Small | 50 | 23 | | | | | 0.001* |
| Medium | 98 | 35 | -0.25 | 0.32 | 0.78 | 0.41-1.45 | |
| Large | 114 | 95 | 0.57 | 0.29 | 1.77 | 1.01-3.12 | |
| Not specified | 101 | 60 | 0.26 | 0.30 | 1.29 | 0.72-2.33 | |
| **Neutered** | | | | | | | **0.02** |
| No | 85 | 69 | | | | | 0.02* |
| Yes | 276 | 142 | -0.46 | 0.19 | 0.63 | 0.43-0.92 | |
| **Working Dog** | | | | | | | **0.6** |
| No | 325 | 185 | | | | | 0.6* |
| Yes | 29 | 19 | 0.14 | 0.31 | 1.15 | 0.63-2.11 | |
| **Given dog treats** | | | | | | | **0.4** |
| Never | 22 | 11 | | | | | |
| Rarely | 52 | 41 | 0.46 | 0.42 | 1.58 | 0.69-3.62 | |
| Sometimes | 170 | 95 | 0.11 | 0.39 | 1.12 | 0.52-2.40 | |
| Often | 113 | 60 | 0.06 | 0.40 | 1.06 | 0.48-2.34 | |
| **Given titbits** | | | | | | | **0.02** |
| Never | 29 | 31 | | | | | |
| Rarely | 124 | 52 | -0.94 | 0.31 | 0.39 | 0.22-0.72 | |
| Sometimes | 150 | 95 | 0.52 | 0.29 | 0.59 | 0.34-1.05 | |
| Often | 49 | 30 | -0.56 | 0.35 | 0.57 | 0.29-1.13 | |
| **Given dog treats** | | | | | | | **0.2** |
| Never/ rarely | 74 | 52 | | | | | 0.2* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Sometimes/ often | 283 | 155 | 0.25 | 0.21 | 0.78 | 0.52-1.17 | 0 |
| **Given titbits** | | | | | | | **0.4** |
| Never/ rarely | 153 | 83 | | | | | 0.4* |
| Sometimes/ often | 199 | 125 | 0.15 | 0.18 | 1.16 | 0.82-1.64 | |
| **Reason for visit** | | | | | | | **0.09** |
| Vaccination/ worming | 151 | 62 | | | | | |
| Presenting complaint | 158 | 107 | 0.50 | 0.20 | 1.65 | 1.12-2.42 | |
| Check up | 13 | 11 | 0.72 | 0.44 | 2.06 | 0.88-4.85 | |
| Work at clinic | 10 | 9 | 0.78 | 0.48 | 2.19 | 0.85-5.66 | |
| Vaccination/ presenting comp | 11 | 11 | 0.89 | 0.45 | 2.44 | 1.00-5.91 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 3 | 2 | 0.48 | 0.93 | 1.62 | 0.26-9.96 | |
| Other | 6 | 7 | 1.04 | 0.58 | 2.84 | 0.92-8.79 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.02** |
| No | 239 | 120 | | | | | 0.02* |
| Yes | 117 | 90 | 0.43 | 0.18 | 1.53 | 1.08-2.18 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.04** |
| No | 297 | 159 | | | | | 0.05* |
| Yes | 58 | 49 | 0.46 | 0.22 | 1.58 | 1.03-2.42 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.1** |
| One off prescription | 4 | 2 | | | | | |
| Up to 5 days | 15 | 18 | 0.88 | 0.93 | 2.40 | 0.38-14.97 | |
| Up to 10 days | 26 | 19 | 0.38 | 0.92 | 1.46 | 0.24-8.82 | |
| Up to 2 weeks | 7 | 6 | 0.54 | 1.03 | 1.71 | 0.23-12.89 | |
| Up to 3 weeks | 1 | 3 | 1.79 | 1.44 | 6.00 | 0.35-101.57 | |
| Over 3 weeks | 1 | 1 | 0.69 | 1.66 | 2.00 | 0.08-51.60 | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 297 | 159 | 0.07 | 0.87 | 1.07 | 0.19-5.91 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.001** |
| **No** | 268 | 128 | | | | | **0.001*** |
| **Yes** | 95 | 83 | 0.60 | 0.18 | 1.83 | 1.27-2.63 | **0.001** |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.1** |
| No | 207 | 107 | | | | | 0.1* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Yes | 148 | 100 | 0.27 | 0.18 | 1.31 | 0.93-1.85 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.2** |
| No | 229 | 120 | | | | | 0.2* |
| Yes | 124 | 84 | 0.26 | 0.18 | 1.29 | 0.91-1.84 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.004** |
| No | 288 | 146 | | | | | 0.005* |
| Yes | 55 | 53 | 0.62 | 0.22 | 1.87 | 1.22-2.86 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.1** |
| One off prescription | 4 | 3 | 0.00 | | | | |
| Up to 5 days | 16 | 16 | 0.29 | 0.84 | 1.33 | 0.26-6.94 | |
| Up to 10 days | 18 | 18 | 0.29 | 0.83 | 1.33 | 0.26-6.83 | |
| Up to 2 weeks | 7 | 10 | 0.64 | 0.91 | 1.90 | 0.32-11.31 | |
| Up to 3 weeks | 3 | 2 | -0.12 | 1.19 | 0.88 | 0.08-9.16 | |
| Over 3 weeks | 4 | 3 | | 1.08 | 1 | 0.12-8.31 | |
| Don’t know | 2 | 0 | | | | | |
| None prescribed | 288 | 146 | -0.39 | 0.77 | 0.68 | 0.14-3.06 | |
| **Left at veterinary premises** | | | | | | | **1.0** |
| No | 271 | 155 | | | | | 1.0* |
| Yes | 29 | 16 | -0.04 | 0.33 | 0.96 | 0.51-1.83 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.8** |
| No | 75 | 45 | | | | | 0.8* |
| Yes | 281 | 161 | -0.05 | 0.21 | 0.95 | 0.63-1.45 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.422** |
| No | 275 | 151 | | | | | 0.5* |
| Yes | 82 | 53 | 0.16 | 0.20 | 1.18 | 0.79-1.75 | 0 |
| **Allowed off lead during walks** | | | | | | | **0.06** |
| No | 81 | 33 | | | | | 0.07* |
| Yes | 277 | 173 | 0.43 | 0.23 | 1.53 | 0.98-2.40 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Other dogs in household** | | | | | | | **0.8** |
| No | 198 | 115 | | | | | 0.9* |
| Yes | 158 | 95 | 0.03 | 0.17 | 1.04 | 0.73-1.46 | |
| **Number of other dogs in household** | | | | | | | **0.9** |
| 0 | 198 | 115 | | | | | |
| 1 | 76 | 44 | 0.00 | 0.22 | 1.00 | 0.64-1.54 | |
| 2 | 32 | 19 | 0.02 | 0.31 | 1.02 | 0.55-1.89 | |
| 3 | 7 | 6 | 0.39 | 0.57 | 1.48 | 0.48-4.50 | |
| 4+ | 9 | 7 | 0.29 | 0.52 | 1.34 | 0.49-3.69 | |
| Not specified | | | | | | | |
| **Other animals in household** | | | | | | | **0.6** |
| No | 197 | 121 | | | | | 0.6* |
| Yes | 159 | 88 | -0.10 | 0.18 | 0.90 | 0.64-1.27 | |
| **Own a cat** | | | | | | | **0.9** |
| No | 263 | 153 | | | | | 0.9* |
| Yes | 93 | 56 | 0.03 | 0.20 | 1.04 | 0.70-1.52 | |
| **Own a bird** | | | | | | | **0.9** |
| No | 330 | 193 | | | | | 0.9* |
| Yes | 26 | 16 | 0.05 | 0.33 | 1.05 | 0.55-2.01 | |
| **Own a rabbit** | | | | | | | **0.9** |
| No | 333 | 196 | | | | | 1.0* |
| Yes | 23 | 13 | -0.04 | 0.36 | 0.96 | 0.48-1.94 | |
| **Own a rodent** | | | | | | | **0.04** |
| No | 324 | 200 | | | | | 0.04* |
| Yes | 32 | 9 | -0.79 | 0.39 | 0.46 | 0.21-0.97 | |
| **Own a reptile or amphibian** | | | | | | | **0.2** |
| No | 351 | 203 | | | | | 0.3* |
| Yes | 5 | 6 | 0.73 | 0.61 | 2.07 | 0.63-6.88 | |
| **Own a fish** | | | | | | | **0.1** |
| No | 343 | 206 | | | | | 0.2* |
| Yes | 13 | 3 | -0.96 | 0.65 | 0.38 | 0.11-1.36 | |
| **Own any other animal or livestock** | | | | | | | **0.5** |
| No | 317 | 190 | | | | | 0.6* |
| Yes | 39 | 19 | -0.21 | 0.29 | 0.81 | 0.46-1.45 | |
| **Where the dog usually sleeps** | | | | | | | **0.9** |
| Outside | 14 | 9 | | | | | |
| Downstairs | 187 | 107 | -0.12 | 0.44 | 0.89 | 0.37-2.13 | |
| Upstairs | 138 | 87 | -0.02 | 0.45 | 0.98 | 0.41-2.36 | |
| Whole house | 15 | 7 | -0.32 | 0.63 | 0.73 | 0.21-2.48 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Outside and downstairs | 1 | 1 | 0.44 | 1.48 | 1.56 | 0.09-28.15 | |
| **Anyone in the household work with farm animals** | | | | | | | **1.0** |
| No | 335 | 194 | | | | | 1.0* |
| Yes | 28 | 17 | 0.02 | 0.32 | 1.02 | 0.54-1.91 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.6** |
| No | 295 | 177 | | | | | 0.6* |
| Yes | 61 | 32 | -0.13 | 0.24 | 0.87 | 0.55-1.39 | |
| **Who took antibiotics** | | | | | | | **0.6** |
| No | 34 | 16 | | | | | |
| Yes | 20 | 15 | 0.47 | 0.46 | 1.59 | 0.65-3.90 | |
| No one/ not specified | 295 | 176 | 0.24 | 0.32 | 1.27 | 0.68-2.36 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.5** |
| No | 248 | 152 | | | | | 0.6* |
| Yes | 109 | 59 | -0.12 | 0.19 | 0.88 | 0.61-1.29 | |
| **Healthcare setting** | | | | | | | **0.4** |
| Human | 33 | 20 | | | | | |
| Animal | 73 | 39 | -0.13 | 0.35 | 0.88 | 0.45-1.74 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 248 | 152 | 0.01 | 0.30 | 1.01 | 0.56-1.83 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.2** |
| No | 254 | 161 | | | | | 0.2* |
| Yes | 102 | 50 | -0.26 | 0.20 | 0.77 | 0.52-1.14 | |
| **Reason for hospital visit** | | | | | | | **0.2** |
| Admission | 12 | 12 | | | | | |
| Visit | 11 | 7 | -0.45 | 0.63 | 0.64 | 0.18-2.20 | |
| Outpatient/ A&E | 78 | 30 | -0.96 | 0.46 | 0.38 | 0.16-0.95 | |
| Other/ not specified | 1 | 1 | 0.00 | 1.47 | 1.00 | 0.06-17.90 | |
| No attendance | 254 | 161 | -0.45 | 0.42 | 0.63 | 0.28-1.45 | |
| **Source of dog** | | | | | | | **0.3** |
| Breeder | 174 | 105 | | | | | |
| Rescue Kennel/ stray | 87 | 39 | -0.29 | 0.23 | 0.74 | 0.47-1.16 | |
| Newspaper/ word of mouth/ internet | 29 | 15 | -0.15 | 0.34 | 0.86 | 0.44-1.67 | |
| Family/friend | 52 | 31 | -0.01 | 0.26 | 0.99 | 0.60-1.64 | |
| Pet shop | 4 | 1 | -0.88 | 1.12 | 0.41 | 0.05-3.76 | |
| Other | 8 | 8 | 0.51 | 0.52 | 1.66 | 0.60-4.55 | |
| Self breed | 5 | 8 | 0.98 | 0.58 | 2.65 | 0.85-8.32 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Fed tinned or packet wet food** | | | | | | | **0.3** |
| Yes | 238 | 131 | | | | | 0.4* |
| No | 122 | 80 | 0.18 | 0.18 | 1.19 | 0.84-1.70 | |
| **Fed dry mixer** | | | | | | | **0.5** |
| No | 289 | 174 | | | | | 0.6* |
| Yes | 71 | 37 | -0.14 | 0.22 | 0.87 | 0.56-1.34 | |
| **Fed dry complete** | | | | | | | **0.1** |
| No | 78 | 58 | | | | | 0.1* |
| Yes | 282 | 153 | -0.32 | 0.20 | 0.73 | 0.49-1.08 | |
| **Fed raw poultry meat** | | | | | | | **0.05** |
| No | 350 | 198 | | | | | 0.075* |
| Yes | 10 | 13 | 0.83 | 0.43 | 2.30 | 0.99-5.34 | 0.053 |
| **Fed cooked poultry meat** | | | | | | | **0.5** |
| No | 284 | 172 | | | | | 0.5* |
| Yes | 76 | 39 | -0.17 | 0.22 | 0.85 | 0.55-1.30 | |
| **Fed raw red meat** | | | | | | | **0.1** |
| No | 354 | 203 | | | | | 0.2* |
| Yes | 6 | 8 | 0.84 | 0.55 | 2.33 | 0.80-6.80 | |
| **Fed cooked red meat** | | | | | | | **0.5** |
| No | 350 | 203 | | | | | 0.6* |
| Yes | 10 | 8 | 0.32 | 0.48 | 1.38 | 0.54-3.55 | |
| **Sex** | | | | | | | **0.9** |
| Male | 176 | 103 | | | | | 1.0* |
| Female | 187 | 108 | -0.01 | 0.17 | 0.99 | 0.70-1.39 | |

Full univariable results for carriage of *E. coli* with resistance to augmentin

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.2** |
| <1 | 54 | 2 | | | | | |
| 1 | 75 | 3 | 0.08 | 0.93 | 1.08 | 0.17-6.69 | |
| 2 | 20 | 3 | 0.99 | 0.94 | 2.70 | 0.43-17.07 | |
| 3 | 40 | 1 | -0.39 | 1.24 | 0.68 | 0.06-7.71 | |
| 4 | 329 | 32 | 0.97 | 0.74 | 2.63 | 0.61-11.28 | |
| **Breed** | | | | | | | **0.9** |
| Pedigree | 405 | 30 | | | | | 0.8* |
| Cross | 114 | 9 | 0.06 | 0.39 | 1.07 | 0.49-2.31 | |
| **Breed Group** | | | | | | | **1.00** |
| Working | 19 | 1 | | | | | |
| Gundog | 182 | 15 | 0.45 | 1.06 | 1.57 | 0.20-12.52 | |
| Hound | 32 | 2 | 0.17 | 1.26 | 1.19 | 0.10-13.9 | |
| Terrier | 51 | 5 | 0.62 | 1.13 | 1.86 | 0.20-16.99 | |
| Utility | 28 | 2 | 0.31 | 1.26 | 1.36 | 0.11.16.05 | |
| Pastoral | 40 | 2 | -0.05 | 1.26 | 0.95 | 0.08-11.14 | |
| Toy | 34 | 1 | -0.58 | 1.44 | 0.56 | 0.03-9.45 | |
| Cross | 114 | 9 | 0.41 | 1.08 | 1.50 | 0.18-12.53 | |
| Not specified | 33 | 4 | 0.83 | 1.15 | 2.30 | 0.24-22.13 | |
| **Breed Size** | | | | | | | **0.6** |
| Small | 68 | 5 | | | | | |
| Medium | 127 | 6 | -0.44 | 0.62 | 0.64 | 0.19-2.18 | |
| Large | 190 | 17 | 0.20 | 0.53 | 1.22 | 0.43-3.43 | |
| Not specified | 148 | 13 | 0.18 | 0.55 | 1.19 | 0.41-3.48 | |
| **Neutered** | | | | | | | **0.3** |
| No | 140 | 14 | | | | | 0.3* |
| Yes | 391 | 27 | -0.37 | 0.34 | 0.69 | 0.35-1.35 | |
| **Working Dog** | | | | | | | **0.7** |
| No | 474 | 36 | | | | | 0.8* |
| Yes | 44 | 4 | 0.18 | 0.55 | 1.20 | 0.41-3.52 | |
| **Given dog treats** | | | | | | | **0.3** |
| Never | 30 | 3 | | | | | |
| Rarely | 85 | 8 | -0.06 | 0.71 | 0.94 | 0.23-3.78 | |
| Sometimes | 252 | 13 | -0.66 | 0.67 | 0.52 | 0.14-1.91 | |
| Often | 157 | 16 | 0.02 | 0.66 | 1.02 | 0.28-3.72 | |
| **Given titbits** | | | | | | | **0.02** |
| Never | 53 | 7 | | | | | |
| Rarely | 170 | 6 | -1.32 | 0.58 | 0.27 | 0.09-0.83 | |
| Sometimes | 221 | 24 | -0.20 | 0.46 | 0.82 | 0.34-2.01 | |
| Often | 76 | 3 | -1.21 | 0.71 | 0.30 | 0.07-1.21 | |
| **Given dog treats** | | | | | | | **0.4** |
| Never/ rarely | 115 | 11 | | | | | 0.4* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Sometimes/ often | 409 | 29 | -0.30 | 0.37 | 0.74 | 0.36-1.53 | |
| **Given titbits** | | | | | | | **0.2** |
| Never/ rarely | 223 | 13 | | | | | 0.2* |
| Sometimes/ often | 297 | 27 | 0.44 | 0.35 | 1.56 | 0.79-3.09 | |
| **Reason for visit** | | | | | | | **0.9** |
| Vaccination/ worming | 198 | 15 | | | | | |
| Presenting complaint | 246 | 19 | 0.02 | 0.39 | 1.02 | 0.51-20.6 | |
| Check up | 22 | 2 | 0.18 | 0.79 | 1.20 | 0.26-5.60 | |
| Work at clinic | 19 | 0 | | | | | |
| Vaccination/ presenting comp | 20 | 2 | 0.28 | 0.79 | 1.32 | 0.28-6.19 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 4 | 1 | 1.19 | 1.15 | 3.30 | 0.35-31.41 | |
| Other | 12 | 1 | 0.10 | 1.07 | 1.10 | 0.13-9.04 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.007** |
| No | 341 | 18 | | | | | 0.01* |
| Yes | 184 | 23 | 0.86 | 0.33 | 2.37 | 1.25-4.50 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.08** |
| No | 427 | 29 | | | | | 0.1* |
| Yes | 95 | 12 | 0.62 | 0.36 | 1.86 | 0.92-3.78 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.1** |
| One off prescription | 5 | 1 | | | | | |
| Up to 5 days | 31 | 2 | -1.13 | 1.32 | 0.32 | 0.02-4.26 | |
| Up to 10 days | 39 | 6 | -0.26 | 1.18 | 0.77 | 0.08-7.77 | |
| Up to 2 weeks | 12 | 1 | -0.88 | 1.51 | 0.42 | 0.02-8.05 | |
| Up to 3 weeks | 3 | 1 | 0.51 | 1.59 | 1.67 | 0.07-37.73 | |
| Over 3 weeks | 1 | 1 | 1.61 | 1.79 | 5.00 | 0.15-166.59 | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 427 | 29 | -1.08 | 1.11 | 0.34 | 0.04-3.00 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.01** |
| **No** | 375 | 21 | | | | | **0.01*** |
| **Yes** | 158 | 20 | 0.82 | 0.33 | 2.26 | 1.19-4.29 | **0.01** |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.2** |
| No | 297 | 17 | | | | | 0.2* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Yes | 227 | 21 | 0.48 | 0.34 | 1.62 | 0.83-3.13 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.3** |
| No | 328 | 21 | | | | | 0.4* |
| Yes | 191 | 17 | 0.33 | 0.34 | 1.39 | 0.72-2.70 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.004** |
| No | 411 | 23 | | | | | 0.009* |
| Yes | 93 | 14 | 0.99 | 0.36 | 2.69 | 1.33-5.43 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.06** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 28 | 4 | REF | | | | |
| Up to 10 days | 32 | 4 | -0.13 | 0.75 | 0.88 | 0.20-3.83 | |
| Up to 2 weeks | 13 | 4 | 0.77 | 0.78 | 2.15 | 0.47-9.99 | |
| Up to 3 weeks | 5 | 0 | | | | | |
| Over 3 weeks | 6 | 1 | 0.15 | 1.21 | 1.17 | 0.11-12.38 | |
| Don’t know | 3 | 0 | | | | | |
| None prescribed | 411 | 23 | -0.94 | 0.58 | 0.39 | 0.13-1.211 | |
| **Left at veterinary premises** | | | | | | | **0.2** |
| No | 399 | 27 | | | | | 0.2* |
| Yes | 40 | 5 | 0.61 | 0.51 | 1.85 | 0.67-5.06 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.6** |
| No | 110 | 10 | | | | | 0.7* |
| Yes | 411 | 31 | -0.19 | 0.38 | 0.83 | 0.39-1.74 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.2** |
| No | 398 | 28 | | | | | 0.3* |
| Yes | 122 | 13 | 0.42 | 0.35 | 1.51 | 0.76-3.01 | |
| **Allowed off lead during walks** | | | | | | | **0.8** |
| No | 105 | 9 | | | | | 0.8* |
| Yes | 418 | 32 | -0.11 | 0.39 | 0.89 | 0.41-1.93 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Other dogs in household** | | | | | | | **0.6** |
| No | 292 | 21 | | | | | 0.6* |
| Yes | 233 | 20 | 0.18 | 0.32 | 1.19 | 0.63-2.25 | |
| **Number of other dogs in household** | | | | | | | **0.5** |
| 0 | 292 | 21 | | | | | |
| 1 | 112 | 8 | -0.01 | 0.43 | 0.99 | 0.43-2.31 | |
| 2 | 44 | 7 | 0.79 | 0.47 | 2.21 | 0.89-5.51 | |
| 3 | 12 | 1 | 0.15 | 1.07 | 1.16 | 0.14-9.34 | |
| 4+ | 15 | 1 | -0.08 | 1.06 | 0.93 | 0.12-7.36 | |
| **Other animals in household** | | | | | | | **0.01** |
| No | 288 | 30 | | | | | 0.01* |
| Yes | 237 | 10 | -0.90 | 0.38 | 0.41 | 0.19-0.85 | |
| **Own a cat** | | | | | | | **0.04** |
| No | 381 | 35 | | | | | 0.04 |
| Yes | 144 | 5 | -0.97 | 0.49 | 0.38 | 0.15-0.98 | |
| **Own a bird** | | | | | | | **0.5** |
| No | 485 | 38 | | | | | 0.8* |
| Yes | 40 | 2 | -0.45 | 0.74 | 0.64 | 0.15-2.74 | |
| **Own a rabbit** | | | | | | | **0.09** |
| No | 489 | 40 | | | | | 0.1 |
| Yes | 36 | 0 | | | | | |
| **Own a rodent** | | | | | | | **0.6** |
| No | 486 | 38 | | | | | 0.8* |
| Yes | 39 | 2 | -0.42 | 0.74 | 0.66 | 0.15-2.82 | |
| **Own a reptile or amphibian** | | | | | | | **0.8** |
| No | 515 | 39 | | | | | 0.6* |
| Yes | 10 | 1 | 0.28 | 1.06 | 1.32 | 0.16-10.58 | |
| **Own a fish** | | | | | | | **0.2** |
| No | 509 | 40 | | | | | 0.6* |
| Yes | 16 | 0 | | | | | |
| **Own any other animal or livestock** | | | | | | | **0.6** |
| No | 472 | 35 | | | | | 0.6* |
| Yes | 53 | 5 | 0.24 | 0.50 | 1.27 | 0.48-3.39 | |
| **Where the dog usually sleeps** | | | | | | | **0.4** |
| Outside | 20 | 3 | | | | | |
| Downstairs | 277 | 17 | -0.89 | 0.67 | 0.41 | 0.11-1.51 | |
| Upstairs | 207 | 18 | -0.55 | 0.67 | 0.58 | 0.16-2.14 | |
| Whole house | 19 | 3 | 0.05 | 0.88 | 1.05 | 0.19-5.87 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Outside and downstairs | 2 | 0 | | | | | |
| **Anyone in the household work with farm animals** | | | | | | | **0.3** |
| No | 483 | 36 | | | | | 0.4* |
| Yes | 40 | 5 | 0.52 | 0.50 | 1.68 | 0.62-4.51 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.4** |
| No | 436 | 36 | | | | | 0.67* |
| Yes | 88 | 5 | -0.37 | 0.49 | 0.69 | 0.26-1.80 | |
| **Who took antibiotics** | | | | | | | **0.2** |
| No | 49 | 1 | | | | | |
| Yes | 31 | 4 | 1.84 | 1.14 | 6.32 | 0.68-59.21 | |
| No one/ not specified | 435 | 36 | 1.40 | 1.02 | 4.06 | 0.54-30.23 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.5** |
| No | 373 | 27 | | | | | 0.5* |
| Yes | 154 | 14 | 0.23 | 0.34 | 1.26 | 0.64-2.46 | |
| **Healthcare setting** | | | | | | | **0.8** |
| Human | 50 | 3 | | | | | |
| Animal | 101 | 11 | 0.60 | 0.67 | 1.82 | 0.48-6.80 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 373 | 27 | 0.19 | 0.69 | 1.21 | 0.35-4.12 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.5** |
| No | 383 | 32 | | | | | 0.6* |
| Yes | 143 | 9 | -0.28 | 0.39 | 0.75 | 0.35-1.62 | |
| **Reason for hospital visit** | | | | | | | **0.7** |
| Admission | 23 | 1 | | | | | |
| Visit | 18 | 0 | | | | | |
| Outpatient/ A&E | 100 | 8 | 0.61 | 1.09 | 1.84 | 0.22-15.45 | |
| Other/ not specified | 2 | 0 | | | | | |
| No attendance | 383 | 32 | 0.65 | 1.04 | 1.92 | 0.25-14.69 | |
| **Source of dog** | | | | | | | |
| Breeder | | | | | | | **0.04** |
| Rescue Kennel/ stray | 262 | 17 | | | | | |
| Newspaper/ word of mouth/ internet | 116 | 10 | 0.28 | 0.41 | 1.33 | 0.59-2.99 | |
| Family/friend | 41 | 3 | 0.12 | 0.65 | 1.13 | 0.32-4.02 | |
| Pet shop | 79 | 4 | -0.25 | 0.57 | 0.78 | 0.26-2.39 | |
| Other | 5 | 0 | | | | | |
| Self breed | 15 | 1 | -0.03 | 1.06 | 1.03 | 0.13-8.25 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| | 9 | 4 | 1.92 | 0.65 | 6.85 | 1.91-24.53 | |
| **Fed tinned or packet wet food** | | | | | | | **0.9** |
| Yes | 343 | 26 | | | | | 0.9* |
| No | 187 | 15 | 0.06 | 0.34 | 1.06 | 0.55-2.05 | |
| **Fed dry mixer** | | | | | | | **0.4** |
| No | 432 | 31 | | | | | 0.4* |
| Yes | 98 | 10 | 0.35 | 0.38 | 1.42 | 0.67-3.00 | |
| **Fed dry complete** | | | | | | | **0.6** |
| No | 125 | 11 | | | | | 0.7* |
| Yes | 405 | 30 | -0.17 | 0.37 | 0.84 | 0.41-1.73 | |
| **Fed raw poultry meat** | | | | | | | **0.8** |
| No | 509 | 39 | | | | | 0.7* |
| Yes | 21 | 2 | 0.22 | 0.76 | 1.24 | 0.28-5.50 | |
| **Fed cooked poultry meat** | | | | | | | **0.2** |
| No | 420 | 36 | | | | | 0.2* |
| Yes | 110 | 5 | -0.63 | 0.49 | 0.53 | 0.20-1.38 | |
| **Fed raw red meat** | | | | | | | **0.3** |
| No | 516 | 41 | | | | | 0.6* |
| Yes | 14 | 0 | | | | | |
| **Fed cooked red meat** | | | | | | | **0.2** |
| No | 512 | 41 | | | | | 0.6* |
| Yes | 18 | 0 | | | | | |
| **Sex** | | | | | | | **0.3** |
| Male | 256 | 23 | | | | | 0.3* |
| Female | 277 | 18 | -0.32 | 0.33 | 0.72 | 0.38-1.37 | |

Full univariable results for carriage of *E. coli* with resistance to chloramphenicol

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.8** |
| <1 | 49 | 7 | | | | | |
| 1 | 73 | 5 | -0.74 | 0.61 | 0.48 | 0.14-1.60 | |
| 2 | 31 | 2 | -0.79 | 0.83 | 0.45 | 0.09-2.32 | |
| 3 | 37 | 4 | -0.28 | 0.66 | 0.76 | 0.21-2.78 | |
| 4 | 327 | 34 | -0.32 | 0.44 | 0.73 | 0.31-1.73 | |
| **Breed** | | | | | | | **0.3** |
| Pedigree | 393 | 42 | | | | | 0.4* |
| Cross | 115 | 8 | -0.43 | 0.40 | 0.65 | 0.30-1.43 | |
| **Breed Group** | | | | | | | **0.2** |
| Working | 18 | 2 | | | | | |
| Gundog | 171 | 26 | 0.31 | 0.77 | 1.37 | 0.30-6.24 | |
| Hound | 29 | 5 | 0.44 | 0.89 | 1.55 | 0.27-8.86 | |
| Terrier | 55 | 1 | -1.81 | 1.25 | 0.16 | 0.01-1.91 | |
| Utility | 27 | 3 | 0.00 | 0.96 | 1.00 | 0.15-6.59 | |
| Pastoral | 39 | 3 | -0.37 | 0.96 | 0.69 | 0.11-4.51 | |
| Toy | 34 | 1 | -1.33 | 1.26 | 0.26 | 0.02-3.12 | |
| Cross | 115 | 8 | -0.47 | 0.83 | 0.63 | 0.12-3.19 | |
| Not specified | 34 | 3 | -0.23 | 0.96 | 0.79 | 0.12-5.2 | |
| **Breed Size** | | | | | | | **0.04** |
| Small | 70 | 3 | | | | | |
| Medium | 123 | 10 | 0.64 | 0.68 | 1.90 | 0.51-7.12 | |
| Large | 179 | 28 | 1.29 | 0.62 | 3.65 | 1.08-12.39 | |
| Not specified | 150 | 11 | 0.54 | 0.67 | 1.71 | 0.46-6.33 | |
| **Neutered** | | | | | | | **0.5** |
| No | 138 | 16 | | | | | 0.5* |
| Yes | 382 | 36 | -0.21 | 0.32 | 0.81 | 0.44-1.51 | |
| **Working Dog** | | | | | | | **<0.001** |
| No | 471 | 39 | | | | | 0.001* |
| Yes | 36 | 12 | 1.39 | 0.37 | 4.03 | 1.94-8.36 | |
| **Given dog treats** | | | | | | | **0.003** |
| Never | 25 | 8 | | | | | |
| Rarely | 82 | 11 | -0.87 | 0.52 | 0.42 | 0.15-1.16 | |
| Sometimes | 250 | 15 | -1.67 | 0.49 | 0.19 | 0.07-0.49 | |
| Often | 155 | 18 | -1.01 | 0.48 | 0.36 | 0.14-0.92 | |
| **Given titbits** | | | | | | | **0.1** |
| Never | 53 | 7 | | | | | |
| Rarely | 165 | 11 | -0.68 | 0.51 | 0.50 | 0.19-1.37 | |
| Sometimes | 223 | 22 | -0.29 | 0.46 | 0.75 | 0.30-1.84 | |
| Often | 67 | 12 | -0.30 | 0.51 | 1.36 | 0.50-3.68 | |
| **Given dog treats** | | | | | | | **0.01** |
| Never/ rarely | 107 | 19 | | | | | 0.01* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Sometimes/ often | 405 | 33 | -0.78 | 0.31 | 0.46 | 0.25-0.84 | |
| **Given titbits** | | | | | | | **0.2** |
| Never/ rarely | 218 | 18 | | | | | 0.3* |
| Sometimes/ often | 290 | 34 | 0.35 | 0.30 | 1.42 | 0.78-2.58 | |
| **Reason for visit** | | | | | | | **0.5** |
| Vaccination/ worming | 198 | 15 | | | | | |
| Presenting complaint | 237 | 28 | 0.44 | 0.33 | 1.56 | 0.81-3.00 | |
| Check up | 21 | 3 | 0.63 | 0.67 | 1.89 | 0.50-7.05 | |
| Work at clinic | 15 | 4 | 1.26 | 0.62 | 3.52 | 1.04-11.94 | |
| Vaccination/ presenting comp | 21 | 1 | -0.46 | 1.06 | 0.63 | 0.08-5.00 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 5 | 0 | | | | | |
| Other | 12 | 1 | 0.10 | 1.07 | 1.10 | 0.13-9.04 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.5** |
| No | 328 | 31 | | | | | 0.5* |
| Yes | 186 | 21 | 0.18 | 0.30 | 1.19 | 0.67-2.14 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.4** |
| No | 416 | 40 | | | | | 0.5* |
| Yes | 95 | 12 | 0.27 | 0.35 | 1.31 | 0.66-2.60 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.7** |
| One off prescription | 5 | 1 | | | | | |
| Up to 5 days | 29 | 4 | -0.37 | 1.22 | 0.69 | 0.06-7.51 | |
| Up to 10 days | 39 | 6 | -0.26 | 1.18 | 0.77 | 0.08-7.77 | |
| Up to 2 weeks | 13 | 0 | | | | | |
| Up to 3 weeks | 3 | 1 | 0.51 | 1.59 | 1.67 | 0.07-37.73 | |
| Over 3 weeks | 2 | 0 | | | | | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 416 | 40 | -0.73 | 1.11 | 0.48 | 0.05-4.22 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.4** |
| No | 363 | 33 | | | | | 0.4* |
| Yes | 159 | 19 | 0.27 | 0.30 | 1.31 | 0.73-2.38 | |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.3** |
| No | 282 | 32 | | | | | 0.4* |
| Yes | 229 | 19 | -0.31 | 0.30 | 0.73 | 0.40-1.32 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | 0.3 |
| No | 314 | 35 | | | | | 0.3* |
| Yes | 193 | 15 | -0.36 | 0.32 | 0.70 | 0.37-1.31 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.9** |
| No | 395 | 39 | | | | | 0.9* |
| Yes | 97 | 10 | 0.04 | 0.37 | 1.04 | 0.50-2.17 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.9** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 29 | 3 | Ref | | | | |
| Up to 10 days | 31 | 5 | 0.44 | 0.77 | 1.56 | 0.34-7.11 | |
| Up to 2 weeks | 16 | 1 | -0.50 | 1.20 | 0.60 | 0.06-6.30 | |
| Up to 3 weeks | 5 | 0 | | | | | |
| Over 3 weeks | 6 | 1 | 0.48 | 1.24 | 1.61 | 0.14-18.26 | |
| Don’t know | 2 | 0 | | | | | |
| None prescribed | 395 | 39 | 0.05 | 0.63 | 0.95 | 0.28-3.28 | |
| **Left at veterinary premises** | | | | | | | **0.56** |
| No | 387 | 39 | | | | | 0.8* |
| Yes | 42 | 3 | -0.34 | 0.62 | 0.71 | 0.21-2.39 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.5** |
| No | 111 | 9 | | | | | 0.6* |
| Yes | 399 | 43 | 0.28 | 0.38 | 1.33 | 0.63-2.81 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.06** |
| No | 392 | 34 | | | | | 0.09* |
| Yes | 117 | 18 | 0.57 | 0.31 | 1.77 | 0.97-3.26 | |
| **Allowed off lead during walks** | | | | | | | **0.2** |
| No | 107 | 7 | | | | | 0.3* |
| Yes | 405 | 45 | 0.53 | 0.42 | 1.70 | 0.74-3.87 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Other dogs in household** | | | | | | | **0.02** |
| No | 292 | 21 | | | | | 0.03* |
| Yes | 222 | 31 | 0.66 | 0.30 | 1.94 | 1.09-3.47 | |
| **Number of other dogs in household** | | | | | | | **<0.001** |
| 0 | 292 | 21 | | | | | |
| 1 | 109 | 11 | 0.34 | 0.39 | 1.40 | 0.62-3.01 | |
| 2 | 46 | 5 | 0.41 | 0.52 | 1.51 | 0.54-4.21 | |
| 3 | 8 | 5 | 2.16 | 0.61 | 8.69 | 2.61-28.91 | |
| 4+ | 12 | 4 | 1.53 | 0.62 | 4.63 | 1.38-15.62 | |
| **Other animals in household** | | | | | | | **0.001** |
| No | 301 | 17 | | | | | 0.001* |
| Yes | 213 | 34 | 1.04 | 0.31 | 2.83 | 1.51-5.19 | |
| **Own a cat** | | | | | | | **<0.001** |
| No | 390 | 26 | | | | | <0.001* |
| Yes | 124 | 25 | 1.11 | 0.30 | 3.02 | 1.68-5.43 | |
| **Own a bird** | | | | | | | **0.7** |
| No | 475 | 48 | | | | | 1.000* |
| Yes | 39 | 3 | -0.27 | 0.62 | 0.76 | 0.23-2.56 | |
| **Own a rabbit** | | | | | | | **0.3** |
| No | 483 | 46 | | | | | 0.4* |
| Yes | 31 | 5 | 0.53 | 0.51 | 1.69 | 0.63-4.57 | |
| **Own a rodent** | | | | | | | **09** |
| No | 477 | 47 | | | | | 0.8* |
| Yes | 37 | 4 | 927453.00 | 0.55 | 1.10 | 0.37-3.21 | |
| **Own a reptile or amphibian** | | | | | | | **1.0** |
| No | 504 | 50 | | | | | 1.0* |
| Yes | 10 | 1 | 0.01 | 1.06 | 1.01 | 0.13-8.04 | |
| **Own a fish** | | | | | | | **0.2** |
| No | 498 | 51 | | | | | 0.4* |
| Yes | 16 | 0 | | | | | |
| **Own any other animal or livestock** | | | | | | | **0.005** |
| No | 467 | 40 | | | | | 0.01* |
| Yes | 47 | 11 | 1.01 | 0.37 | 2.69 | 1.31-5.68 | |
| **Where the dog usually sleeps** | | | | | | | **0.2** |
| Outside | 20 | 3 | | | | | |
| Downstairs | 264 | 30 | -0.28 | 0.65 | 0.76 | 0.21-2.70 | |
| Upstairs | 209 | 16 | -0.67 | 0.67 | 0.51 | 0.14-1.90 | |
| Whole house | 20 | 2 | -0.41 | 0.97 | 0.67 | 0.10-4.43 | |
| Outside and downstairs | 1 | 1 | 1.90 | 1.54 | 6.67 | 0.32-137.41 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Anyone in the household work with farm animals** | | | | | | | **0.3** |
| No | 473 | 46 | | | | | 0.3* |
| Yes | 39 | 6 | 0.46 | 0.46 | 1.58 | 0.64-3.94 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.9*** |
| No | 429 | 43 | | | | | 1.0* |
| Yes | 85 | 8 | -0.06 | 0.40 | 0.94 | 0.43-2.07 | |
| **Who took antibiotics** | | | | | | | **0.9** |
| No | 46 | 4 | | | | | |
| Yes | 31 | 4 | 0.39 | 0.74 | 1.48 | 0.35-6.38 | |
| No one/ not specified | 482 | 43 | 0.14 | 0.55 | 1.16 | 0.40-3.34 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.07** |
| No | 369 | 31 | | | | | 0.08* |
| Yes | 147 | 21 | 0.53 | 0.30 | 1.70 | 0.95-3.06 | |
| **Healthcare setting** | | | | | | | **0.6** |
| Human | 44 | 9 | | | | | |
| Animal | 100 | 12 | -0.53 | 0.48 | 0.59 | 0.23-1.49 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 369 | 31 | -0.89 | 0.41 | 0.41 | 0.18-0.92 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.2** |
| No | 373 | 42 | | | | | 0.3* |
| Yes | 142 | 10 | -0.47 | 0.37 | 0.63 | 0.31-1.28 | |
| **Reason for hospital visit** | | | | | | | **0.4** |
| Admission | 21 | 3 | | | | | |
| Visit | 18 | 0 | | | | | |
| Outpatient/ A&E | 101 | 7 | -0.72 | 0.73 | 0.49 | 0.12-2.03 | |
| Other/ not specified | 2 | 0 | | | | | |
| No attendance | 373 | 42 | -0.24 | 0.64 | 0.79 | 0.23-2.75 | |
| **Source of dog** | | | | | | | **0.02** |
| Breeder | 244 | 35 | | | | | |
| Rescue Kennel/ stray | 121 | 5 | -1.24 | 0.49 | 0.29 | 0.11-0.75 | |
| Newspaper/ word of mouth/ internet | 43 | 1 | -1.82 | 1.03 | 0.16 | 0.02-1.21 | |
| Family/friend | 78 | 5 | -0.81 | 0.50 | 0.45 | 0.17-1.18 | |
| Pet shop | 5 | 0 | | | | | |
| Other | 14 | 2 | 0.00 | 0.78 | 1.00 | 0.22-4.57 | |
| Self breed | 10 | 3 | 0.74 | 0.68 | 2.09 | 0.55-7.97 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Fed tinned or packet wet food** | | | | | | | **0.9** |
| Yes | 335 | 34 | | | | | 1.0* |
| No | 184 | 18 | -0.04 | 0.31 | 0.96 | 0.53-1.75 | |
| **Fed dry mixer** | | | | | | | **0.01** |
| No | 414 | 49 | | | | | 0.009* |
| Yes | 105 | 3 | -1.42 | 0.60 | 0.24 | 0.07-0.79 | |
| **Fed dry complete** | | | | | | | **0.8** |
| No | 123 | 13 | | | | | 0.9* |
| Yes | 396 | 39 | -0.07 | 0.34 | 0.93 | 0.48-1.80 | |
| **Fed raw poultry meat** | | | | | | | **<0.001** |
| No | 505 | 43 | | | | | <0.001* |
| Yes | 14 | 9 | 2.02 | 0.46 | 7.55 | 3.09-18.45 | |
| **Fed cooked poultry meat** | | | | | | | **0.6** |
| No | 416 | 40 | | | | | 0.6* |
| Yes | 103 | 12 | 0.19 | 0.35 | 1.21 | 0.61-2.39 | |
| **Fed raw red meat** | | | | | | | **08** |
| No | 506 | 51 | | | | | 1.0* |
| Yes | 13 | 1 | -0.27 | 1.05 | 0.76 | 0.10-5.95 | |
| **Fed cooked red meat** | | | | | | | **0.8** |
| No | 503 | 50 | | | | | 0.7* |
| Yes | 16 | 2 | 0.23 | 0.76 | 1.26 | 0.28-5.63 | |
| **Sex** | | | | | | | **0.1** |
| Male | 259 | 20 | | | | | 0.1* |
| Female | 263 | 32 | 0.45 | 0.30 | 1.58 | 0.88-2.83 | |

Full univariable results for carriage of *E. coli* with resistance to ciprofloxacin

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.2** |
| <1 | 54 | 2 | | | | | |
| 1 | 77 | 1 | -1.05 | 1.24 | 0.35 | 0.03-3.97 | |
| 2 | 33 | 0 | | | | | |
| 3 | 39 | 2 | 0.33 | 1.02 | 1.38 | 0.19-10.26 | |
| 4 | 338 | 23 | 0.61 | 0.72 | 1.38 | 0.42-8.01 | |
| **Breed** | | | | | | | **0.1** |
| Pedigree | 410 | 25 | | | | | 0.2* |
| Cross | 120 | 3 | -0.89 | 0.62 | 0.41 | 0.12-1.38 | |
| **Breed Group** | | | | | | | **0.03** |
| Working | 16 | 4 | | | | | |
| Gundog | 185 | 12 | -1.35 | 0.63 | 0.26 | 0.07-0.90 | |
| Hound | 32 | 2 | -1.39 | 0.92 | 0.25 | 0.04-1.51 | |
| Terrier | 55 | 1 | -2.62 | 1.15 | 0.07 | 0.01-0.70 | |
| Utility | 27 | 3 | -0.81 | 0.83 | 0.44 | 0.09-2.25 | |
| Pastoral | 40 | 2 | -1.61 | 0.92 | 0.20 | 0.03-1.20 | |
| Toy | 34 | 1 | -2.14 | 1.16 | 0.12 | 0.01-1.14 | |
| Cross | 120 | 3 | -2.30 | 0.81 | 0.10 | 0.02-0.49 | |
| Not specified | 37 | 0 | | | | | |
| **Breed Size** | | | | | | | **0.03** |
| Small | 71 | 2 | | | | | |
| Medium | 127 | 6 | 0.52 | 0.83 | 1.68 | 0.33-8.53 | |
| Large | 190 | 17 | 1.16 | 0.76 | 3.18 | 0.72-14.10 | |
| Not specified | 158 | 3 | -0.39 | 0.92 | 0.67 | 0.11-4.12 | |
| **Neutered** | | | | | | | **0.5** |
| No | 145 | 9 | | | | | 0.5* |
| Yes | 399 | 19 | -0.27 | 0.42 | 0.77 | 0.34-1.73 | |
| **Working Dog** | | | | | | | **0.06** |
| No | 488 | 22 | | | | | 0.07* |
| Yes | 43 | 5 | 0.95 | 0.52 | 2.58 | 0.93-7.15 | |
| **Given dog treats** | | | | | | | **0.3** |
| Never | 31 | 2 | | | | | |
| Rarely | 85 | 8 | 0.38 | 0.82 | 1.46 | 0.29-7.25 | |
| Sometimes | 256 | 9 | -0.61 | 0.80 | 0.54 | 0.11-2.64 | |
| Often | 164 | 9 | -0.16 | 0.81 | 0.85 | 0.18-4.13 | |
| **Given titbits** | | | | | | | **0.03** |
| Never | 57 | 3 | | | | | |
| Rarely | 173 | 3 | -1.11 | 0.83 | 0.33 | 0.06-1.68 | |
| Sometimes | 231 | 14 | 0.14 | 0.65 | 1.15 | 0.32-4.14 | |
| Often | 71 | 8 | 0.76 | 0.70 | 2.14 | 0.54-8.44 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Given dog treats** | | | | | | | **0.08** |
| Never/ rarely | 116 | 10 | | | | | 0.1* |
| Sometimes/ often | 420 | 18 | -0.70 | 0.41 | 0.50 | 0.22-1.11 | |
| **Given titbits** | | | | | | | **0.02** |
| Never/ rarely | 230 | 6 | | | | | 0.03* |
| Sometimes/ often | 302 | 22 | 1.03 | 0.47 | 2.79 | 1.11-7.00 | |
| **Reason for visit** | | | | | | | **0.5** |
| Vaccination/ worming | 207 | 6 | | | | | |
| Presenting complaint | 248 | 17 | 0.86 | 0.48 | 2.36 | 0.92-6.11 | |
| Check up | 22 | 2 | 1.14 | 0.85 | 3.14 | 0.60-16..49 | |
| Work at clinic | 17 | 2 | 1.40 | 0.85 | 4.06 | 0.76-21.67 | |
| Vaccination/ presenting comp | 21 | 1 | 0.50 | 1.10 | 1.64 | 0.19-14.30 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 5 | 0 | | | | | |
| Other | 13 | 0 | | | | | |
| **Medication prescribed during most recent visit** | | | | | | | **0.1** |
| No | 345 | 14 | | | | | 0.2* |
| Yes | 193 | 14 | 0.58 | 0.39 | 1.79 | 0.83-3.83 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.07** |
| No | 437 | 19 | | | | | 0.08* |
| Yes | 98 | 9 | 0.75 | 0.42 | 2.11 | 0.93-4.81 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.3** |
| One off prescription | 6 | 0 | | | | | |
| Up to 5 days | 29 | 4 | Ref | | | | |
| Up to 10 days | 42 | 3 | -0.66 | 0.80 | 0.52 | 0.11-2.49 | |
| Up to 2 weeks | 11 | 2 | 0.28 | 0.94 | 1.32 | 0.21-8.25 | |
| Up to 3 weeks | 4 | 0 | | | | | |
| Over 3 weeks | 2 | 0 | | | | | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 437 | 19 | -1.15 | 0.58 | 0.32 | 0.10-0.99 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.03** |
| **No** | 382 | 14 | | | | | 0.04* |
| **Yes** | 164 | 14 | 0.85 | 0.39 | 2.33 | 1.09-5.00 | 0.03 |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.03** |
| No | 304 | 10 | | | | | 0.03* |
| Yes | 230 | 18 | 0.87 | 0.40 | 2.38 | 1.08-5.25 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.002** |
| No | 339 | 10 | | | | | 0.004* |
| Yes | 190 | 18 | 1.17 | 0.40 | 3.21 | 1.45-7.10 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.008** |
| No | 417 | 17 | | | | | 0.01* |
| Yes | 96 | 11 | 1.03 | 0.40 | 2.81 | 1.28-6.19 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.5** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 29 | 3 | Ref | | | | |
| Up to 10 days | 30 | 6 | 0.66 | 0.75 | 1.93 | 0.44-8.47 | |
| Up to 2 weeks | 16 | 1 | -0.50 | 1.20 | 0.60 | 0.06-6.30 | |
| Up to 3 weeks | 5 | 0 | | | | | |
| Over 3 weeks | 6 | 1 | 0.48 | 1.24 | 1.61 | 0.14-18.26 | |
| Don’t know | 3 | 0 | | | | | |
| None prescribed | 417 | 17 | -0.93 | 0.66 | 0.39 | 0.11-1.42 | |
| **Left at veterinary premises** | | | | | | | **0.9** |
| No | 408 | 18 | | | | | 1.0* |
| Yes | 43 | 2 | 0.05 | 0.76 | 1.05 | 0.24-4.70 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.6** |
| No | 113 | 7 | | | | | 0.6* |
| Yes | 422 | 20 | -0.27 | 0.45 | 0.77 | 0.32-1.85 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.2** |
| No | 408 | 18 | | | | | 0.3* |
| Yes | 126 | 9 | 0.48 | 0.42 | 1.62 | 0.71-3.69 | |
| **Allowed off lead during walks** | | | | | | | **0.8** |
| No | 109 | 5 | | | | | 1.0* |
| Yes | 427 | 23 | 0.16 | 0.50 | 1.17 | 0.44-3.16 | |
| **Other dogs in household** | | | | | | | **0.08** |
| No | 302 | 11 | | | | | 0.1* |
| Yes | 236 | 17 | 0.68 | 0.40 | 1.97 | 0.91-4.30 | |
| **Number of other dogs in household** | | | | | | | **0.006** |
| 0 | 302 | 11 | | | | | |
| 1 | 116 | 4 | -0.05 | 0.59 | 0.95 | 0.30-3.03 | |
| 2 | 46 | 5 | 1.09 | 0.56 | 2.98 | 0.99-8.98 | |
| 3 | 11 | 2 | 1.91 | 0.83 | 4.99 | 0.99-25.28 | |
| 4+ | 13 | 3 | 1.85 | 0.71 | 6.34 | 1.57-25.49 | |
| **Other animals in household** | 306 | 12 | | | | | 0.2* |
| No | 232 | 15 | 0.50 | 0.40 | 1.65 | 0.76-3.59 | |
| Yes | | | | | | | |
| **Own a cat** | 402 | 14 | | | | | 0.01 |
| No | 136 | 13 | 1.01 | 0.40 | 2.74 | 1.26-5.98 | |
| Yes | | | | | | | |
| **Own a bird** | 498 | 25 | | | | | 1.0* |
| No | 40 | 2 | 0.00 | 0.75 | 1.00 | 0.23-4.36 | |
| Yes | | | | | | | |
| **Own a rabbit** | 504 | 25 | | | | | 0.7* |
| No | 34 | 2 | 0.17 | 0.76 | 1.19 | 0.27-5.22 | |
| Yes | | | | | | | |
| **Own a rodent** | 497 | 27 | | | | | 0.2* |
| No | 41 | 0 | | | | | |
| Yes | | | | | | | |
| **Own a reptile or amphibian** | | | | | | | **0.5** |
| No | 528 | 26 | | | | | 0.4* |
| Yes | 10 | 1 | 0.71 | 1.07 | 2.03 | 0.25-16.47 | |
| **Own a fish** | | | | | | | **0.4** |
| No | 517 | 27 | | | | | 1.0* |
| Yes | 16 | 0 | | | | | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Own any other animal or livestock** | | | | | | | **0.4** |
| No | 484 | 23 | | | | | 0.5* |
| Yes | 54 | 4 | 0.44 | 0.56 | 1.56 | 0.52-4.98 | |
| **Where the dog usually sleeps** | | | | | | | **0.1** |
| Outside | 20 | 3 | | | | | |
| Downstairs | 281 | 13 | -1.18 | 0.68 | 0.31 | 0.08-1.17 | |
| Upstairs | 216 | 9 | -1.28 | 0.71 | 0.28 | 0.07-1.11 | |
| Whole house | 19 | 3 | 0.05 | 0.88 | 1.05 | 0.19-5.87 | |
| Outside and downstairs | 2 | 0 | | | | | |
| **Anyone in the household work with farm animals** | | | | | | | **0.05** |
| No | 496 | 23 | | | | | 0.06* |
| Yes | 40 | 5 | 0.99 | 0.52 | 2.70 | 0.97-7.47 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.4** |
| No | 447 | 25 | | | | | 0.6* |
| Yes | 90 | 3 | -0.52 | 0.62 | 0.60 | 0.18-2.02 | |
| **Who took antibiotics** | | | | | | | **0.8** |
| No | 48 | 2 | | | | | |
| Yes | 34 | 1 | -0.35 | 1.25 | 0.71 | 0.06-8.10 | |
| No one/ not specified | 446 | 25 | 0.30 | 0.75 | 1.35 | 0.31-5.86 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.8** |
| No | 381 | 19 | | | | | 0.8* |
| Yes | 159 | 9 | 0.13 | 0.42 | 1.14 | 0.50-2.56 | |
| **Healthcare setting** | | | | | | | **1.0** |
| Human | 50 | 3 | | | | | |
| Animal | 106 | 6 | -0.06 | 0.73 | 0.94 | 0.23-3.93 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 381 | 19 | -0.18 | 0.64 | 0.83 | 0.24-2.91 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.8** |
| No | 394 | 21 | | | | | 1.0* |
| Yes | 145 | 7 | -0.10 | 0.45 | 0.91 | 0.38-2.18 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Reason for hospital visit** | | | | | | | **1.0** |
| Admission | 23 | 1 | | | | | |
| Visit | 17 | 1 | 0.30 | 1.45 | 1.35 | 0.08-23.20 | |
| Outpatient/ A&E | 103 | 5 | 0.11 | 1.12 | 1.12 | 0.12-10.02 | |
| Other/ not specified | 2 | 0 | | | | | |
| No attendance | 394 | 21 | 0.20 | 0.10 | 1.23 | 0.16-9.52 | |
| **Source of dog** | | | | | | | **0.09** |
| Breeder | 264 | 15 | | | | | |
| Rescue Kennel/ stray | 121 | 5 | -0.32 | 0.53 | 0.73 | 0.26-2.05 | |
| Newspaper/ word of mouth/ internet | 42 | 2 | -0.18 | 0.77 | 0.84 | 0.18-3.80 | |
| Family/friend | 81 | 2 | -0.83 | 0.76 | 0.43 | 0.10-1.94 | |
| Pet shop | 5 | 0 | | | | | |
| Other | 15 | 1 | 0.16 | 1.07 | 1.17 | 0.15-9.49 | |
| Self breed | 10 | 3 | 1.66 | 0.71 | 5.28 | 1.31-21.22 | |
| **Fed tinned or packet wet food** | | | | | | | **0.1** |
| Yes | 347 | 22 | | | | | 0.2* |
| No | 196 | 6 | -0.73 | 0.47 | 0.48 | 0.19-1.21 | |
| **Fed dry mixer** | | | | | | | **0.5** |
| No | 439 | 24 | | | | | 0.6* |
| Yes | 104 | 4 | -0.35 | 0.55 | 0.70 | 0.24-2.07 | |
| **Fed dry complete** | | | | | | | **0.3** |
| No | 127 | 9 | | | | | 0.4* |
| Yes | 416 | 19 | -0.44 | 0.42 | 0.64 | 0.28-1.46 | |
| **Fed raw poultry meat** | | | | | | | **<0.001** |
| No | 525 | 23 | | | | | 0.004* |
| Yes | 18 | 5 | 1.85 | 0.55 | 6.34 | 2.16-18.58 | |
| **Fed cooked poultry meat** | | | | | | | **0.8** |
| No | 433 | 23 | | | | | 1.0* |
| Yes | 110 | 5 | -0.16 | 0.50 | 0.86 | 0.32-2.30 | |
| **Fed raw red meat** | | | | | | | **0.4** |
| No | 529 | 28 | | | | | 1.0* |
| Yes | 14 | 0 | | | | | |
| **Fed cooked red meat** | | | | | | | **0.9** |
| No | 526 | 27 | | | | | 0.6* |
| Yes | 17 | 1 | 0.14 | 1.05 | 1.15 | 0.15-8.93 | |
| **Sex** | | | | | | | **0.03** |
| Male | 271 | 8 | | | | | 0.03* |
| Female | 275 | 20 | 0.90 | 0.43 | 2.46 | 1.07-5.69 | |

Full univariable results for carriage of *E. coli* with resistance to nalidixic acid

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.1** |
| <1 | 52 | 4 | | | | | |
| 1 | 75 | 3 | -0.65 | 0.78 | 0.52 | 0.11-2.42 | |
| 2 | 33 | 0 | | | | | |
| 3 | 39 | 2 | -0.41 | 0.89 | 0.37 | 0.12-3.83 | |
| 4 | 325 | 36 | 0.36 | 0.55 | 1.44 | 0.49-4.21 | |
| **Breed** | | | | | | | **0.3** |
| Pedigree | 397 | 38 | | | | | 0.3* |
| Cross | 116 | 7 | -0.46 | 0.42 | 0.63 | 0.27-1.45 | |
| **Breed Group** | | | | | | | **0.04** |
| Working | 15 | 5 | | | | | |
| Gundog | 178 | 19 | -1.14 | 0.57 | 0.32 | 0.10-0.98 | |
| Hound | 31 | 3 | -1.24 | 0.80 | 0.29 | 0.06-1.38 | |
| Terrier | 55 | 1 | -2.91 | 1.13 | 0.05 | 0.01-0.50 | |
| Utility | 27 | 3 | -1.10 | 0.80 | 0.33 | 0.07-1.59 | |
| Pastoral | 38 | 4 | -1.15 | 0.74 | 0.32 | 0.07-1.34 | |
| Toy | 32 | 3 | -1.27 | 0.79 | 0.28 | 0.06-1.33 | |
| Cross | 116 | 7 | -1.71 | 0.65 | 0.18 | 0.05-0.64 | |
| Not specified | 37 | 0 | | | | | |
| **Breed Size** | | | | | | | **0.04** |
| Small | 69 | 4 | | | | | |
| Medium | 124 | 9 | 0.22 | 0.62 | 1.25 | 0.37-4.22 | |
| Large | 182 | 25 | 862681.00 | 0.56 | 2.37 | 0.80-7.6 | |
| Not specified | 154 | 7 | -0.24 | 0.64 | 0.78 | 0.22-2.77 | |
| **Neutered** | | | | | | | **0.3** |
| No | 139 | 15 | | | | | 0.3* |
| Yes | 388 | 30 | -0.33 | 0.33 | 0.72 | 0.37-1.37 | |
| **Working Dog** | | | | | | | **0.003** |
| No | 476 | 34 | | | | | 0.007* |
| Yes | 39 | 9 | 1.17 | 0.41 | 3.23 | 1.45-7.22 | |
| **Given dog treats** | | | | | | | **0.6** |
| Never | 29 | 4 | | | | | |
| Rarely | 84 | 9 | -0.25 | 0.64 | 0.78 | 0.22-2.71 | |
| Sometimes | 247 | 18 | -0.64 | 0.59 | 0.53 | 0.17-1.67 | |
| Often | 159 | 14 | -0.45 | 0.60 | 0.64 | 0.20-2.08 | |
| **Given titbits** | | | | | | | **0.1** |
| Never | 57 | 3 | | | | | |
| Rarely | 167 | 9 | 0.02 | 0.68 | 1.02 | 0.27-3.91 | |
| Sometimes | 223 | 22 | 0.63 | 0.63 | 1.87 | 0.54-6.48 | |
| Often | 69 | 11 | 1.01 | 0.68 | 2.75 | 0.72-10.49 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Given dog treats** | | | | | | | **0.3** |
| Never/ rarely | 113 | 13 | | | | | 0.3* |
| Sometimes/ often | 406 | 32 | -0.38 | 0.35 | 0.69 | 0.35-1.35 | |
| **Given titbits** | | | | | | | **0.04** |
| Never/ rarely | 224 | 12 | | | | | 0.04* |
| Sometimes/ often | 292 | 32 | 0.72 | 0.35 | 2.05 | 1.03-4.06 | |
| **Reason for visit** | | | | | | | **0.6** |
| Vaccination/ worming | 201 | 12 | | | | | |
| Presenting complaint | 238 | 27 | 0.64 | 0.36 | 1.90 | 0.94-3.85 | |
| Check up | 22 | 2 | 0.42 | 0.80 | 1.52 | 0.32-7.25 | |
| Work at clinic | 17 | 2 | 0.68 | 0.80 | 1.97 | 0.41-9.54 | |
| Vaccination/ presenting comp | 20 | 2 | 0.52 | 0.80 | 1.67 | 0.35-8.02 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 5 | 0 | | | | | |
| Other | 13 | 0 | | | | | |
| **Medication prescribed during most recent visit** | | | | | | | **0.02** |
| No | 338 | 21 | | | | | 0.02* |
| Yes | 183 | 24 | 0.75 | 0.31 | 2.11 | 1.14-3.90 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.3** |
| No | 423 | 33 | | | | | 0.3* |
| Yes | 96 | 11 | 0.38 | 0.37 | 1.47 | 0.72-3.01 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.643** |
| One off prescription | 6 | 0 | | | | | |
| Up to 5 days | 28 | 5 | Ref | | | | |
| Up to 10 days | 41 | 4 | -0.60 | 0.71 | 0.55 | 0.13-2.22 | |
| Up to 2 weeks | 11 | 2 | 0.02 | 0.91 | 1.02 | 0.17-6.05 | |
| Up to 3 weeks | 4 | 0 | | | | | |
| Over 3 weeks | 2 | 0 | | | | | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 423 | 33 | -0.83 | 0.52 | 0.44 | 0.16-1.21 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.2** |
| **No** | 369 | 27 | | | | | 0.2* |
| **Yes** | 160 | 18 | 0.43 | 0.32 | 1.54 | 0.82-2.87 | |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.3** |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| No | 292 | 22 | | | | | 0.4* |
| Yes | 225 | 23 | 0.31 | 0.31 | 1.36 | 0.74-2.50 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.1** |
| No | 326 | 23 | | | | | 0.1* |
| Yes | 186 | 22 | 0.52 | 0.31 | 1.66 | 0.91-3.09 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.1** |
| No | 403 | 32 | | | | | 0.1* |
| Yes | 94 | 13 | 0.55 | 0.35 | 1.74 | 0.88-3.44 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.02** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 29 | 3 | Ref | | | | |
| Up to 10 days | 30 | 6 | 0.66 | 0.75 | 1.93 | 0.44-8.47 | |
| Up to 2 weeks | 16 | 1 | -0.50 | 1.20 | 0.60 | 0.06-6.30 | |
| Up to 3 weeks | 5 | 0 | | | | | |
| Over 3 weeks | 4 | 3 | 1.98 | 0.98 | 7.25 | 1.07-49.03 | |
| Don’t know | 3 | 0 | | | | | |
| None prescribed | 402 | 32 | -0.26 | 0.63 | 0.77 | 0.22-2.66 | |
| **Left at veterinary premises** | | | | | | | **0.7** |
| No | 394 | 32 | | | | | 0.8* |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.5** |
| No | 109 | 11 | | | | | 0.6* |
| Yes | 409 | 33 | -0.22 | 0.36 | 0.80 | 0.39-1.63 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.9** |
| No | 393 | 33 | | | | | 0.9* |
| Yes | 124 | 11 | 0.05 | 0.36 | 1.06 | 0.52-2.15 | |
| **Allowed off lead during walks** | | | | | | | **0.7** |
| No | 106 | 8 | | | | | 0.8* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Yes | 413 | 37 | 0.17 | 0.40 | 1.19 | 0.54-2.62 | |
| **Other dogs in household** | | | | | | | **0.07** |
| No | 294 | 19 | | | | | 0.09* |
| Yes | 227 | 26 | 0.57 | 0.31 | 1.77 | 0.96-3.28 | |
| **Number of other dogs in household** | | | | | | | **0.001** |
| 0 | 294 | 19 | | | | | |
| 1 | 114 | 6 | -0.21 | 0.48 | 0.81 | 0.32-2.09 | |
| 2 | 43 | 8 | 1.06 | 0.45 | 2.88 | 1.19-6.98 | |
| 3 | 10 | 3 | 1.54 | 0.70 | 4.64 | 1.18-18.29 | |
| 4+ | 12 | 4 | 1.64 | 0.62 | 5.16 | 1.52-17.52 | |
| **Other animals in household** | | | | | | | **0.6** |
| No | 295 | 23 | | | | | 0.6* |
| Yes | 226 | 221 | 0.18 | 0.31 | 1.19 | 0.64-2.21 | |
| **Own a cat** | | | | | | | **0.1** |
| No | 388 | 28 | | | | | 0.2* |
| Yes | 133 | 16 | 0.51 | 0.33 | 1.67 | 0.87-3.18 | |
| **Own a bird** | | | | | | | **0.9** |
| No | 482 | 41 | | | | | 1.0* |
| Yes | 39 | 3 | -0.10 | 0.62 | 0.90 | 0.27-3.05 | |
| **Own a rabbit** | | | | | | | **0.6** |
| No | 487 | 42 | | | | | 1.0* |
| Yes | 34 | 2 | -0.38 | 0.75 | 0.68 | 0.16-2.94 | |
| **Own a rodent** | | | | | | | **0.05** |
| No | 48 | 44 | | | | | 0.06* |
| Yes | 41 | 0 | | | | | |
| **Own a reptile or amphibian** | | | | | | | **0.9** |
| No | 511 | 43 | | | | | 0.6* |
| Yes | 10 | 1 | 0.05 | 1.06 | 1.05 | 0.13-8.41 | |
| **Own a fish** | | | | | | | **0.8** |
| No | 506 | 43 | | | | | 1.0* |
| Yes | 15 | 1 | -0.24 | 1.04 | 0.78 | 0.10-6.08 | |
| **Own any other animal or livestock** | | | | | | | **0.4** |
| No | 469 | 38 | | | | | 0.4* |
| Yes | 52 | 6 | 0.35 | 0.46 | 1.42 | 0.57-3.53 | |
| **Where the dog usually sleeps** | | | | | | | **0.4** |
| Outside | 19 | 4 | | | | | |
| Downstairs | 273 | 21 | 1.01 | 0.59 | 0.37 | 0.11-1.17 | |
| Upstairs | 208 | 17 | -0.95 | 0.61 | 0.39 | 0.12-1.27 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Whole house | 19 | 3 | -0.29 | 0.83 | 0.75 | 0.15-3.81 | |
| Outside and downstairs | 2 | 0 | | | | | |
| **Anyone in the household work with farm animals** | | | | | | | **0.2** |
| No | 480 | 39 | | | | | 0.2* |
| Yes | 39 | 6 | 0.64 | 0.47 | 1.89 | 0.76-4.75 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.6** |
| No | 433 | 39 | | | | | 0.7* |
| Yes | 87 | 6 | -0.27 | 0.45 | 0.77 | 0.31-1.86 | 0 |
| **Who took antibiotics** | | | | | | | **0.5** |
| No | 45 | 5 | | | | | |
| Yes | 34 | 1 | -1.33 | 1.12 | 0.26 | 0.03-2.37 | |
| No one/ not specified | 432 | 39 | 0.21 | 0.50 | 0.81 | 0.30-2.17 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.8** |
| No | 369 | 31 | | | | | 0.9* |
| Yes | 154 | 14 | 0.08 | 0.34 | 1.08 | 0.56-2.09 | |
| **Healthcare setting** | | | | | | | **0.7** |
| Human | 47 | 6 | | | | | |
| Animal | 104 | 8 | -0.51 | 0.57 | 0.60 | 0.20-1.83 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 369 | 31 | -0.42 | 0.47 | 0.66 | 0.26-1.66 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.5** |
| No | 380 | 35 | | | | | 0.6* |
| Yes | 142 | 10 | -0.27 | 0.37 | 0.76 | 0.37-1.58 | |
| **Reason for hospital visit** | | | | | | | **0.9** |
| Admission | 22 | 2 | | | | | |
| Visit | 17 | 1 | -0.44 | 1.27 | 0.65 | 0.05-7.75 | |
| Outpatient/ A&E | 101 | 7 | -0.27 | 0.84 | 0.76 | 0.15-3.92 | |
| Other/ not specified | 2 | 0 | | | | | |
| No attendance | 380 | 35 | 0.01 | 0.76 | 1.01 | 0.23-4.49 | |
| **Source of dog** | | | | | | | **0.2** |
| Breeder | 257 | 22 | | | | | |
| Rescue Kennel/ stray | 115 | 11 | 0.11 | 0.39 | 1.12 | 0.52-2.38 | |
| Newspaper/ word of mouth/ internet | 42 | 2 | -0.59 | 0.76 | 0.56 | 0.13-2.45 | |
| Family/friend | 80 | 3 | -0.83 | 0.63 | 0.44 | 0.13-1.50 | |
| Pet shop | 4 | 1 | 1.02 | 1.14 | 2.92 | 0.31-27.27 | |
| Other | 14 | 2 | 0.51 | 0.79 | 1.67 | 0.36-782 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Self breed | 10 | 3 | 1.25 | 0.69 | 3.50 | 0.90-13.68 | |
| **Fed tinned or packet wet food** | | | | | | | **0.5** |
| Yes | 338 | 31 | | | | | 0.6* |
| No | 188 | 14 | -0.21 | 0.33 | 0.81 | 0.42-1.56 | |
| **Fed dry mixer** | | | | | | | **0.3** |
| No | 424 | 39 | | | | | 0.4* |
| Yes | 102 | 6 | -0.45 | 0.45 | 0.64 | 0.26-1.55 | |
| **Fed dry complete** | | | | | | | **0.1** |
| No | 121 | 15 | | | | | 0.1* |
| Yes | 405 | 30 | -0.51 | 0.33 | 0.6 | 0.31-1.15 | |
| **Fed raw poultry meat** | | | | | | | **<0.001** |
| No | 511 | 37 | | | | | <0.001* |
| Yes | 15 | 8 | 2.00 | 0.47 | 7.37 | 2.93-18.5 | |
| **Fed cooked poultry meat** | | | | | | | **0.7** |
| No | 419 | 37 | | | | | 0.8* |
| Yes | 107 | 8 | -0.17 | 0.40 | 0.85 | 0.38-1.87 | |
| **Fed raw red meat** | | | | | | | **0.3** |
| No | 512 | 45 | | | | | 0.6* |
| Yes | 14 | 0 | | | | | |
| **Fed cooked red meat** | | | | | | | **0.7** |
| No | 509 | 44 | | | | | 1.0* |
| Yes | 17 | 1 | -0.38 | 1.04 | 0.68 | 0.09-5.23 | 0.7 |
| **Sex** | | | | | | | **0.01** |
| Male | 265 | 14 | | | | | 0.02* |
| Female | 264 | 31 | 0.80 | 0.33 | 2.22 | 1.16-4.27 | |

Full univariable results for carriage of *E. coli* with resistance to tetracycline

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.9** |
| <1 | 39 | 17 | | | | | |
| 1 | 57 | 21 | -0.17 | 0.39 | 0.85 | 0.40-10.80 | |
| 2 | 24 | 0 | -0.15 | 0.49 | 0.86 | 0.33-2.23 | |
| 3 | 27 | 14 | 0.17 | 0.44 | 1.19 | 0.50-2.81 | |
| 4 | 253 | 108 | -0.02 | 0.31 | 0.98 | 0.53-1.81 | |
| **Breed** | | | | | | | **0.1** |
| Pedigree | 299 | 136 | | | | | 0.1* |
| Cross | 94 | 29 | -0.39 | 0.24 | 0.68 | 0.43-1.08 | |
| **Breed Group** | | | | | | | **0.3** |
| Working | 11 | 9 | | | | | |
| Gundog | 128 | 69 | -0.42 | 0.47 | 0.66 | 0.26-1.67 | |
| Hound | 23 | 11 | -0.54 | 0.58 | 0.58 | 0.19-1.82 | |
| Terrier | 43 | 13 | -1.00 | 0.55 | 0.37 | 0.13-1.09 | |
| Utility | 22 | 8 | -0.81 | 0.61 | 0.44 | 0.13-1.47 | |
| Pastoral | 31 | 11 | -0.84 | 0.57 | 0.43 | 0.14-1.33 | |
| Toy | 25 | 10 | -0.72 | 0.58 | 0.49 | 0.16-1.54 | |
| Cross | 94 | 29 | -0.98 | 0.50 | 0.38 | 0.14-1.00 | |
| Not specified | 28 | 9 | -0.93 | 0.59 | 0.39 | 0.12-1.25 | |
| **Breed Size** | | | | | | | **0.01** |
| Small | 53 | 20 | | | | | |
| Medium | 100 | 33 | -0.13 | 0.33 | 0.87 | 0.46-1.67 | |
| Large | 129 | 78 | 0.47 | 0.30 | 1.60 | 0.89-2.88 | |
| Not specified | 123 | 38 | -0.20 | 0.32 | 0.82 | 0.44-1.54 | |
| **Neutered** | | | | | | | **0.2** |
| No | 102 | 52 | | | | | 0.2* |
| Yes | 301 | 117 | -0.27 | 0.20 | 0.76 | 0.51-1.13 | |
| **Working Dog** | | | | | | | **0.004** |
| No | 366 | 144 | | | | | 0.008* |
| Yes | 25 | 23 | 0.85 | 0.31 | 2.34 | 1.29-4.25 | |
| **Given dog treats** | | | | | | | **0.2** |
| Never | 18 | 15 | | | | | |
| Rarely | 65 | 28 | -0.66 | 0.42 | 0.52 | 0.23-1.17 | |
| Sometimes | 186 | 79 | -0.67 | 0.37 | 0.51 | 0.24-1.06 | |
| Often | 127 | 46 | -0.83 | 0.39 | 0.43 | 0.20-0.93 | |
| **Given titbits** | | | | | | | **1.0** |
| Never | 43 | 17 | | | | | |
| Rarely | 124 | 52 | 0.06 | 0.33 | 1.06 | 0.55-2.03 | |
| Sometimes | 171 | 74 | 0.09 | 0.32 | 1.09 | 0.59-2.04 | |
| Often | 57 | 22 | -0.02 | 0.38 | 0.98 | 0.46-2.06 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Given dog treats** | | | | | | | **0.2** |
| Never/ rarely | 83 | 43 | | | | | 0.2* |
| Sometimes/ often | 313 | 125 | -0.26 | 0.22 | 0.77 | 0.51-1.18 | |
| **Given titbits** | | | | | | | **0.9** |
| Never/ rarely | 167 | 69 | | | | | 0.9* |
| Sometimes/ often | 228 | 96 | 0.02 | 0.19 | 1.02 | 0.71-1.47 | |
| **Reason for visit** | | | | | | | **0.2** |
| Vaccination/ worming | 160 | 53 | | | | | |
| Presenting complaint | 182 | 83 | 0.32 | 0.21 | 1.38 | 0.92-2.06 | |
| Check up | 16 | 8 | 0.41 | 0.46 | 1.51 | 0.61-3.73 | |
| Work at clinic | 11 | 8 | 0.79 | 0.49 | 2.20 | 0.84-5.75 | |
| Vaccination/ presenting comp | 11 | 11 | 1.10 | 0.45 | 3.02 | 1.24-7.36 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 4 | 1 | -0.28 | 1.13 | 0.75 | 0.08-6.90 | |
| Other | 8 | 5 | 0.63 | 0.59 | 1.89 | 0.59-6.02 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.3** |
| No | 257 | 102 | | | | | 0.3* |
| Yes | 140 | 67 | 0.19 | 0.19 | 1.21 | 0.83-1.75 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.2** |
| No | 326 | 130 | | | | | 0.2* |
| Yes | 70 | 37 | 0.28 | 0.23 | 1.33 | 0.85-2.07 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.7** |
| One off prescription | 5 | 1 | | | | | |
| Up to 5 days | 20 | 13 | 1.18 | 1.15 | 3.25 | 0.34-31.08 | |
| Up to 10 days | 30 | 15 | 0.92 | 1.14 | 2.50 | 0.27-23.36 | |
| Up to 2 weeks | 8 | 5 | 1.14 | 1.23 | 3.12 | 0.28-35.16 | |
| Up to 3 weeks | 2 | 2 | 1.61 | 1.48 | 5.00 | 0.27-91.52 | |
| Over 3 weeks | 2 | 0 | | | | | |
| Don’t know | 3 | 1 | 0.51 | 1.59 | 1.67 | 0.07-37.73 | |
| None prescribed | 326 | 130 | 0.69 | 1.10 | 1.99 | 0.23-17.23 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.02** |
| No | 291 | 105 | | | | | **0.02*** |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Yes | 114 | 64 | 0.44 | 0.19 | 1.56 | 1.07-2.27 | |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.1** |
| No | 230 | 84 | | | | | 0.1* |
| Yes | 166 | 82 | 0.30 | 0.19 | 1.35 | 0.94-1.95 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.06** |
| No | 256 | 93 | | | | | 0.07* |
| Yes | 137 | 71 | 0.36 | 0.19 | 1.43 | 0.98-2.07 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.07** |
| No | 314 | 120 | | | | | 0.08* |
| Yes | 68 | 39 | 0.41 | 0.23 | 1.50 | 0.96-2.35 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.09** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 18 | 14 | | | | | |
| Up to 10 days | 22 | 14 | -0.20 | 0.49 | 0.82 | 0.31-2.15 | |
| Up to 2 weeks | 9 | 8 | 0.13 | 0.60 | 1.14 | 0.35-3.72 | |
| Up to 3 weeks | 3 | 2 | -0.15 | 0.98 | 0.86 | 0.13-5.85 | |
| Over 3 weeks | 4 | 3 | -0.04 | 0.84 | 0.96 | 0.18-5.03 | |
| Don’t know | 2 | 0 | | | | | |
| None prescribed | 314 | 120 | -0.71 | 0.37 | 0.49 | 0.24-1.02 | |
| **Left at veterinary premises** | | | | | | | **0.3** |
| No | 304 | 122 | | | | | 0.4* |
| Yes | 29 | 16 | 0.32 | 0.33 | 1.37 | 0.72-2.62 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.8** |
| No | 84 | 36 | | | | | 0.8* |
| Yes | 315 | 127 | -0.06 | 0.23 | 0.94 | 0.60-1.46 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.003** |
| No | 315 | 111 | | | | | 0.005* |
| Yes | 82 | 53 | 0.61 | 0.21 | 1.83 | 1.22-2.76 | |
| **Allowed off lead during walks** | | | | | | | **0.09** |
| No | 88 | 26 | | | | | 0.1* |
| Yes | 311 | 139 | 0.41 | 0.25 | 1.51 | 0.94-2.45 | |
| **Other dogs in household** | | | | | | | **0.02** |
| No | 232 | 81 | | | | | 0.03* |
| Yes | 165 | 88 | 0.42 | 0.18 | 1.53 | 1.06-2.19 | |
| **Number of other dogs in household** | | | | | | | **0.02** |
| 0 | 232 | 81 | | | | | |
| 1 | 82 | 38 | 0.28 | 0.23 | 1.33 | 0.84-2.10 | |
| 2 | 29 | 22 | 0.78 | 0.31 | 2.17 | 1.18-4.00 | |
| 3 | 5 | 8 | 1.52 | 0.58 | 4.58 | 1.46-14.41 | |
| 4+ | 11 | 5 | 0.26 | 0.55 | 1.30 | 0.44-3.86 | |
| **Other animals in household** | | | | | | | **0.5** |
| No | 227 | 91 | | | | | 0.5* |
| Yes | 170 | 77 | 0.12 | 0.19 | 1.13 | 0.79-1.62 | |
| **Own a cat** | | | | | | | **0.6** |
| No | 295 | 121 | | | | | 0.6* |
| Yes | 102 | 47 | 0.12 | 0.21 | 1.12 | 0.75-1.68 | |
| **Own a bird** | | | | | | | **0.6** |
| No | 366 | 157 | | | | | 0.7* |
| Yes | 31 | 11 | -0.19 | 0.36 | 0.83 | 0.41-1.69 | |
| **Own a rabbit** | | | | | | | **0.3** |
| No | 369 | 160 | | | | | 0.4* |
| Yes | 28 | 8 | -0.42 | 0.41 | 0.66 | 0.29-1.48 | |
| **Own a rodent** | | | | | | | **0.3** |
| No | 365 | 159 | | | | | 0.3* |
| Yes | 32 | 9 | -0.44 | 0.39 | 0.65 | 0.30-1.38 | |
| **Own a reptile or amphibian** | | | | | | | **0.2** |
| No | 391 | 163 | | | | | 0.3* |
| Yes | 6 | 5 | 0.69 | 0.61 | 2.00 | 0.60-6.64 | |
| **Own a fish** | | | | | | | **0.04** |
| No | 382 | 167 | | | | | 0.05* |
| Yes | 15 | 1 | -1.88 | 1.04 | 0.15 | 0.02-1.16 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Own any other animal or livestock** | | | | | | | **0.04** |
| No | 363 | 144 | | | | | 0.05* |
| Yes | 34 | 24 | 0.58 | 0.28 | 1.78 | 1.02-3.11 | |
| **Where the dog usually sleeps** | | | | | | | **0.4** |
| Outside | 13 | 10 | | | | | |
| Downstairs | 202 | 92 | -0.52 | 0.44 | 0.59 | 0.25-1.40 | |
| Upstairs | 166 | 59 | -0.77 | 0.45 | 0.46 | 0.19-1.11 | |
| Whole house | 15 | 7 | -0.50 | 0.62 | 0.61 | 0.18-2.05 | |
| Outside and downstairs | 1 | 1 | 0.26 | 1.48 | 1.30 | 0.07-23.43 | |
| **Anyone in the household work with farm animals** | | | | | | | **0.1** |
| No | 368 | 151 | | | | | 0.1* |
| Yes | 27 | 18 | 0.49 | 0.32 | 1.62 | 0.87-3.0 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.6** |
| No | 334 | 138 | | | | | 0.6* |
| Yes | 63 | 30 | 0.14 | 0.24 | 1.15 | 0.71-1.86 | |
| **Who took antibiotics** | | | | | | | **0.7** |
| No | 33 | 17 | | | | | |
| Yes | 23 | 12 | 0.01 | 0.46 | 1.01 | 0.41-2.52 | |
| No one/ not specified | 333 | 138 | -0.22 | 0.32 | 0.80 | 0.43-1.49 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.3** |
| No | 276 | 124 | | | | | 0.4* |
| Yes | 123 | 45 | -0.21 | 0.21 | 0.81 | 0.54-1.22 | |
| **Healthcare setting** | | | | | | | **0.7** |
| Human | 39 | 14 | | | | | |
| Animal | 81 | 31 | 0.06 | 0.38 | 1.07 | 0.51-2.23 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 276 | 124 | 0.22 | 0.33 | 1.25 | 0.66-2.39 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.2** |
| No | 285 | 130 | | | | | 0.2* |
| Yes | 113 | 39 | -0.28 | 0.21 | 0.76 | 0.50-1.15 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Reason for hospital visit** | | | | | | | **0.6** |
| Admission | 17 | 7 | | | | | |
| Visit | 14 | 4 | -0.37 | 0.72 | 0.69 | 0.17-2.86 | |
| Outpatient/ A&E | 81 | 27 | -0.21 | 0.50 | 0.81 | 0.30-2.16 | |
| Other/ not specified | 1 | 1 | 0.88 | 1.48 | 2.43 | 0.13-44.50 | |
| No attendance | 285 | 130 | 0.10 | 0.46 | 1.11 | 0.45-2.74 | |
| **Source of dog** | | | | | | | **0.3** |
| Breeder | 189 | 90 | | | | | |
| Rescue Kennel/ stray | 100 | 26 | -0.61 | 0.25 | 0.55 | 0.33-0.92 | |
| Newspaper/ word of mouth/ internet | 31 | 13 | -0.13 | 0.35 | 0.88 | 0.44-1.76 | |
| Family/friend | 59 | 24 | -0.16 | 0.27 | 0.85 | 0.50-1.46 | |
| Pet shop | 4 | 1 | -0.64 | 1.13 | 0.53 | 0.06-4.77 | |
| Other | 10 | 6 | 0.23 | 0.53 | 1.26 | 0.44-3.57 | |
| Self breed | 8 | 5 | 0.27 | 0.58 | 1.31 | 0.42-4.13 | |
| **Fed tinned or packet wet food** | | | | | | | **0.4** |
| Yes | 264 | 105 | | | | | 0.4* |
| No | 138 | 64 | 0.15 | 0.19 | 1.17 | 0.80-1.69 | |
| **Fed dry mixer** | | | | | | | **0.4** |
| No | 322 | 141 | | | | | 0.4* |
| Yes | 80 | 28 | -0.22 | 0.24 | 0.80 | 0.50-1.28 | |
| **Fed dry complete** | | | | | | | **0.1** |
| No | 89 | 47 | | | | | 0.2* |
| Yes | 313 | 122 | -0.30 | 0.30 | 0.74 | 0.49-1.11 | |
| **Fed raw poultry meat** | | | | | | | **0.004** |
| No | 392 | 156 | | | | | 0.008* |
| Yes | 10 | 13 | 1.18 | 0.43 | 3.27 | 1.40-7.61 | |
| **Fed cooked poultry meat** | | | | | | | **0.6** |
| No | 319 | 137 | | | | | 0.7* |
| Yes | 83 | 32 | -0.11 | 0.23 | 0.90 | 0.57-1.41 | |
| **Fed raw red meat** | | | | | | | **0.3** |
| No | 394 | 163 | | | | | 0.4 |
| Yes | 8 | 6 | 0.59 | 0.58 | 1.81 | 0.62-5.31 | |
| **Fed cooked red meat** | | | | | | | **0.9** |
| No | 389 | 164 | | | | | 1.0* |
| Yes | 13 | 5 | -0.09 | 0.53 | 0.91 | 0.32-2.60 | |
| **Sex** | | | | | | | **0.6** |
| Male | 200 | 79 | | | | | 0.6* |
| Female | 205 | 90 | 0.11 | 0.18 | 1.11 | 0.78-1.59 | |

Full univariable results for carriage of *E. coli* with resistance to trimethoprim

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.8** |
| <1 | 43 | 13 | | | | | |
| 1 | 61 | 17 | -0.08 | 0.42 | 0.92 | 0.41-2.09 | |
| 2 | 28 | 5 | -0.53 | 0.58 | 0.59 | 0.19-1.84 | |
| 3 | 30 | 11 | 0.19 | 0.47 | 1.21 | 0.48-3.07 | |
| 4 | 273 | 88 | 0.06 | 0.34 | 1.07 | 0.55-2.07 | |
| **Breed** | | | | | | | **0.7** |
| Pedigree | 335 | 100 | | | | | 0.8* |
| Cross | 93 | 30 | 0.08 | 0.24 | 1.08 | 0.68-1.73 | |
| **Breed Group** | | | | | | | **0.5** |
| Working | 13 | 7 | | | | | |
| Gundog | 144 | 53 | -0.38 | 0.50 | 0.68 | 0.26-1.81 | |
| Hound | 27 | 7 | -0.73 | 0.63 | 0.48 | 0.14-1.66 | |
| Terrier | 49 | 7 | -1.33 | 0.62 | 0.27 | 0.08-0.89 | |
| Utility | 24 | 6 | -0.77 | 0.65 | 0.46 | 0.13-1.67 | |
| Pastoral | 33 | 9 | -0.68 | 0.60 | 0.51 | 0.16-1.64 | |
| Toy | 28 | 7 | 0.77 | 0.63 | 0.46 | 0.13-1.60 | |
| Cross | 93 | 30 | -0.51 | 0.51 | 0.60 | 0.22-1.64 | |
| Not specified | 29 | 8 | -0.67 | 0.62 | 0.51 | 0.15-1.71 | |
| **Breed Size** | | | | | | | **0.09** |
| Small | 60 | 13 | | | | | |
| Medium | 109 | 24 | 0.02 | 0.38 | 1.02 | 0.48-2.14 | |
| Large | 148 | 59 | 0.61 | 0.34 | 1.84 | 0.94-3.60 | |
| Not specified | 123 | 38 | 0.35 | 0.36 | 1.43 | 0.71-2.85 | |
| **Neutered** | | | | | | | **0.5** |
| No | 115 | 39 | | | | | 0.5* |
| Yes | 323 | 95 | -0.14 | 0.22 | 0.87 | 0.56-1.33 | |
| **Working Dog** | | | | | | | **0.2** |
| No | 396 | 114 | | | | | 0.2* |
| Yes | 33 | 15 | 0.46 | 0.33 | 1.58 | 0.83-3.01 | |
| **Given dog treats** | | | | | | | **0.2** |
| Never | 21 | 12 | | | | | |
| Rarely | 68 | 25 | -0.44 | 0.43 | 0.64 | 0.28-1.50 | |
| Sometimes | 203 | 62 | -0.63 | 0.39 | 0.53 | 0.25-1.15 | |
| Often | 139 | 34 | -0.85 | 0.41 | 0.43 | 0.19-0.95 | |
| **Given titbits** | | | | | | | **1.0** |
| Never | 47 | 13 | | | | | |
| Rarely | 135 | 41 | 0.09 | 0.36 | 1.10 | 0.54-2.23 | |
| Sometimes | 186 | 59 | 0.14 | 0.35 | 1.15 | 0.58-2.26 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Often | 59 | 20 | 0.20 | 0.41 | 1.23 | 0.55-2.72 | |
| **Given dog treats** | | | | | | | **0.08** |
| Never/ rarely | 89 | 37 | | | | | 0.1* |
| Sometimes/ often | 342 | 96 | -0.39 | 0.23 | 0.68 | 0.43-1.05 | |
| **Given titbits** | | | | | | | **0.7** |
| Never/ rarely | 182 | 54 | | | | | 0.7* |
| Sometimes/ often | 245 | 79 | 0.08 | 0.20 | 1.09 | 0.73-1.61 | |
| **Reason for visit** | | | | | | | **0.2** |
| Vaccination/ worming | 175 | 38 | | | | | |
| Presenting complaint | 196 | 69 | 0.48 | 0.23 | 1.62 | 1.04-2.53 | |
| Check up | 18 | 6 | 0.43 | 0.50 | 1.54 | 0.57-4.12 | |
| Work at clinic | 14 | 5 | 0.50 | 0.55 | 1.64 | 0.56-4.84 | |
| Vaccination/ presenting comp | 13 | 9 | 1.16 | 0.47 | 3.19 | 1.27-8.00 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 3 | 2 | 1.12 | 0.93 | 3.07 | 0.50-19.01 | |
| Other | 9 | 4 | 0.72 | 0.63 | 2.05 | 0.60-7.00 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.3** |
| No | 279 | 80 | | | | | 0.3* |
| Yes | 153 | 54 | 0.21 | 0.20 | 1.23 | 0.83-1.83 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.5** |
| No | 351 | 105 | | | | | 0.5* |
| Yes | 79 | 28 | 0.17 | 0.25 | 1.18 | 0.73-1.92 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.6** |
| One off prescription | 5 | 1 | | | | | |
| Up to 5 days | 25 | 8 | 0.47 | 1.17 | 1.60 | 0.16-15.80 | |
| Up to 10 days | 33 | 12 | 0.60 | 1.15 | 1.82 | 0.19-17.19 | |
| Up to 2 weeks | 8 | 5 | 1.14 | 1.23 | 3.12 | 0.28-35.16 | |
| Up to 3 weeks | 2 | 2 | 1.61 | 1.48 | 5.00 | 0.27-91.52 | |
| Over 3 weeks | 2 | 0 | | | | | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 351 | 105 | 0.40 | 1.10 | 1.50 | 0.17-12.94 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.2** |
| **No** | 310 | 86 | | | | | 0.2* |
| **Yes** | 130 | 48 | 0.29 | 0.21 | 1.33 | 0.88-2.00 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.1** |
| No | 249 | 65 | | | | | 0.1* |
| Yes | 183 | 65 | 0.31 | 0.20 | 1.36 | 0.92-2.02 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.4** |
| No | 272 | 77 | | | | | 0.5* |
| Yes | 156 | 52 | 0.16 | 0.21 | 1.18 | 0.79-1.76 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.3** |
| No | 336 | 98 | | | | | 0.4* |
| Yes | 78 | 29 | 0.24 | 0.25 | 1.27 | 0.79-2.06 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.3** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 24 | 8 | Ref | | | | |
| Up to 10 days | 23 | 13 | 0.53 | 0.54 | 1.70 | 0.59-4.85 | |
| Up to 2 weeks | 12 | 5 | 0.22 | 0.67 | 1.25 | 0.34-4.65 | |
| Up to 3 weeks | 4 | 1 | -0.29 | 1.19 | 0.75 | 0.07-7.73 | |
| Over 3 weeks | 4 | 3 | 0.81 | 0.87 | 2.25 | 0.41-12.28 | |
| Don’t know | 2 | 0 | | | | | |
| None prescribed | 336 | 98 | -0.13 | 0.42 | 0.88 | 0.38-2.01 | |
| **Left at veterinary premises** | | | | | | | **0.7** |
| No | 329 | 97 | | | | | 0.9* |
| Yes | 36 | 9 | -0.16 | 0.39 | 0.85 | 0.39-1.82 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.6** |
| No | 94 | 26 | | | | | 0.6* |
| Yes | 336 | 106 | 0.13 | 0.25 | 1.14 | 0.70-1.85 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.05** |
| No | 335 | 91 | | | | | 0.06* |
| Yes | 95 | 40 | 0.44 | 0.22 | 1.55 | 1.00-2.40 | |
| **Allowed off lead during walks** | | | | | | | **0.2** |
| No | 93 | 21 | | | | | 0.2* |
| Yes | 339 | 111 | 0.37 | 0.27 | 1.45 | 0.86-2.44 | |
| **Other dogs in household** | | | | | | | **0.4** |
| No | 243 | 70 | | | | | 0.4* |
| Yes | 189 | 64 | 0.16 | 0.20 | 1.18 | 0.80-1.73 | |
| **Number of other dogs in household** | | | | | | | **0.4** |
| 0 | 243 | 70 | | | | | |
| 1 | 93 | 27 | 0.01 | 0.26 | 1.01 | 0.61-1.67 | |
| 2 | 39 | 12 | 0.07 | 0.36 | 1.07 | 0.53-2.15 | |
| 3 | 7 | 6 | 1.09 | 0.57 | 2.98 | 0.97-9.14 | |
| 4+ | 11 | 5 | 0.46 | 0.56 | 1.58 | 0.53-4.69 | |
| **Other animals in household** | | | | | | | **0.06** |
| No | 253 | 65 | | | | | 0.07* |
| Yes | 180 | 67 | 0.37 | 0.20 | 1.45 | 0.98-2.14 | |
| **Own a cat** | | | | | | | **0.1** |
| No | 326 | 90 | | | | | 0.1* |
| Yes | 107 | 42 | 0.35 | 0.22 | 1.42 | 0.93-2.18 | |
| **Own a bird** | | | | | | | **0.4** |
| No | 403 | 120 | | | | | 0.4* |
| Yes | 30 | 12 | 0.30 | 0.36 | 1.34 | 0.67-2.70 | |
| **Own a rabbit** | | | | | | | **0.3** |
| No | 408 | 121 | | | | | 0.3* |
| Yes | 25 | 11 | 0.39 | 0.38 | 1.48 | 0.71-3.10 | |
| **Own a rodent** | | | | | | | **0.5** |
| No | 400 | 124 | | | | | 0.7* |
| Yes | 33 | 8 | -0.25 | 0.41 | 0.78 | 0.35-1.74 | |
| **Own a reptile or amphibian** | | | | | | | **0.8** |
| No | 425 | 129 | | | | | 0.7* |
| Yes | 8 | 3 | 0.21 | 0.68 | 1.24 | 0.32-4.73 | |
| **Own a fish** | | | | | | | **0.3** |
| No | 419 | 130 | | | | | 0.4* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Yes | 14 | 2 | -0.78 | 0.76 | 0.46 | 0.10-2.05 | |
| **Own any other animal or livestock** | | | | | | | **0.3** |
| No | 392 | 115 | | | | | 0.3* |
| Yes | 41 | 17 | 0.35 | 0.31 | 1.41 | 0.77-2.58 | |
| **Where the dog usually sleeps** | | | | | | | **0.7** |
| Outside | 16 | 7 | | | | | |
| Downstairs | 222 | 72 | -0.30 | 0.47 | 0.74 | 0.29-1.87 | |
| Upstairs | 177 | 48 | -0.48 | 0.48 | 0.62 | 0.24-1.59 | |
| Whole house | 16 | 6 | -0.15 | 0.66 | 0.86 | 0.24-3.12 | |
| Outside and downstairs | 1 | 1 | 0.83 | 1.49 | 2.29 | 0.12-41.99 | |
| **Anyone in the household work with farm animals** | | | | | | | **0.4** |
| No | 398 | 121 | | | | | 0.5* |
| Yes | 32 | 13 | 0.29 | 0.34 | 1.34 | 0.68-2.63 | |
| **Anyone in the household taken antibiotics** | | | | | | | **1.0** |
| No | 361 | 111 | | | | | 1.0* |
| Yes | 71 | 22 | 0.01 | 0.27 | 1.01 | 0.60-1.70 | |
| **Who took antibiotics** | | | | | | | **0.9** |
| No | 38 | 12 | | | | | |
| Yes | 26 | 9 | 0.09 | 0.51 | 1.10 | 0.40-2.97 | |
| No one/ not specified | 360 | 111 | -0.24 | 0.35 | 0.98 | 0.49-1.93 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.6** |
| No | 303 | 97 | | | | | 0.6* |
| Yes | 131 | 37 | -0.13 | 0.22 | 0.88 | 0.57-1.36 | |
| **Healthcare setting** | | | | | | | **0.9** |
| Human | 40 | 13 | | | | | |
| Animal | 88 | 24 | -0.18 | 0.39 | 0.84 | 0.39-1.82 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 303 | 97 | -0.02 | 0.34 | 0.99 | 0.51-1.92 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.7** |
| No | 315 | 100 | | | | | 0.7* |
| Yes | 118 | 34 | -0.10 | 0.23 | 0.91 | 0.58-1.41 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Reason for hospital visit** | | | | | | | **0.8** |
| Admission | 17 | 7 | | | | | |
| Visit | 14 | 4 | -0.37 | 0.72 | 0.69 | 0.17-2.86 | |
| Outpatient/ A&E | 85 | 23 | -0.42 | 0.51 | 0.66 | 0.24-1.77 | |
| Other/ not specified | 2 | 0 | | | | | |
| No attendance | 315 | 100 | -0.26 | 0.46 | 0.77 | 0.31-1.91 | |
| **Source of dog** | | | | | | | **0.3** |
| Breeder | 211 | 68 | | | | | |
| Rescue Kennel/ stray | 100 | 26 | -0.21 | 0.26 | 0.81 | 0.48-1.34 | |
| Newspaper/ word of mouth/ internet | 36 | 8 | -0.37 | 0.41 | 0.69 | 0.31-1.56 | |
| Family/friend | 67 | 16 | -0.30 | 0.31 | 0.74 | 0.40-1.36 | |
| Pet shop | 4 | 1 | -0.25 | 1.13 | 0.78 | 0.09-7.06 | |
| Other | 9 | 7 | 0.88 | 0.52 | 2.41 | 0.87-6.73 | |
| Self breed | 8 | 5 | 0.66 | 0.59 | 1.94 | 0.61-6.13 | |
| **Fed tinned or packet wet food** | | | | | | | **0.5** |
| Yes | 286 | 83 | | | | | 0.5* |
| No | 151 | 51 | 0.15 | 0.20 | 1.16 | 0.78-1.74 | |
| **Fed dry mixer** | | | | | | | **0.3** |
| No | 350 | 113 | | | | | 0.3* |
| Yes | 87 | 21 | -0.29 | 0.27 | 0.75 | 0.44-1.26 | |
| **Fed dry complete** | | | | | | | **0.6** |
| No | 102 | 34 | | | | | 0.6* |
| Yes | 335 | 100 | -0.11 | 0.23 | 0.90 | 0.57-1.40 | |
| **Fed raw poultry meat** | | | | | | | **0.005** |
| No | 425 | 123 | | | | | 0.01* |
| Yes | 12 | 11 | 1.15 | 0.43 | 3.17 | 1.36-7.35 | |
| **Fed cooked poultry meat** | | | | | | | **0.3** |
| No | 345 | 111 | | | | | 0.4* |
| Yes | 92 | 23 | -0.25 | 0.26 | 0.78 | 0.47-1.29 | |
| **Fed raw red meat** | | | | | | | **0.6** |
| No | 427 | 130 | | | | | 0.7* |
| Yes | 10 | 4 | 0.27 | 0.60 | 1.31 | 0.41-4.26 | |
| **Fed cooked red meat** | | | | | | | **0.5** |
| No | 422 | 131 | | | | | 0.8* |
| Yes | 15 | 3 | -0.44 | 0.64 | 0.64 | 0.18-2.26 | |
| **Sex** | | | | | | | **0.2** |
| Male | 221 | 58 | | | | | 0.2* |
| Female | 219 | 76 | 0.28 | 0.20 | 1.32 | 0.90-1.95 | |

Full univariable results for carriage of ESBL (TEM or CTX-M) producing *E. coli*

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.2** |
| <1 | 53 | 3 | | | | | |
| 1 | 77 | 1 | -1.47 | 1.14 | 0.23 | 0.02-2.27 | |
| 2 | 33 | 0 | | | | | |
| 3 | 41 | 0 | | | | | |
| 4 | 341 | 20 | 0.04 | 0.64 | 1.04 | 0.30-3.61 | |
| **Breed** | | | | | | | **0.2** |
| Pedigree | 414 | 21 | | | | | 0.3* |
| Cross | 120 | 3 | -0.71 | 0.63 | 0.49 | 0.14-1.68 | |
| **Breed Group** | | | | | | | **0.6** |
| Working | 18 | 2 | | | | | |
| Gundog | 186 | 11 | -0.63 | 0.81 | 0.53 | 0.11-2.59 | |
| Hound | 33 | 1 | -1.30 | 1.26 | 0.27 | 0.02-3.22 | |
| Terrier | 54 | 2 | -1.10 | 1.04 | 0.33 | 0.04-2.54 | |
| Utility | 28 | 2 | -0.44 | 1.04 | 0.64 | 0.08-4.98 | |
| Pastoral | 41 | 1 | -1.52 | 1.26 | 0.22 | 0.02-2.58 | |
| Toy | 33 | 2 | -0.61 | 1.04 | 0.55 | 0.07-4.20 | |
| Cross | 120 | 3 | -1.49 | 0.95 | 0.23 | 0.04-1.44 | |
| Not specified | 37 | 0 | | | | | |
| **Breed Size** | | | | | | | **0.2** |
| Small | 71 | 2 | | | | | |
| Medium | 127 | 6 | 0.52 | 0.83 | 1.68 | 0.33-8.53 | |
| Large | 194 | 13 | 0.87 | 0.77 | 2.38 | 0.52-10.80 | |
| Not specified | 158 | 3 | -0.39 | 0.92 | 0.67 | 0.11-4.12 | |
| **Neutered** | | | | | | | **0.2** |
| No | 150 | 4 | | | | | 0.3* |
| Yes | 398 | 20 | 0.63 | 0.56 | 1.88 | 0.63-5.60 | |
| **Working Dog** | | | | | | | **1.0** |
| No | 489 | 21 | | | | | 1.0* |
| Yes | 46 | 2 | 0.01 | 0.76 | 1.01 | 0.23-4.45 | |
| **Given dog treats** | | | | | | | **0.3** |
| Never | 30 | 3 | | | | | |
| Rarely | 87 | 6 | -0.37 | 0.74 | 0.69 | 0.16-2.93 | |
| Sometimes | 255 | 10 | -0.94 | 0.69 | 0.39 | 0.10-1.50 | |
| Often | 168 | 5 | -1.21 | 0.76 | 0.30 | 0.07-1.31 | |
| **Given titbits** | | | | | | | **0.5** |
| Never | 56 | 4 | | | | | |
| Rarely | 167 | 9 | -0.28 | 0.62 | 0.75 | 0.22-2.55 | |
| Sometimes | 238 | 7 | -0.89 | 0.64 | 0.41 | 0.12-1.46 | |
| Often | 75 | 4 | -0.29 | 0.73 | 0.75 | 0.18-3.12 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Given dog treats** | | | | | | | **0.07** |
| Never/ rarely | 117 | 9 | | | | | 0.08* |
| Sometimes/ often | 423 | 15 | -0.77 | 0.43 | 0.46 | 0.20-1.08 | |
| **Given titbits** | | | | | | | **0.2** |
| Never/ rarely | 223 | 13 | | | | | 0.3* |
| Sometimes/ often | 313 | 11 | -0.51 | 0.42 | 0.60 | 0.27-1.37 | |
| **Reason for visit** | | | | | | | **0.2** |
| Vaccination/ worming | 208 | 5 | | | | | |
| Presenting complaint | 250 | 15 | 0.91 | 0.52 | 2.50 | 0.89-6.98 | |
| Check up | 24 | 0 | | | | | |
| Work at clinic | 17 | 2 | 1.59 | 0.87 | 4.89 | 0.88-27.13 | |
| Vaccination/ presenting comp | 22 | 0 | | | | | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 4 | 1 | 2.34 | 1.21 | 10.40 | 0.98-110.59 | |
| Other | 12 | 1 | 1.24 | 1.13 | 3.47 | 0.37-32.06 | |
| **Medication prescribed during most recent visit** | | | | | | | **0.3** |
| No | 346 | 13 | | | | | 0.4* |
| Yes | 196 | 11 | 0.40 | 0.42 | 1.49 | 0.66-3.40 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **0.07** |
| No | 440 | 16 | | | | | 0.1* |
| Yes | 99 | 8 | 0.80 | 0.45 | 2.22 | 0.93-5.34 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **0.2** |
| One off prescription | 6 | 0 | | | | | |
| Up to 5 days | 32 | 1 | REF | | | | |
| Up to 10 days | 40 | 5 | 1.39 | 1.12 | 4.00 | 0.44-35.98 | |
| Up to 2 weeks | 11 | 2 | 1.76 | 1.27 | 5.82 | 0.48-70.62 | |
| Up to 3 weeks | 4 | 0 | | | | | |
| Over 3 weeks | 2 | 0 | | | | | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 440 | 16 | 0.15 | 1.05 | 1.16 | 0.15-9.06 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **0.01** |
| **No** | 385 | 11 | | | | | 0.02* |
| **Yes** | 165 | 13 | 1.01 | 0.42 | 2.76 | 1.21-6.28 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.06** |
| No | 306 | 8 | | | | | 0.08* |
| Yes | 234 | 14 | 0.83 | 0.45 | 2.29 | 0.94-5.55 | |
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.06** |
| No | 340 | 9 | | | | | 0.07* |
| Yes | 196 | 12 | 0.84 | 0.45 | 2.31 | 0.96-5.59 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.03** |
| No | 421 | 13 | | | | | 0.05* |
| Yes | 99 | 8 | 0.96 | 0.46 | 2.62 | 1.06-6.48 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **0.1** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 2 | 4 | Ref | | | | |
| Up to 10 days | 34 | 2 | -0.89 | 0.90 | 0.41 | 0.07-2.42 | |
| Up to 2 weeks | 15 | 2 | -0.69 | 0.92 | 0.93 | 0.15-5.70 | |
| Up to 3 weeks | 5 | 0 | | | | | |
| Over 3 weeks | 7 | 0 | | | | | |
| Don’t know | 2 | 0 | | | | | |
| None prescribed | 421 | 13 | -1.53 | 0.60 | 0.21 | 0.07-0.71 | |
| **Left at veterinary premises** | | | | | | | **0.8** |
| No | 410 | 16 | | | | | 0.7* |
| Yes | 43 | 2 | 0.18 | 0.77 | 1.19 | 0.27-5.36 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.1** |
| No | 112 | 8 | | | | | 0.2* |
| Yes | 426 | 16 | -0.64 | 0.45 | 0.53 | 0.22-1.26 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.7** |
| No | 407 | 19 | | | | | 0.8* |
| Yes | 130 | 5 | -0.19 | 0.51 | 0.82 | 0.30-2.25 | |
| **Allowed off lead during walks** | | | | | | | **0.2** |
| No | 107 | 7 | | | | | 0.3* |
| Yes | 434 | 16 | -0.57 | 0.47 | 0.56 | 0.23-1.40 | |
| **Other dogs in household** | | | | | | | **0.6** |
| No | 301 | 12 | | | | | 0.7* |
| Yes | 241 | 12 | 0.22 | 0.42 | 1.25 | 0.55-2.83 | |
| **Number of other dogs in household** | | | | | | | **0.005** |
| 0 | 301 | 12 | | | | | |
| 1 | 117 | 3 | -0.44 | 0.65 | 0.60 | 0.18-2.32 | |
| 2 | 49 | 2 | 0.02 | 0.78 | 1.02 | 0.22-4.71 | |
| 3 | 10 | 3 | 2.02 | 0.72 | 7.52 | 1.83-30.93 | |
| 4+ | 14 | 2 | 1.28 | 0.81 | 3.58 | 0.73-17.57 | |
| **Other animals in household** | | | | | | | **0.4** |
| No | 307 | 11 | | | | | 0.5* |
| Yes | 235 | 12 | 0.35 | 0.43 | 1.43 | 0.62-3.29 | |
| **Own a cat** | | | | | | | **0.02** |
| No | 404 | 12 | | | | | 0.03* |
| Yes | 138 | 11 | 0.99 | 0.43 | 2.68 | 1.16-6.22 | |
| **Own a bird** | | | | | | | **0.8** |
| No | 502 | 21 | | | | | 0.7* |
| Yes | 40 | 2 | 0.18 | 0.76 | 1.20 | 0.27-5.28 | |
| **Own a rabbit** | | | | | | | **0.6** |
| No | 508 | 21 | | | | | 0.67* |
| Yes | 34 | 2 | 0.35 | 0.76 | 1.42 | 0.32-6.32 | |
| **Own a rodent** | | | | | | | **0.2** |
| No | 501 | 23 | | | | | 0.4* |
| Yes | 41 | 0 | | | | | |
| **Own a reptile or amphibian** | | | | | | | **0.5** |
| No | 531 | 23 | | | | | 1.0* |
| Yes | 11 | 0 | | | | | |
| **Own a fish** | | | | | | | **0.4** |
| No | 526 | 23 | | | | | 1.0* |
| Yes | 16 | 0 | | | | | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Own any other animal or livestock** | | | | | | | **0.3** |
| No | 485 | 22 | | | | | 0.5* |
| Yes | 57 | 1 | -0.95 | 1.03 | 0.39 | 0.05-2.92 | |
| **Where the dog usually sleeps** | | | | | | | **0.5** |
| Outside | 23 | 0 | | | | | |
| Downstairs | 279 | 15 | Ref | | | | |
| Upstairs | 218 | 7 | -0.52 | 0.47 | 0.60 | 0.24-1.49 | |
| Whole house | 20 | 2 | 0.62 | 0.79 | 1.86 | 0.40-8.71 | |
| Outside and downstairs | 2 | 0 | | | | | |
| **Anyone in the household work with farm animals** | | | | | | | **0.1** |
| No | 499 | 20 | | | | | 0.1* |
| Yes | 41 | 4 | 0.89 | 0.57 | 2.43 | 0.79-7.46 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.6** |
| No | 451 | 21 | | | | | 0.8* |
| Yes | 90 | 3 | -0.33 | 0.63 | 0.72 | 0.21-2.45 | |
| **Who took antibiotics** | | | | | | | **0.6** |
| Family member | 49 | 1 | | | | | |
| Pet | 33 | 2 | 1.09 | 1.25 | 2.97 | 0.26-34.09 | |
| No one/ not specified | 450 | 21 | 0.83 | 1.03 | 2.29 | 0.30-17.37 | |
| **Anyone in the household work in healthcare** | | | | | | | **1.0** |
| No | 383 | 17 | | | | | 1.0* |
| Yes | 161 | 7 | -0.21 | 0.46 | 0.98 | 0.40-2.41 | |
| **Healthcare setting** | | | | | | | **1.0** |
| Human | 51 | 2 | | | | | |
| Animal | 107 | 5 | 0.18 | 0.85 | 1.19 | 0.22-6.35 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 383 | 17 | 0.12 | 0.76 | 1.13 | 0.25-5.04 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.5** |
| No | 399 | 16 | | | | | 0.5* |
| Yes | 144 | 8 | 0.33 | 0.44 | 1.39 | 0.58-3.31 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Reason for hospital visit** | | | | | | | **0.9** |
| Admission | 23 | 1 | | | | | |
| Visit | 17 | 1 | 0.30 | 1.45 | 1.35 | 0.08-23.20 | |
| Outpatient/ A&E | 102 | 6 | 0.30 | 1.10 | 1.35 | 0.16-11.79 | |
| Other/ not specified | 2 | 0 | | | | | |
| No attendance | 399 | 16 | -0.08 | 1.05 | 0.92 | 0.12-7.26 | |
| **Source of dog** | | | | | | | **0.4** |
| Breeder | 265 | 14 | | | | | |
| Rescue Kennel/ stray | 123 | 3 | -0.77 | 0.65 | 0.46 | 0.13-1.64 | |
| Newspaper/ word of mouth/ internet | 43 | 1 | -0.82 | 1.05 | 0.44 | 0.06-3.43 | |
| Family/friend | 80 | 3 | -0.34 | 0.65 | 0.71 | 0.20-2.53 | |
| Pet shop | 5 | 0 | | | | | |
| Other | 15 | 1 | 0.23 | 1.07 | 1.26 | 0.16-10.25 | |
| Self breed | 11 | 2 | 1.24 | 0.82 | 3.44 | 0.70-17.04 | |
| **Fed tinned or packet wet food** | | | | | | | **0.5** |
| Yes | 352 | 17 | | | | | 0.7* |
| No | 195 | 7 | -0.30 | 0.46 | 0.74 | 0.30-1.82 | |
| **Fed dry mixer** | | | | | | | **0.4** |
| No | 442 | 21 | | | | | 0.6* |
| Yes | 105 | 3 | -0.51 | 0.63 | 0.60 | 0.18-2.05 | |
| **Fed dry complete** | | | | | | | **0.5** |
| No | 129 | 7 | | | | | 0.6* |
| Yes | 418 | 17 | -0.29 | 0.46 | 0.75 | 0.30-1.85 | |
| **Fed raw poultry meat** | | | | | | | **<0.001** |
| No | 530 | 18 | | | | | <0.001* |
| Yes | 17 | 6 | 2.34 | 0.53 | 10.39 | 3.66-29.48 | |
| **Fed cooked poultry meat** | | | | | | | **0.9** |
| No | 437 | 19 | | | | | 1.0* |
| Yes | 110 | 5 | 0.04 | 513820.00 | 1.05 | 0.38-2.86 | |
| **Fed raw red meat** | | | | | | | **0.4** |
| No | 533 | 24 | | | | | 1.0* |
| Yes | 14 | 0 | | | | | |
| **Fed cooked red meat** | | | | | | | **0.4** |
| No | 529 | 24 | | | | | 1.0* |
| Yes | 18 | 0 | | | | | |
| **Sex** | | | | | | | **0.9** |
| Male | 267 | 12 | | | | | 1.0* |
| Female | 283 | 12 | -0.06 | 0.42 | 0.94 | 0.42-2.14 | |

Full univariable results for carriage of AmpC β-lactamase producing *E. coli*

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Age** | | | | | | | **0.5** |
| <1 | 51 | 5 | 0 | | | | |
| 1 | 73 | 5 | -0.36 | 0.66 | 0.70 | 0.19-2.54 | |
| 2 | 33 | 0 | | | | | |
| 3 | 39 | 2 | -0.65 | 0.86 | 0.52 | 0.10-2.84 | |
| 4 | 333 | 28 | -0.15 | 0.51 | 0.86 | 0.32-2.32 | |
| **Breed** | | | | | | | **0.4** |
| Pedigree | 404 | 31 | 0 | | | | 0.5* |
| Cross | 117 | 6 | -0.40 | 0.46 | 0.67 | 0.27-1.64 | |
| **Breed Group** | | | | | | | **0.4** |
| Working | 20 | 0 | | | | | |
| Gundog | 181 | 16 | 0 | | | | |
| Hound | 32 | 2 | -0.35 | 0.77 | 0.71 | 0.16-3.22 | |
| Terrier | 50 | 6 | 0.31 | 0.50 | 1.36 | 0.50-3.65 | |
| Utility | 28 | 2 | -0.21 | 0.78 | 0.81 | 0.18-3.71 | |
| Pastoral | 38 | 4 | 0.17 | 0.59 | 1.19 | 0.38-3.76 | |
| Toy | 35 | 0 | | | | | |
| Cross | 117 | 6 | -0.54 | 0.49 | 0.58 | 0.22-1.53 | |
| Not specified | 33 | 4 | 0.32 | 0.59 | 1.37 | 0.43-4.36 | |
| **Breed Size** | | | | | | | **0.8** |
| Small | 68 | 5 | 0 | | | | |
| Medium | 125 | 8 | -0.14 | 0.59 | 0.87 | 0.27-2.76 | |
| Large | 190 | 17 | 0.20 | 0.53 | 1.22 | 0.43-3.43 | |
| Not specified | 151 | 10 | -0.10 | 0.57 | 0.90 | 0.30-2.74 | |
| **Neutered** | | | | | | | **0.02** |
| No | 137 | 17 | 0 | | | | 0.03* |
| Yes | 395 | 23 | -0.76 | 0.00 | 0.47 | 0.24-0.90 | |
| **Working Dog** | | | | | | | **0.8** |
| No | 473 | 37 | 0 | | | | 1.0* |
| Yes | 45 | 3 | -0.16 | 0.62 | 0.85 | 0.25-2.87 | 0.8 |
| **Given dog treats** | | | | | | | **0.7** |
| Never | 32 | 1 | 0 | | | | |
| Rarely | 88 | 5 | 0.60 | 1.11 | 1.82 | 0.20-16.16 | |
| Sometimes | 245 | 20 | 0.96 | 1.04 | 2.61 | 0.34-20.13 | |
| Often | 159 | 14 | 1.04 | 1.05 | 2.82 | 0.36-22.20 | |
| **Given titbits** | | | | | | | **0.05** |
| Never | 52 | 8 | 0 | | | | |
| Rarely | 170 | 6 | -1.47 | 0.56 | 0.23 | 0.08-0.69 | |
| Sometimes | 226 | 19 | -0.60 | 0.45 | 0.55 | 0.23-1.32 | |
| Often | 72 | 7 | -0.46 | 0.55 | 0.63 | 0.22-1.85 | |
| **Given dog treats** | | | | | | | **0.2** |
| Never/ rarely | 120 | 6 | 0 | | | | 0.3* |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| Sometimes/ often | 404 | 34 | 0.52 | 0.45 | 1.68 | 0.69-4.10 | |
| **Given titbits** | | | | | | | **0.3** |
| Never/ rarely | 222 | 14 | 0 | | | | 0.4* |
| Sometimes/ often | 298 | 26 | 0.32 | 0.34 | 1.38 | 0.71-2.71 | 0 |
| **Reason for visit** | | | | | | | **0.5** |
| Vaccination/ worming | 202 | 11 | 0 | | | | |
| Presenting complaint | 241 | 24 | 0.60 | 0.38 | 1.83 | 0.87-3.82 | |
| Check up | 22 | 2 | 0.51 | 0.80 | 1.67 | 0.35-8.02 | |
| Work at clinic | 19 | 0 | | | | | |
| Vaccination/ presenting comp | 20 | 2 | 0.61 | 0.90 | 1.84 | 0.38-8.87 | |
| Vaccination/ check up | 1 | 0 | | | | | |
| Presenting comp/ check up | 4 | 1 | 1.52 | 1.16 | 4.59 | 0.47-44.61 | |
| Other | 13 | 0 | | | | | |
| **Medication prescribed during most recent visit** | | | | | | | **<0.001** |
| No | 346 | 13 | 0 | | | | <0.001* |
| Yes | 180 | 27 | 1.38 | 0.35 | 3.99 | 2.01-7.93 | |
| **Antibiotic prescribed during most recent visit** | | | | | | | **<0.001** |
| No | 433 | 23 | 0 | | | | <0.001* |
| Yes | 90 | 17 | 1.27 | 0.34 | 3.56 | 1.83-6.93 | |
| **Length of prescription of antibiotic given at most recent visit** | | | | | | | **<0.001** |
| One off prescription | 5 | 1 | 0 | | | | |
| Up to 5 days | 28 | 5 | -0.11 | 1.20 | 0.89 | 0.09-9.35 | |
| Up to 10 days | 39 | 6 | -0.26 | 1.18 | 0.77 | 0.08-7.77 | |
| Up to 2 weeks | 11 | 2 | 0.10 | 1.34 | 0.91 | 0.07-12.52 | |
| Up to 3 weeks | 2 | 2 | 1.61 | 1.48 | 5.00 | 0.27-91.52 | |
| Over 3 weeks | 1 | 1 | 1.61 | 1.79 | 5.00 | 0.15-166.60 | |
| Don’t know | 4 | 0 | | | | | |
| None prescribed | 433 | 23 | -1.33 | 1.12 | 0.27 | 0.03-2.37 | |
| **Prescribed any antibiotic in previous three months** | | | | | | | **<0.001** |
| **No** | 383 | 13 | 0 | | | | <0.001* |
| **Yes** | 151 | 27 | 1.66 | 0.35 | 5.27 | 2.65-10.48 | |
| **Received any veterinary treatment (excluding most recent visit)in the last three months** | | | | | | | **0.007** |
| No | 333 | 16 | 0 | | | | 0.009* |
| Yes | 186 | 22 | 0.95 | 0.35 | 2.60 | 1.30-5.19 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Medication prescribed in the last three months (excluding most recent visit)** | | | | | | | **0.007** |
| No | 333 | 16 | 0 | | | | 0.009* |
| Yes | 186 | 22 | 0.90 | 0.34 | 2.46 | 1.26-4.80 | |
| **Antibiotic prescribed in the last three months (excluding most recent visit)** | | | | | | | **<0.001** |
| No | 416 | 18 | 0 | | | | <0.001* |
| Yes | 88 | 19 | 1.61 | 0.35 | 4.99 | 2.52-9.89 | |
| **Length of prescription of antibiotic given in the last three months (excluding most recent visit)** | | | | | | | **<0.001** |
| One off prescription | 7 | 0 | | | | | |
| Up to 5 days | 27 | 5 | 0 | | | | |
| Up to 10 days | 30 | 6 | 0.08 | 0.66 | 1.08 | 0.30-3.95 | |
| Up to 2 weeks | 11 | 6 | 1.08 | 0.70 | 2.95 | 0.74-11.69 | |
| Up to 3 weeks | 4 | 1 | 0.30 | 1.22 | 1.35 | 0.12-14.73 | |
| Over 3 weeks | 7 | 0 | | | | | |
| Don’t know | 2 | 0 | | | | | |
| None prescribed | 416 | 18 | -1.45 | 0.54 | 0.23 | 0.08-0.68 | |
| **Left at veterinary premises** | | | | | | | **0.5** |
| No | 400 | 26 | 0 | | | | 0.5* |
| Yes | 41 | 4 | 0.41 | 0.56 | 1.50 | 0.50-4.51 | |
| **Allowed close contact with other dogs during walks** | | | | | | | **0.3** |
| No | 109 | 11 | 0 | | | | 0.3* |
| Yes | 414 | 28 | -0.40 | 0.37 | 0.67 | 0.32-1.39 | |
| **Regular contact with wild or farm animals during walks** | | | | | | | **0.9** |
| No | 396 | 30 | 0 | | | | 1.0* |
| Yes | 126 | 9 | -0.06 | 0.39 | 0.94 | 0.44-2.04 | |
| **Allowed off lead during walks** | | | | | | | **0.7** |
| No | 107 | 7 | 0 | | | | 0.8* |
| Yes | 418 | 32 | 0.16 | 0.43 | 1.17 | 0.50-2.72 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Other dogs in household** | | | | | | | **0.1** |
| No | 286 | 27 | 0 | | | | 0.1* |
| Yes | 240 | 13 | -0.56 | 0.35 | 0.57 | 0.29-1.14 | |
| **Number of other dogs in household** | | | | | | | **0.3** |
| 0 | 286 | 27 | 0 | | | | |
| 1 | 114 | 6 | -0.58 | 0.46 | 0.56 | 0.22-1.39 | |
| 2 | 46 | 5 | 0.14 | 0.51 | 1.15 | 0.42-3.14 | |
| 3 | 13 | 0 | | | | | |
| 4+ | 16 | 0 | | | | | |
| **Other animals in household** | | | | | | | **0.03** |
| No | 289 | 29 | 0 | | | | 0.03* |
| Yes | 236 | 11 | 0.77 | 0.36 | 0.46 | 0.23-0.95 | |
| **Own a cat** | | | | | | | **0.2** |
| No | 383 | 33 | 0 | | | | 0.3* |
| Yes | 142 | 7 | -0.56 | 0.43 | 0.57 | 0.25-1.32 | |
| **Own a bird** | | | | | | | **0.2** |
| No | 484 | 39 | 0 | | | | 0.3* |
| Yes | 41 | 1 | -1.20 | 1.03 | 0.3 | 0.04-2.26 | |
| **Own a rabbit** | | | | | | | **0.3** |
| No | 490 | 39 | 0 | | | | 0.5* |
| Yes | 35 | 1 | -1.02 | 1.03 | 0.36 | 0.05-2.69 | |
| **Own a rodent** | | | | | | | **0.2** |
| No | 485 | 39 | 0 | | | | 0.3* |
| Yes | 40 | 1 | -1.17 | 1.03 | 0.31 | 0.04-2.32 | |
| **Own a reptile or amphibian** | | | | | | | **0.4** |
| No | 514 | 40 | | | | | 1.0* |
| Yes | 11 | 0 | | | | | |
| **Own a fish** | | | | | | | **0.3** |
| No | 509 | 40 | | | | | 0.6* |
| Yes | 16 | 0 | | | | | |
| **Own any other animal or livestock** | | | | | | | **0.3** |
| No | 469 | 38 | 0 | | | | 0.4 |
| Yes | 56 | 2 | -0.82 | 0.74 | 0.44 | 0.10-1.88 | |
| **Where the dog usually sleeps** | | | | | | | **0.8** |
| Outside | 21 | 2 | 0 | | | | |
| Downstairs | 274 | 20 | -0.27 | 0.78 | 0.77 | 0.17-3.50 | |
| Upstairs | 210 | 15 | -0.29 | 0.79 | 0.75 | 0.16-3.51 | |
| Whole house | 19 | 3 | 0.51 | 0.97 | 1.66 | 0.25-11.02 | |
| Outside and downstairs | 2 | 0 | | | | | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Anyone in the household work with farm animals** | | | | | | | **0.2** |
| No | 480 | 39 | 0 | | | | 0.4* |
| Yes | 44 | 1 | -1.27 | 1.02 | 0.28 | 0.04-2.09 | |
| **Anyone in the household taken antibiotics** | | | | | | | **0.5** |
| No | 437 | 35 | 0 | | | | 0.7* |
| Yes | 88 | 5 | -0.34 | 0.49 | 0.71 | 0.27-1.86 | |
| **Who took antibiotics** | | | | | | | **0.6** |
| Family member | 48 | 2 | 0 | | | | |
| Pet | 32 | 3 | 0.81 | 0.94 | 2.25 | 0.36-14.23 | |
| No one/ not specified | 436 | 35 | 0.66 | 0.74 | 1.92 | 0.45-8.26 | |
| **Anyone in the household work in healthcare** | | | | | | | **0.5** |
| No | 370 | 30 | 0 | | | | 0.6* |
| Yes | 158 | 10 | -0.25 | 0.38 | 0.78 | 0.37-1.64 | |
| **Healthcare setting** | | | | | | | **0.9** |
| Human | 49 | 4 | 0 | | | | |
| Animal | 106 | 6 | -0.37 | 0.67 | 0.69 | 0.19-2.57 | |
| Both | 2 | 0 | | | | | |
| Not specified | 1 | 0 | | | | | |
| Does not work in healthcare | 370 | 30 | -0.01 | 0.55 | 0.99 | 0.34-2.94 | |
| **Anyone in the household attended hospital in last month** | | | | | | | **0.5** |
| No | 384 | 31 | 0 | | | | 0.6* |
| Yes | 143 | 9 | -0.25 | 0.39 | 0.78 | 0.36-1.68 | |
| **Reason for hospital visit** | | | | | | | **1.0** |
| Admission | 23 | 1 | 0 | | | | |
| Visit | 17 | 1 | 0.30 | 1.45 | 1.35 | 0.08-23.20 | |
| Outpatient/ A&E | 101 | 7 | 0.47 | 1.09 | 1.59 | 0.19-13.60 | |
| Other/ not specified | 2 | 0 | | | | | |
| No attendance | 384 | 31 | 0.62 | 1.04 | 1.86 | 0.24-14.21 | |
| **Source of dog** | | | | | | | **0.6** |
| Breeder | 258 | 21 | 0 | | | | |
| Rescue Kennel/ stray | 118 | 8 | -0.18 | 0.43 | 0.83 | 0.36-1.94 | |
| Newspaper/ word of mouth/ internet | 41 | 3 | -0.11 | 0.64 | 0.90 | 0.26-3.15 | |
| Family/friend | 80 | 3 | -0.77 | 0.63 | 0.46 | 0.13-1.58 | |
| Pet shop | 5 | 0 | | | | | |
| Other | 14 | 2 | 0.56 | 0.79 | 1.76 | 0.37-8.24 | |
| Self breed | 11 | 2 | 0.80 | 0.80 | 2.23 | 0.46-10.75 | |

| Variable | - (n) | + (n) | Coefficient | SE | OR | 95% CI | P-value |
|---|---|---|---|---|---|---|---|
| **Fed tinned or packet wet food** | | | | | | | **1.00** |
| Yes | 343 | 26 | 0 | | | | 1.0* |
| No | 188 | 14 | -0.02 | 0.34 | 0.98 | 0.50-1.93 | |
| **Fed dry mixer** | | | | | | | **0.5** |
| No | 432 | 31 | 0 | | | | 0.5* |
| Yes | 99 | 9 | 0.24 | 0.39 | 1.27 | 0.58-2.75 | |
| **Fed dry complete** | | | | | | | **0.6** |
| No | 125 | 11 | 0 | | | | 0.6* |
| Yes | 406 | 29 | -0.21 | 0.37 | 0.81 | 0.39-1.67 | |
| **Fed raw poultry meat** | | | | | | | **0.2** |
| No | 508 | 40 | | | | | 0.4* |
| Yes | 23 | 0 | | | | | |
| **Fed cooked poultry meat** | | | | | | | **0.7** |
| No | 425 | 31 | 0 | | | | 0.7* |
| Yes | 106 | 9 | 0.15 | 0.39 | 1.16 | 0.54-2.52 | |
| **Fed raw red meat** | | | | | | | **0.3** |
| No | 519 | 38 | 0 | | | | 0.3* |
| Yes | 12 | 2 | 0.82 | 0.78 | 2.28 | 0.49-10.54 | |
| **Fed cooked red meat** | | | | | | | **0.1** |
| No | 516 | 37 | 0 | | | | 0.1* |
| Yes | 15 | 3 | 1.03 | 0.65 | 2.79 | 0.77-10.07 | |
| **Sex** | | | | | | | **0.1** |
| Male | 255 | 24 | 0 | | | | 0.1* |
| Female | 279 | 16 | -0.50 | 0.33 | 0.61 | 0.32-1.17 | |

**Appendix Four**

Published paper - Wedley, A. L., T. W. Maddox, C. Westgarth, K. P. Coyne, G. L. Pinchbeck, N. J. Williams and S. Dawson (2011).
Prevalence of antimicrobial-resistant *Escherichia coli* in dogs in a cross-sectional, community-based study.
Veterinary Record **168**(13): 354.