



**Human Contact with Dogs and the Risk of
Acquiring Antimicrobial Resistant and Extended-
Spectrum β -Lactamase-producing *Escherichia coli***

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by

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Abstract

Human Contact with Dogs and the Risk of Acquiring Antimicrobial Resistant and Extended-Spectrum β -Lactamase-producing *Escherichia coli*

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The increasing global disease burden due to antimicrobial-resistant (AMR) infections is a significant public health concern. Multi-drug resistant (MDR) isolates, including those resistant to third generation cephalosporins (3GC), are of particular concern in human and veterinary medicine. Previous studies report faecal carriage of AMR *E. coli* in up to 63.0% of UK dogs. However, there is currently a paucity of data regarding community carriage rates AMR *E. coli* in UK human populations, particularly those with high levels of contact with dogs. The aims of this study were to determine the prevalence and risk factors for faecal carriage of AMR and extended-spectrum β -lactamase (ESBL)-producing *E. coli* in humans working with dogs in multiple environments, and in-contact kennelled dogs. The study also aimed to characterise and compare isolates of human and canine origin.

Two concurrent cross-sectional studies were undertaken collecting faecal samples from 229 human and 296 canine participants across 69 premises in the North of England. All *E. coli* isolates were subjected to antimicrobial susceptibility testing and phylogenetic grouping, while resistant isolates were also subjected to PCR assays in order to detect resistance genes. Isolates displaying ESBL, AmpC or MDR phenotypes were also subjected to whole genome sequencing. Mixed effect logistic regression models were utilised in order to assess risk factors for carriage of AMR *E. coli* using questionnaire-derived data.

Faecal carriage of AMR *E. coli* was high in the human (68.7%) and canine (58.9%) populations sampled, with resistance to ampicillin, trimethoprim and tetracycline most prevalent. An increased prevalence of resistance to amoxycylav and 3GCs was noted in dogs (10.6% and 9.7%) when compared to humans (3.1% and 4.2%), while the prevalence of ESBL-producing *E. coli* was higher in humans (3.1%) than dogs (0.003%). Molecular characterisation of *E. coli* isolates indicated that in the canine population phylogroup B1 isolates predominated, while in humans B2 isolates were most prevalent. Multi-locus sequence typing identified a large number of sequence types (ST) indicating a high level of diversity, and, while the most prevalent STs varied between host species, many were identified in both humans and dogs. Numerous *bla* genes conferring resistance to 3GCs were identified in both species. In humans, *bla*_{CTX-M-15} was most prevalent (n=4), while in dogs *bla*_{CMY-2} predominated (n=23), and was commonly associated with IncI1 ST23 (n=10) plasmids. Potential intra-species transmission of 3GC-resistant and MDR *E. coli* between individuals was identified on multiple premises and of additional concern, was the potential transmission of a CMY-2-producing *E. coli* ST372 strain between host species on the same premises.

Risk factors associated with each resistance outcome varied between host species, however, some patterns did emerge. Prior hospitalisation was identified as a risk factor for multiple resistance outcomes in both species, and contact with an owner working in a human hospital was a risk factor for 3GC resistance in dogs. The role of diet was also highlighted as a risk factor in both species; feeding of a raw food diet to dogs in particular was associated with multiple outcomes, including carriage of MDR *E. coli*. Additionally, contact with farm animals was associated with multiple outcomes in both species, while dogs with owners working in farming were additionally at increased risk of carriage of MDR *E. coli*. Interestingly, the effect of antimicrobial treatment was variable.

Our findings indicate dogs may act as a reservoir of resistance determinants to in-contact humans, however transmission may be bidirectional. The potential role of dogs in the complex epidemiology of antimicrobial resistance alongside other healthcare, animal and environment-associated risk factors, highlights the need for multidisciplinary approaches to address this issue effectively.

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Abbreviations used

3GC	Third generation cephalosporin
95% CI	95% confidence interval
AC	Amoxyclav
AIEC	Adherent-invasive <i>E. coli</i>
AMR	Antimicrobial-resistant
AMP	Ampicillin
ASPIC	Antimicrobial Stewardship in Primary Care
AST	Antimicrobial susceptibility testing
BSAC	British Society for Antimicrobial Chemotherapy
BSAVA	British Small Animal Veterinary Association
cAmpC	Chromosomal AmpC
CHL	Chloramphenicol
Class 2ber	Functional class of β -lactamases which hydrolyse third generation cephalosporins and confer resistance to β -lactamase inhibitors
CIP	Ciprofloxacin
DAEC	Diffuse-adherent <i>E. coli</i>
EAEC	Enteraggregative <i>E. coli</i>
EHEC	Enterohaemorrhagic <i>E. coli</i>
EIEC	Enteroinvasive <i>E. coli</i>
EMBA	Eosin methylene blue agar
EPEC	Enteropathogenic <i>E. coli</i>
ESBL	Extended-spectrum β -lactamase
ETEC	Enterotoxigenic <i>E. coli</i>
ExPEC	Extra-intestinal pathogenic <i>E. coli</i>
APEC	Avian pathogenic <i>E. coli</i>
ICC	Intraclass correlation coefficient
IRT	Inhibitor-resistant TEM
MDR	Multi-drug resistant
MLST	Multi-locus sequence typing
NA	Nalidixic acid
NICE	National Institute for Health and Care Excellence
NMEC	Neonatal meningitis-associated <i>E. coli</i>
OIE	World Organisation for Animal Health
OR	Odds Ratio
pAmpC	Plasmid-mediated AmpC
PHE	Public Health England
ST	Sequence type
TET	Tetracycline
TM	Trimethoprim
UPEC	Uropathogenic <i>E. coli</i>
UTI	Urinary tract infection
VMD	Veterinary Medicines Directorate
WHO	World Health Organisation

Chapter 1

Introduction and Literature Review

1.1 Introduction

Antimicrobial resistance is a significant issue globally, impacting on both human and veterinary patients and the emergence of multi-drug resistant (MDR) isolates, displaying resistance to three or more antimicrobial classes, such as those producing extended-spectrum β -lactamases (ESBL) are of particular concern. Clinically, ESBL-producing isolates have been identified as a major cause of nosocomial and community-acquired infections in humans (Doi et al., 2013; Leistner et al., 2014; Rogers et al., 2011) and animals (Huber et al., 2013; Timofte et al., 2011). The interaction between humans and animals may allow for zoonotic transfer of antimicrobial-resistant (AMR) bacteria and as such resistance in these populations should be considered jointly if antimicrobial resistance is to be tackled effectively (O'Neill, 2016).

To date, the focus of antimicrobial resistance research in humans has largely been aimed at clinical isolates; however, exposure of gastrointestinal commensals to antimicrobials used for treatment of clinical infections selects for the acquisition of resistance determinants, which may be transferred to previously susceptible pathogenic bacteria. There is currently a paucity of data regarding carriage of AMR *E. coli* in the general population of the United Kingdom and the impact of animal contact on this carriage needs further investigation.

1.2 *Escherichia coli*

First identified in 1885, *Escherichia coli* (*E. coli*) represent a versatile and genetically diverse Gram-negative bacterial species of the family *Enterobacteriaceae* (Escherich, 1989). The diverse nature of the species is due to both recombination and high rates of horizontal gene transfer (Touchon et al., 2009). Despite this genomic plasticity, *E. coli* has distinct genetic sub-structure made up of eight phylogroups (A, B1, B2, C, D, E, F and *Escherichia* clade I), defined by the presence or absence of genes such as *chuA*, *yjaA* and *gadA* (Clermont et al., 2000; Clermont et al., 2013; Doumith et al., 2012). Of these groups, B2 is the most diverse leading some to postulate it having subspecies status (Lescat et al., 2009; Tenailon et al., 2010). *E. coli* is a common commensal of the lower gastrointestinal tract humans and other mammals (Berg, 1996; Gordon and Cowling, 2003), however expression of virulence-associated genes by pathogenic strains can also lead to gastrointestinal and extra-intestinal disease in humans and animals (Beutin, 1999; Johnson and Russo, 2002; Lanz et al., 2003; Norris et al., 2000). In addition, commensal strains have the potential to act as opportunistic pathogens in immunocompromised individuals (Packey and Sartor, 2009).

1.2.1 Role as a pathogen

Prior to 1940, all *E. coli* strains were considered commensal organisms; however, the association of the bacterium with multiple outbreaks of gastrointestinal disease in this decade led to a paradigm shift when considering the role of this bacterium in disease (Bray, 1945; Chaudhuri and Henderson, 2012). Pathogenicity in this species is due to the presence of virulence genes coding for factors such as adhesins and toxins. Interestingly, these genes likely evolved primarily to improve adaptation to the bacteria's ecological niche and cause disease secondarily (Le Gall et al., 2007). To date, six distinct pathotypes have been identified which cause enteric disease (Clements et al., 2012; Kaper et al., 2004; Nataro and Kaper, 1998; Windahl et al., 2014):

1. **Enteropathogenic *E. coli* (EPEC)** are associated with attaching effacing lesions in the ileum and the cause of the initial outbreaks in the 1940s.
2. **Enterohaemorrhagic *E. coli* (EHEC)** are associated with haemorrhagic colitis and more severe sequelae such as haemolytic uraemic syndrome.
3. **Enterotoxigenic *E. coli* (ETEC)** are a major cause of infant diarrhoea in the developing world as well as traveller's diarrhoea.
4. **Enteroinvasive *E. coli* (EIEC)** is the only pathotype able to replicate within gut epithelial cells and is closely related to *Shigella* species, but causing milder clinical signs.
5. **Diffuse-adherent *E. coli* (DAEC)** are defined by diffuse adherence to enterocytes.
6. **Enteroregative *E. coli* (EAEC)** cause persistent diarrhoea in both children and adults without the secretion of heat labile or stable toxins.

The presence of another pathotype associated with Crohn's Disease, known as adherent-invasive *E. coli* (AIEC) has also been postulated (Darfeuille-Michaud, 2002). These intra-intestinal pathotypes most commonly belong to phylogroups A, B1 and E (Clermont et al., 2011). An additional pathotype, extra-intestinal pathogenic *E. coli* (ExPEC), principally comprises *E. coli* of phylogroups B2 and D (Clermont et al., 2011; Johnson and Stell, 2000; Picard et al., 1999). This pathotype contains three subgroups (Johnson and Russo, 2002; Kaper et al., 2004; Nataro and Kaper, 1998):

1. **Uropathogenic *E. coli* (UPEC)** are the most common cause of urinary tract infections (UTIs) including cystitis and pyelonephritis in both humans and dogs.
2. **Neonatal meningitis-associated *E. coli* (NMEC)** are the most common cause of Gram-negative neonatal meningitis in humans, while cases of infant bacteraemia due to these bacteria are increasing.

3. **Avian pathogenic *E. coli* (APEC)** are associated with colibacillosis in poultry species, most commonly presenting as air-sacculitis, bacteraemia and pericarditis.

In contrast to commensal and intestinal pathogenic *E. coli*, ExPEC strains commonly share numerous extra-intestinal virulence determinants which are located on pathogenicity associated islands (Johnson and Russo, 2002). The virulence factors associated with ExPEC include toxins, such as haemolysin; adhesins, such as fimbrial pap proteins enabling colonisation of mucosal surfaces (Johnson et al., 2003a); nutrient acquisition systems, such as the iron siderophore ironN (Bäumler et al., 1998; Hantke et al., 2003); and host evasion mechanisms, including production of polysaccharide capsules and outer membrane serum survival proteins offering complement resistance, such as that coded by the *iss* gene (Johnson et al., 2003a; Johnson et al., 2008c).

1.2.2 *Role as a commensal bacterium*

E. coli is the predominant aerobe in the gastrointestinal tract of humans (Berg, 1996) and can additionally be found in secondary habitats such as watercourses and their associated sediment (Gordon et al., 2002). Commensal *E. coli* do not adhere to the enterocytes of their hosts but rather reside in and derive nutrients from the mucus layer covering these cells (Poulsen et al., 1994). While the bacteria benefit from the stable ecological niche provided by their host, they may also provide colonisation resistance against intestinal pathogens (Hudault et al., 2001; van der Waaij et al., 1971; Vollaard and Clasener, 1994). Despite the ubiquitous role of *E. coli* as a commensal, diversity exists in the bacterial population, not only between hosts but also within a single host. At a single time point, an individual host will carry a predominant, resident strain constituting over 50% of the *E. coli* present. These resident strains commonly colonise for a period of months to years and are joined by transient strains which are present for days to weeks (Caugant et al., 1981; Sears et al., 1950; Tenaillon et al., 2010). The diversity in commensal *E. coli* between hosts can be caused by numerous factors including species, environment and hygiene, which can influence both the prevalence and the phylogroups of *E. coli* present. In humans and dogs, the prevalence of *E. coli* is over 90%, but this reduces to 10% when considering some animals such as reptiles (Gordon and Cowling, 2003; Mentula et al., 2005; Penders et al., 2006). Some studies also suggest in humans commensal *E. coli* belonging to phylogroup A predominate, whereas in animals group B1 is the dominant phylogroup (Tenaillon et al., 2010), however more extensive studies are required to investigate these findings further. The potential species differences in commensal *E. coli* populations are not due to host-specific strains, few of which have been identified (Clermont et al., 2008b; Escobar-Páramo et al.,

2006). They are more likely related to the differing diet, gastrointestinal anatomy, environments and geographical locations of these host species.

The presence of commensal *E. coli* in the gastrointestinal niche means it will inevitably be exposed to antimicrobials used in the treatment of clinical infections. This exposure will increase the selection pressure for the development or acquisition of antimicrobial resistance genes. The exposure of commensal *E. coli* to antimicrobials combined with ease of culture and the high concentration of *E. coli* excreted in faeces mean that *E. coli* is considered a good indicator for the reservoir of antimicrobial resistance circulating in the gastrointestinal commensal population (De Graef et al., 2004; van den Bogaard and Stobberingh, 2000).

1.3 Antimicrobials

Antimicrobials are considered the 'most successful form of chemotherapy developed in the 20th Century' (Wright, 2007). Indeed, the development of penicillin for clinical use in the 1940s heralded a golden age of antimicrobial discovery and development (Fleming, 1932; Florey et al., 1943). Over the next 20 years, more than twenty different classes of antimicrobials including tetracyclines, cephalosporins and quinolones were marketed and became integral to the treatment of bacterial infections (Coates et al., 2011). These drugs remain an essential tool for the treatment of infections but, given the increased prevalence of antimicrobial resistance, the discovery void since the first report of lipopeptides in 1987 is of concern (Silver, 2011).

1.3.1 Mechanisms of action

Antimicrobials can be classified as either bacteriostatic, those which inhibit bacterial growth or bacteriocidal, those which kill the bacterial cells, dependent on their mechanism of action and the concentration at which they are administered (Pankey and Sabath, 2004). Antimicrobial drugs target essential components of bacterial metabolism and five main targets have been described: cell wall synthesis, DNA synthesis, folic acid metabolism, protein synthesis and RNA synthesis (Coates et al., 2002; Hooper, 2001). The specific mechanisms of action of antimicrobials pertinent to this study are detailed below.

1.3.1.1 β -lactams

This group of antimicrobials comprises penicillin derivatives, cephalosporins, monobactams and carbapenems. Different classes of β -lactams have differing structures and spectra of

activity; however, all contain a conserved central β -lactam ring (Rang et al., 2016). These antimicrobials are bacteriocidal, targeting cell wall synthesis in order to exert their effect. Peptidoglycan is an essential component of the bacterial cell wall, comprising linear amino sugars cross-linked by peptide side chains. Peptidoglycan is synthesised endogenously in three stages by bacteria, with β -lactams inhibiting the final stage of synthesis, transpeptidation. These antimicrobials have a high affinity for transpeptidases, known as penicillin binding proteins and form covalent bonds at the active site of these enzymes, preventing normal crosslinking of the peptide side chains of peptidoglycan (Spratt, 1980; Tipper, 1985). The absence of crosslinking weakens the cell wall, leaving the bacterium susceptible to changes in osmotic pressure it could otherwise withstand, resulting in cell lysis (Walsh, 2000).

1.3.1.2 *Quinolones*

Quinolone antimicrobials are bacteriocidal and inhibit DNA synthesis in bacterial cells. First generation quinolones such as nalidixic acid were first approved for use during the 1960's and have now largely been superseded by fluoroquinolones, which have increased potency and a wider spectrum of activity (Drlica and Zhao, 1997). Fluoroquinolones target two enzymes DNA gyrase and Topoisomerase IV, involved in DNA synthesis. These complex enzymes are comprised of two pairs of subunits, GyrA and GyrB in the case of DNA gyrase and ParC and ParE in the case of Topoisomerase IV (Drlica and Zhao, 1997). DNA gyrase is responsible for the initiation of DNA replication by formation of negative supercoils in bacterial DNA, while Topoisomerase IV is the principle enzyme required for decatenation of daughter chromosomal DNA following replication (Hooper, 1999). Fluoroquinolones bind to enzyme-DNA complexes, inhibiting progression of DNA synthesis and can lead to the accumulation of double stranded DNA breaks ultimately resulting in bacterial cell death (Hooper, 2001). DNA gyrase is more susceptible to fluoroquinolones in Gram-negative bacteria, whereas Topoisomerase IV is the main target in Gram-positives (Jacoby, 2005). Currently, numerous fluoroquinolones are approved for parenteral or topical use in human and veterinary medicine (DataPharm, 2016; NOAH, 2016). They are however, considered highly critically important antimicrobials for human and animal health and as such, their use should be restricted (OIE, 2015; WHO, 2017).

1.3.1.3 *Tetracyclines*

The tetracycline family are bacteriostatic antimicrobials, which inhibit bacterial protein synthesis. The first generation tetracyclines such as chlortetracycline were discovered in the

1940s and were naturally occurring metabolites of *Streptomyces* bacteria. Following this discovery, multiple second generation semi-synthetic tetracyclines were developed. These drugs bind reversibly to the 30S subunit of the 70S bacterial ribosome blocking the A site. This prevents amino-acyl tRNA binding to the complementary mRNA and thus inhibits translation and synthesis of peptides (Chopra and Roberts, 2001; Nguyen et al., 2014). The reversible nature of drug binding at this site may explain the bacteriostatic nature of these drugs (Chopra et al., 1992). Tetracyclines preferentially inhibit protein synthesis in prokaryote cells, as eukaryotic cells possess 80S rather than 70S ribosomes; however, the presence of 70S ribosomes in mitochondria mean tetracyclines can inhibit mitochondrial protein synthesis (Riesbeck et al., 1990; van den Bogert and Kroon, 1981). Following emergence of resistance to tetracyclines in the late 1980s, there was renewed interest in further tetracycline development. This led to the release of the glycylicycline, tigecycline in 2005 with further drugs currently in Phase 3 clinical trials (Paratek Pharmaceutical Inc, 2015; Petersen et al., 1999).

1.3.1.4 Trimethoprim

Trimethoprim was first used clinically in 1962 and is widely used in human medicine for the treatment of UTIs (Hawker et al., 2014). This drug interrupts the bacterial folic acid metabolism pathway by binding to the active site of dihydrofolate reductase (DHFR). This enzyme is required for reduction of dihydrofolic acid to tetrahydrofolic acid, an important step in the synthesis of thymidine in both prokaryote and eukaryote cells (Huovinen, 1987). The preferential action of this drug on prokaryote cells is due to a markedly higher affinity for bacterial DHFR than the human enzyme (Burchall, 1979; Hitchings, 1973). Trimethoprim is bacteriostatic when used alone, but is commonly used in combination with a sulphonamide drug due to the reported synergistic bacteriocidal action of potentiated sulphonamides (Hitchings, 1973). Interestingly, potentiated sulphonamides are widely prescribed in equine veterinary medicine, but in human medicine they are generally restricted for the treatment of toxoplasmosis or nocardiosis (Hughes et al., 2013; Rang et al., 2016)

1.3.2 Use of antimicrobials in the UK

1.3.2.1 Dogs

In the UK, accurate data regarding the volume of antimicrobials prescribed by veterinarians in practice is not available, however data regarding national sales of antimicrobials are published annually by the Veterinary Medicines Directorate (VMD) (VMD, 2015). Data from

this report indicate that sales of antimicrobials for veterinary treatment have remained stable over the last five years, and sales of tetracyclines accounted for 40% of veterinary antimicrobial sales in 2014. This national data is not representative of UK small animal prescribing as 369 (86%) of the 429 tonnes of antimicrobials sold were exclusively for food-producing animals (VMD, 2015). However, studies have been undertaken, which investigate antimicrobial prescribing in companion animal species. The proportion of dogs receiving antimicrobials during consultations at UK first opinion veterinary practices ranges between 35.0% and 45.1%; although there was a difference in antimicrobial prescribing between locations, suggesting this could influence prescribing practices (Mateus et al., 2011; Radford et al., 2011). Throughout first opinion veterinary practice, β -lactam antimicrobials are most commonly prescribed, with amoxycylav dispensed most frequently for dogs in the UK and Europe (De Briyne et al., 2014; Hughes et al., 2012; Mateus et al., 2011; Radford et al., 2011; Singleton et al., 2017; Summers et al., 2014). In a study by De Briyne et al. (2014), Europe-wide use of critically important antimicrobials for the treatment of dogs was low. Within the study, there was however some national variation in prescribing rates. The UK ranked second only to Sweden, which had the lowest rates of prescribing of these drugs. Interestingly, Sweden has a high level of antimicrobial stewardship nationally, and between 2006 and 2013 prescription of antimicrobials for use in dogs has reduced by 46% (SWEDRES-SVARM, 2013). While studies indicate that the selection of antimicrobial classes for treatment of infections by UK small animal veterinarians is largely appropriate, up to 26% of prescriptions are out with the recommended dose range and animals are not always weighed prior to dispensing of medications (Hughes et al., 2012; Summers et al., 2014). This inaccurate dosing has previously been highlighted as a driver for the dissemination of antimicrobial resistance and is of concern (Zhou et al., 2000). Furthermore, although vets report antimicrobial susceptibility testing (AST) as one of the most important factors governing selection of antimicrobials, 60.3% of UK vets recruited to a recent study reported seldom or never undertaking AST compared to 22.0% in Sweden (De Briyne et al., 2013). The empiric choice of antimicrobial treatment commonly undertaken in UK first opinion veterinary practice highlights the need for clear practice guidelines on antimicrobial use. The British Small Animal Veterinary Association (BSAVA) is currently promoting antimicrobial stewardship amongst its members including promotion of practice policies for empirical therapy, increased use of AST and careful consideration of prophylactic antimicrobial treatment (BSAVA, 2016).

1.3.2.2 Humans

Antimicrobials prescribed in the UK are largely prescribed for community-based patients, with general practitioners prescribing 74.0% of the antimicrobials dispensed (PHE, 2015). While no data exists describing the total proportion of community-based patients receiving antimicrobials, 34.1% of hospital patients received antimicrobial treatment (HPA, 2012). In community and hospital settings, penicillins alone or in combination with a β -lactamase inhibitor were the most frequently prescribed antimicrobial, making up 45% of total prescriptions. However, in contrast to first opinion veterinary practice, drug-inhibitor combinations are not used widely in general practice and are not recommended for first line use. Rather, these drugs are reserved for conditions such as acute pyelonephritis, cellulitis or sepsis (PHE, 2015; PHE, 2016). European data suggest national variation in prescribing rates similar to those seen for veterinary antimicrobial prescribing. However, in contrast to the veterinary sector where the UK was commonly ranked low, the UK is a middle to high prescribing country among human physicians (ECDC, 2015). Within the EU, the UK was highlighted as having the highest year on year increase in defined daily doses of penicillins throughout Europe (De Briyne et al., 2014; ECDC, 2015), in keeping with a worrying trend indicating a 6.5% increase in consumption of antimicrobials in the UK from 2011-2014 (PHE, 2015). Given the global emergence and dissemination of antimicrobial resistance, guidelines have been issued recommending appropriate antimicrobial choices for clinical syndromes and when they are required (PHE, 2016). It is of concern therefore, that in a recent study, 51% of general practitioners prescribed antimicrobials for coughs, colds and viral sore throats where antibiotics is not required (Hawker et al., 2014). This study also indicated a variation between practices, mirroring those previously reported in veterinary antimicrobial prescribing (Mateus et al., 2011; Radford et al., 2011). Within the UK, 99% of Clinical Commissioning Groups have written dedicated antimicrobial use policies in order to improve antimicrobial stewardship (PHE, 2015). Reports, however, suggest that numerous factors including clinical aspects of a case, the doctor-patient relationship and time constraints of the consultation all influence prescribing patterns and need to be considered with respect to antimicrobial stewardship (Shallcross and Davies, 2014). To this end, the TARGET toolkit was developed by the Antimicrobial Stewardship in Primary Care (ASPIC) collaboration, aiming to influence both practitioner and patient attitudes to antimicrobial prescribing (RCGP, 2016). These tools complement both the UK Five Year Antimicrobial Resistance Strategy, as well as current National Institute for Health and Care Excellence (NICE)

guidelines to promote antimicrobial stewardship at a national and practice level (DoH and DEFRA, 2013; NICE, 2015).

1.4 Antimicrobial resistance mechanisms

Antimicrobial resistance is considered one of the greatest challenges currently facing clinicians in human and veterinary medicine globally, though the ability of bacteria to resist the effects of antibiotic compounds is not a new occurrence. Many environmental bacteria produce antimicrobial compounds as a result of secondary metabolism during nutrient shortage (Vining, 1990). These compounds appear to have a hormetic effect; at low concentrations they may act as signalling molecules involved in quorum sensing, which maintain the homeostasis of bacterial populations (Fajardo and Martínez, 2008; Linares et al., 2006), while at higher concentrations they may also act as defence molecules in order to out-compete other bacteria within their ecological niche (D'Costa et al., 2007). The need for self-protection against these antimicrobials has led to the co-evolution of compensatory resistance mechanisms against these compounds (Huang et al., 2005; Pootoolal et al., 2002). Furthermore, some efflux pumps utilised by bacteria to export antimicrobial compounds have other physiological roles including exporting waste products such as bile, from the bacteria cell (Pidcock, 2006). These resistance mechanisms have preceded the clinical use of antimicrobials; indeed Abraham and Chain reported the first discovery of penicillinase in 1940, a year before penicillin was first used clinically (Abraham and Chain, 1988; Cooksey, 2006). While clinical use of antimicrobials has therefore not led to the evolution of these resistance genes (Livermore, 2003), extensive use of antimicrobials in both human and veterinary medicine has provided the selection pressure necessary for promotion and dissemination of these resistance mechanisms within and between bacterial populations (Levy and Marshall, 2004).

Antimicrobial resistance can be considered as either intrinsic or acquired. Intrinsic resistance is the ability of all members of a bacterial species to resist the action of an antimicrobial drug due to an inherent structural or functional characteristic of that species (Blair et al., 2014). Commonly these mechanisms include exclusion of the compound from the bacterial cell such as impermeability of the Gram-negative outer cell membrane to vancomycin (Tsuchido and Takano, 1988), or the absence of a susceptible target, encountered for triclosan resistance in *Pseudomonas* species (Zhu et al., 2010). In contrast, acquired resistance is the resistance of a microorganism to an antimicrobial to which it was originally sensitive.

This resistance can be mediated by three different mechanisms (Blair et al., 2014; Wright, 2011):

1. Reduction of intracellular drug concentrations by decreased cellular permeability or increased efflux
2. Modification of the antimicrobial target site by mutation or protection
3. Inactivation of the antimicrobial by hydrolysis or modification

Development of acquired resistance can occur in two ways; random chromosomal mutation and horizontal transfer of mobile genetic elements. Chromosomal mutation occurs via uncorrected nucleotide base substitutions and commonly only affect a single antimicrobial class, but if the mutation is in a gene affecting membrane permeability, such as those coding an efflux pump or porin, the effects could be more wide ranging effects (Livermore, 2003). Horizontal gene transfer occurs by the transmission of mobile genetic elements such as plasmids, transposons and bacteriophages between bacteria of the same, or different species (Livermore, 2003). Dissemination of these mobile genetic elements may occur by transformation, conjugation or transduction (Ochman et al., 2000). In the *Enterobacteriaceae*, plasmid transmission via conjugation is the most common form of horizontal gene transfer (Tenover, 2006). Many plasmids carry multiple resistance genes and exposure to one antimicrobial may therefore co-select for resistance to another. As such, many bacterial isolates carrying plasmid-mediated resistance genes display a MDR phenotype (displaying resistance to three or more antimicrobial classes), making the treatment of clinical infections caused by these bacteria very challenging (Paterson, 2000).

The specific mechanisms of resistance to the antimicrobials of interest in this study are detailed below.

1.4.1 β -lactam resistance

The predominant mechanism of resistance to β -lactam antimicrobials in Gram-negative bacteria is production of β -lactamase enzymes. First described in 1940 (Abraham and Chain, 1988), these enzymes hydrolyse the central β -lactam ring conserved in this class of antimicrobials, breaking open the ring rendering the drug inactive. Over 890 unique β -lactamase protein sequences have been identified (Bush and Jacoby, 2010) and these enzymes are most commonly encoded by genes located on transferrable plasmids, however they can also be chromosomal in origin (Livermore, 1995). While numerous classification systems have been proposed for these enzymes, two have predominated. Firstly, the Ambler

system, classifying the enzymes into four groups (A-D) based on their amino acid sequences. Groups A, C and D are defined as serine β -lactamases and members of Group B are defined as metallo- β -lactamases which require metal ions, commonly zinc, as enzymatic cofactors (Ambler, 1980; Hall and Barlow, 2005). Secondly, a functional classification system has been defined which categorises enzymes into three clusters (Groups 1-3) based on similarities in substrate and inhibitor profiles (Bush et al., 1995; Bush and Jacoby, 2010).

The most commonly encountered plasmid-mediated β -lactamase produced by *Enterobacteriaceae* is the TEM-1 enzyme (Livermore, 1995). This enzyme belongs to functional class 2b and confers resistance to broad spectrum penicillins, as well as early generation cephalosporins (Bush and Jacoby, 2010). Penicillin resistance due to TEM-1 was first described in 1965 (Anderson and Datta, 1965; Datta and Kontomichalou, 1965) and is still the most common cause of ampicillin resistance within *E. coli* (Livermore, 1995). In contrast, SHV-1 is the most common β -lactamase identified in *Klebsiella pneumoniae* (*K. pneumoniae*) isolates. Indeed in this species, SHV-1 enzymes can be encoded chromosomally or be plasmid-bound. A precursor of this class of enzymes is found universally in *K. pneumoniae*, suggesting the plasmid-mediated SHV enzymes were likely derived from this species (Chaves et al., 2001; Stürenburg and Mack, 2003).

1.4.1.1 Extended-spectrum β -lactamases

First reported in 1983 (Knothe et al., 1983), ESBLs are defined phenotypically by their ability to confer resistance to penicillins alongside oxyimino-cephalosporins and monobactams, while retaining susceptibility to β -lactam inhibitors, such as clavulanic acid or tazobactam. Four main classes of enzymes are described which are associated with such resistance; TEM, SHV, CTX-M and OXA enzymes. All of these enzymes belong to groups 2be and 2d of the Bush-Jacoby-Medeiros functional classification scheme (Bush et al., 1995), and are members of Ambler molecular class A with the exception of the OXA enzymes which belong to class D (Ambler et al., 1991). Genes conferring an ESBL phenotype are located on mobile genetic elements such as plasmids, which facilitate the transfer of resistance between commensal and pathogenic bacteria of the same or different species. Similarly, resistance genes to other antimicrobial class such as aminoglycosides and fluoroquinolones may also be located on these plasmids. As such, bacteria harbouring ESBL genes may display MDR phenotypes (Lavigne et al., 2006; Paterson and Bonomo, 2005).

1.4.1.1.1 TEM and SHV enzymes

The first ESBL enzyme, designated SHV-2, was described in 1985 (Kliebe et al., 1985). This enzyme differed from SHV-1 by a single amino acid substitution at position 238, with glycine replaced by serine. No further ESBL enzymes were reported until 1988 when Sougakoff et al. described a TEM variant, designated TEM-3, which also had an extended spectrum of activity. This enzyme displayed a high level of homology with the parent TEM-2 enzyme from which it was derived; differing by only two amino acid substitutions (Sougakoff et al., 1988). Following the discovery of these initial enzyme variants, over 150 TEM and SHV-type ESBLs have been reported (Bush and Palzkill, 2015). These enzyme variants all share a high level of homology with their parent enzymes and only require a small number point mutations within the *bla* gene in order to enhance their spectrum of activity (Bradford, 2001).

TEM and SHV enzymes have been identified in *E. coli* isolates from humans and animals, including dogs, globally (Bradford, 2001; Briñas et al., 2003; Teshager et al., 2000; Tuerena et al., 2016) and were the most prevalent ESBL enzymes throughout the 1990's before being superseded by the cefotaximase (CTX-M) enzymes in the early part of the 21st Century (Livermore et al., 2007; Valentin et al., 2014).

In addition to the TEM and SHV mutants conferring an ESBL phenotype, some TEM and SHV variants confer resistance to β -lactamase inhibitors including clavulanic acid and sulbactam (Bonomo et al., 1997; Chaibi et al., 1999). Most commonly identified in Europe, over 50 of these inhibitor-resistant TEM (IRT) enzymes have been reported (Bush and Palzkill, 2015), differing in structure to the parent TEM-1 and TEM-2 enzymes by one to three amino acid substitutions (Bradford, 2001). IRTs do not generally convey resistance to oxyimino-cephalosporins; however, some variants, such as TEM-50, have amino acid substitutions common to both ESBLs and IRT enzymes. These enzymes, are assigned to the functional class 2ber by Bush and Jacoby (2010), due to their ability to confer resistance to both third generation cephalosporins (3GC) and β -lactamase inhibitors (Robin et al., 2005; Sirot et al., 1997).

1.4.1.1.2 CTX-M enzymes

A non-TEM, non-SHV ESBL was first reported in faecal *E. coli* from a laboratory dog in 1988 (Matsumoto et al., 1988). Following reports of a similar enzyme found in clinical *E. coli* isolates, these enzymes were ascribed the name CTX-M due to their propensity to hydrolyse cefotaxime over ceftazidime (Bauernfeind et al., 1990). Distinct from TEM and SHV enzymes,

genes encoding the CTX-M enzymes demonstrate a high level of homology to *bla*_{AmpC} genes of *Kluyvera* species suggesting they may originate from chromosomal genes from this bacterial genus (Barthélémy et al., 1992; Bonnet, 2004; Cantón et al., 2012; Oliver et al., 2001). Currently more than 160 distinct ESBL enzymes have been identified in multiple bacterial genera, including *Escherichia*, *Klebsiella* and *Enterobacter* species (Bush and Palzkill, 2015). These enzymes are categorised into five clusters; CTX-M1, CTX-M2, CTX-M8, CTX-M9 and CTX-M25 based on their amino acid sequences (Livermore et al., 2007). CTX-M enzymes have a worldwide distribution and, while there is some geographic influence determining which enzymes predominate, CTX-M-14 and 15 are the most important globally (Cantón et al., 2012). CTX-M-15, a member of the CTX-M1 cluster, is of particular interest due to its pattern of dissemination, linked to the spread of the pandemic *E. coli* O25b:H4 sequence type (ST) 131 clone, with which it is commonly associated (Coque et al., 2008; Woodford et al., 2011). The UK has mirrored the global pattern for CTX-M emergence; since the presence of CTX-M-9 was first confirmed in 2000, CTX-M enzymes have become the predominant ESBL group identified in both hospital and community-acquired infections (Alobwede et al., 2003; Munday et al., 2004; Woodford et al., 2004). Furthermore, *bla*_{CTX-M-15} has become the most prevalent ESBL gene in humans in the UK (Livermore and Hawkey, 2005). CTX-M enzymes have also been identified from canine clinical isolates (Steen and Webb, 2007; Timofte et al., 2011; Timofte et al., 2014b), as well from faeces of healthy dogs globally including the United Kingdom (Damborg et al., 2015; Rocha-Gracia et al., 2015; Wedley, 2012), indicating the dissemination of these enzymes is not merely restricted to human populations. In contrast to humans however, CTX-M-1 appears to be the predominant CTX-M enzyme in canine clinical and non-clinical isolates (Dierikx et al., 2012; Wedley, 2012; Carattoli et al., 2005), although CTX-M-15 associated with *E. coli* O25b:H4 ST131 has been identified in dogs (Huber et al., 2013; Pomba et al., 2009; Timofte et al., 2014b; Timofte et al., 2014a).

1.4.1.1.3 OXA enzymes

The OXA group of enzymes are most commonly produced by *Pseudomonas aeruginosa* rather than members of the *Enterobacteriaceae*. These enzymes are distinct from TEM, SHV and CTX-M enzymes phylogenetically, belonging to molecular class D and functional class 2d (Ambler, 1980; Bush and Jacoby, 2010). The most prevalent OXA-type β -lactamase in the *Enterobacteriaceae* is OXA-1, a non-ESBL enzyme which has been identified in up to 10% of *E. coli* isolates (Livermore, 1995). OXA-type ESBLs are commonly derived from OXA-2 or OXA-10 β -lactamases and differ in structure to these parent enzymes by one or two amino acid

substitutions, mirroring the evolution of the TEM and SHV enzymes (Danel et al., 1995; Danel et al., 1997; Danel et al., 1999). These enzymes confer a high level of resistance to ceftazidime with potential activity against β -lactamase inhibitors (Bush et al., 1995).

Not all OXA-type enzymes confer an ESBL phenotype; many have hydrolytic activity against carbapenems. Of concern currently is OXA-48 a carbapenemase first isolated in Turkey (Poirel et al., 2004), which has been identified in numerous *Enterobacteriaceae* including *E. coli* and *K. pneumoniae*. Isolates carrying this enzyme are commonly MDR and frequently also carry genes encoding non-OXA ESBL enzymes on the same plasmid. Indeed, clinical isolates displaying this MDR phenotype have been recovered from both humans and dogs (Pfeifer et al., 2012; Stolle et al., 2013).

1.4.1.2 AmpC β -lactamases

In addition to the β -lactamases described above, AmpC enzymes are also of concern. These molecular class C enzymes are distinct from the ESBLs described above, but do have a corresponding serine active site (Ambler et al., 1991). They hydrolyse both oxyimino and 7- α -methoxy-cephalosporins, but are also largely resistant to β -lactamase inhibitors, with the exception of the combination of piperacillin and tazobactam, to which they largely remain susceptible (Bauernfeind et al., 1996a; Bauernfeind et al., 1996b; Philippon et al., 2002). The *bla*_{AmpC} genes are located on the chromosome of many Gram-negative genera; in many bacterial species constitutive AmpC production is present at low levels and is inducible in the presence of β -lactams or clavulanic acid (Hanson and Sanders, 1999; Sanders, 1987; Weber and Sanders, 1990). However, the absence of the *ampR* promoter in *E. coli* means that this induction mechanism is not present in this species (Honoré et al., 1986). Additionally, mutation of the *ampD* promoter region can lead to hyper production of AmpC enzymes in multiple Gram-negative species and is the most common cause of AmpC mediated resistance reported in clinical *E. coli* isolates (Jacoby, 2009).

Transferable *bla*_{AmpC} genes were first reported in 1989 (Bauernfeind et al., 1989; Bauernfeind et al., 1990) and confirmed to be plasmid-bound in 1990 (Papanicolaou et al., 1990). Sequencing of these genes indicated they were closely related to chromosomal *bla*_{AmpC} genes of *Citrobacter* and *Enterobacter* species (Bauernfeind et al., 1996a; Bauernfeind et al., 1996b). To date more than 30 plasmid-mediated AmpC (pAmpC) enzymes have been described and have been categorised into six groups (CITM, DHA, ACC, EBC, FOX and MOX) (Pérez-Pérez and Hanson, 2002). Previous studies suggest *bla*_{CMY-2}, a member of the CITM

group, is the most prevalent of these genes in humans and dogs in the UK and worldwide (Ewers et al., 2012; Wedley et al., 2010; Woodford et al., 2007).

1.4.2 Quinolone resistance

As previously described, the quinolone antimicrobials target the DNA gyrase and DNA topoisomerase IV enzymes in order to prevent bacterial DNA synthesis. It is therefore unsurprising that resistance mechanisms against quinolone drugs target these enzymes. In the case of Gram-negative bacteria including *E. coli*, mutations to genes encoding the DNA gyrase enzyme are most common (Drlica and Zhao, 1997). Single chromosomal mutations led to amino acid substitutions in the quinolone-resistance determining region (QRDR) of the GyrA subunit of DNA gyrase, with mutation hot spots at amino acids 83 and 87 (Ruiz, 2003; Vila et al., 1994). The target alteration caused by these mutations reduces drug affinity and therefore binding of the antimicrobial to the DNA-DNA gyrase complex (Willmott and Maxwell, 1993). Once this initial mutation has reduced the susceptibility of DNA gyrase to the nalidixic acid, additional point mutations in *gyrA*, *gyrB* or *parC* can further enhance the level of resistance to fluoroquinolones (Barnard and Maxwell, 2001; Vila et al., 1994). Further chromosomally encoded mechanisms of resistance to quinolones involve the reduction of the intracellular concentration of these drugs. The cell membrane of *E. coli* contains three main porins OmpA, OmpC and OmpF, which mediate entry of quinolones into the bacterial cell (Hirai et al., 1986). Reduction in the expression of OmpF on the outer membrane has been linked to reduction in uptake and therefore resistance to some quinolones. This resistance is mirrored by reduced susceptibility to other antimicrobials such as β -lactams and tetracycline (Cohen et al., 1989). The chromosomal loci (MarRAB or SoxRS) that regulate OmpF expression also regulate the expression of multiple efflux pumps (Aleksun and Levy, 1997). Therefore, mutations in these loci reduce the cellular concentration of quinolones not only by reducing permeability of the cell to the drug but also by active removal from the bacterial cell.

In addition to these chromosomal mechanisms of resistance, numerous plasmid-mediated quinolone resistance mechanisms have also been described. The first such mechanism of was first described in 1998 (Martínez-Martínez et al., 1998) and the gene responsible was designated the quinolone resistance (*qnr*) gene (Tran and Jacoby, 2002). The Qnr proteins are pentapeptide-repeat proteins, which interact with DNA gyrase and DNA topoisomerase IV preventing quinolone-enzyme binding and leading to consequential drug inhibition (Tran and Jacoby, 2002). Although these proteins only provide low level quinolone resistance, they

appear to facilitate the development of further chromosomal mutations, mirroring the effect of chromosomal *gyrA* mutation (Martínez-Martínez et al., 2003).

To date six families of Qnr proteins have been described; QnrA, QnrB, QnrC, QnrD, QnrS and QnrVC, with over 70 allelic variants reported (Ruiz et al., 2012b). The original function of qnr proteins is unknown, but it has been postulated that they may have served to protect against endogenous DNA gyrase inhibitors (Garrido et al., 1988). Studies suggest these genes may have originated from waterborne Gram-negative microorganisms, with chromosomal proteins of *Shewanella* species displaying over 70% homology with QnrA proteins (Poirel et al., 2005). However, the Qnr proteins are diverse and it is unlikely this is the sole ancestor. Indeed, Ellington and Woodford (2006) hypothesize the role of QnrA as an antitoxin in a plasmid-encoded toxin-antitoxin addiction system analogous to the CcdB/CcdA system located on the F plasmid of *E. coli*. Plasmids carrying *qnrA*, *qnrB* and *qnrS*, genes have been identified globally in isolates of *E. coli* of both human and canine origin and are commonly associated with ESBL genes (Ma et al., 2009; Paterson et al., 2000; Pomba et al., 2009; Shaheen et al., 2013).

The second plasmid-mediated mechanism for quinolone resistance is via enzyme inactivation. Robicsek et al. (2006a) described an aminoglycoside acetyl transferase with additional activity against some fluoroquinolones. This AAC(6′)-Ib-cr variant contains two amino acid substitutions, which enable acetylation of fluoroquinolones, such as ciprofloxacin and norfloxacin while retaining activity against aminoglycosides (Robicsek et al., 2006a). The enzyme has no effect on some quinolones such as nalidixic acid, due to the absence of an amino-nitrogen on the structural piperazinyl ring of these drugs, and furthermore, the resistance conferred to fluoroquinolones is low level. However, when present it has the capacity to increase the selection of chromosomal mutants in similar way to *qnr* genes. Interestingly, when *qnr* and *aac(6′)-Ib-cr* genes are located on the same plasmid, enhanced resistance to fluoroquinolones is observed (Robicsek et al., 2006a). This gene variant is most commonly identified in *E. coli* and is frequently associated with plasmids carrying multiple resistance genes including those carrying *bla*_{CTX-M-15}, associated with the pandemic *E. coli* O25b:H4 ST131 clone (Ruiz et al., 2012a; Timofte et al., 2014b). AAC(6′)-Ib-cr has been found in isolates from humans and companion animals globally and is now more prevalent than Qnr enzymes (Guo et al., 2015; Park et al., 2006; Pomba et al., 2009). In addition, the gene has also been demonstrated in the chromosome of some *E. coli* strains (Ruiz et al., 2012a).

The final mechanism of resistance to quinolone antimicrobials are plasmid-mediated efflux pumps such as quinolone efflux pump QepA, which was first described in 2007 (Périchon et al., 2007; Yamane et al., 2007). The preferred substrates for this pump are ciprofloxacin and norfloxacin, but it has negligible effects on nalidixic acid and other hydrophobic quinolones (Yamane et al., 2007). A second variant of this efflux pump QepA2 has also been described. This enzyme differs from QepA by only two amino acid substitutions and its spectrum of activity is very similar (Cattoir et al., 2008). In common with other plasmid-mediated quinolone resistance mechanisms, this pump confers only low level resistance. The prevalence of *qepA*-type resistance genes is currently low (~1%) in human clinical *E. coli* isolates globally (Ciesielczuk et al., 2013; Kim et al., 2009a; Yamane et al., 2008), however higher levels have been reported in *E. coli* isolated from pig-faeces in China (Deng et al., 2013; Liu et al., 2008). A further efflux pump OqxAB has also been described; first reported in 2003, this is a multi-drug efflux pump capable of removing chloramphenicol, trimethoprim and quinolones from the bacterial cell (Hansen et al., 2007; Sørensen et al., 2003). While this efflux pump has been found on plasmids in clinical *E. coli* isolates, it is most commonly found on the *K. pneumoniae* chromosome (Kim et al., 2009b; Rodriguez-Martinez et al., 2013).

1.4.3 Tetracycline resistance

To date, more than forty-five tetracycline resistance genes have been described; this resistance is most commonly acquired and is conferred by three main mechanisms, drug efflux pumps, ribosomal protection proteins, and drug inactivation (Roberts, 2005). Thus far, 12 tetracycline resistance genes have been identified from isolates of *E. coli* (Roberts, 2016a; Roberts, 2016b) with efflux pumps, such as *tet(A)* and *tet(B)*, the most numerous (Nguyen et al., 2014). Whereas it was once postulated that *E. coli* was only capable of carrying one of these genes (Jones et al., 1992; Mendez et al., 1980), this has since been disproved in isolates of both human and animal origin (Bryan et al., 2004; Olowe et al., 2013; Tuckman et al., 2007).

Drug efflux pumps are the most prevalent mechanism of resistance to tetracyclines within the *Enterobacteriaceae*, including *E. coli*. These efflux pumps are categorised into seven groups based on their amino acid structure, with over 74% homology between proteins in the same group (Thaker et al., 2010). All bar one of the efflux pumps identified in *E. coli* belong to Group One. These proteins contain twelve transmembrane helices and export the tetracycline molecule in exchange for a proton across a concentration gradient (Yamaguchi et al., 1990). Two genes encode this efflux mechanism, one coding the efflux protein and

another coding a repressor. In the absence of tetracycline, the repressor blocks transcription of the efflux protein, however binding of tetracycline to the repressor leads to induction of efflux protein transcription. This system requires very low tetracycline concentrations and is the one of the most sensitive systems of this type described (Hillen and Berens, 1994). Group One efflux proteins are commonly located on transposons present in large plasmids. These plasmids frequently contain further antimicrobial and heavy metal resistance genes and as such, exposure to any of these additional compounds will co-select for tetracycline resistance (Chopra and Roberts, 2001).

Three ribosomal protection proteins have been identified in *E. coli* and of these Tet(M) is the most extensively characterised (Connell et al., 2003). These cytoplasmic proteins reduce the susceptibility of the bacterial cell to tetracycline by binding to the ribosome in order to prevent antimicrobial-ribosomal complexes forming and therefore prevent tetracycline-mediated inhibition of protein synthesis. In the presence of these proteins, bound tetracycline is also released from the ribosome (Burdett, 1996). These proteins have a wider spectrum of resistance to tetracyclines than the efflux proteins discussed above, with the exception of Tet(B), but are less prevalent (Chopra and Roberts, 2001).

The third mechanism of tetracycline resistance in *E. coli* is drug inactivation, a single enzyme Tet(X) has been identified in this species. This protein is a monooxygenase, which is able to hydroxylate the drug, reducing its affinity for the ribosome and leads to drug decomposition (Yang et al., 2004). This enzyme is active against earlier generation tetracyclines but has less of an effect against newer drugs such as tigecycline (Grossman, 2009). An additional tetracycline-specific resistance mechanism, mutation to 16S rRNA has been reported in some bacterial species, but does not appear to be present in *E. coli* (Nguyen et al., 2014; Nonaka et al., 2005). Intrinsic mechanisms for resistance such as multi-drug efflux pumps may also be active against tetracycline; these have a wide range of substrates and are not tetracycline specific (Piddock, 2006).

Resistance to tetracycline is widespread in *E. coli* from both humans and companion animals, and carriage rates of up to 50% have been reported in healthy individuals from both species (Bonten et al., 1992; Bryan et al., 2004; Costa et al., 2008; Schmidt et al., 2015; Wedley et al., 2011). Furthermore, in a study of 1729 human and animal derived clinical *E. coli* isolates dating from 1950-2002, tetracycline resistance was the most prevalent resistance phenotype reported (Tadesse et al., 2012). This resistance is most commonly associated with resistance

determinants *tet(A)* and *tet(B)* in both dogs and humans (Costa et al., 2008; Tuckman et al., 2007; Wedley et al., 2011).

1.4.4 Trimethoprim resistance

Inhibition of bacterial dihydrofolate reductase (DHFR) by trimethoprim is mediated competitive inhibition, and resistance to this drug is most commonly conferred by chromosomal or plasmid-bound variants of the bacterial *dhfr* gene. Two mechanisms of chromosomally derived resistance have been described. Firstly, mutations leading to an absence of bacterial thymidylate synthetase precipitate a bacterial requirement for exogenous thymine. This renders the DHFR enzyme largely redundant and as such, bacteria are unaffected by trimethoprim binding (Huovinen et al., 1995). This mechanism has been described in clinical isolates of *E. coli* and confers a low level of resistance (Maskell et al., 1976). High-level resistance can also be conferred chromosomally through overproduction of the DHFR enzyme; this mechanism is complex and requires multiple mutational events involving both the structural gene and the promoter region. While this high-level resistance has been reported, it is not common in *E. coli* (Huovinen et al., 1995).

The most prevalent mechanism of trimethoprim resistance encountered in *E. coli*, is the production of an additional DHFR enzyme with reduced affinity for trimethoprim, which therefore resists inhibition by the drug (Heikkilä et al., 1990). To date, almost twenty exogenous *dfr* genes have been described and they are typically associated with gene cassettes on plasmids or transposons (Blahna et al., 2006; Cocchi et al., 2007; Heikkilä et al., 1993). The DHFR enzymes have been categorized into three families based on their amino acid sequences, and members of Family 1 and Family 2 share high level of homology, while Family Three is more diverse (Huovinen et al., 1995). The most widespread *dfr* gene amongst Gram-negative bacteria is *dfrA1* and predominates in isolates of both human and animal origin (Blahna et al., 2006; Seputiene et al., 2010). This high prevalence is likely due to the successful spread of its carrier, transposon Tn7, via high frequency insertion into the chromosome of many bacteria including *E. coli* (Craig, 1991). Faecal carriage of trimethoprim-resistant *E. coli* is common in both humans and animals, with levels of up to 40% reported in healthy dogs (Schmidt et al., 2015; Wedley et al., 2011). Few reports describe the carriage of trimethoprim resistance by humans in the UK, though carriage rates of up to 36% have been described in healthy individuals in the Netherlands (Bonten et al., 1992; London et al., 1994).

1.5 Epidemiology of extended-spectrum β -lactamases

1.5.1 Dogs

1.5.1.1 Prevalence of ESBL-producing *E. coli*

Clinically, ESBL-producing isolates have been identified in numerous canine diagnostic samples including, urine, joint fluid, lung tissue and purulent discharges (Gibson et al., 2010b). These isolates are commonly MDR and as such, reduce the treatment options available (Dierikx et al., 2012; Huber et al., 2013). ESBL-producing *E. coli* is most frequently associated with UTIs (Dierikx et al., 2012), and numerous studies indicate that approximately 3% of isolates causing UTI in dogs are ESBL-producers (Dierikx et al., 2012; Huber et al., 2013; Shaheen et al., 2011). However, O' Keefe et al. (2010) report a prevalence of 7.3% in urinary isolates in a veterinary referral hospital in the US. Studies by both Dierikx et al. (2012) and Shaheen et al. (2011) report the a high prevalence of *bla*_{CTX-M-1} among ESBL-producing clinical isolates in Holland and the US, though Huber et al. (2013) identified *bla*_{CTX-M-15} in all ESBL-producing isolates causing UTIs from dogs in Switzerland. Furthermore, though they do not predominate, some clinical isolates from dogs in the UK and overseas have been identified as the MDR *E. coli* O25b:H4 ST131 human pandemic clone suggesting transfer of clinical isolates between species may occur (Ewers et al., 2010; Johnson et al., 2009b; Pomba et al., 2009; Timofte et al., 2014b).

Whilst awareness of the role ESBL-producing isolates play in clinical disease is vital, knowledge of ESBL production by commensal bacteria is also of importance due to the ability of these organisms to act as a reservoir of resistance determinants. First reported in Japan in 1988, canine faecal carriage of ESBL-producing *E. coli* has since been identified in diverse canine populations across the globe (Matsumoto et al., 1988). Within the UK, a small number of studies have investigated the faecal carriage of AMR *E. coli* in healthy dogs. Wedley et al. (2011) reported a prevalence of AMR, AmpC and ESBL-producing *E. coli* of 29.0%, 7.1% and 0.5%, respectively in 183 healthy community based dogs, while Schmidt et al. (2015) reported corresponding carriage rates of 63.0%, 16.4% and 1.3% in 73 healthy Labrador retriever show dogs. These studies may indicate that the overall prevalence of antimicrobial resistance and AmpC-producing *E. coli* are increasing in the UK, while carriage of ESBL-producing *E. coli* remains low in healthy animals. However, caution is advised when comparing these two studies due to their differing sample populations. Two further UK studies have been undertaken investigating carriage rates in hospitalised and vet-visiting dogs and the populations sampled offer a direct comparison to the study undertaken by

Wedley et al. (2011). Tuerena et al. (2016) found that 50.1% of 333 faecal samples from hospitalised dogs carried AMR *E. coli*, while 8.6% and 14% carried AmpC and ESBL-producers, respectively. Similarly, Wedley (2012) describes increased carriage rates of 44.8%, 7.1% and 4.1% respectively in 581 vet visiting dogs. These studies suggest that animals visiting veterinary practices may face an increased selection pressure for the development of antimicrobial resistance, which is further exacerbated during a period of hospitalisation. Within all of these studies, resistance to ampicillin was reported most frequently with resistance to tetracyclines and trimethoprim also prevalent in non-hospitalised dogs (Schmidt et al., 2015; Tuerena et al., 2016; Wedley et al., 2011; Wedley, 2012). Resistance to 3GCs was markedly higher in hospitalised animals when compared to other comparable populations and is likely representative of a differing selection pressures in these groups of animals. This may be attributable to an increased use of 3GCs in a hospital environment, mirroring prescribing patterns seen in human medicine (PHE, 2015; Tuerena et al., 2016; Wedley et al., 2011; Wedley, 2012). The predominant mechanism conferring resistance to 3GCs in UK dogs appears to be AmpC-mediated resistance conferred by *bla*_{CMY-2}. There is however, some discrepancy over the most common mechanism mediating ESBL-production. Wedley (2011; 2012) reports the predomination of *bla*_{CTX-M-1}, while *bla*_{CTX-M-15} is the most prevalent ESBL genotype described by Tuerena et al. (2016). This may indicate a temporal change in the prevalence of ESBL genes in the UK canine population, or signify the potential difference in circulating strains between community and hospitalised populations.

Out with the UK, numerous studies have investigated the prevalence of carriage of AmpC and ESBL-producing *E. coli* in healthy dogs, indicating variable carriage rates of 0.0-20.0% and 0.0-30.0% respectively (Table 1.1). Of particular concern were the levels of carriage of both these resistance mechanisms in the Netherlands, which were markedly higher than those reported elsewhere in Europe (Hordijk et al., 2013). Interestingly raw food diets, often associated with faecal carriage of AMR *E. coli* (Baede et al., 2015; Schmidt et al., 2015; Wedley, 2012) are popular in the Netherlands and up to 60% of animals participating in a longitudinal study by Baede et al. (2015) were fed this diet, compared to 4.0% reported by Wedley (2012) in the UK. In contrast to the findings from the UK, the prevalence of ESBLs in Europe was higher than AmpC enzymes in all studies where both were investigated. However, throughout Europe the predominant genes causing an AmpC or ESBL phenotype in healthy dogs were *bla*_{CTX-M-1} and *bla*_{CMY-2}, mirroring the findings of Wedley et al. (2011; 2012) (Table 1.1). Throughout the rest of the world, the prevalence of carriage was again variable. No resistance to 3GCs was detected in healthy Chilean dogs (n=15) by Moreno et

al. (2008), mirroring the absence of resistance to these antimicrobials reported in healthy dogs in Japan (Harada et al., 2012). In Canada, Murphy et al. (2009) report a prevalence of carriage of ESBL-producing *E. coli* of 1.1% with no AmpC-producing isolates identified, mirroring the findings in many other western countries (Costa et al., 2008; Damborg et al., 2015). Meanwhile faecal carriage rates in Tunisia and Kenya of 12.1% and 21.7%, respectively were reported (Albrechtova et al., 2012; Ben Sallem et al., 2011). While *bla*_{CTX-M-1} and *bla*_{CMY-2} remain prevalent in most countries, *bla*_{CTX-M-15} associated with *aac(6')-Ib-cr* was highly prevalent in Kenya (Albrechtova et al., 2012).

When considering carriage rates in animals receiving veterinary treatment, there is an inevitable increase in the prevalence of resistance described (Table 1.1), due to the increased selection pressure for development and acquisition of resistance determinants (Levy and Marshall, 2004). Indeed, Damborg et al. (2011) report AmpC-producing *E. coli* in 61.5% of dogs receiving antimicrobial treatment for pyoderma in Denmark, while 35.0% and 30.0% of Dutch diarrhoeic dogs carried AmpC and ESBL-producing *E. coli*, respectively (Hordijk et al., 2013). Meanwhile a lower prevalence of carriage (7.6% AmpC and 0.0% ESBL) was described in Swedish dogs on admission to surgical wards at a referral hospital (Johard et al., 2015). These studies demonstrate a predominance of AmpC over ESBL-producing *E. coli* in dogs treated with antimicrobials. This difference is likely attributable to the use of cephalexin for the treatment of pyoderma in the study by Damborg et al. (2011), alongside the frequent use of amoxycylav for empiric treatment of infections in small animal practice globally (Hughes et al., 2012; Pleydell et al., 2012). Both of these drugs have been shown to increase selection for AmpC-producing *Enterobacteriaceae* (Damborg et al., 2011; Seiffert et al., 2013a). Interestingly, while *bla*_{CMY-2} remains the most prevalent pAmpC, *bla*_{CTX-M-14} was the most prevalent ESBL in diarrhoeic dogs. This may suggest circulating strains differ between sick and healthy animals in the same environment. In contrast the previous studies, Moreno et al. (2008) reported faecal carriage of ESBL-producing *E. coli* in 33.3% of pets treated with enrofloxacin, but an absence of AmpC-producing isolates. The frequent co-localisation of ESBL and fluoroquinolone resistance genes on the same plasmid may offer an explanation for this discrepancy with other studies where fluoroquinolones were not prescribed (Lavigne et al., 2006). The studies discussed indicate a variation in prevalence and resistance mechanisms observed globally. This variation is likely due to many factors including true national variation in carriage rates and circulating ESBL-producing strains as well differing selection and microbiological methods used in different studies, which may have affected detection rates.

Table 1.1 Global reports of faecal carriage of pAmpC and ESBL-producing *E. coli* isolated from non-UK dogs

Population	N	Prevalence (%)		ESBL/AmpC genes identified	Country	Notes	Reference
		AmpC	ESBL				
Healthy owned and kennelled dogs	49	2.0	6.1	<i>bla</i> _{CTX-M-1} (n=3) <i>bla</i> _{CMY-2} (n=1)	Italy		Carattoli et al. (2005)
Healthy dogs	39	0.0	10.3	<i>bla</i> _{TEM-52b} (n=3) <i>bla</i> _{CTX-M-1} (n=1)	Portugal	No antimicrobials previously	Costa et al. (2004)
Healthy vet visiting dogs	39	0	2.6	<i>bla</i> _{CTX-M-1} (n=1)	Portugal	No antimicrobials within 4 months	Costa et al. (2008)
Faecal samples in parks	209	0.0	1.9	<i>bla</i> _{CTX-M-1} (n=3) <i>bla</i> _{CTX-M-15} (n=2)	Denmark		Damborg et al. (2015)
Dogs with pyoderma	13	61.5	0.0	<i>bla</i> _{CMY-2} (n=8)	Denmark	Dogs treated with cephalexin	Damborg et al. (2011)
Nursing home and vet visiting dogs	174	NA	2.5		Switzerland	Specific genes not typed	Gandolfi-Decristophoris et al. (2013)
Healthy Dogs	368	6.25	13.5	<i>bla</i> _{CTX-M-1} (n=32) <i>bla</i> _{CMY-2} (n=23) <i>bla</i> _{CTX-M-15} (n=5) <i>bla</i> _{SHV-12} (n=3) <i>bla</i> _{CTX-M-32} (n=1) <i>bla</i> _{CTX-M-3} (n=1)	France	3 dogs expressed both AmpC and ESBL <i>E. coli</i> 8 isolates carried <i>bla</i> _{CTX-M-Group 9} but genes were not typed	Haenni et al. (2014)
Healthy dogs	20	20.0	30.0	<i>bla</i> _{CTX-M-1} (n=5) <i>bla</i> _{CMY-2} (n=3)	Netherlands	Previous history unknown	Hordijk et al. (2013)
Diarrhoeic dogs	20	35.0	30.0	<i>bla</i> _{CTX-M-1} (n=5) <i>bla</i> _{CTX-M-14} (n=14) <i>bla</i> _{CMY-2} (n=3)	Netherlands	Faecal samples submitted for diagnostic testing	Hordijk et al. (2013)
Surgical admissions	66	7.6	0.0	<i>bla</i> _{CMY-2} (n=4)	Sweden	2 dogs were <i>bla</i> _{CMY-2} positive 16 months post op	Johard et al. (2015)
Dogs in the community	216	NA	21.7	<i>bla</i> _{CTX-M-15} (n=47)	Kenya	All isolates also carried <i>aac(6')-Ib-cr</i>	Albrechtova et al. (2012)
Healthy vet visiting dogs	41	2.4	12.1	<i>bla</i> _{CTX-M-1} (n=5) <i>bla</i> _{CMY-2} (n=1)	Tunisia	<i>Bla</i> _{CTX-M-1} most prevalent amongst humans Tunisia	Ben Sallem et al. (2013)
Owned dogs	34	0.0	0.0	Not typed	Japan		Harada et al. (2012)
Healthy dogs	15	0.0	0.0		Chile		Moreno et al. (2008)
Enrofloxacin treated dogs and cats	15	0.0	33.3	<i>bla</i> _{CTX-M-1} (n=4) <i>bla</i> _{CTX-M-14} (n=1) <i>bla</i> _{PER-2} (n=1)	Chile		Moreno et al. (2008)
Vet visiting dogs	188	1.1	0.0	<i>bla</i> _{CMY-2} (n=2)	Canada	No antimicrobials within 6 weeks	Murphy et al. (2009)

1.5.1.2 Risk factors for canine carriage of ESBL-producing *E. coli*

Multiple studies have investigated potential risk factors associated with carriage of AMR and ESBL-producing *E. coli* in dogs. The risk factors identified by these studies can largely be split into two sections, lifestyle and veterinary treatment.

Animals in areas of high dog density such as those attending a doggy day care or housed in kennel environments are reported to be at increased risk of carriage of ampicillin-resistant (Procter et al., 2014) and 3GC-resistant (Belas et al., 2014) *E. coli*. Given inter-dog transmission of *E. coli* previously reported (Johnson et al., 2008b), it is unsurprising that animals in dog-dense locations may be more likely to share faecal *E. coli* isolates including those harbouring resistance determinants. The canine diet has also been reported to have an impact on carriage rates of resistant *E. coli*, with consumption of raw food diets highlighted as a risk factor for ciprofloxacin and amoxycylav-resistant *E. coli* as well as resistance to 3GCs and MDR phenotypes (Schmidt et al., 2015; Wedley, 2012). Interestingly, Procter et al. (2014) also reported that feeding commercial dry or home-cooked diets was protective. Given the presence of AMR *Enterobacteriaceae* previously reported in uncooked food products (Dhanji et al.; Finley et al., 2008; Nilsson, 2015); this may suggest that the cooking of these food products prevents the transmission and colonisation by these resistant bacteria which occurs following ingestion. In addition to foodstuffs, the consumption of water from a toilet bowl has also been highlighted as a potential risk factor for carriage of fluoroquinolone-resistant bacteria (Stenske et al., 2009).

The signalment of an animal also appears to have some influence on carriage rates, with large breed dogs more likely to carry AMR and MDR isolates than small breed dogs (Procter et al., 2014; Wedley, 2012). This is likely related to behavioural differences between these breeds, as off lead walking has also been reported to increase the risk of carriage of resistant *E. coli* (Wedley, 2012), and anecdotal evidence suggests these breeds are more likely to be fed raw food diets. Similarly, Wedley (2012) reported that neutered animals were at increased risk of carriage of ESBL-producing *E. coli*, it is however possible that this is an indicator of levels of veterinary treatment, with neutered animals more likely to have received additional veterinary treatment, including antimicrobials, than those who are not. The same author also reported an association between AMR *E. coli* carriage and contact with farm animals. Given farm animals have previous been highlighted as a potential reservoir of antimicrobial resistance determinants (Carattoli, 2008; Costa et al., 2009b), it is not

surprising that contact with these animals, their faeces, or the farm environment may lead to acquisition of resistant bacteria.

Certain aspects of veterinary treatment have also been associated with carriage of AMR *E. coli*; hospitalisation is predominantly associated with multi-drug resistance (Gibson et al., 2011a; Gibson et al., 2011b; Hamilton et al., 2013), but has also been identified as a risk factor for ampicillin, amoxycylav and nalidixic acid resistance (Ogeer-Gyles et al., 2006). There is some discrepancy over the duration of hospitalisation associated with carriage of MDR *E. coli*; Hamilton et al. (2013) report hospitalisation of greater than three days duration, while (Gibson et al., 2011a) report a duration greater than six days. It is likely that this difference is due to differences in the hospitals and study populations in the two studies and therefore makes any generalisation difficult. Treatment with antimicrobials has been commonly identified as a driver for antimicrobial resistance and as such, numerous studies have found that antimicrobial treatment is a risk factor for antimicrobial resistance in canine commensal *E. coli* (Damborg et al., 2011; Gronvold et al., 2010; Lawrence et al., 2013; Wedley, 2012). Commonly, use of a particular antimicrobial is associated with resistance in the same class; Lawrence et al. (2013) report the use of cefovecin selecting for resistance to β -lactams, particularly resistance mediated by *bla*_{CMY-2}. The use of cephalexin is associated with both MDR and AmpC-producing isolates (Damborg et al., 2011; Gibson et al., 2011a). Similarly, the use of enrofloxacin is linked with recovery of quinolone-resistant isolates from treated dogs (Ogeer-Gyles et al., 2006). In addition, the use of this drug was also associated with the carriage of MDR and ESBL-producing *E. coli* (Moreno et al., 2008; Trott et al., 2004). The lifestyle and veterinary risk factors identified by previous studies suggest a complex relationship between animals, humans and the environment, which influences the acquisition and dissemination of antimicrobial resistance.

1.5.2 Humans

1.5.2.1 Prevalence of ESBL-producing *E. coli*

Since 2001, an increasing trend of nosocomial and community-acquired infections associated with ESBL-producing *Enterobacteriaceae* has been identified in the UK and Ireland (Fennell et al., 2012; Livermore et al., 2008). The majority of infections associated with ESBL production are UTIs or bacteraemias caused by *E. coli* and *K. pneumoniae*. Current data suggest that in England 11.1% of bacteraemias caused by *E. coli* in 2014 displayed resistance to 3GCs, increasing from 8% the previous year. However the mechanism of resistance was not reported (PHE, 2015). In addition, 10-20% of community and hospital-derived *E. coli*

isolates from UTIs displayed resistance to these antimicrobials, (PHE, 2015). While coincident increasing trends in the prevalence of resistance have also been seen in Europe, extensive national variation in the prevalence of invasive *E. coli* isolates resistant to 3GCs is reported, ranging from 3.3% in Iceland to 40.4% in Bulgaria (ECDC, 2015). In contrast to dogs, 3GC-resistant infections in humans are most commonly mediated by the production of ESBLs rather than pAmpC enzymes (Jacoby, 2009). Globally, the highest prevalence of nosocomial infections caused by ESBL-producing *E. coli* is reported in Latin America and Asia, while North America has the lowest prevalence (Pitout and Laupland, 2008). The *bla*_{CTX-M} genes are the most frequently identified ESBL genes in clinical isolates (Livermore et al., 2007; Livermore et al., 2008). In the UK, Woodford et al. (2004) report the predominance of *bla*_{CTX-M-15} in clinical isolates of both community and hospital origin, highlighting the predominance of this gene domestically. This finding has been mirrored by numerous further studies in the UK and overseas and is linked to the global dissemination of the MDR pandemic *E. coli* O25b:H4 ST131 clone (Moubareck et al., 2005; Nicolas-Chanoine et al., 2008; Peirano et al., 2010; Rogers et al., 2011; Song et al., 2009; Yumuk et al., 2008).

Due to the importance of ESBL-producing *Enterobacteriaceae* as pathogens, most UK studies focus on clinical isolates and papers reporting faecal carriage of ESBL-producing *Enterobacteriaceae* in the community are scarce. There are however, two UK-based studies, which have reported faecal carriage of ESBL-producing *E. coli* in the community. Wickramasinghe et al. (2012) reported a faecal prevalence of ESBL carriage of 11.3% (n=723) in GP and hospital outpatients in Birmingham and highlighted a carriage rate 2.8 times higher in patients originating from the Middle East and South Asia versus those from Europe (22.8% vs. 8.1%). The study also identified *bla*_{CTX-M-15} as the most prevalent β -lactamase gene conferring resistance. Munday (2004) reported a prevalence of 1.9% (n=1000) in community and hospitalised patients, with *bla*_{CTX-M-9} most prevalent. Direct comparison of these two studies is difficult, Wickramasinghe et al. (2012), report a sample prevalence, while Munday reports an isolate prevalence. However, the differences in reported prevalence between the two studies may indicate a temporal increase in the faecal carriage of ESBL-producing *Enterobacteriaceae*, or may be due to geographic or demographic differences in the study populations. Furthermore, while these studies may offer some insight into community carriage rates of ESBL-producing *E. coli* they exclude healthy individuals and as such, only give an insight into community-based patient carriage of these bacteria. Of interest however, is the molecular epidemiology of ESBL-producing isolates in each study. While *bla*_{CTX-M-9} was the most prevalent gene reported in 2004 (Munday et al., 2004), *bla*_{CTX-M-15} was most

prevalent in 2012 (Wickramasinghe et al., 2012), mirroring the clinical dissemination of *bla*_{CTX-M} genes in the UK over the same period (Hawkey and Jones, 2009; Livermore et al., 2007).

More widespread studies sampling healthy volunteers have been undertaken throughout Europe, indicating carriage rates of 2.0-8.6% (Table 1.2). These studies suggest regional and national variations in carriage of ESBL-producing *Enterobacteriaceae*, and of concern is the increased level of carriage by healthy individuals in the Netherlands when compared to other countries (Reuland et al., 2016). The predominant genes associated with an ESBL phenotype throughout Europe were the *bla*_{CTX-M} genes, with *bla*_{CTX-M-15} and *bla*_{CTX-M-1} dominating in all countries except Spain, where *bla*_{CTX-M-14} and *bla*_{SHV-12} were most commonly identified (Reuland et al., 2016; Rodrigues et al., 2016; Valverde et al., 2008; Valenza et al., 2014). Numerous studies have also investigated faecal colonisation of hospitalised and community-based patients with ESBL-producing bacteria and report higher carriage rates in these patients (Table 1.2). Reuland et al. (2013) detected ESBL-producing *Enterobacteriaceae* in 10.1% of diarrhoeic patients (n=720) and *bla*_{CTX-M-15} was identified most frequently, mirroring the findings of Wickramasinghe et al. (2012) in the UK. Valverde et al. (2004) report ESBL-producing *Enterobacteriaceae* carriage in 7.0% of Spanish patients, double that of healthy volunteers and Stomdahl et al (2011) detected an increase of the same magnitude in Swedish patients when compared to a healthy population (6.8% vs 3.0%).

Throughout the rest of the world, the prevalence of carriage in healthy volunteer populations was again variable. Studies in Lebanon, Japan and Tunisia reported faecal carriage rates of 2.4%, 6.4% and 7.3% respectively and identified CTX-M-15 and CTX-M-1 as the predominant enzymes, mirroring reports from Europe (Ben Sallem et al., 2011; Luvsansharav et al., 2011; Moubareck et al., 2005). However, reports from the rest of Asia and Latin America indicate these regions have increased rates of carriage. In Latin America, Villar et al. (2013) report faecal carriage of ESBL-producing *Enterobacteriaceae* in 26.8% (n=164) of healthy volunteers in Argentina, while Colquechagua et al. (2015) detected ESBL-producing *E. coli* in the faeces of 64.2% (n=235) of children in Peru. While in Asia, 20.3% (n=290) of healthy Korean volunteers carried ESBL-producing *E. coli* in faeces (Ko et al., 2013b) and 58.2% of healthy adult participants (n=141) carried CTX-M β -lactamase-producing *Enterobacteriaceae* (Sasaki et al., 2010).

Table 1.2 Reports of faecal carriage of ESBL-producing *E. coli* isolated from humans in Europe

Population	n	Prevalence (%)	Prevalent ESBL genes (% of isolates)	Country	Notes	Reference
Healthy volunteers	232	3.5	Not reported	Germany	Conference attendees	Meyer et al. (2012)
Healthy volunteers	100	3.0	<i>bla</i> _{CTX-M-1} (n=11)	Sweden	No details of genes carried by participant types	Strömdahl et al. (2011)
Hospital patients	118	6.8	<i>bla</i> _{CTX-M-9} (n=4) <i>bla</i> _{SHV} (n=1)			
Healthy volunteers	105	6.6	<i>bla</i> _{CTX-M-14} (n=2) <i>bla</i> _{CTX-M-1} (n=1) <i>bla</i> _{SHV-12} (n=2)	Spain	<i>E. coli</i> only No antimicrobials within 3 months	Vinué et al. (2009)
Healthy volunteers	108	3.7	<i>bla</i> _{CTX-M-14} (n=1) <i>bla</i> _{CTX-M-2-like} (n=1)	Spain		Valverde et al. (2004)
Hospital patients	286	7.0	<i>bla</i> _{CTX-M-14} (n=9) <i>bla</i> _{SHV-12} (n=7) <i>bla</i> _{CTX-M-9} (n=6)	Spain		Valverde et al. (2004)
Adult population of Amsterdam	1695	8.6	<i>bla</i> _{CTX-M-15} (n=60) <i>bla</i> _{CTX-M-1} (n=26) <i>bla</i> _{CTX-M-14} (n=19) <i>bla</i> _{CTX-M-15} (n=34)	Netherlands	Excluded hospitalised and terminally ill patients	Reuland et al. (2016)
Community patients	720	10.1	<i>bla</i> _{CTX-M-14} (n=8) <i>bla</i> _{SHV-12} (n=8) <i>bla</i> _{CTX-M-15} (n=14)	Netherlands	Patients all had gastroenteritis	Reuland et al. (2013)
Healthy volunteers	586	5.8	<i>bla</i> _{CTX-M-1} (n=10) <i>bla</i> _{CTX-M-14} (n=7)	Switzerland	Workers in meat processing company	Geser et al. (2012a)
Healthy children	112	2.7	<i>bla</i> _{CTX-M-1} (n=1) <i>bla</i> _{SHV-12} (n=1)	Portugal	<i>E. coli</i> only	Guimaraes et al. (2009)
Healthy adults	1033	5.1	Not reported	Netherlands		Huijbers et al. (2013)
Healthy adults	3344	6.3	<i>bla</i> _{CTX-M-15} (n=97) <i>bla</i> _{CTX-M-1} (n=51) <i>bla</i> _{CTX-M-14} (n=31)	Germany	<i>E. coli</i> only Contact with gastroenteritis patients	Valenza et al. (2014)
Healthy volunteers	199	2.0	<i>bla</i> _{CTX-M-14} (n=4) <i>bla</i> _{CTX-M-27} (n=2)	Portugal	First report of <i>bla</i> _{CTX-M-27} in Portugal Two isolates carried <i>bla</i> _{CTX-M-14} and <i>bla</i> _{CTX-M-27}	Rodrigues et al. (2016)

1.5.2.2 Risk factors for human carriage of ESBL -producing *E. coli*

The majority of studies considering risk factors related to ESBL-producing bacteria are concerned with clinical infections. However, risk factors for faecal carriage of ESBL-producing *E. coli* have been reported in a small number of studies and as previously discussed in dogs can be split into two broad classes: medical and non-medical. Most commonly identified as a risk factor for colonisation with resistant bacteria is prior antimicrobial treatment, with the use of β -lactamase inhibitor combinations and critically important antimicrobials such as 3GCs highlighted as a particular risk (Friedmann et al., 2009; Harris et al., 2007; Razazi et al., 2012; Reuland et al., 2016). Prior hospitalisation, particularly in higher dependency wards such as intensive care or step down units (Ko et al., 2013b; Zahar et al., 2010), alongside a short duration between discharge and readmission (Zahar et al., 2010) also increase the risk and duration of intestinal colonisation with ESBL-producing bacteria. Indeed, patients who are hospitalised on numerous occasions and in higher dependency wards will not only be more likely to receive medical treatment including antimicrobials that will select for resistant commensal *Enterobacteriaceae*, but will also be more likely to encounter resistant nosocomial isolates, which may colonise. Additionally, Razazi et al. (2012) report undergoing surgery to be associated with ESBL-producing *E. coli* carriage however, this association may be due to the hospitalisation and potential antimicrobial treatment associated with surgeries rather than the procedure itself. A small number of studies also highlighted chronic conditions including diabetes and skin disease as risk factors for carriage of ESBL-producing isolates (Harris et al., 2007; Huijbers et al., 2014). Often chronic conditions such as diabetes are associated with increased risk of infection (Muller et al., 2005), as such these patients will be exposed to more antimicrobial treatment than healthy individuals.

Of the non-medical variables associated with increased risk of intestinal carriage of ESBL-producing *Enterobacteriaceae*, travel to a country in South and South East Asia was identified most frequently (Angelin et al., 2015; Östholm-Balkhed et al., 2013; Reuland et al., 2016; Tängdén et al., 2010). Given the high prevalence of colonisation of individuals from these countries with resistant bacteria (Ko et al., 2013b; Sasaki et al., 2010), this association is not surprising. Similarly, having a relative with an infection caused by an ESBL-producing isolate also increases the risk of carriage and is likely due to transmission of the resistant organism between individuals (Rodríguez-Baño et al., 2008). In addition, two studies identified an increased risk of colonisation related to increasing age (Harris et al., 2007; Östholm-Balkhed et al., 2013). This factor may however be a proxy for medical treatment; given the increasing

number of co-morbidities and chronic conditions requiring treatment as one ages (Davis et al., 2011), it is likely that older individuals have increased medical treatment, which may have increased their risk of carriage. Indeed, antimicrobial prescribing is increased in patients over 65 when compared to other adults in the UK, Europe and the US (Haeseker et al., 2012; Lee et al., 2014; Majeed and Moser, 1999). Finally, contact with animals has been reported to increase the risk of faecal carriage of resistant *Enterobacteriaceae*. Huijbers et al. (2014) report increased contact with broiler chickens as a risk factor, while Meyer et al. (2012) report owning a pet increases the risk of intestinal colonisation with 3GC-resistant *E. coli*. These reports suggest that farm and companion animals could act as a reservoir of resistant bacteria.

1.6 Dog ownership and the transfer of resistance

Current data suggest the UK dog population totals nine million dogs, with 24% of households owning at least one dog (PFMA, 2015). This ownership has been reported to have numerous potential physical and mental health benefits including increased physical activity and stress reduction in pet owners versus non pet owners (Beetz et al., 2012; Christian et al., 2013). In addition to these positive aspects of dog ownership however, is the potential for transfer of zoonoses between owners and pets. Indeed, Westgarth et al. (2008) highlighted a number of situations which may play an important role in this transfer, including the frequent use of the kitchen as a pet sleeping area, allowing pets to lick the hands or face, manual disposal of pet faeces and a potential lack of hand hygiene after handling pets (Westgarth et al., 2008). The high levels of contact between owners and pets alongside increasing levels of resistance in human and animal populations may therefore provide an opportunity for dissemination of resistance determinants between these two populations.

While numerous studies have identified ESBL genes of human importance in companion animals (Ewers et al., 2014; Ewers et al., 2010; Pomba et al., 2009; Timofte et al., 2014b), the evidence for transmission of resistance between these species is sparse, with only a few studies investigating transmission dynamics between humans and dogs. Carvalho et al. (2016) reported isolation of MDR *E. coli* with identical macro-restriction pulsed field gel electrophoresis (PFGE) profiles from 9.5% (n=42) of dog-owner pairs in Brazil indicating sharing of strains between owners and their pets. This is in agreement with a previous study reporting transfer of *E. coli* between owners and their dogs in 8.8% (n=34) of Japanese households sampled. Furthermore, this study reported that resistant *E. coli* carried by dog owners to be more similar to their dogs than non-dog owners (Harada et al., 2012). Johnson

et al. (2008b) report within-household sharing of faecal *E. coli* in 68% of pet-owning households. While sharing of *E. coli* strains between humans and pets occurred in 17% of households, intra-species sharing was more prevalent (pet-pet sharing in 58% and human-human sharing in 31% of households). These studies all indicate the potential of transmission between owners and pets, although no temporal relationships were described and as such directionality cannot be ascertained.

Two longitudinal studies have been undertaken however which may offer more insight; Damborg et al. (2009) sampled all members of eight dog owning households at ten time points over a six month period. In this study, 19 *E. coli* clones were shared within six households, seven of which indicated inter-species transmission. While for six of these clones, transmission could not be proven, the authors inferred a transmission event from a human to a dog for a single clone, due to the previous shedding patterns of individuals in this household. The final study offers the most robust evidence for transmission of *E. coli* between humans and dogs. Johnson et al. (2008a) sampled a five person, one dog household for a period of three years. During this time period both a human and the dog had UTIs independently caused by two separate *E. coli* clones. During each of these infections, four out of five other household members also became colonised by these strains. The *E. coli* strain causing the canine UTI was isolated first from the dog, then other household members the following week. This temporal pattern of isolation suggests dog to human transmission of this clone occurred. The direction of transmission of the human UTI-causing clone was however not obvious as both the patient and dog were colonised by this isolate on the first sampling date (Johnson and Clabots, 2006; Johnson et al., 2008a).

These studies suggest that while dogs may act as a reservoir of resistance determinants, direction of transmission is not always clear and may occur in both directions and as such, this dynamic relationship requires further investigation.

1.7 Study aims

While faecal carriage rates of AMR and ESBL-producing *E. coli* have been described in hospitalised and community human populations, there is a paucity of data describing carriage of these bacteria in healthy humans in the UK. Previous studies suggest the mechanisms of resistance identified in bacterial isolates from dogs mirror those recognised in humans and other animal species. These animals have therefore been suggested as a potential source of resistance to in-contact humans. To date, no studies have examined the

risk of carriage of AMR and ESBL-producing bacteria in humans with high levels of contact with dogs or the potential transfer of resistant bacteria between these two populations.

The primary aim of this project was to determine the prevalence and risk factors for carriage of ESBL-producing and AMR *E. coli* in humans working with dogs in different environments including staff at boarding kennels, rescue kennels and veterinary practices, as well as in-contact kennelled dogs. Additionally, characterisation of *E. coli* isolates was undertaken in order to investigate the potential for sharing of *E. coli* strains, mobile genetic elements and resistance genes between in-contact human and canine populations. In order to achieve these aims, concurrent cross-sectional studies sampling humans and kennelled dogs within the same premises were undertaken and their results are presented in Chapters Three to Six.

Chapter 2

Materials and Methods

2.1 Study population

2.1.1 Selection of premises

2.1.1.1 Humans working with dogs

Veterinary practices, boarding kennels and rescue centres were identified as premises where people may work with, and therefore have a high level of contact with dogs. These premises formed the study population. Given the nature of the study, premises located within a restricted geographical area, within a 75 mile radius of the University of Liverpool, Leahurst Campus were identified as the sampling frame. Due to the diverse types of premises involved in the study details of appropriate premises were sourced in three ways:

1. Veterinary premises were identified from a list practices located within the geographical area of interest which, in the 2014 Register of Veterinary Practice Premises, indicated that they treated dogs.
2. Local council registers of licensed boarding establishments were used to identify boarding premises.
3. An absence of published lists of rescue kennels necessitated that internet searches for appropriate establishments was undertaken in order to identify rescue kennels based within the study area.

From these defined lists, premises were randomly selected for participation in the study via random number generation. Premises were excluded if they were identified as home boarding establishments rather than kennelled premises. Following sample size calculations, an initial aim to recruit 15 individuals from 22 premises was identified. Ethical approval for this study was granted by the University of Liverpool Veterinary Research Ethics Committee in May 2014 and recruitment was initiated in June 2014.

2.1.1.2 Kennelled dogs

Due to the concurrent nature of sampling of human and canine participants required in this project, premises identified to participate in the study above were also invited to take part in an additional cross-sectional study investigating kennelled dogs. The pre-requisite that kennelled dogs were required for this study necessitated that veterinary premises were excluded from recruitment. Ethical approval for this study was granted by the University of Liverpool Veterinary Research Ethics Committee in April 2014 and recruitment initiated in conjunction with the study above in June 2014.

2.1.2 Participant recruitment

2.1.2.1 Humans

Randomly selected premises were initially contacted by telephone in order to identify the correct person to address. Following contact with the identified individual, the principal investigator explained the aims of the study and gave a brief summary of what it entailed. Additional provision of written information was offered to premises via fax or email. Where the appropriate individual could not be contacted after three attempts, or an invitation for premises participation was declined, an additional premises was randomly selected for participation. Due to low response rates, a total of 369 premises were contacted and recruitment of 69 premises was required. Recruited premises were visited by the principal investigator in order to meet with staff members, alongside delivery of sample packs. Potential participants were provided with information about the study verbally and in written form. In order to maximise the response rate, participation in the study was anonymous leading to an absence of written consent forms. It was therefore made overt to participants during recruitment meetings and in written documentation that provision of a faecal sample and submission of the corresponding questionnaire constituted consent for their data to be used in the study (Appendix I). All sample packs contained a unique reference number, which could be provided to the principal investigator should a participant wish to withdraw their data or sample from the study.

2.1.2.2 Kennelled Dogs

Staff at kennel premises were provided with information about the canine study during their recruitment visit and provided with information about recruitment protocols. In the case of boarding kennels, recruitment was undertaken by kennel staff during admittance to the kennels. Kennel employees were asked to approach owners of all animals admitted to the kennels over an agreed time period and to avoid selection of particular clients. Owners were provided with a sample pack detailing the study and asked to provide written informed consent (Appendix II). Written informed consent for the sampling of rescue dogs was provided to the principal investigator by the appropriate member of staff and animals were randomly selected for sampling.

2.2 Sample collection

For both studies fresh pea-sized faecal samples were collected on a sterile swab and enclosed within a sealed leak proof bag within a sample pack with a completed questionnaire.

Disposable gloves were provided to all participants for collection of samples. Completed sample packs were collected from an anonymous cooled drop box on a pre-arranged date (no longer than 48 hours post sample collection).

2.3 Processing of faecal samples

2.3.1 Isolation of *E. coli*

Following collection from participating premises, human and canine faecal samples were transported directly to the University of Liverpool, Leahurst Campus and processed immediately. The complete sample was added to 1ml of brain heart infusion broth with 5% glycerol and homogenised by vortexing. This faecal homogenate was then split and 500µl was added to 4.5ml buffered peptone water for aerobic overnight enrichment at 37°C for 18-24 hours. The remaining 500µl of faecal homogenate was then stored at -80°C.

Following overnight enrichment, broths were streaked onto eosin methylene blue agar (EMBA) using a 5µl disposable sterile loop. In order to detect antimicrobial-resistant *Escherichia coli* (*E. coli*), an EMBA plate was inoculated with the enriched faecal homogenate using a sterile cotton-wool swab and rotary plating device in order to achieve uniform semi-confluent growth and four antimicrobial discs applied (ampicillin 10µg, amoxyclav 30 µg, ciprofloxacin 1 µg, trimethoprim 2.5 µg) as previously described (Bartoloni et al., 1998). Additionally, broths were streaked onto two further EMBA plates containing third generation cephalosporins (3GC) in order to screen for potential ESBL-producing isolates. These plates were supplemented with cefotaxime (1µg/ml) or ceftazidime (1µg/ml), respectively (Liebana et al., 2006). All four plates were then incubated aerobically for 18-24 hours at 37°C. In any case where no growth was detected on all of the plates for a given sample, repeat enrichment and agar plating of this sample was undertaken.

2.3.2 Selection of *E. coli* isolates

A maximum of seven colonies from each faecal sample were selected for further testing; Three colonies with morphology resembling *E. coli* were randomly selected from the plain EMBA plate, while a single colony was selected from each of the plates supplemented with cefotaxime and ceftazidime. Where bacterial growth was present inside the zone of inhibition around antimicrobial discs, a single colony was selected. Where no defined zone of inhibition was present due to bacterial growth reaching the surface of the antimicrobial disc, a loopful of colonies was selected and streaked onto a plain EMBA plate and incubated at 37°C for 18-24 hours in order that a single colony could be selected. The selected colonies

were then streaked onto nutrient agar using a disposable 5µl sterile loop and incubated at 37°C for 18-24 hours under aerobic conditions.

2.3.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was undertaken on all selected isolates following guidelines produced by the British Society of Antimicrobial Chemotherapy (BSAC) (BSAC, 2013). Following overnight incubation 1-2 colonies were selected from the nutrient agar plate and homogenised in 3ml sterile distilled water in order to achieve turbidity equal to a 0.5 McFarland standard. The suspension was then diluted one in ten by transfer of 500µl into 4.5ml of sterile distilled water. The diluted suspension was inoculated onto a single Iso-Sensitest agar plate using a sterile cotton-wool swab and rotary plater in order to achieve semi-confluent growth. Following inoculation, seven antimicrobial discs were applied to each plate (ampicillin 10 µg, amoxycylav 30 µg, ciprofloxacin 1 µg, chloramphenicol 30µg, nalidixic acid 30µg tetracycline 30 µg and trimethoprim 2.5 µg). Plates were incubated at 37°C aerobically overnight and zones of inhibition were measured in millimetres and recorded. Isolates were categorised as resistant or non-resistant following comparison with clinical breakpoints published in BSAC guidelines (BSAC, 2015). Where isolates displayed an intermediate resistance phenotype they were categorised as non-resistant.

2.3.4 Phenotypic confirmation of ESBL-producing *E. coli*

All isolates selected from ESBL screening plates containing ceftazidime and cefotaxime were additionally subjected to further testing for ESBL-production using the combination disc diffusion method (M'Zali et al., 2000). Briefly, 1-2 colonies were selected and suspended in 3ml of distilled water to achieve a turbidity equal to a 0.5 MacFarland Standard. This suspension was then inoculated onto an Iso-Sensitest agar plate using a sterile cotton-wool swab in order to achieve semi-confluent growth. Three pairs of antimicrobial discs containing ceftazidime (30µg), cefotaxime (30µg) and cefpodoxime (30µg) with and without clavulanic acid (10µg) were directly applied to the inoculated plates, which were then incubated aerobically at 37°C for 18-24 hours. An increase in the diameter of the zone of inhibition in the presence of clavulanic acid of at least 5mm for any of the three pairs of discs was confirmatory for ESBL production. Resistance to the 3GC discs with a difference of less than 5mm when clavulanic acid was present is indicative of another mechanism of 3GC resistance including production of AmpC or inhibitor-resistant TEM β-lactamases.

2.3.5 Phenotypic confirmation of AmpC-producing *E. coli*

Isolates resistant to amoxycylav were additionally tested for production of AmpC enzymes. A bacterial suspension of turbidity equal to a 0.5 MacFarland Standard, was inoculated onto Iso-Sensitest agar using a rotary plater to achieve semi-confluent growth. Three antimicrobial discs containing 10µg cefpodoxime plus AmpC inducer (A), 10µg cefpodoxime plus AmpC inducer plus ESBL inhibitor (B) and 10µg cefpodoxime plus AmpC inducer, ESBL inhibitor and AmpC inhibitors (C), were applied to the inoculated plate. Plates were incubated at 37°C for 18-24 hours. AmpC production was confirmed when the zone of inhibition around disc C was at least 5mm larger than disc A and B (Halstead et al., 2012).

2.3.6 Conjugation experiments

Isolates phenotypically resistant to 3GCs, which displayed an ESBL or multi-drug resistant phenotype were selected to undergo conjugation mating experiments to determine their ability to transfer resistance to a plasmid-negative, lactose-negative *E. coli* 26R793 recipient as previously described by Karczmarczyk et al. (2011b). Briefly, donor and recipient isolates were cultured separately in nutrient broth for 18 hours at 37°C. Equal volumes of donor and recipient broths were then mixed and incubated for 18 hours at 37°C without shaking. Broths were then streaked onto MacConkey agar containing a combination of 100 µg/ml rifampin and either 50 µg/ml nalidixic acid, 50 µg/ml trimethoprim, 2 µg/ml ceftazidime or 2 µg/ml of cefotaxime. Where present, three lactose negative transconjugant colonies were selected for subculture on nutrient agar using a 5µl sterile loop. Plates were incubated for 18 hours at 37°C.

All culture media was sourced from Lab M Ltd, Bury, UK, antimicrobial discs were obtained from MAST Group Ltd., Bootle, UK, and cefotaxime and ceftazidime powder was sourced from Sigma-Aldrich Company Ltd., Gillingham, UK.

2.3.7 Isolate storage

Long-term storage of isolates was undertaken at -80°C using Microbank™ cryovials (Pro-Lab Diagnostics U.K, Cheshire UK). Briefly, colonies from a pure isolate culture were inoculated into the cryopreservative fluid in order to achieve a turbidity of approximately 4 McFarland standard and inverted numerous times to ensure binding of the organisms to Microbank™ beads. Excess cryopreservative was then removed using a sterile pipette. Isolates were recovered by removing a single bead from the cryovial using sterile forceps and inoculation of the bead onto nutrient agar for aerobic incubation at 37°C for 18-24 hours.

2.4 PCR

All isolates with the morphological appearance of *E. coli* were confirmed by examination for the presence of the *uidA* gene (McDaniels et al., 1996) and confirmed *E. coli* isolates underwent PCR-based phylogenetic grouping (Clermont et al., 2013). PCR assays were additionally used to assess carriage of antimicrobial resistance genes and identification of isolates belonging to O25b-ST131 (Clermont et al., 2009).

2.4.1 DNA extraction

Cell lysates were prepared by inoculation of one to three colonies of a pure culture of each isolate from nutrient agar into 500µl sterile molecular grade water in 1.5ml Eppendorf tubes. Suspensions were then vortexed and heated at 100°C for 20 minutes. Lysates were stored at 4°C prior to analysis, followed by long term storage at -20°C.

2.4.2 PCR substrates and primers

All PCR assays were undertaken using 5x FIREPol® 12.5mMCl₂ Master Mix Ready to Load (Solis Biodyne, Tartu, Estonia), comprising 1U FIREPol® DNA polymerase, 80mM Tris-HCl, 20mM (NH₄)₂SO₄, 0.02% w/v Tween-20, 2.5mM MgCl₂ and 200µM of each dNTP per reaction plus blue and yellow dye. Reactions testing for the presence of *uidA* and antimicrobial resistance genes were undertaken in reaction volumes of 25µl constituting 4µl of master mix, 5pmol of each primer and 1µl of DNA lysate with the addition of sterile molecular grade water (Sigma-Aldrich, Dorset, UK) to make up the reaction required reaction volume, with the exception of the detection of *bla*_{AmpC} genes where 5µl of DNA lysate was added. PCR assays relating to phylogenetic grouping and detection of *E. coli* O25b-ST131 were undertaken in reactions containing 4µl of master mix, 5pmol of each primer, 3µl of DNA lysate and sterile molecular grade water making up a reaction volume of 20µl. A lysate of a bacterial isolate known to carry the gene of interest and sterile molecular grade water were included as a positive and negative control in each PCR run. All primers were synthesised by Eurofins MWG Operon (Ebersberg, Germany) and PCR reactions were undertaken using ABI 2720 Thermal Cyclers (Applied Biosystems, California, USA). The details of individual assays performed, primer sequences, reaction conditions and references are detailed in the appropriate chapters.

2.4.3 Visualisation of PCR products

PCR amplification products were identified from their size using gel electrophoresis. All electrophoresis was undertaken using peqGREEN-stained 2% agarose gels (35-250ml), prepared using high pure low EEO agarose (Biogene Ltd, Cambridgeshire, UK) dissolved in 1 x Tris-acetate-EDTA buffer (Sigma-Aldrich, Dorset, UK) with the addition of 5µl peqGREEN (Peqlab, Fareham, UK) per 100ml of gel prior to pouring. Once set, 10µl of a 100-bp ladder (Solis Biodyne, Tartu, Estonia) was added into the first well of each gel and 10µl of each PCR product was loaded into wells in groups of eight followed by a ladder. All gels were run in an appropriately sized electrophoresis tank containing 1 x Tris-acetate-EDTA buffer. Gels containing PCR products from simplex PCR assays were run at a potential difference of 120V for 30, 60 and 90 minutes for small (35ml), medium (150ml) and large gels (250ml) respectively. In the case of gels containing multiplex PCR products the potential difference was reduced to 100V and run times extended. PCR products were visualised under ultraviolet (UV) transillumination using the UVIttec gel documentation system (UVIttec, Cambridge, UK). Gel images were printed for analysis and saved using UVIPromV (UVIttec, Cambridge, UK).

2.5 Whole genome sequencing

All isolates of *E. coli* displaying an ESBL phenotype alongside a selection of MDR and AmpC-producing, alongside all isolates of human and canine origin from two premises were selected for whole genome sequencing.

2.5.1 DNA extraction

Selected isolates were recovered from cold storage as previously described and DNA extraction was undertaken from overnight cultures using the QIAamp® DNA Mini Kit (Qiagen, Manchester, UK) according to manufacturer's instructions described overleaf.

1. A loopful of pure colonies of the desired isolates was inoculated into 5ml of LB media and incubated at 37°C for 18 hours with shaking.
2. Bacteria were harvested from 2ml of culture in a 2ml Eppendorf by centrifugation at 7500 revolutions per minute (rpm) for 10 minutes at room temperature and the supernatant removed using a sterile pipette.
3. The bacterial pellet was resuspended with 180µl of Buffer ATL and vortexed, then incubated for 30 minutes at 37°C
4. 20µl of Proteinase K was added to the suspension, which was then mixed by vortexing and incubated for 60 minutes at 56°C with periodic vortexing during incubation to ensure efficient lysis.
5. The suspensions were then briefly centrifuged to remove condensation from the Eppendorf lid, and 4µl of RNAase A (100mg/ml) was added, followed by vortexing for 15 seconds and a two minute incubation at room temperature.
6. 200µl of Buffer AL was added, followed by an incubation at 70°C for 10 minutes.
7. 200µl of 100% ethanol was then added to the mixture and the solution was pulse vortexed to mix followed by brief centrifugation.
8. The resulting suspension was pipetted into a QIAamp® Mini spin column and centrifuged at 8000rpm for one minute at room temperature and the flow through discarded.
9. 500µl of Buffer AW1 was added to the spin column and centrifuged at room temperature for one minute at 8000rpm and the flow through discarded.
10. 500µl of Buffer AW2 was added and the spin column centrifuged for three minutes at 14000rpm at room temperature.
11. 50µl of Buffer AE was pipetted onto the column membrane and the column rested at room temperature for one minute prior to centrifuging at 8000rpm at room temperature for one minute to elute the genomic DNA.

A Nanodrop spectrophotometer (Thermo Fisher Scientific, Cheshire, UK) was used to quantify the concentration of DNA within the resulting suspension and assess the purity of the DNA extract using 260/230 and 260/280 ratios. The extracts were then stored at -20°C prior to sequencing.

2.5.2 Sequencing

Whole genome sequencing of all isolates was performed by the Sheppard Lab at the University of Swansea using methods previously described (Culebro et al., 2016; McNally et al., 2016). Briefly, sequence libraries were prepared using the Nextera XT v2 library preparation kit (Illumina, San Diego, USA) and sequenced on MiSeq desktop sequencers (Illumina, San Diego, USA) using MiSeq v3 cartridges. Genome assemblies were undertaken on the 300 bp short read pair end data using the *de novo* assembly algorithm SPAdes v3.3 (Bankevich et al., 2012). Assembled genomes were uploaded to the BIGSdb database and sequences aligned to the *E. coli* K12 reference strain (accession number U00096) using MAFFT (Kato and Standley, 2013), to produce an alignment of 5,451 kbp on a gene by gene

basis. The alignment was concatenated into a contiguous sequence for each isolate genome including gaps (Sheppard et al., 2012).

Whole genome sequencing results of individual isolates were returned in FASTA file format for analysis. In addition, an FAS file containing isolate genomes aligned in MUSCLE (Edgar, 2004) using the seven Achtman MLST loci (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) was also returned.

2.5.3 Analysis of sequencing results

Nucleotide sequences of all isolates were uploaded in FASTA format to the online tools Resfinder 2.1 (Zankari et al., 2012) and Virulence finder 1.5 (Joensen et al., 2014), in order to ascertain carriage of plasmid mediated resistance genes and virulence determinant. In each case, a 90% threshold for identification was set and a minimum query length of 60% was utilised. In addition, nucleotide sequences were also uploaded to the online Comprehensive Antibiotic Resistance Database (CARD) Resistance Gene Identifier (RGI) (<https://card.mcmaster.ca/>) to search for carriage of chromosomal quinolone resistance genes using the basic local alignment search tool (BLAST) Expect values (Jia et al., 2017; McArthur et al., 2013).

In the case of plasmid replicon identification, the online PlasmidFinder 1.3 tool (Carattoli et al., 2014) was utilised using an identity threshold of 95%. For isolates harbouring IncA/C, IncF, IncI1, and IncN replicons plasmid Multi-locus Sequence Typing (pMLST) was utilised by comparing the sequence of replicon specific loci with allelic variants published within the PubMLST database (<https://pubmlst.org/plasmid/>). A plasmid ST was then allocated based on published allelic combinations. In cases where alleles for all loci were identified but no ST match was found, the ST was nominally described as a potentially novel ST.

In silico serotyping of isolates was undertaken by uploading isolate nucleotide sequences to SerotypeFinder 1.1 (Joensen et al., 2015). The presence of O-antigen and flagellin genes was used to designate the O and H serogroup of each isolate using a 90% identity threshold and a query length of 60%.

E. coli MLST was undertaken by extracting the sequences of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) and comparing them to those published within the *E. coli* MLST database (<http://enterobase.warwick.ac.uk/>). An allele number was assigned to each loci and an ST designated based on the allelic profile. In cases where incomplete allelic

profiles were identified, the MLST database was manually checked and if only a single ST contained the alleles present an isolate was nominally allocated to this ST.

Chapter 3

The prevalence and risk factors associated with faecal carriage of antimicrobial-resistant and extended-spectrum β -lactamase-producing *Escherichia coli* in people working with dogs

3.1 Introduction

The issue of antimicrobial resistance is recognised globally as an increasing problem in both human and veterinary medicine (Levy and Marshall, 2004; Wieler et al., 2011). The appearance of resistant clinical isolates has led to reduced efficacy of recognised treatment regimens and an associated increase in patient morbidity and mortality (Melzer and Petersen, 2007). The impact of antimicrobial resistance therefore has multiple medical, societal and economic costs (Cosgrove and Carmeli, 2003; Cosgrove, 2006; Roberts et al., 2009).

Escherichia coli (*E. coli*) and other members of the *Enterobacteriaceae* frequently exist as commensals within the gastrointestinal tract of humans and animals (Berg, 1996). Due to their ecological niche, these bacteria are commonly exposed to antimicrobials used for the treatment of clinical infections, and therefore face an increased selection pressure to acquire antimicrobial resistance (Edlund et al., 1994; Jakobsson et al., 2010; Nyberg et al., 2007). Resistance genes acquired by these bacteria may then be transferred to other commensals or pathogenic variants within the intestinal tract by the transfer of mobile genetic elements, such as plasmids or transposons (Blake et al., 2003; Karami et al., 2007). Furthermore, bacteria carrying these resistance genes may be transferred to other individuals in the community (Mesa et al., 2006). *E. coli* is readily isolated from faecal samples of both humans and animals, and can therefore provide a good indication of the reservoir of resistance present in the gastrointestinal commensal population (van den Bogaard and Stobberingh, 2000).

Of particular concern currently, are the extended-spectrum beta-lactamase (ESBL) enzymes. These enzymes have an extended spectrum of activity against oxyimino-cephalosporins, such as cefotaxime and ceftazidime, but largely remain susceptible to β -lactamase inhibitors such as clavulanic acid (Philippon et al., 1989). In addition, plasmids encoding ESBL genes commonly carry genes conferring resistance to other antimicrobial classes, including aminoglycosides and quinolones (Lavigne et al., 2006; Paterson and Bonomo, 2005) and therefore, display multi-drug resistant (MDR) phenotypes (resistance to three or more antimicrobial classes). Clinically, ESBL-producing isolates have been identified as a major cause of nosocomial infections (Leistner et al., 2015; Wieler et al., 2011), but are also responsible for a large number of community-acquired infections (Doi et al., 2013; Ho et al., 2007; Pitout et al., 2005; Rogers et al., 2011). ESBL-producing *Enterobacteriaceae* have therefore been identified as a significant and increasing issue for human health both in the

UK (PHE, 2015) and throughout the world (ECDC, 2015). Similarly, ESBL-producing *E. coli* are commonly reported as causing clinical infections in dogs (Huber et al., 2013; Timofte et al., 2011; Warren et al., 2001).

Carriage of ESBL-producing *E. coli* as part of the gastrointestinal flora has also been reported in humans and animals (Pallecchi et al., 2004; Pallecchi et al., 2007; Wedley et al., 2011), and numerous risk factors have been identified for the carriage of these isolates in human patients. Previous medical treatment including hospitalisation and antimicrobial treatment are widely accepted as risk factors for carriage of ESBL-producing isolates (Harris et al., 2007; Ko et al., 2013a; Reuland et al., 2016). Other non-medical risk factors include travel to Asia and pet ownership for carriage in healthy European volunteers (Östholm-Balkhed et al., 2013; Reuland et al., 2016; Tängdén et al., 2010). There are however, no studies investigating risk factors for carriage of ESBL-producing *E. coli* in a healthy UK population.

Additionally, there is currently a paucity of information regarding the prevalence of carriage of these bacteria in asymptomatic individuals in the UK. Community-based patient surveys to assess carriage rates in York and Birmingham have indicated a variable prevalence 1.9-11.3% (Munday et al., 2004; Wickramasinghe et al., 2012). More widespread studies of healthy volunteers throughout Europe indicate national variation in carriage rate of 2.0-7.6% (Leflon-Guibout et al., 2008; Meyer et al., 2012; Reuland et al., 2016; Rodrigues et al., 2016). Globally, reports suggest the highest prevalence of human carriage is in South Asia and Latin America; however official data for these regions is scarce.

More information is available regarding faecal carriage of ESBL-producing *E. coli* in UK dogs and indicates carriage of 0.5-4.1% in non-hospitalised animals (Schmidt et al., 2015; Wedley et al., 2011; Wedley, 2012), rising to 14.0% in hospitalised pets (Tuerena et al., 2016). Faecal carriage of such resistant isolates by the canine population, combined with frequent and close contact with humans, highlights their potential to act of reservoirs of these resistant bacteria (Guardabassi et al., 2004; Meyer et al., 2012).

The aim of this study was to identify the prevalence and risk factors for faecal carriage of ESBL-producing and antimicrobial-resistant (AMR) *E. coli* in people working with dogs in the North of England.

3.2 Materials and methods

3.2.1 Study population

The study population recruited for this study included people who work with dogs in veterinary practices, boarding kennels and rescue centres in the North of England within a 75 mile radius of the University of Liverpool, Leahurst Campus. Lists of appropriate premises were sourced in multiple ways; in the case of veterinary practices, premises were sourced from a list of all practices in the study area which, in the 2014 Register of Veterinary Practice Premises, indicated that they treated dogs. Local council registers of licensed boarding establishments were used to identify boarding premises and, due to the absence of a published list of rescue kennels, internet searches for appropriate establishments was undertaken in order to identify rescue kennels based in the geographic area of interest. From these defined lists, premises were randomly selected for participation in the study via random number generation. Any boarding premises which undertook home boarding rather than kennelling were excluded from the study.

Previous work investigating ESBL carriage in community populations in the UK has identified a carriage rate of 1.9-11.3% (Munday et al., 2004; Wickramasinghe et al., 2012). Sample size calculations were therefore based on an expected prevalence within this population of 10% indicating a sample size (n) of 136 would be required to estimate prevalence with a precision of 5% and 95% confidence. Due to the clustered nature of the sampling strategy used, adjustment of this sample size was required. Intraclass correlation coefficients (ICC) have not been calculated for carriage of AMR *E. coli* by people working with animals. However, premises-level ICCs have been reported for clusters of animals with several veterinary infectious diseases as well as carriage of ESBL-producing *E. coli* in veterinary practices. These are commonly reported to be 0.05-0.15 (Gandolfi-Decristophoris et al., 2013; McDermott and Schukken, 1994; Otte and Gumm, 1997), suggesting that 5-15% of the risk of infectious disease or carriage of ESBL-producing *E. coli* is related to premises rather than individual level factors. Thus, assuming an intraclass correlation coefficient (ρ) of 0.1 and an estimated sample size per cluster (m) of 15, an adjusted total (n^1) of 326 faecal samples would be required from 22 premises.

$$n^1 = n(1+\rho(m-1))$$

In order to allow for an estimated response rate of around 50%, where possible the aim was to recruit 30 participants per premises, in premises where the staff numbers were lower than

this the aim was to recruit all staff members present. The inclusion criteria for participants in this study were any member of staff or volunteer working at the premises over the age of 16 with no additional exclusion criteria.

A five-page questionnaire was designed using Microsoft Word 2010 (Microsoft Corporation) (Appendix I). This questionnaire was mostly comprised of questions with tick box responses and data were collected relating to participant age, diet, travel history, employment patterns and duration, animal contact both at work and at home, as well as previous medical history including antimicrobial treatments and hospitalisations. Space was also given at the end of the questionnaire for any additional information which the participant felt was pertinent. In order to ensure participant anonymity, no questions were asked which could lead to identification of participants. The questionnaire was reviewed by supervisors involved in the study as well as being reviewed and piloted by researchers not involved in the study prior to submission to the University of Liverpool Veterinary Research Ethics Committee. Ethical approval was granted by the Committee in May 2014 and recruitment of premises began in June 2014; data collection continued until June 2015.

3.2.2 *Sample collection and E. coli isolation*

Recruited premises were visited in order to provide information about the study and also supply the premises with sample packs. Participants were asked to provide a pea-sized faecal sample on a swab and complete the enclosed questionnaire, which would be collected from an anonymous drop box within the premises on a pre-arranged date (no longer than 48 hours after sample collection). In order to encourage participation and maximise the response rate, participation was anonymous, meaning that written informed consent could not be obtained. Therefore, it was made clear to participants both during recruitment meetings and on written information provided to them in study packs (Appendix I), that by providing a faecal sample and submitting a questionnaire they were providing informed consent for their data to be used in the study. Contents of all sample packs contained a unique reference number in order to ensure samples and questionnaires were correctly matched.

Laboratory protocols were initially evaluated on a small pilot study involving a convenience sample of 11 participants, in order to ensure sufficient bacterial growth would be yielded from small samples on swabs and the batch collection of samples would not cause any problems with sample processing. These data were excluded from the main study.

Following collection of samples from premises they were transported directly to the University of Liverpool and processed immediately. The complete sample was added to 1ml of brain heart infusion broth with 5% glycerol and homogenised. The faecal homogenate was then split and 500µl was enriched aerobically overnight at 37°C in 4.5ml buffered peptone water, with the remainder being stored at -80°C. Following enrichment the faecal homogenate was streaked onto three eosin methylene blue agar (EMBA) plates, one containing cefotaxime (1µg/ml), one containing ceftazidime (1µg/ml) and one containing no antimicrobials (Liebana et al., 2006). Additionally, an EMBA plate was inoculated with the enriched faecal homogenate in order to achieve uniform semi-confluent growth and four antimicrobial discs directly applied (ampicillin 10µg, amoxycylav 30 µg, ciprofloxacin 1 µg, trimethoprim 2.5 µg) as previously described (Bartoloni et al., 1998). All plates were then incubated aerobically overnight at 37°C. Three colonies which resembled *E. coli* morphologically were then randomly selected from the plain EMBA, one colony from each of the plates which contained cefotaxime and ceftazidime and a single colony growing within the inhibition zone of each of the antimicrobial discs were also selected and sub-cultured on nutrient agar and incubated overnight at 37°C. All presumptive isolates of *E. coli* were subjected to a PCR assay to detect the presence of the *uidA* gene and confirm the isolate as being *E. coli* (McDaniels et al., 1996). All media was sourced from Lab M Ltd Bury, UK, antimicrobial discs were obtained from MAST Group Ltd., Bootle, UK, and cefotaxime and ceftazidime powder was sourced from Sigma–Aldrich Company Ltd., Gillingham, UK.

3.2.3 Antimicrobial susceptibility testing

All isolates sub-cultured on nutrient agar underwent further antimicrobial susceptibility testing against a panel of seven antimicrobials. Inocula containing the isolates were prepared as according to the British Society for Antimicrobial Chemotherapy (BSAC) guidelines (BSAC, 2013). Iso-Sensitest agar plates were then inoculated with each isolate and a panel of seven antimicrobial discs applied (ampicillin 10 µg, amoxycylav 30 µg, ciprofloxacin 1 µg, chloramphenicol 30µg, nalidixic acid 30µg tetracycline 30 µg and trimethoprim 2.5 µg). Following overnight incubation at 37°C the diameter of the zone of inhibition around each disc was measured in millimetres and recorded.

3.2.4 Phenotypic confirmation of ESBL production

All isolates selected from EMBA plates containing ceftazidime and cefotaxime were subjected to testing for ESBL production using the combination disc diffusion method (M'Zali

et al., 2000). Briefly three pairs of antimicrobial discs containing ceftazidime, cefotaxime and cefpodoxime with and without clavulanic acid were applied to an Iso-Sensitest agar plate inoculated for confluent bacterial growth and incubated overnight at 37°C. An increase in the diameter of the zone of inhibition in the presence of clavulanic acid of at least 5mm for any of the three pairs of discs was confirmatory for ESBL production. Resistance to the third generation cephalosporin (3GC) discs with a difference of less than 5mm when clavulanic acid was present is indicative of another mechanism of resistance and could be suggestive of AmpC production.

3.2.5 Statistical analysis

All questionnaire and microbiological data were entered into a database (Microsoft Excel 2010, Microsoft Corporation) and the dataset was reviewed to ensure accuracy of input. Isolate antimicrobial susceptibility data was coded as a binary variable either resistant (1) or non-resistant (0) using BSAC breakpoint values (BSAC, 2015). Data were exported into R (www.r-project.org) for analysis.

3.2.5.1 Analysis of prevalence

Within this prevalence study the sample level data was considered the unit of interest such that a sample with at least one resistant *E. coli* isolate was considered resistant. The antimicrobial resistance outcomes considered were antimicrobial resistance (resistance to any of the antimicrobials tested), resistance to each of the seven antimicrobials tested, 3GC resistance, multi-drug resistance (presence of an *E. coli* isolate resistant to at least three antimicrobial classes), and phenotypically confirmed ESBL-mediated resistance.

Data obtained within this study was clustered within premises so in order to accurately approximate the prevalence (and confidence intervals) of resistance within this population the prevalence of each outcome was estimated using separate logistic regression models including premises as a random effect to account for clustering at this level. The true prevalence (P_T) was calculated using the beta coefficient (β_0) from the intercept-only random effects model constructed for each outcome using the formula below.

$$P_T = \frac{e^{\beta_0}}{1 + e^{\beta_0}}$$

In the case of prevalence estimates, 95% confidence intervals were calculated as a function of the calculated standard error of the beta coefficient from the intercept-only logistic

regression model for each outcome. Confidence intervals for all other proportions were calculated as the proportion $\pm 1.96 \times$ standard error.

Further examination of the resistance phenotypes displayed by *E. coli* isolates was undertaken in order to assess which antimicrobials isolates were more likely to display co-resistance to. Following calculation of binary distance matrices, hierarchical cluster analysis was undertaken using Ward's method and performed in R using the graphics package (R Core Team, 2015).

3.2.5.2 Risk factor analysis

Potential risk factors were identified from data provided in questionnaires and examined for association with the resistance outcomes defined above. All explanatory variables derived from questionnaires were binary or categorical in nature and data were analysed using the lme4 (Bates et al., 2014), lmerTest (Hothorn et al., 2015) and mcgvc (Wood, 2016) packages.

Initial univariable analysis was conducted on all explanatory variables using separate mixed effect logistic regression models for each outcome. These models included individual premises as a random effect to account for the clustering of data at this level. Variables were considered for inclusion into a multivariable model if they showed some association with that outcome, indicated by a likelihood ratio test statistic (LRTS) of $p < 0.25$ when compared to the null model. Where response variable numbers were low, polytomous variables were collapsed into a smaller number of categories when appropriate. Where low response numbers resulted in categories containing no responses, initial screening of variables was undertaken using Fisher's exact test. If a potential association between this variable and the outcome was present ($p < 0.25$), then an artificial positive was placed in the category that would otherwise contain no data, allowing estimation of its association with the outcome of interest and as such, its inclusion in the multivariable model. This adjusted dataset was only used while this variable remained in the multivariable model.

In order to avoid effects of collinearity, if any variables were found to have a correlation coefficient of ≥ 0.7 , only the variable with the smallest p-value was incorporated into the multivariable model. In cases where nesting of variables occurred (for example being a meat eater and consuming beef ≥ 3 times a week), a categorical variable incorporating both these variables was created to assess their independent association with the outcome. Only variables which remained significant were included in the multivariable model.

The final models were constructed by a manual backwards stepwise elimination, where variables were retained in the model if their exclusion resulted in a LRTS of $p < 0.05$ or if confounding, evidenced by change in the β coefficient of other explanatory variables of greater than 25%, was present. For variables retained in the model, biologically plausible interaction terms and the effect of random slopes were tested and retained if found to be significant ($p < 0.05$). All variables considered for initial inclusion in the model were then added back into the model and tested for significance, in order to ensure no significant or confounding variables had been inadvertently excluded. For each of the final models, an ICC was calculated in order to assess the amount of variation due to the premises versus sample level factors. This was calculated using the latent variable approach, assuming that the total variance at the level one unit of interest is equal to $\pi^2/3$ (Goldstein et al., 2002). Final models were checked for premises, which may have had an increased influence by plotting premises level residuals against the overall mean. Where a significant difference was detected, data from these premises were checked for any errors.

3.3 Results

3.3.1 Study population

A total of 363 premises were contacted in order to recruit a total of 69 premises into the study and at least one participant was successfully recruited from 66 of these premises. A summary of premises recruited is provided in Table 3.1. Personnel numbers at the recruited premises ranged from two to forty and the median number of participants recruited from these premises was three (Interquartile Range (IQR) 2-4), hence the need to increase the total number of practices sampled from the 22 originally planned. In total 229 participants were recruited to the study, of which 227 samples yielded bacterial growth and 220 usable questionnaires were received. Participants whose faecal samples elicited no growth were excluded from prevalence and risk factor analysis. The overall individual response rate for the study, based on 696 individuals present at the recruited premises, was 32.9%. The locations of premises recruited for the study can be found in Figure 3.1.

Table 3.1 Summary of premises types recruited in a cross-sectional study of people working with dogs in the North of England

Premises Type	Number Contacted	Number Recruited	Percent	95%CI	Number of Participants
Boarding Kennel	161	25	15.5	9.9-21.1	86
Rescue Centre	27	8	29.6	12.4-46.9	24
Veterinary Practice	175	36	20.6	14.6-26.6	119

Figure 3.1 Geographical location of premises in a cross-sectional study of people working with dogs in the North of England



3.3.2 Survey results

3.3.2.1 Demographics and the home environment

Participants aged 26-55 represented approximately equal proportions of the people sampled in this study with people aged over 65 the least prevalent (4.5% 95% CI 1.8-7.3). Due to the desire to keep samples anonymous no further personal demographic data was requested from the participants. Most participants (92.7% 95% CI 89.3-96.2) reported travel outside of the United Kingdom, with 64.7% (95% CI 58.1-71.3) of these participants identifying a European destination. Nearly a quarter (22.3% 95% CI 16.8-27.8) of respondents had received antimicrobial treatment in the last six months, while only 5.9% (95% CI 2.8-9.0) had been hospitalised over this period. Contact with animals at home was reported by 90.0% (95%CI 86-94) of respondents and contact with companion animals was most prevalent (89.5% 95%CI 85.5-93.6). Additional information regarding participant demographics, medical treatment and residential contact with animals can be found in Table 3.2.

Table 3.2 Summary of participant demographics, medical treatment and residential animal contact in a cross-sectional study of 220 people working with dogs in the North of England

		Percent	95% CI
Age	16-25	18.2	13.1-23.3
	26-35	22.3	16.8-27.8
	36-45	20.0	14.7-25.3
	46-55	24.1	18.4-29.7
	56-65	10.9	6.8-15.0
	>65	4.5	1.8-7.3
Diet	Vegetarian	9.1	5.3-12.9
	Non-Vegetarian	90.9	87.1-94.7
Meat consumption (>3 times weekly)	Beef	44.1	37.5-50.7
	Lamb	17.3	12.3-22.3
	Pork	26.8	21.0-32.7
	Poultry	71.8	65.9-77.8
	Fish	30.5	24.4-36.5
Travel outside of UK	3m	26.4	20.5-32.2
	4-6m	13.6	9.1-18.2
	7-9m	5.9	2.8-9.0
	10-12m	7.3	3.8-10.7
	>12m	39.5	33.1-46.0
	Never	7.3	3.8-10.7
Travel Destination	Africa	2.0	0.1-3.9
	Asia	2.5	0.3-4.6
	Australasia	2.0	0.1-3.9
	Europe	64.7	58.1-71.3
	Middle East	1.5	0-3.1
	North America	6.9	3.4-10.3
	South America	0.5	0-1.4
	Not specified	20.1	14.6-25.6
Antimicrobial treatment within last 6 months	1m	5.0	2.1-7.9
	2-3m	9.1	5.3-12.9
	4-6m	7.3	3.8-10.7
	None	77.7	72.2-83.2
Hospitalisation within last 6 months	1m	2.3	0.3-4.2
	2-3m	1.8	0.1-3.6
	4-6m	1.8	0.1-3.6
	None	94.1	91.0-97.2
Animal Contact	Companion Animals	89.5	85.5-93.6
	Dogs	75.0	69.3-80.7
	Cats	50.0	43.4-56.6
	Small Mammals	20.9	15.5-26.3
	Horses	17.7	12.7-22.8
	Other	10.5	6.4-14.5
	Farm Animals	19.1	13.9-24.3
	Cattle	5.9	2.8-9.0
	Sheep	6.8	3.5-10.1
	Pigs	1.4	0-2.9
	Poultry	15.5	10.7-20.2

3.3.2.2 Work environment

Samples from participants employed at veterinary practices represented more than half of those obtained (52.3% 95%CI 45.7-58.9). Most of the participants (96.4% 95% CI 93.9-98.8) were employed at the recruited premises and of these, 77.4% (95%CI 71.7-83.0) worked at the premises full time. The median duration of employment within the premises recruited was 63 months (IQR 20.0-147.5). Direct contact with animals was reported by 95% (95% CI 92.1-97.9) of respondents and 94.5% (95%CI 91.5-97.5) had direct contact with dogs. Further details of premises types, work patterns and work environment are detailed in Table 3.3.

Table 3.3 Summary of employment and work-based animal contact provided by participants in a cross-sectional study of 220 people working with dogs in the North of England

		Percent	95% CI
Premises Type	Boarding Kennel	38.2	31.8-44.6
	Rescue Centre	9.5	5.7-13.4
	Veterinary Practice	52.3	45.7-58.9
Employment	Staff	96.4	93.9-98.8
	Volunteer	3.6	1.2-6.1
Staff Work Pattern	Full-time	77.4	71.7-83.0
	Part-time	22.6	17.0-28.3
Animals On Site	Cats	86.4	81.8-90.9
	Small Mammals	56.4	49.8-62.9
	Horses	15.5	10.7-20.2
	Farm Animals	16.4	11.5-21.3
	Reptiles	3.6	1.2-6.1
	Birds	5.9	2.8-9.0
Animal Contact	Wildlife	0.9	0-2.2
	Direct Contact-Dogs	94.5	91.5-97.5
	Direct Contact-Other Animals	73.6	67.8-79.5

3.3.3 Sample prevalence of carriage of antimicrobial-resistant *E. coli*

At least one isolate of *E. coli* was identified from 220 (96.9% 95%CI 94.7-99.2) faecal samples, indicating a high prevalence of faecal carriage of *E. coli*. In total, 364 non-duplicate isolates of *E. coli* were identified based on their antimicrobial susceptibility profiles. The median number of isolates recovered per sample was two (IQR 1-2). Isolates with resistance to at least one antimicrobial were recovered from 154 (67.8% 95% CI 61.8-73.9) samples, with resistance identified to all antimicrobials tested. The highest prevalence of resistance identified was to ampicillin, trimethoprim and tetracycline. MDR *E. coli* were recovered from 54 (23.8% 95%CI 18.2-29.3) samples. ESBL-producing *E. coli* were isolated from seven (3.1% 95% CI 0.8-5.3) samples obtained from seven different premises. Additionally, resistance to critically important fluoroquinolone antimicrobials was identified faecal *E. coli* from 17 (7.5% 95% CI 4.1-10.9) individuals located at 29 premises. Table 3.4 details the sample prevalence

of resistance to each antimicrobial tested, MDR phenotypes, resistance to a 3GC and presence of a phenotypic ESBL-producing *E. coli* after correction for clustering within premises.

Table 3.4 The number and sample prevalence of AMR faecal *E. coli* (adjusted for clustering) within a cross-sectional study of 227 people working with dogs in the North of England

Resistance Outcome	Number of positive samples	Adjusted Prevalence	95% CI
Any resistance	154	68.7	61.1-75.4
Ampicillin	128	56.8	49.3-63.9
Amoxycylav	14	3.1	0.6-16.5
Chloramphenicol	27	7.8	3.5-16.5
Ciprofloxacin	17	5.5	1.8-15.8
Nalidixic Acid	42	16.2	10.4-24.4
Tetracycline	73	32.1	25.7-39.2
Trimethoprim	76	33.5	27.1-40.6
Multi-drug resistance	54	23.6	18.0-30.3
Third-generation Cephalosporin resistance	18	4.2	1.0-15.5
ESBL phenotype	7	3.1	1.5-6.3

3.3.4 *E. coli* resistance phenotypes

Of the 364 *E. coli* isolates recovered in this study 219 (60.2% 95 CI 55.1-65.2) were found to be resistant to at least one antimicrobial. Susceptibility of isolates was tested against five antimicrobial classes and 17.3% (n=63) were found to be MDR. Two isolates, both from participants working in veterinary practices were resistant to all antimicrobial drugs against which they were tested.

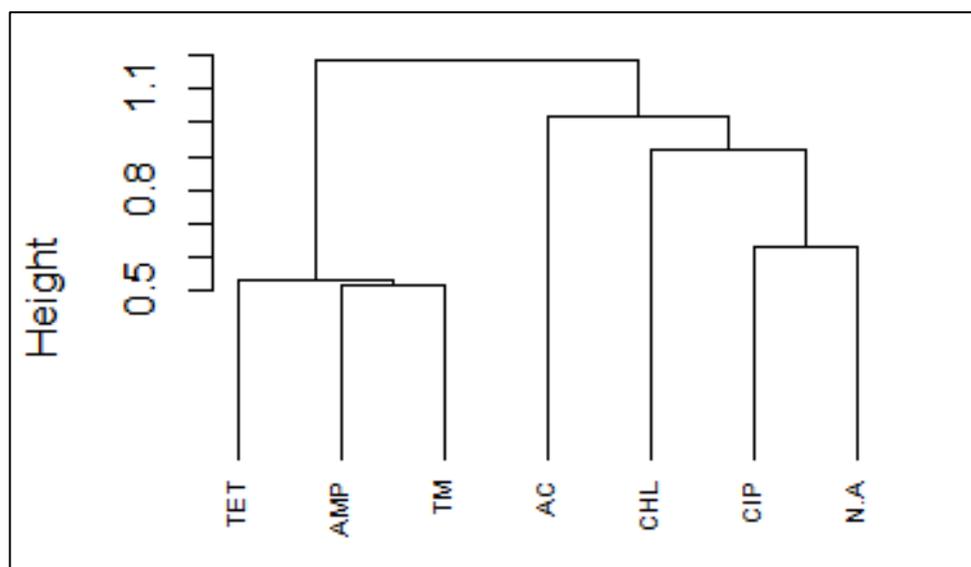
Thirty-eight different resistance profiles were identified from a possible 127 different resistant profiles, indicating diversity within this *E. coli* population. The most commonly identified phenotype amongst the AMR isolates was to ampicillin alone (24.2%) and the ten most commonly identified phenotypes represented 73.1% (n=160) of the resistant *E. coli* isolated in this study. Details of these resistance profiles can be found in Table 3.5.

Table 3.5 The ten most frequently identified resistance phenotypes identified among the 219 AMR *E. coli* isolates

Resistance Phenotype	Number of Isolates (%)	95% CI
AMP	53 (24.2)	18.5-29.9
AMP TET TM	23 (10.5)	6.4-14.6
AMP TET	19 (8.7)	5.0-12.4
TM	17 (7.8)	4.2-11.3
AMP TM	14 (5.67)	2.8-8.6
AMP CIP NA	8 (3.65)	1.8-6.1
CHL	8 (3.65)	1.8-6.1
TET TM	7 (3.2)	0.9-5.5
AMP AC	6 (2.7)	0.6-4.9
AMP CHL TET	5 (2.3)	0.3-4.3

Key: AMP=ampicillin; AC=amoxyclov; CHL=chloramphenicol; TET=tetracycline; TM=trimethoprim

Cluster analysis evaluated the presence of co-resistance to antimicrobials and indicated a propensity for some isolates to display co-resistance to antimicrobials within the same antimicrobial class. This is highlighted by the proximity of quinolone antimicrobials to each other on the dendrogram. Interestingly however, ampicillin displays a propensity for co-resistance with trimethoprim and tetracycline rather than amoxyclov (Figure 3.2).

Figure 3.2 Antimicrobial agents clustered by resistance patterns for 364 isolates of human faecal *E. coli*

Key: AMP=ampicillin; AC=amoxyclov; CHL=chloramphenicol; CIP=ciprofloxacin; N.A=nalidixic acid; TET=tetracycline; TM=trimethoprim; Isolates resistant to one antimicrobial are more likely to display resistance to an antimicrobial on close branches of the dendrogram. For example, an isolate resistant to ciprofloxacin is more likely to be resistant to nalidixic acid than to chloramphenicol.

A total of nine (2.5% 95% CI 0.9-4.1%) ESBL-producing isolates were identified phenotypically, of which seven displayed resistance to all three 3GC tested. The two remaining isolates retained susceptibility to ceftazidime. Six distinct resistance phenotypes were identified and six isolates were identified as being MDR. All isolates retained

susceptibility to at least one of the antimicrobials tested. Further details of the ESBL-producing isolates identified phenotypically in this study can be found in Table 3.6.

Table 3.6 Details of resistance phenotypes displayed by ESBL-producing *E. coli* isolates within in a cross-sectional study of 220 people working with dogs in the North of England

Isolate	Sample	Premise Type	Antimicrobial resistance phenotype
399	88	Veterinary Practice	AMP CAZ CPD CTX NA TET TM
421	95	Veterinary Practice	AMP CAZ CPD CTX NA TET TM
422	95	Veterinary Practice	AMP CAZ CPD CTX TET TM
677	148	Boarding Kennel	AMP CAZ CPD CTX NA TET TM
690	149	Veterinary Practice	AMP CPD CTX TM
697	149	Veterinary Practice	AMP CAZ CHL CPD CTX TET
712	152	Boarding Kennel	AMP CPD CTX
766	163	Rescue Centre	AMP CAZ CIP CPD CTX NA TET TM
1118	229	Veterinary Practice	AMP CAZ CPD CTX TM

Key: AMP ampicillin; CAZ ceftazidime; CIP ciprofloxacin; CPD cefpodoxime; CTX cefotaxime; NA nalidixic acid; TET tetracycline; TM trimethoprim

3.3.5 Risk factor analysis

3.3.5.1 Univariable analysis

Initial univariable analysis identified a large number of potential explanatory variables associated with the eleven resistance outcomes examined, however only a small number of significant associations were identified (Table 3.7). When considering age as an explanatory variable, collapse of the six categories into three was necessary due to low numbers of responses in some groups. Participant exposure to antimicrobial treatment within one, three or six months of sampling were significantly correlated with each other. Where present in conjunction, antimicrobial treatment within three months of sampling was the most significant variable and was therefore selected for inclusion in multivariable models. In the case of tetracycline resistance, a categorical variable considering antimicrobial treatment less than one month, two to three months, or greater than three months previously was the most significant variable. Similarly, participant hospitalisation within one, three and six months of sampling were significantly correlated and where present simultaneously, hospitalisation within one month of sampling was most significant and included in the multivariable model. Nested explanatory variables were identified for five resistance outcomes; antimicrobial resistance, 3GC, amoxyclav, nalidixic acid and trimethoprim resistance. Associations with farm animal contact and specific farm animal species were identified in conjunction in the case of antimicrobial resistance and resistance to 3GCs, nalidixic acid and trimethoprim. Polytomous variables containing these nested variables were therefore created for each of the outcomes in order to assess which variables were the

best fit for each model. In the case of trimethoprim resistance, workplace contact with farm animals ($p=0.115$) was identified as the variable with the best fit for the model and was therefore solely included. However, for 3GC, nalidixic acid and antimicrobial resistance, individual animal species were identified as being most significant and therefore included in the corresponding multivariable model. Workplace contact with sheep was found to be significantly associated with 3GC ($p=0.003$), while contact with poultry in the home environment ($p=0.025$) and household contact with dairy cattle ($p=0.066$) were associated with nalidixic acid and antimicrobial resistance, respectively. In addition, being a non-vegetarian and regular consumption of specific types of meat were identified in conjunction for 3GC, amoxycylav and trimethoprim resistance. When considering trimethoprim and amoxycylav resistance, regular consumption of poultry ($p=0.013$) and pork ($p=0.051$), respectively, were considered the best fit for the model, while in the case of 3GC resistance, a categorical variable containing consumption of meat combined with regular consumption of lamb and pork ($p=0.078$) was most significant. These variables were therefore selected for inclusion in the multivariable model for the corresponding outcome. The complete results of univariable analyses including polytomous variables created from nested variables are located in Appendix I.

Table 3.7 Variables considered for inclusion in a multivariable models of risk factors for faecal carriage of AMR *E. coli* in 220 people working with dogs in the North of England

		ANY	MDR	3GC	ESBL	AMP	AC	CHL	CIP	NA	TET	TM
Demographics and medical history	Age			Grey								Grey
	Antimicrobials <1 month					Grey					Black	
	Antimicrobials <3 months	Grey				Black						
	Antimicrobials < 6 months	Grey				Grey	Grey			Grey		
	Antimicrobials Categorical					Grey					Black	
	Hospitalisation < 1 month		Black								Black	Black
	Hospitalisation <3 months	Grey	Black								Grey	
	Hospitalisation <6 months		Grey									
	Household member hosp.	Grey										
	International travel	Grey										
Diet	Meat eater			Grey			Grey					Black
	Beef >3 times weekly			Grey								
	Lamb >3 times weekly		Grey	Grey	Black	Black						
	Pork >3 times weekly			Grey		Black	Grey	Grey				
	Poultry >3 times weekly	Grey				Grey	Grey				Grey	Black
	Fish >3 times weekly	Grey				Black						
	Game >3 times weekly	Grey			Black	Grey						
	Eat salad >3 times weekly			Grey		Grey			Grey	Grey		
	Animal contact at home	Dogs			Grey	Black						
Cats								Grey		Black	Grey	
Small Mammals				Grey			Grey					
Horses							Grey					Grey
Fish						Grey						
Reptiles			Grey			Grey					Grey	Grey
Ornamental birds				Black			Grey					
Farm Animals		Grey								Grey		
Cattle												Grey
Dairy		Grey										
Beef		Grey										Grey
Poultry										Black		
Work environment		Premises Type	Black				Grey			Black		
	Hours of work						Grey	Black	Grey			
	Duration of employment	Grey	Grey									
	Handling dogs	Grey								Grey		
	Handling other animals	Grey			Grey				Grey		Grey	
	Reception work											
	Cleaning kennels				Black							
	Office work								Grey			
Animal contact at work	Cats										Grey	Grey
	Horses											Grey
	Small Mammals	Black					Grey					
	Reptiles										Grey	
	Ornamental Birds											
	Farm Animals			Black	Grey							Grey
	Cattle											Grey
	Sheep			Black			Black					Grey
Poultry									Grey		Grey	
Wildlife							Grey					

Key: ANY=resistance to at least one antimicrobial; MDR= multi-drug resistance; 3GC=third generation cephalosporin; AMP=ampicillin; AC=amoxyclovax; CHL=chloramphenicol; CIP=ciprofloxacin; NA=nalidixic acid; TET=tetracycline; TM=trimethoprim; Grey=LRT p<0.25; Black=LRT p<0.05

With the exception of prior hospitalisation, regular consumption of lamb, and working with sheep, which were significantly associated with multi-drug ($p=0.009$), tetracycline ($p=0.047$) and trimethoprim ($p=0.031$) resistance; an ESBL phenotype ($p=0.003$) and ampicillin resistance ($p=0.037$); and 3GC ($p=0.003$) and amoxycylav ($p=0.047$) resistance respectively, no explanatory variables were associated with more than one outcome. When considering carriage of phenotypic ESBL-producing *E. coli*, univariable analysis revealed participants who cleaned kennels as part of their job role ($p=0.021$) were at higher risk of carriage, while having contact with dogs in the home ($p=0.012$) or regularly consuming game ($p=0.044$) were protective. The low prevalence of amoxycylav-resistant and phenotypic ESBL-producing *E. coli* identified in this study precluded model construction for these outcomes and therefore relationships between these outcomes and potential explanatory variables could not be explored further.

3.3.5.2 Multivariable analysis

Mixed effect logistic regression was undertaken for nine resistance outcomes and a model successfully constructed for all outcomes. Five of the final models included multiple explanatory variables, while only a single explanatory variable remained in the models examining resistance to antimicrobial resistance, chloramphenicol, nalidixic acid and multidrug resistance (Table 3.8). For those models which contained multiple explanatory variables no significant interaction terms were identified, however in the case of nalidixic acid a significant random slope effect was present ($p<0.001$). This suggests that the effect of contact with poultry differed between different premises. The inclusion of this random slope did not alter the protective nature of the factor; it did however increase the magnitude of the effect.

Models constructed indicated that the proportion of variance attributed to the premises was between 2.5% and 25.0% for all outcomes except chloramphenicol (44.0%) and 3GC resistance (88.7%). Premises level residual plots for these outcomes showed that six and thirteen premises, respectively were significantly different from the overall mean (Appendix I). Further examination of data from these outlying premises identified that all contained at least one sample positive for these resistance outcomes. Given the low prevalence of both chloramphenicol and 3GC resistance, it is not unexpected that premises within which samples were positive for these outcomes would be outliers. All data was from these premises was however rechecked prior to acceptance of the model. Examination of residuals

from all other outcomes indicated no individual premises were significantly different from the mean.

The models constructed for each outcome show little overlap in the risk factors identified (Table 3.8). No single risk factor was identified as being associated with all of the outcomes examined and furthermore, only two explanatory variables were identified as being a risk factor for more than one outcome. Multi-drug ($p=0.009$), tetracycline ($p=0.047$) and trimethoprim resistance ($p=0.033$) were associated with hospitalisation within the last month, while antimicrobial treatment within the last three months was a risk factor for ampicillin resistance ($p=0.017$). Tetracycline resistance was also associated with recent antimicrobial treatment ($p=0.043$) with participants who had received antimicrobials within the last two to three months, were significantly less to carry resistance (Wald $p=0.017$) than those who had received antimicrobials within the last month. Aspects of participant diets were however highlighted as being a risk factor for multiple outcomes; regular consumption of pork was a risk factor for ampicillin resistance ($p=0.015$), while consumption of poultry increased the risk of carriage of tetracycline ($p=0.024$) and trimethoprim ($p=0.014$) resistance. Regular consumption of salad leaves was associated with ciprofloxacin resistance ($p=0.032$). Contact with differing types of non-canine animals was also identified as a risk factor for numerous outcomes. Ciprofloxacin resistance was the only outcome for which the type of premises had a significant association ($p<0.001$), with work at both veterinary practices (Wald $p=0.040$) and rescue centres (Wald $p=0.006$) associated with increased risk of ciprofloxacin resistance.

Table 3.8 Multivariable logistic regression models of risk factors associated with antimicrobial resistance in faecal *E. coli* obtained from a cross-sectional study of 220 people working with dogs in the North of England

Resistance outcome and variables	Category	β	SE	OR	95% CI	LRT P value	Variance (ICC)																																																																																																																																																																																																																																																																														
Antimicrobial Resistance																																																																																																																																																																																																																																																																																					
Work with small mammals	No	(Ref)	-	-	-	0.049	0.16 (0.046)																																																																																																																																																																																																																																																																														
	Yes	-0.62	0.32	0.54	0.29-1.00			Ampicillin								Antimicrobials in last 3 months	No	(Ref)	-	-	-	0.017	0.10 (0.030)	Yes	1.02	0.45	2.78	1.15-6.72	Regular pork consumption	No	(Ref)	-	-	-	0.015		Yes	0.79	0.33	2.21	1.15-4.25	Chloramphenicol								Work full time	No	(Ref)	-	-	-	0.045	2.58 (0.440)	Yes	1.33	0.77	3.80	0.84-17.18	Ciprofloxacin								Premises type	Boarding	(Ref)	-	-	-	<0.001	0.94 (0.222)	Rescue	4.21	1.54	67.57	3.28-1392	0.006*	Vet	2.44	1.19	11.50	1.12-117.7	0.040*	Contact with cats at home	No	(Ref)	-	-	-	0.017		Yes	-1.47	0.67	0.23	0.06-0.86	Regular salad consumption	No	(Ref)	-	-	-	0.032		Yes	1.53	0.81	4.60	0.94-22.45	Nalidixic Acid								Contact with poultry at home†	No	(Ref)	-	-	-	<0.001	1.10 (0.250)	Yes	-12.26	7.00	5.0E-06	0.00-4.28	Tetracycline								Antimicrobial Treatment	<1m	(Ref)	-	-	-	0.043	0.28 (0.078)	2-3m	-2.28	0.96	0.10	0.02-0.67	0.017*	>3m	-1.19	0.70	0.30	0.08-1.20	0.090	Hospitalised in last month	No	(Ref)	-	-	-	0.047		Yes	2.38	1.36	10.80	0.76-153.83	Regular poultry consumption	No	(Ref)	-	-	-	0.024		Yes	0.85	0.39	2.33	1.09-4.98	Trimethoprim								Hospitalised in last month	No	(Ref)	-	-	-	0.033	0.097 (0.029)	Yes	2.19	1.17	8.96	0.91-88.36	Regular poultry consumption	No	(Ref)	-	-	-	0.014		Yes	0.89	0.37	2.42	1.17-5.02	Multi-drug resistance								Hospitalised in last month	No	(Ref)	-	-	-	0.009	0.085 (0.025)	Yes	2.61	1.15	13.64	1.45-128.8	3rd Generation Cephalosporin Resistance								Work with sheep	No	(Ref)	-	-	-	0.032	25.74 (0.887)	Yes	4.1	2.06	60.31	1.06-3433	Contact with birds at home	No	(Ref)	-	-	-	0.027		Yes	3.18
Ampicillin																																																																																																																																																																																																																																																																																					
Antimicrobials in last 3 months	No	(Ref)	-	-	-	0.017	0.10 (0.030)																																																																																																																																																																																																																																																																														
	Yes	1.02	0.45	2.78	1.15-6.72			Regular pork consumption	No	(Ref)	-	-	-	0.015		Yes	0.79	0.33	2.21	1.15-4.25	Chloramphenicol								Work full time	No	(Ref)	-	-	-	0.045	2.58 (0.440)	Yes	1.33	0.77	3.80	0.84-17.18	Ciprofloxacin								Premises type	Boarding	(Ref)	-	-	-	<0.001	0.94 (0.222)	Rescue	4.21	1.54	67.57	3.28-1392	0.006*	Vet	2.44	1.19	11.50	1.12-117.7	0.040*	Contact with cats at home	No	(Ref)	-	-	-	0.017		Yes	-1.47	0.67	0.23	0.06-0.86	Regular salad consumption	No	(Ref)	-	-	-	0.032		Yes	1.53	0.81	4.60	0.94-22.45	Nalidixic Acid								Contact with poultry at home†	No	(Ref)	-	-	-	<0.001	1.10 (0.250)	Yes	-12.26	7.00	5.0E-06	0.00-4.28	Tetracycline								Antimicrobial Treatment	<1m	(Ref)	-	-	-	0.043	0.28 (0.078)	2-3m	-2.28	0.96	0.10	0.02-0.67	0.017*	>3m	-1.19	0.70	0.30	0.08-1.20	0.090	Hospitalised in last month	No	(Ref)	-	-	-	0.047		Yes	2.38	1.36	10.80	0.76-153.83	Regular poultry consumption	No	(Ref)	-	-	-	0.024		Yes	0.85	0.39	2.33	1.09-4.98	Trimethoprim								Hospitalised in last month	No	(Ref)	-	-	-	0.033	0.097 (0.029)	Yes	2.19	1.17	8.96	0.91-88.36	Regular poultry consumption	No	(Ref)	-	-	-	0.014		Yes	0.89	0.37	2.42	1.17-5.02	Multi-drug resistance								Hospitalised in last month	No	(Ref)	-	-	-	0.009	0.085 (0.025)	Yes	2.61	1.15	13.64	1.45-128.8	3rd Generation Cephalosporin Resistance								Work with sheep	No	(Ref)	-	-	-	0.032	25.74 (0.887)	Yes	4.1	2.06	60.31	1.06-3433	Contact with birds at home	No	(Ref)	-	-	-	0.027		Yes	3.18	1.47	24.14	1.35-431.5																		
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	Yes	-1.47	0.67	0.23	0.06-0.86			Regular salad consumption	No	(Ref)	-	-	-	0.032		Yes	1.53	0.81	4.60	0.94-22.45	Nalidixic Acid								Contact with poultry at home†	No	(Ref)	-	-	-	<0.001	1.10 (0.250)	Yes	-12.26	7.00	5.0E-06	0.00-4.28	Tetracycline								Antimicrobial Treatment	<1m	(Ref)	-	-	-	0.043	0.28 (0.078)	2-3m	-2.28	0.96	0.10	0.02-0.67	0.017*	>3m	-1.19	0.70	0.30	0.08-1.20	0.090	Hospitalised in last month	No	(Ref)	-	-	-	0.047		Yes	2.38	1.36	10.80	0.76-153.83	Regular poultry consumption	No	(Ref)	-	-	-	0.024		Yes	0.85	0.39	2.33	1.09-4.98	Trimethoprim								Hospitalised in last month	No	(Ref)	-	-	-	0.033	0.097 (0.029)	Yes	2.19	1.17	8.96	0.91-88.36	Regular poultry consumption	No	(Ref)	-	-	-	0.014		Yes	0.89	0.37	2.42	1.17-5.02	Multi-drug resistance								Hospitalised in last month	No	(Ref)	-	-	-	0.009	0.085 (0.025)	Yes	2.61	1.15	13.64	1.45-128.8	3rd Generation Cephalosporin Resistance								Work with sheep	No	(Ref)	-	-	-	0.032	25.74 (0.887)	Yes	4.1	2.06	60.31	1.06-3433	Contact with birds at home	No	(Ref)	-	-	-	0.027		Yes	3.18	1.47	24.14	1.35-431.5																																																																																													
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*P values are from the Wald Chi squared test; †model includes random slope

3.4 Discussion

The main aim of this study was to estimate the prevalence and risk factors for carriage of AMR and ESBL-producing *E. coli* in people working with dogs. Although the potential role of companion animals as a reservoir of antimicrobial resistance has been considered by other studies (Carattoli et al., 2005; Maddox et al., 2012; Wedley et al., 2011), these have focussed on animal carriage. To the author's knowledge this is the first study of its kind investigating faecal carriage of ESBL-producing *E. coli* in those working with dogs.

The prevalence of faecal carriage of ESBL-producing *E. coli* in this study was 3.1% and ESBL-producing *E. coli* was isolated from personnel working at all types of premises recruited. Few other reports of ESBL carriage in similar populations of individuals exist, making any comparison challenging. Two UK-based studies (Munday et al., 2004; Wickramasinghe et al., 2012) have investigated faecal carriage of ESBL-producing *E. coli* in the community, however both recruited medical patients and only one reported carriage levels of ESBL-producing *E. coli* at the sample level. Wickramasinghe et al. (2012) reported an overall prevalence of carriage of 11.3% in GP and hospital outpatients and also highlighted the difference in carriage rates between different ethnic groups, with those originating from the Middle East and South Asia having a higher carriage rate than those from Europe (22.8% vs. 8.1%). The ethnically diverse nature of the population in this study may have made it more likely for these participants to have travelled to areas with a higher prevalence of ESBL carriage and this has previously been highlighted as a risk factor for ESBL carriage (Kuenzli et al., 2014; Meyer et al., 2012; Östholm-Balkhed et al., 2013; Tängdén et al., 2010). Furthermore, all samples collected by Wickramasinghe et al. (2012) were submitted for diagnostic testing, suggesting the participants were undergoing medical treatment. These participants may therefore have been exposed to further risk factors such as prior hospitalisation or antimicrobial treatment (Kalter et al., 2010; Reuland et al., 2016; Seidman et al., 2009; Tham et al., 2013), and therefore do not offer a direct comparison to the population in this study. Munday et al. (2004) reported an isolate prevalence of 1.9% similar to that identified in this study. However, given the global trend for increasing ESBL carriage over the last two decades (Janvier et al., 2011; Nicolas-Chanoine et al., 2012; Pallecchi et al., 2007; Strömdahl et al., 2011), and the inclusion of other *Enterobacteriaceae*, any comparison should be evaluated with caution. The *E. coli* isolated within this study were sourced from faecal samples and do not represent the clinical picture of antimicrobial resistance in the UK, where the prevalence of resistance to 3GCs in invasive isolates is over 10% (ECDC, 2015; PHE, 2015).

Out with the United Kingdom, more comparable studies in mainland Europe have identified varying carriage rates of ESBL-producing *E. coli* in the general population of 0.6-7.6% (Leflon-Guibout et al., 2008; Meyer et al., 2012; Reuland et al., 2016; Rodrigues et al., 2016), supporting the findings of this study but also indicating national variance in carriage rates. Further studies in northern Europe have also investigated the role of animals in human carriage of ESBL producing *E. coli*. These studies focussed on farm personnel and reported carriage rates of 2.5-19.1% in individuals living or working on cattle, pig and poultry farms (Dahms et al., 2015; Hammerum et al., 2014; Schmithausen et al., 2015; Huijbers et al., 2014). The farm environment does not offer a direct comparison to those working with companion animals and the wide variation of ESBL carriage in these studies highlights how the differing farming systems employed, as well as the animal species present, can impact on antimicrobial resistance (Akwar et al., 2008; Benedict et al., 2015; Morley et al., 2011; Thibodeau et al., 2008). Indeed, intensive farming systems such as those employed on pig and poultry farms often employ prophylactic or metaphylactic antimicrobial treatment regimens and sales of antimicrobials for these species are the highest among veterinary species (VMD, 2015). Despite differences between farming and companion animal work, these studies highlight the potential for transfer of ESBL-producing *E. coli* between humans and animals. Indeed, Huijbers et al. (2014) report an increased prevalence of carriage of 27.1% in those individuals who had had a high level of contact with live broilers. Furthermore, identical ESBL-producing isolates were detected from pigs and staff on numerous farms by Hammerum et al. (2014), indicating potential transfer between species in these premises. The environment of those working with companion animals is different to farming and therefore comparisons with these studies should be viewed with caution. They do, however, further highlight the potential for transfer of resistant bacteria between humans and animals.

The prevalence of AMR faecal *E. coli* identified in this study was considerably higher than ESBL carriage, however, comparison with other research is challenging due to the low number of studies considering AMR *E. coli* in healthy volunteer adult populations. Resistance to at least one antimicrobial was identified in 68.7% of samples and carriage of MDR *E. coli* in 23.6%. Susceptibility testing on varying numbers and classes of antimicrobials in previous studies mean that a comparison of overall resistance is unfeasible. Similarly, many studies considering MDR *E. coli* describe prevalence at the isolate level or only consider the prevalence of multi-drug resistance in ESBL-producing isolates (Bartoloni et al., 2006; Rooney et al., 2009). Previous studies outside of the UK have identified a high prevalence of

resistance to unpotentiated penicillins (49.6-78.0%), tetracyclines (55.1-79%) and trimethoprim, alone or in combination (43.0-53.0%), in healthy volunteer populations (Bonten et al., 1992; Gulay et al., 2000; Nijsten et al., 1996; van den Bogaard et al., 2001). These studies largely indicate a higher prevalence of carriage of resistance to these antimicrobials than reported here. This may be attributed to differing participant demographics in each study, national variations in both antimicrobial prescribing (Goossens et al., 2005) and prevalence of resistance (ECDC, 2015), and the change in prescribing patterns since these studies were undertaken. A high prevalence of resistance to these antimicrobials has also been identified in companion animals (Costa et al., 2008; Maddox et al., 2012; Wedley et al., 2011), indicating similar selection pressures in both human and animal populations.

One further finding in this study is the low prevalence of resistance to amoxycylav which is mirrored in previous human studies (Domínguez et al., 2002; Stürmer et al., 2004). In human medicine, amoxycylav is not generally the empiric treatment of choice for primary care physicians and is reserved for treatment of emergent cases such as sepsis, pyelonephritis or animals bites (PHE, 2015). In contrast, amoxycylav is the most commonly prescribed antimicrobial in UK small animal veterinary practice (Radford et al., 2011) and faecal carriage of amoxycylav-resistant *E. coli* in 7-29% of dogs has previously been reported (Schmidt et al., 2015; Tuerena et al., 2016; Wedley, 2012). Whilst contact with amoxycylav and animals carrying amoxycylav-resistant *E. coli* are likely to be higher in individuals working with dogs than in the general population, this study indicates this exposure does not appear to be driving selection for increased amoxycylav resistance in in-contact humans. However, the increased faecal carriage rates of amoxycylav-resistant *E. coli* in dogs, does highlight a potential reservoir of genes conferring resistance to this drug and, given the complex epidemiology of antimicrobial resistance across multiple species, an integrated research strategy which includes multiple species, populations and environments is therefore warranted.

A high level of diversity in resistance was identified in this study with 38 different resistance phenotypes detected. However, the ten most prevalent phenotypes were heavily represented, with almost three-quarters (73.1%) of isolates displaying these resistance profiles. Cluster analysis highlighted patterns of co-resistance seen in these resistance profiles and the reasons for this are likely multiple. The presence of multiple resistance genes on mobile elements such as plasmids means that resistance to one antimicrobial can

additionally select for resistance to another. In this study, co-selection for resistance to ampicillin, trimethoprim and tetracycline by this mechanism is a likely explanation for the high level of co-resistance observed to these antimicrobials. Secondly, cross-resistance to antimicrobials within the same class is common due to the mechanism of resistance targeting a conserved element of these drugs. For example, resistance mechanisms against quinolone antimicrobials target DNA gyrase enzymes reducing antimicrobial-enzyme binding (Drlica and Zhao, 1997; Tran and Jacoby, 2002). Hence commonly in isolates resistant to ciprofloxacin, there is also resistance to nalidixic acid. Unlike the other antimicrobials tested, fluoroquinolones are defined as critically important for human health by the World Health Organisation (WHO, 2017) and therefore use of these drugs is reserved for critically ill patients or those suffering from MDR infections. Due to the healthy population of interest in this study, exposure to fluoroquinolone antimicrobials is likely low and as such, resistance to these antimicrobials occurs at a lower rate than the other antimicrobial classes tested. This means that although there were numerous fluoroquinolone-resistant phenotypes identified in this study they are represented in low numbers.

Interestingly, although resistance mechanisms to β -lactam antimicrobials target the conserved β -lactam ring present in drugs in this class, amoxycylav and ampicillin do not cluster together (Figure 2). As expected, isolates displaying resistance to amoxycylav did also display co-resistance to ampicillin (Majiduddin et al., 2002), however the low prevalence of amoxycylav resistance meant that ampicillin resistance was more commonly identified in conjunction with resistance to tetracycline and trimethoprim.

Multivariable analysis identified numerous risk factors for the outcomes of interest in this study; however there was very little overlap between each of these outcomes. The identification of recent hospitalisation as a significant risk factor for tetracycline, trimethoprim and multi-drug resistance is not unexpected. Previous reports indicate a high prevalence of faecal carriage of resistant *E. coli* in hospitalised versus healthy individuals (Ko et al., 2012; Overdeest et al., 2011) and recent hospitalisation has previously been highlighted as a risk factor for faecal carriage of resistant *E. coli* and AMR infections. (Bartoloni et al., 1998; Ben-Ami et al., 2009; Ko et al., 2013a; Luvsansharav et al., 2012; Siedelman et al., 2012). This increased risk is likely due to both nosocomial spread of resistant organisms and increased exposure to antimicrobials in hospital environments. These findings highlight the potential for hospitalised patients once discharged, to act as a reservoir of both AMR bacteria and resistance determinants in the wider community. Surprisingly, prior

hospitalisation was not identified as a risk factor for the carriage of 3GC resistance. However, the low prevalence of this outcome alongside low numbers of participants reporting recent hospitalisation may mean there is a lack of statistical power to detect any effect.

Within the population sampled, recent antimicrobial treatment was identified as being associated with ampicillin and tetracycline resistance. The use of antimicrobials has previously been highlighted as a risk factor for the development of antimicrobial resistance in human and veterinary medicine (Kalter et al., 2010; Maddox et al., 2011; Reuland et al., 2016; Stenske et al., 2009; Wedley, 2012), by providing a direct selection pressure for the development of resistance either by chromosomal mutation or acquisition mobile genetic elements (Blair et al., 2014; Levy, 2002). While an association with only two resistance outcomes is initially surprising, narrow spectrum beta-lactams were the antimicrobials most frequently administered to the participants in this study, mirroring the national picture in the UK, where penicillins are the most commonly prescribed antimicrobials in general practice (PHE, 2015). The predominant use of this antimicrobial class by the study population would therefore drive resistance to ampicillin in particular. In addition, the previous findings that resistance to ampicillin and tetracycline were commonly identified in conjunction may indicate this use of ampicillin within this study population lead to co-selection for tetracycline resistance.

The potential importance of diet in the development and dissemination of antimicrobial resistance has also been highlighted in this study. Frequent consumption of pork and poultry were identified as being risk factors for carriage of ampicillin or tetracycline and trimethoprim resistance respectively. Previous studies have also highlighted the potential for pork and poultry to act as a reservoir for pathogenic and non-pathogenic AMR *E. coli* (Jakobsen et al., 2010; Manges et al., 2007; Johnson et al., 2007). The pig and poultry industries rely on more intensive production systems than other meat industries in the UK and the use of metaphylactic or prophylactic treatment regimens is widespread throughout Europe (Callens et al., 2012; Stevens et al., 2007). As such, the use of antimicrobials in these intensive systems is higher per animal than in extensive systems, where treatment of individual animals is more appropriate (VMD, 2015). Indeed, in 2015 antimicrobials for treatment of pigs and poultry exclusively, represented over 70% of antimicrobials sold for use in food producing animals in the UK, with tetracyclines the most frequently purchased antimicrobial in the poultry industry (VMD, 2016). The selective pressure for development of resistance in these animal populations is greater and therefore may lead to contamination

of meat with AMR *E. coli* during processing. Indeed during random testing in 2015, 38% of *E. coli* isolates from caecal samples of randomly selected healthy pigs at slaughter were found to be resistant to ampicillin (VMD, 2016) highlighting these animals as a potential reservoir of resistance.

Regular consumption of salad leaves was also highlighted as risk factor for ciprofloxacin resistance. While antimicrobials are not used commonly in agriculture, this is a plausible risk factor. The use of manure as fertiliser in many agricultural systems provides a potential route for transfer of resistant organisms and mobile genetic elements to the environment (Thanner et al., 2016). Indeed, manure has previously been described as a potential source of antimicrobial resistance genes to soil bacteria (Binh et al., 2008; Wolters et al., 2014) and transfer of *E. coli* from manure to plants for human consumption has been reported (Solomon et al., 2002). Additionally, contaminated water used for crop irrigation has also been highlighted as a source of foodborne pathogens and may therefore be a reservoir of AMR *E. coli* (Steele and Odumeru, 2004; Park et al., 2012). The use of agricultural pesticides may also co-select for antibiotic resistance in *E. coli* (Kurenbach et al., 2015) and therefore add to the environmental reservoir of resistance determinants. Uncooked vegetables and salad leaves have previously been linked with outbreaks of foodborne disease including *E. coli* O157:H7 (Evans et al., 2003; Jenkins et al., 2015; Solomon et al., 2002), and as such it is plausible that these food products may also act as a source of AMR *E. coli*.

Contact with various non-canine animals was also highlighted as having an association with several outcomes. In all cases bar two, the association involved animals within the home rather than the work environment and highlights the potential differences in these two environments. These differences may include both the level and type of physical interaction with animals, which is likely higher in a home environment as well as consideration of biosecurity and hygiene practices, which may be considered more in a work environment than in the home. Indeed, ownership of pet animals has previously been reported as being associated carriage of AMR *E. coli* (Meyer et al., 2012). Contact with both ornamental birds in the home and sheep at work were identified as risk factors for resistance to 3GCs. Conversely, contact with small mammals, cats and poultry were protective against any resistance, ciprofloxacin and nalidixic acid resistance respectively.

While some of these relationships are simple to interpret, others are more challenging. Enrofloxacin, a fluoroquinolone is currently the only authorised parenteral antimicrobial for use in ornamental birds (NOAH, 2016). Co-localisation of resistance genes to both ESBLs and

fluoroquinolones on plasmids (Lavigne et al., 2006; Paterson and Bonomo, 2005) means the use of this drug in birds may also select for resistance to 3GCs. Hence, contact with this drug and droppings of animals treated with it may lead to development or transfer of 3GC-resistant *E. coli* to these owners. The protective nature of cat ownership on ciprofloxacin resistance is interesting. While authorised for use in cats, fluoroquinolones have been associated with retinotoxic effects leading to blindness in this species (NOAH, 2016; Ramirez et al., 2011). The use of this drug in this species may therefore be lower than for some animal species (Radford et al., 2011), hence reducing owner contact with the drug and bacteria resistant to it when compared to owners of other species. Another factor, which may influence this apparent relationship, is differing demographics between cat owners and owners of other animal species. Murray et al. (2010) describe the profiles of cat owners, indicating they are less likely to own dogs, and also more likely to live in semi urban areas than dog owners. Factors such as time spent outdoors and potential exposure to environmental AMR *E. coli* may therefore be lower in this population than in owners of dogs and give the appearance of cats offering protection. The relationship between ovine contact and increasing risk of 3GC resistance is challenging. 3GCs are not approved for use in the sheep farming industry in the UK (NOAH, 2016), so this association may be a proxy for variables not considered here, such as time spent outdoors, land use in the local area or exposure to environmental sources of resistance that may influence carriage rates. Similarly, explaining the protective nature of working with small mammals of carriage of AMR *E. coli* is not simple. To the author's knowledge, no studies have investigated carriage of AMR *E. coli* by small mammals kept as pets, however wild rodents have been shown to carry AMR *E. coli* (Williams et al., 2011) and suggested as potential sentinels for the environmental transmission of antimicrobial resistance (Furness et al., 2017). As such, further investigation of the role of domestic and wild small mammals in the epidemiology of antimicrobial resistance is warranted.

Age of the participants was not identified as a risk factor for antimicrobial resistance in this study. While there is a lack of data on the impact of age on non-ESBL AMR *E. coli*, two previous studies have indicated increasing age is a risk factor for ESBL carriage, with those aged over 65 most at risk (Östholm-Balkhed et al., 2013; Toner et al., 2015). Results in this study suggest this may not be true for non-ESBL AMR *E. coli*, however, the lack of participants over 65 recruited for this study may mean this study lacks the statistical power to detect this effect. Due to the anonymous nature of data collection in this study, no comment can be made on the impact of any other demographic data in this population. The tasks undertaken

at work were also shown not to have a significant effect on carriage of resistant *E. coli*. While it may be expected that those coming into higher levels of contact with animals would have higher carriage rates than those carrying out more administrative roles, the structure of the premises sampled meant that job roles were less restricted. As such, a large proportion of those sampled carried out both administrative and non-administrative tasks and therefore the impact of these roles on carriage was hard to assess. The types of premises sampled did however have an impact on carriage of ciprofloxacin resistance, with those working in veterinary surgeries or rescue centres at higher risk. Given fluoroquinolones are defined as an antimicrobial critically important for human and veterinary medicine (OIE, 2015; WHO, 2017), it is unlikely that these antimicrobials will be encountered at high levels in boarding establishments. In contrast, those working in veterinary practices may be more likely to encounter animals with MDR infections requiring use of these drugs. Of further concern, is the increased risk of carriage by staff in rescue centres. Dogs in rescue centres are commonly surrendered by their owners and have unknown histories. Therefore, the proportion of dogs with chronic ongoing problems including MDR infections is likely higher than in the canine population presenting at either boarding or veterinary premises. The high density of dogs within rescue centres may also facilitate spread of resistant bacteria between dogs and potentially dogs and humans more readily, leading to even higher risk of carriage in personnel working in these environments.

The inclusion of premises as a random effect during data analysis was undertaken due to the clustered nature of the sampling strategy in this study. One would expect participants working in the same premises to be more similar to each other than those in other clusters, as individuals sharing the same environment are more likely to have similar contacts and exposures. On examination, the ICCs of the models constructed indicate that the proportion of the variation attributed to premises varied from 2.9% to 88.7% but, with the exception of 3GC resistance, were in the range previously reported for some infectious diseases (McDermott and Schukken, 1994; Otte and Gumm, 1997). In outcomes where the prevalence was higher, the proportion of variance explained at the premises level was low. This is not unexpected as the more ubiquitous an outcome, the less likely there is to be variation between clusters. Conversely, where the prevalence of an outcome was low, for instance 3GC resistance, the proportion of variance attributed to the premises was much higher (88.7%). Within these premises, there may be specific exposures or animal populations which warrant further investigation.

Several potential limitations were identified in this study. Recruitment of participants was challenging, and 97 fewer samples than originally required were obtained, this was due to both lower staff numbers at recruited premises and an initial overestimation of the response rate achieved (32.9%). Compliance with faecal sampling, even for diagnostic reasons, is commonly poor due to the nature of the sample being requested (Hynam et al., 1995). Therefore, multiple methods were employed to encourage participation, including face to face meetings to discuss the research being undertaken and anonymous sampling. Sterile swabs were also provided for sample collection, which has previously been identified as a preferred method of sampling, rather than requesting whole stool samples (Ellis et al., 2007). This study showed an improvement to the 25% response rate reported by Reuland et al. (2016), which could be indicative of improved compliance due to the sampling methods utilised. In order to increase participant numbers, a larger number of premises were recruited and as such, the data displays a lower level of clustering for ESBL carriage than originally anticipated, with a calculated ICC of 3×10^{-13} which is likely attributed to the low prevalence of ESBL-producing *E. coli*. Similarly, the calculated ICC for carriage of AMR *E. coli* was lower than originally estimated, and was calculated to be 0.05. The reduction in cluster sizes to some extent mitigates the reduced sample size in this study, and therefore it would still appear likely that the prevalence of faecal ESBL-producing *E. coli* in this population is low.

Recruitment methods employed in this study may have introduced some selection bias. Despite random selection of premises, the participants in this study may have been influenced by previous exposure to, or interest in, antimicrobial resistance and therefore not be entirely representative of the population of interest. Due to the anonymised nature of sampling, the demographics of the study population cannot be fully compared to the target population in order to ensure it is representative. However, given the population of interest this was the most practical recruitment method.

Previous studies have shown that duration of carriage of ESBL-producing *E. coli* is variable in humans (Alsterlund et al., 2012; Tandé et al., 2010), here the carriage rate is reported at a single time point and further research investigating longitudinal carriage in this population could provide further information about the potential for community spread of these resistant bacteria. The lack of a control population and limited geographic area within this study makes the comparison of results with the general population challenging. Given the lack of data currently available, one cannot say if this population is at an increased risk of

carriage based on this prevalence alone and further investigation of community carriage of ESBL-producing *E. coli* in the UK is warranted.

The findings of this study indicate that while the prevalence of ESBL carriage is low for people working with dogs, the overall carriage of AMR and MDR *E. coli* is high. The importance of both human-animal contact and diet as risk factors for carriage of AMR *E. coli* have been described, suggesting the potential for exchange of resistance determinants between humans, animal species and the environment. The widespread carriage of AMR commensal *E. coli* in this population is therefore of concern. These bacteria may act as a reservoir of resistance genes to pathogenic bacteria and play a role in dissemination of antimicrobial resistance between humans and animals in the community.

Chapter 4

The prevalence of faecal carriage of antimicrobial-resistant and extended-spectrum β -lactamase-producing *Escherichia coli* in kennelled dogs

4.1 Introduction

Escherichia coli (*E. coli*) is a Gram-negative bacterial species of the family *Enterobacteriaceae*, and a commensal of the lower gastrointestinal tract of humans and animals, including dogs (Berg, 1996; Gordon and Cowling, 2003). Commensal *E. coli* strains play an important role in maintaining the intestinal mucosal barrier against potentially pathogenic bacteria (Dethlefsen et al., 2007; Tlaskalova-Hogenova et al., 2004). However, expression of virulence determinants by pathogenic strains may lead to gastrointestinal and extra-intestinal disease including, but not limited to diarrhoea, urinary tract infections and neonatal septicaemia in both humans and animals (Beutin, 1999; Johnson and Russo, 2002; Lanz et al., 2003; Norris et al., 2000).

Antimicrobial resistance is an increasing challenge faced by both medical and veterinary clinicians globally (Levy and Marshall, 2004; Wieler et al., 2011), with significant societal and economic implications (Cosgrove and Carmeli, 2003; Paladino et al., 2002). Reduced susceptibility of clinical *E. coli* isolates to commonly prescribed antimicrobials has been reported in multiple species (Farrell et al., 2003; Huber et al., 2013; Lanz et al., 2003; Sader et al., 2014; Wagner et al., 2014), and of increasing concern is the emergence of multi-drug resistant (MDR) isolates, resistant to three or more antimicrobial classes, in both human and veterinary patients (Gibson et al., 2010a; Ikram et al., 2015; Sidjabat et al., 2006b; Wagner et al., 2014). The dissemination of such isolates has been implicated in nosocomial infections in hospitalised dogs (Gibson et al., 2010b; Sanchez et al., 2002), and is linked to increased morbidity and mortality in human patients (Melzer and Petersen, 2007).

As a member of the commensal microflora, non-pathogenic strains of *E. coli* are exposed to antimicrobials used for the treatment of bacterial infections in patients, driving the acquisition and dissemination of antimicrobial resistance determinants in this population (Edlund et al., 1994; Jakobsson et al., 2010; Nyberg et al., 2007). These resistance genes may be acquired either through chromosomal mutation or transfer of mobile genetic elements such as plasmids (Blake et al., 2003; Karami et al., 2007), and hence these bacteria may therefore act as a potential reservoir for resistance genes. This antimicrobial exposure, combined with ease of isolation of *E. coli* from faecal samples of dogs, means that *E. coli* can act as a good indicator bacteria for the reservoir of antimicrobial resistance circulating in the gastrointestinal commensal population (De Graef et al., 2004; van den Bogaard and Stobberingh, 2000).

The World Health Organisation (WHO) and the World Organisation for Animal Health (OIE), have defined antimicrobials which are of critical importance to both human and veterinary medicine. Included in these lists are third generation cephalosporins (3GCs), fluoroquinolones, macrolides and glycopeptides (OIE, 2015; WHO, 2017). Currently, resistance mediated by extended-spectrum beta-lactamase (ESBL) enzymes is particularly concerning. These plasmid mediated enzymes were first reported by Kliebe et al. (1985) and confer resistance to a wide range of β -lactams including 3GCs, such as cefotaxime and ceftazidime, while remaining susceptible to beta-lactamase inhibitors such as clavulanic acid (Philippon et al., 1989). ESBL-producing isolates commonly display MDR phenotypes due to the propensity for carriage of multiple resistance genes, including those conferring resistance to fluoroquinolones and aminoglycosides, on a single plasmid (Lavigne et al., 2006; Paterson and Bonomo, 2005). Of further concern, are the plasmid-mediated AmpC enzymes, which additionally confer resistance to β -lactamase inhibitors, further limiting treatment options and masking the presence of ESBL production (Jacoby, 2009).

Clinically, ESBL-producing isolates have been identified as a major cause of nosocomial (Leistner et al., 2015; Wieler et al., 2011) and community-acquired infections in humans (Doi et al., 2013; Ho et al., 2007; Pitout et al., 2005; Rogers et al., 2011) and dogs (Huber et al., 2013; Timofte et al., 2011; Warren et al., 2001). However, ESBL production by commensal *E. coli* is also of concern. First reported in 1988 (Matsumoto et al., 1988), canine faecal carriage of ESBL-producing *E. coli* has been reported in up to 4.1% of non-hospitalised dogs in the UK (Schmidt et al., 2015; Wedley et al., 2011; Wedley, 2012). These reports however focus solely on privately owned dogs residing in residential properties. Furthermore, global investigation of carriage rates in kennelled dogs is limited to a single study reporting a prevalence of 7.0% in South Korea (Harada et al., 2011), which may not be comparable to kennels in the UK. Kennel environments commonly house a high density of animals, and given regular, close contact between kennel staff and these animals, present a potential site for transmission of ESBL-mediated resistance between humans and dogs (Guardabassi et al., 2004; Meyer et al., 2012).

The aim of this study was to identify the prevalence of faecal carriage of ESBL-producing and antimicrobial-resistant (AMR) *E. coli* in dogs housed in kennels in the North of England.

4.2 Materials and methods

4.2.1 Study population

For this study, dogs kennelled at boarding establishments and rescue centres were recruited in the North of England within a 75 mile radius of the University of Liverpool, Leahurst Campus. Premises were selected for inclusion in the study via random number generation from registers of licensed boarding kennels and internet searches for rescue centres as previously described (Chapter Three). Boarding and rescue establishments were recruited simultaneously for this study and the study in Chapter Three.

The prevalence of faecal carriage of ESBL-producing *E. coli* in community dog populations in the UK has previously been estimated as 0.5-4.1% (Schmidt et al., 2015; Wedley et al., 2011; Wedley, 2012). A sample size for this study was therefore calculated based on an expected prevalence of 4% within this population, indicating 60 dogs (n) would be required to estimate prevalence with a precision of 5% and 95% confidence. Adjustment of this sample size estimate due to the clustered nature of data collection indicated an adjusted sample size (n^1) of 174 dogs would be required. This calculation assumed an intraclass correlation coefficient (ρ) of 0.1 and an estimated sample size per cluster (m) of 20.

$$n^1 = n(1+\rho(m-1))$$

In order to allow for a response rate of around 70%, the aim was to recruit 30 participants per premises where possible. However, due to the varied size of premises involved in the study, where this was not possible, the number of sample packs left was tailored to the boarding capacity of the kennel premises in order to ensure compliance. Staff members at participating premises were asked to collect a single voided faecal sample from dogs kennelled on the premises. Dogs were excluded from the study if they were sharing a kennel and the identity of the dog which was being sampled could not be confirmed e.g. by sampling during exercise.

Sample packs were assembled and contained an owner information sheet describing the aims of the study and what participating would entail, a written consent form for owners to sign and a questionnaire (Appendix II). The two-part questionnaire was designed using Microsoft Word 2010 (Microsoft Corporation). The first part of this questionnaire was to be completed by the owner on arrival at the kennels. This section was two-sided and comprised a small number of questions relating to dog signalment and duration of stay in kennels. Once

completed, this was returned with the faecal sample. Part Two was a longer five-page questionnaire and was mostly comprised of questions with tick box responses and data were collected relating to ownership of the dog, veterinary history, the home environment (including animal housing, diet and contact with other animals), and a short section relating to owner contact with animal and healthcare environments. Space was provided at the end of both questionnaires for any additional information which the participant wished to provide. All paperwork was reviewed by researchers involved in the study prior to submission to the University of Liverpool Veterinary Research Ethics Committee. Ethical approval was granted in April 2014 and recruitment of premises began in June 2014, continuing until June 2015.

4.2.2 Sample collection and *E. coli* isolation

All premises were visited in order to provide information about the study, explain participant recruitment protocols and distribute sample packs. In the case of boarding kennels, recruitment of participants was carried out by the kennel personnel during admittance of the animal. Following provision of written informed consent, the owner was requested to complete part one of the questionnaire on site and was provided part two to complete at their convenience with a prepaid return addressed envelope. Informed consent for sampling at rescue kennels was given by the appropriate staff member and a member of kennel staff also completed the questionnaire. Staff members at boarding kennels and rescue centres were asked to collect a fresh pea-sized faecal sample from participating dogs on a swab, mirroring sample collection methods for humans, reported in Chapter Three. Samples with enclosed questionnaires were collected from the premises at a pre-arranged time, no longer than 48 hours after sample collection. Contents of all sample packs contained a unique reference number in order to ensure samples and questionnaires were correctly matched when returned.

Once received, samples were transported directly in a cool box to the University of Liverpool for immediate processing. The complete sample was homogenised with 1ml brain heart infusion broth containing 5% glycerol. Half of the faecal homogenate was then enriched aerobically in 4.5ml buffered peptone water for 18-24 hours at 37°C, with the remainder stored at -80°C. In order to identify AMR *E. coli*, the enriched faecal homogenate was inoculated on to the surface of an eosin methylene blue agar (EMBA) plate and four antimicrobial discs (ampicillin 10µg, amoxycylav 30 µg, ciprofloxacin 1 µg, trimethoprim 2.5 µg) applied (Bartoloni et al., 1998). In order to screen for ESBL-producing *E. coli*, the

homogenate was streaked onto a two EMBA plates, one containing cefotaxime (1µg/ml), one containing ceftazidime (1µg/ml) (Liebana et al., 2006). Finally, to enable non-selective culture of *E. coli*, an EMBA plate containing no antimicrobials was streaked with the faecal homogenate. All plates were then incubated aerobically at 37°C for 18-24 hours and colonies resembling *E. coli* morphologically were then selected for further testing. From the EMBA plate with antimicrobials discs, a single colony growing within the inhibition zone of each of the antimicrobial discs was selected. Where growth had occurred on the plates containing cefotaxime and ceftazidime a single colony was selected, and three colonies were randomly selected from the plain EMBA. All isolates were then sub-cultured on nutrient agar for 18-24 hours at 37°C prior to further testing. All presumptive isolates of *E. coli* were confirmed using a PCR assay to detect the presence of the *E. coli* specific *uidA* gene (McDaniels et al., 1996). All media was sourced from Lab M Ltd Bury, UK, antimicrobial discs were obtained from MAST Group Ltd., Bootle, UK, and cefotaxime and ceftazidime powder was sourced from Sigma–Aldrich Company Ltd., Gillingham, UK.

4.2.3 Antimicrobial susceptibility testing

All *E. coli* isolates were subjected to further antimicrobial susceptibility in accordance with the British Society for Antimicrobial Chemotherapy (BSAC) guidelines (BSAC, 2013). An Iso-Sensitest agar plate was inoculated with each isolate and a panel of seven antimicrobial discs applied (ampicillin 10 µg, amoxycylav 30 µg, ciprofloxacin 1 µg, chloramphenicol 30µg, nalidixic acid 30µg tetracycline 30 µg and trimethoprim 2.5 µg). Following incubation for 18-24 hours at 37°C the diameter of the zone of inhibition around each disc was measured in millimetres and recorded.

4.2.4 Phenotypic confirmation of ESBL production

The combination disc diffusion test (M'Zali et al., 2000) was performed on all isolates selected from EMBA plates containing a 3GC. Briefly, three pairs of antimicrobial discs were applied to Iso-Sensitest agar plates inoculated with each isolate. The paired discs used were cefotaxime (30µg) and cefotaxime/clavulanic acid (30/10µg), cefpodoxime (30µg) and cefpodoxime/clavulanic acid (30/10µg), ceftazidime (30µg) and ceftazidime/clavulanic acid (30/10µg). Plates were incubated for 18-24 hours at 37°C and the diameter of each of the zones of inhibition was recorded in millimetres and compared to BSAC breakpoints (BSAC, 2015). Resistance to any of the 3GC discs alone, with an increase of at least 5mm in the diameter of the zone of inhibition in the presence of clavulanic acid confirmed ESBL

production. A difference of less than 5mm indicates another potential mechanism conferring 3GC resistance.

4.2.5 Statistical analysis

All data were entered into a database (Microsoft Excel 2010, Microsoft Corporation) and the dataset was coded and reviewed to confirm data input accuracy. Antimicrobial susceptibility data of isolates were compared to BSAC breakpoint values (BSAC, 2015) and coded as a binary variable either resistant (1) or non-resistant (0). Data were then exported into R (www.r-project.org) for analysis.

The resistance outcomes considered were antimicrobial resistance (presence of an *E. coli* isolate resistant to any of the antimicrobials tested), resistance to each of the seven antimicrobials tested, 3GC resistance, multi-drug resistance (presence of an *E. coli* isolate resistant to at least three antimicrobial classes) and phenotypically confirmed ESBL production.

Due to the sampling strategy employed in this study, data were clustered within premises; therefore, logistic regression models were built including premises as a random effect to account for clustering at this level as previously described in Chapter Three. The true prevalence (P_T) was calculated using the beta coefficient (β_0) from the intercept-only random effects model constructed for each outcome using the formula below. Confidence intervals for prevalence estimates were calculated as a function of the standard error of the beta coefficient from the intercept-only models.

$$P_T = \frac{e^{\beta_0}}{1 + e^{\beta_0}}$$

Confidence intervals for questionnaire data were calculated as the proportion +/- 1.96 x standard error.

Further examination of the resistance phenotypes displayed by *E. coli* isolates was undertaken in order to assess which antimicrobials isolates were more likely to display co-resistance to. Following calculation of binary distance matrices, hierarchical cluster analysis was undertaken using Ward's method and performed in R using the graphics package (R Core Team, 2015).

4.3 Results

4.3.1 Study population

In total, 188 boarding and rescue premises were contacted and 33 agreed to participate in this study and the location of these sites is detailed in Chapter Three. At least one dog was recruited from 32 of the premises, and the median number of dogs enrolled per premises was eight (Interquartile Range (IQR) 5.0-9.0 dogs). A single faecal sample was collected from each of the 296 kennelled dogs enrolled in the study. All of the samples yielded bacterial growth and usable questionnaires were returned with 292 of these samples.

4.3.2 Survey Results

4.3.2.1 Dog characteristics and veterinary treatment

The median age of the dogs sampled in this study was five years (IQR 2.5-8.0 years). Approximately equal numbers of male (52.6% 95% CI 46.8-58.4) and female (47.4% 95% CI 41.6-53.2) dogs were sampled, of which 62.9% (95% CI 57.1-68.6) were neutered. Over two-thirds of the dogs sampled were reported as being pure breeds (69.1% 95% CI 63.8-74.4). Fifty-five different breeds were represented in the study and Labrador retrievers were the most prevalent, representing 8.2% (95%CI 5.0-11.4) of the animals recruited.

Over half of the dogs' sampled (53.8% 95% CI 47.8-59.8) had received veterinary treatment within the last six months, of which 35.6% (95% CI 27.4-43.8) were for routine reasons, including vaccination, preventative parasite treatment and routine neutering. Owners reported 17.5% (95% CI 12.9-22.0) of dogs had received antimicrobial treatment in the last six months, while only 4.2% (95% CI 1.7-6.6) had been hospitalised over this period. Further information regarding dog characteristics and veterinary treatment administered can be found in Table 4.1.

Table 4.1 Summary of dog characteristics and veterinary treatment in a cross-sectional survey of 296 kennelled dogs in the North of England

		Percent	95% CI
Sex	Male Entire	21.7	16.8-26.6
	Male Neutered	32.0	26.4-37.5
	Female Entire	15.4	11.1-19.7
	Female Neutered	30.9	25.4-36.4
Breed	Pure Breed	69.1	63.8-74.4
	Crossbreed	30.9	25.6-36.2
Breed Size	Toy	3.5	1.4-5.6
	Small	20.4	15.8-25.1
	Medium	36.7	31.1-42.2
	Large	36.3	30.8-41.9
	Giant	3.1	1.1-5.1
Veterinary Treatment	<1m	28.4	23.0-33.8
	2-3m	13.6	9.5-17.8
	4-6m	8.0	4.7-11.2
	>6m	46.2	40.2-52.2
	Not Specified (<6m)	3.8	1.5-6.1
Antimicrobial Treatment	1m	8.6	5.2-11.9
	2-3m	5.2	2.6-7.9
	4-6m	3.7	1.5-6.0
	>6m	82.5	78.0-87.1
Hospitalisation	1m	0.4	0-1.1
	2-3m	1.9	0.2-3.5
	4-6m	1.9	0.2-3.5
	>6m	95.8	93.4-98.3

4.3.2.2 Kennel and home environment

Of the dogs sampled, 64.4% (95%CI 58.9-69.9) were recruited from boarding kennels and most (66.5% 95% CI 59.7-73.2) were identified as overnight boarders. The median duration of kennelling was 10.0 days (IQR 3.0-175.5), with 77.9% (95%CI 73.1-82.6) of dogs kennelled individually.

When within their full-time residence, most dogs were housed either indoors (49.8% 43.8-55.8) or in a kennel environment (44.2% 95% CI 38.2-50.2), with a small number housed outdoors. Only 32.3% (95% CI 26.7-38.0) of animals sampled had direct contact with other dogs and approximately a quarter of dogs (25.8% 95% CI 20.5-31.0) had regular contact with other animals. Further details about kennelling and the home environment are reported in Table 4.2.

Table 4.2 Summary of kennelling and details of the home environment provided by respondents in a cross-sectional survey of 296 kennelled dogs in the North of England

		Percent	95% CI
Premises Type	Boarding Kennel	64.4	58.9-69.9
	Rescue Centre	35.6	30.1-41.1
Boarder Type	Overnight Boarder	43.3	37.5-49.0
	Rescue	34.9	29.5-40.4
	Kennel Resident	17.3	12.9-21.7
	Day Boarder	4.5	2.1-6.9
Number of dogs per kennel	1 Dog	77.9	73.1-82.6
	2 Dogs	15.6	11.4-19.8
	3 Dogs	1.0	0-2.2
	4+ Dogs	5.5	2.9-8.2
Full-time Housing	Indoor	49.8	43.8-55.8
	Kennel	44.2	5.9-12.9
	Outdoor	9.4	38.2-50.2
Diet	Complete Dry	56.9	51.0-62.9
	Wet food	18.7	14.0-23.4
	Dry Mixer	82.8	78.2-87.3
	Raw Meat and Bones	9.0	5.6-12.4
	Cooked Meat	7.1	4.0-10.2
Residential Animal Contact	Direct contact-Dogs	32.3	26.7-38.0
	Direct contact-Other Animals	25.8	20.5-31.0
	Cat	15.5	11.2-19.9
	Horse	10.2	6.6-13.9
	Farm	8.7	5.3-12.1
	Small Mammals	4.5	2.0-7.1
	Wildlife	9.8	6.3-13.4
	Other	1.9	0.2-3.5

4.3.3 Sample prevalence of carriage of antimicrobial-resistant *E. coli*

A total of 508 non-duplicate isolates of *E. coli* were identified from the 296 faecal samples obtained in this study, the median number recovered per sample was one (IQR 1-2). The isolates were classified based on their antimicrobial resistance phenotypes. Resistance was identified against all antimicrobials which were tested and isolates with resistance to at least one antimicrobial were isolated from 168 (56.8% 95% CI 51.1-62.4) samples. Isolates displaying an MDR phenotype were recovered from 66 (22.3% 95% CI 17.5-27.0) samples. Over half of the samples (n=153) demonstrated resistance to ampicillin, and resistance to trimethoprim (n=119) or tetracycline (n=86) was also prevalent. The sample prevalence of resistance to each antimicrobial tested, multi-drug resistance, 3GC resistance and an ESBL phenotype after correction for clustering are detailed in Table 4.3.

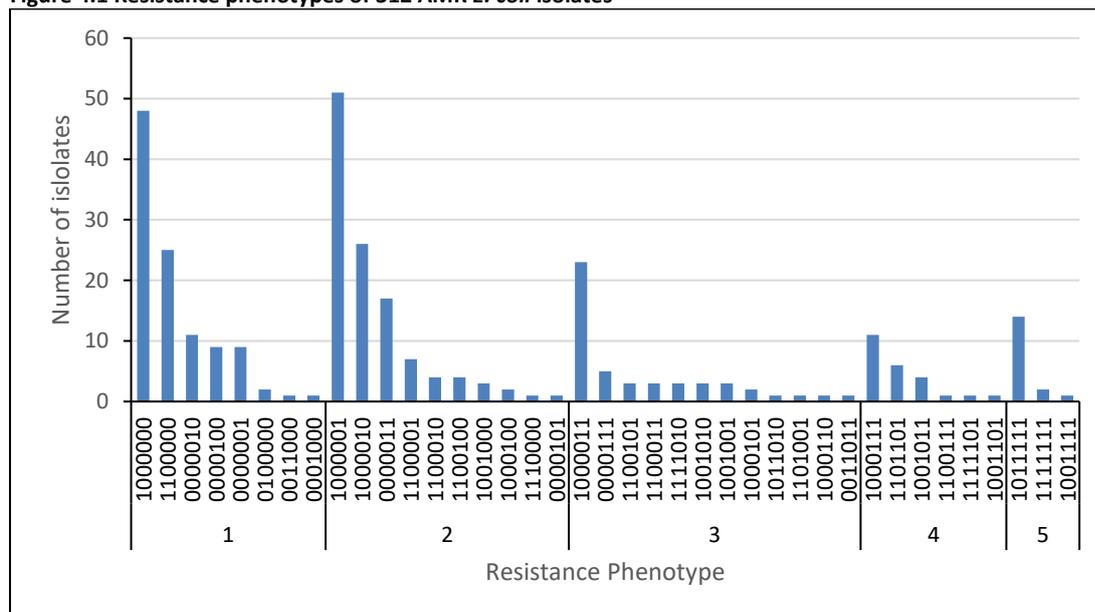
Table 4.3 The number and sample prevalence of AMR faecal *E. coli* (adjusted for clustering) within in a cross-sectional study of 296 kennelled dogs in the North of England

Resistance Outcome	Boarding premises (n=190)		Rescue premises (n=106)		All premises (n=296)	
	n	Adjusted Prevalence (95% CI)	n	Adjusted Prevalence (95% CI)	n	Adjusted Prevalence (95% CI)
Any resistance	102	55.8 (41.1-69.6)	66	65.8 (43.2-82.9)	168	58.9 (46.3-70.4)
Ampicillin	91	48.4 (30.4-66.9)	62	60.6 (30.2-84.5)	153	51.9 (35.7-67.7)
Amoxycylav	25	10.7 (5.7-19.2)	26	13.4 (3.4-40.4)	51	10.6 (5.6-19.0)
Chloramphenicol	28	11.1 (5.8-20.3)	25	15.7 (15.6-15.8)	53	12.3 (7.0-20.8)
Ciprofloxacin	13	0.01 (0.0-1.6)	9	4.0 (0.5-24.6)	22	0.1 (0.0-3.4)
Nalidixic Acid	19	0.6 (0.01-37.5)	17	3.0 (0.1-47.1)	36	1.1 (0.1-19.5)
Tetracycline	53	23.7 (23.6-23.8)	33	28.5 (14.2-48.9)	86	25.4 (16.1-37.6)
Trimethoprim	64	25.9 (25.8-25.9)	55	54.5 (35.0-72.8)	119	34.4 (22.3 48.9)
Multi-drug resistance	35	11.4 (4.4-26.4)	31	17.3 (4.2-50.1)	66	12.8 (5.7-25.9)
Third-generation cephalosporin resistance	27	9.7 (4.5-19.8)	23	10.8 (2.4-37.1)	50	9.7 (4.6-18.8)
ESBL phenotype	10	0.001 (0.0-1.3)	0	0.0 (0.0-0.0)	10	0.003 (0.0-0.45)

4.3.4 *E. coli* resistance phenotypes

Of the 508 *E. coli* isolates recovered in this study 312 (61.6% 95% CI 57.42-65.9) were found to be resistant to at least one antimicrobial. These isolates most commonly displayed resistance against one (34.0% 95% CI 28.7-39.2) or two (37.2% 95% CI 31.8-42.5) antimicrobial classes, with two isolates found to be resistant to all antimicrobials against which they were tested. Thirty-eight resistant phenotypes were detected within this study but over three-quarters (75.3%) of the isolates were represented by the ten most commonly identified phenotypes. Co-resistance to ampicillin and trimethoprim (16.3%) and resistance to ampicillin alone (15.4%) were the most prevalent phenotypes. However, MDR phenotypes were commonly identified and a total of 90 isolates representing 21 different MDR phenotypes were recovered. Details of the resistance phenotypes identified can be found in Figure 4.1.

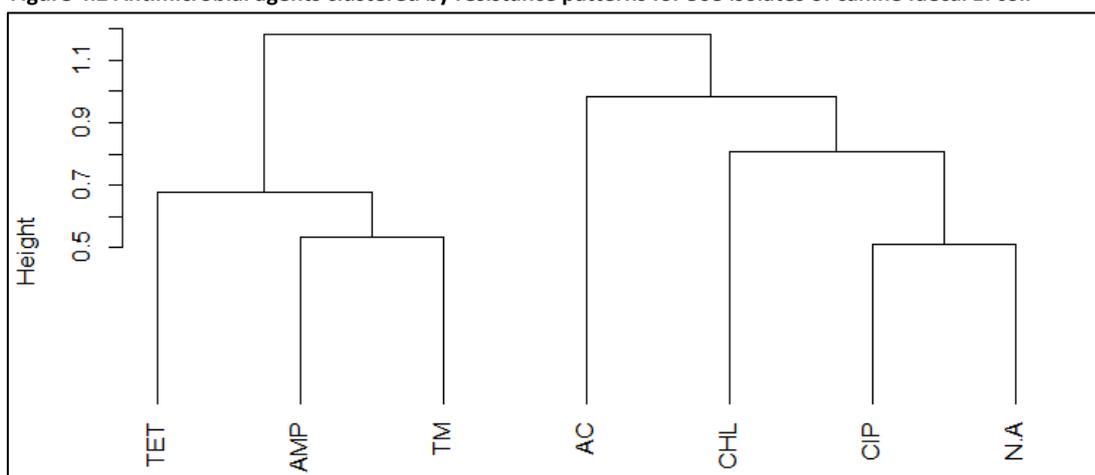
Figure 4.1 Resistance phenotypes of 312 AMR *E. coli* isolates



The profiles indicate resistance (1) or susceptibility (0) to each antimicrobial agent in the following order: ampicillin, amoxyclav, ciprofloxacin, nalidixic acid, chloramphenicol, tetracycline and trimethoprim. The profiles are ordered from left according to the number of antimicrobial classes (numbered one to five) against which the phenotype confers resistance.

Cluster analysis of isolates using Ward’s method evaluated patterns of co-resistance to antimicrobials (Figure 4.2). The findings mirrored those seen with human isolates in Chapter Three and again highlight the propensity for co-resistance to antimicrobials in the same class, highlighted by the proximity of quinolone antimicrobials as well as a tendency for isolates to display co-resistance to ampicillin and trimethoprim or tetracycline.

Figure 4.2 Antimicrobial agents clustered by resistance patterns for 508 isolates of canine faecal *E. coli*



Key: AMP=Ampicillin; AC=Amoxyclav; CHL=chloramphenicol; CIP=ciprofloxacin; N.A=nalidixic acid; TET=tetracycline; TM=trimethoprim; Isolates resistant to one antimicrobial are more likely to display resistance to an antimicrobial on close branches of the dendrogram. For example, an isolate resistant to ciprofloxacin is more likely to be resistant to nalidixic acid than to trimethoprim.

4.3.5 ESBL-producing isolates

In total, 12 (2.3% 95%CI 1.3-3.7) non-duplicate ESBL-producing isolates were identified phenotypically from ten dogs at four premises. All of the dogs carrying ESBL-producing *E. coli* were located at boarding kennels, while no ESBL-producing isolates were recovered from rescue dogs. All of these isolates displayed resistance to cefpodoxime and cefotaxime on paired disc diffusion testing, while resistance to ceftazidime was identified in nine isolates. Co-resistance to all three 3GCs was most commonly identified (n=9). Antimicrobial susceptibility testing of these isolates indicated that all isolates, as anticipated, displayed resistance to ampicillin while five isolates displayed a MDR phenotype. Isolates were most commonly resistant to two (n=6) or three (n=5) antimicrobial classes and no isolates displayed pan-resistance to all antimicrobials tested. A total of seven different resistance phenotypes were represented by the ESBL-producing isolates recovered in this study, with three resistance profiles identified on more than one occasion. Interestingly however, three isolates displaying the same resistance phenotype were isolated from three dogs kennelled at the same premises. Further details regarding the resistance phenotypes displayed by ESBL-producing isolates in this study can be located in Table 4.4.

Table 4.4 Details of resistance phenotypes displayed by ESBL-producing *E. coli* isolates within in a cross-sectional study of kennelled dogs in the North of England

Isolate	Sample	Premises	Premises Type	Antimicrobial resistance phenotype
139	37	7	Boarding	AMP CAZ CPD CTX NA TM
151	38	7	Boarding	AMP CAZ CPD CTX
193	38	7	Boarding	AMP CAZ CPD CTX NA
159	39	7	Boarding	AMP CAZ CPD CTX NA
194	39	7	Boarding	AMP CAZ CPD CTX TET
173	41	7	Boarding	AMP CAZ CPD CTX NA
178	42	7	Boarding	AMP AC CAZ CIP CPD CTX NA TET
191	43	7	Boarding	AMP CAZ CPD CTX TET TM
528	106	29	Boarding	AMP CAZ CPD CTX NA TET
540	109	29	Boarding	AMP CPD CTX TET
714	145	36	Boarding	AMP CPD CTX TET TM
1328	284	55	Boarding	AMP CPD CTX TET

Key: AMP Ampicillin; AC Amoxyclav; CAZ Ceftazidime; CIP Ciprofloxacin; CPD Cefpodoxime; CTX Cefotaxime; NA Nalidixic Acid; TET Tetracycline; TM Trimethoprim

4.4 Discussion

This study aimed to estimate the faecal carriage rate of AMR and ESBL-producing *E. coli* in kennelled dogs in the North of England. Previous studies investigating carriage of AMR *E. coli* in UK dogs have been reported, however studies have been restricted to those receiving

veterinary treatment (Wedley, 2012), specific breeds (Schmidt et al., 2015), or communities in restricted geographic regions (Wedley et al., 2011). To the author's knowledge, this is the first study investigating the prevalence of faecal AMR and ESBL-producing *E. coli* in UK kennelled dogs in the community.

The prevalence of AMR *E. coli* in this study was 58.9% (95% CI 46.3-70.4) mirroring the prevalence reported by Schmidt et al. (2015) in healthy Labrador retrievers, but markedly higher than a previous study investigating community dogs in the North West of England which reported a prevalence of 29% (95% CI 22.4-35.5) (Wedley et al., 2011). Both of these studies used the same laboratory methods reported here and as such, direct comparisons can be undertaken. The explanation for the increased prevalence of AMR reported both in this study and by Schmidt et al. (2015) over that by Wedley et al. (2011) is likely two-fold. Firstly, as antimicrobial resistance is recognised as a growing problem globally in both human and animal populations (Weese, 2008; Seiffert et al., 2013b; Levy and Marshall, 2004; Wieler et al., 2011), the temporal difference in the studies would likely have an impact on the reported prevalence. Given the five-year difference in data collection between these studies, it is not unexpected that the prevalence of resistance has increased in canine populations in this time. Secondly, the dogs within this study were kennelled at the time of sampling whereas household sampling was undertaken by Wedley et al. (2011). It has previously been reported that dogs in kennel environments have a higher prevalence of faecal AMR *E. coli* carriage than those within home environments (De Graef et al., 2004). This difference can likely be attributed to the close proximity of animals to one another and increased animal density within the kennel environment which could lead to transmission of resistant isolates between individuals. Additionally, outbreaks of disease within a kennel, for example infectious canine tracheobronchitis, may lead to the requirement for metaphylactic treatment of a group of animals housed together. Gastrointestinal commensals of these animals will therefore be under mutual selection pressure for development of resistance which individually housed dogs would not.

Comparison of overall prevalence of AMR *E. coli* with studies other than those reported above is challenging due to the varied use of selective or non-selective isolation methods, the number of isolates selected for susceptibility testing and antimicrobials tested (Costa et al., 2008; Murphy et al., 2009). Similarly, many studies have reported prevalence of resistance at an isolate level rather than considering the sample as the unit of interest (Carattoli et al., 2005; De Graef et al., 2004; Harada et al., 2012). However, while this means

direct comparisons cannot always be made similar patterns of resistance were reported as in this study. Previous studies report resistance to ampicillin, trimethoprim and tetracycline as being most prevalent in community animals (Costa et al., 2008; De Graef et al., 2004; Schmidt et al., 2015; Wedley et al., 2011), as well as those presenting to veterinary practices (Harada et al., 2011; Murphy et al., 2009; Wedley, 2012), supporting the findings reported here. Additionally, a sample prevalence of resistance to amoxycylav of 10.6% (95% CI 5.6-19.0) was identified, mirroring results previously reported in healthy dogs (3.8-18%) (Murphy et al., 2009; Schmidt et al., 2015; Wedley et al., 2011), and indicating faecal carriage of amoxycylav-resistant *E. coli* in dogs is higher than that reported for in-contact humans (Chapter Three). This finding is not unexpected given the frequent use of amoxycylav in small animal veterinary practice when compared to human medicine. Indeed, β -lactams are reported as the most frequently prescribed antimicrobial class approved for use in companion animals, with amoxycylav the most commonly prescribed antimicrobial drug (Hughes et al., 2012; Radford et al., 2011; Singleton et al., 2017). This use leads to an increased selection pressure on gut commensals to develop resistance to this drug, either by chromosomal mutation or acquisition of plasmid mediated resistance genes (Blair et al., 2014; Levy, 2002). In contrast, in human medicine use of amoxycylav is generally reserved for emergent cases such as sepsis or animal bites (PHE, 2015). Isolates resistant to amoxycylav were additionally found to be co-resistant to ampicillin, which is not unexpected. β -lactamase enzymes produced by amoxycylav-resistant isolates target the β -lactam ring conserved in all antimicrobials in this class therefore allowing cross-resistance between these drugs (Majiduddin et al., 2002).

A similar pattern of cross resistance, although at much lower levels, can be observed for the quinolone antimicrobials, where resistance to ciprofloxacin is most commonly preceded by nalidixic acid resistance. The low prevalence of resistance to ciprofloxacin reported here has been described previously (Schmidt et al., 2015; Wedley et al., 2011), and where present is commonly associated with MDR isolates. This finding can be explained by the limited number of fluoroquinolones currently approved for systemic use in dogs (NOAH, 2016) and the categorisation of these drugs as critically important to both human and animal health (OIE, 2015; WHO, 2017). Fluoroquinolones are therefore, used in reduced frequency to other antimicrobials (Hughes et al., 2012; Radford et al., 2011; Singleton et al., 2017) and are reserved as a 'last-line' treatment in clinical practice where resistance to other antimicrobials is present (Platell et al., 2010).

The prevalence of MDR *E. coli* in this study was 12.8%, in agreement with previous studies in healthy dogs where MDR *E. coli* was isolated from 11.0-15.0% of dogs (Murphy et al., 2009; Wedley et al., 2011). Interestingly however, the prevalence of MDR *E. coli* in a more recent study involving dogs recruited at dog shows reported an increased frequency; double that reported here (Schmidt et al., 2015). The reasons for this difference are likely to be due to the differing demographics of the populations sampled. Both populations of dogs sampled were in environments of high dog density; however, direct contact between different dogs is much more likely in the population sampled by Schmidt et al. due to the nature of dog shows. Similarly, the show dogs were all of a single breed and likely to be high value dogs. It may therefore be likely these animals would receive more frequent veterinary treatment including antimicrobial treatments. The diet and lifestyle of these dogs may also be different than those sampled within this study.

The prevalence of resistance to 3GCs in this study was 9.7% and is supported by previous studies which report a prevalence of up to 16.0% in healthy dogs (Ben Sallem et al., 2013; Gandolfi-Decristophoris et al., 2013; Murphy et al., 2009; Schmidt et al., 2015; Wedley et al., 2011). The lower prevalence of resistance to this class of drugs when compared to other antimicrobials is not unexpected given that only a single injectable 3GC, cefovecin, is approved for systemic use in dogs (NOAH, 2016). The first line use of this drug in dogs is infrequent, due to both its categorisation as a critically important antimicrobial (OIE, 2015), and the cost of the drug which may be prohibitive, particularly in larger dogs and in charity run rescue centres.

However, the prevalence of ESBL-producing isolates was lower than previously reported in both vet visiting and healthy dogs (Damborg et al., 2015; Gandolfi-Decristophoris et al., 2013; Schaufler et al., 2015; Wedley et al., 2011). Within this study, the prevalence of phenotypic ESBL-producing isolates only are reported; other mechanisms of resistance such as AmpC enzymes are resistant to both third-generation cephalosporins and clavulanic acid and as such have the ability to mask ESBL production. This could prevent the phenotypic detection of some ESBL-producing isolates, reducing the apparent prevalence. Further molecular characterisation of all 3GC-resistant isolates could elucidate if this phenomenon has occurred. The use of other veterinary antimicrobial treatments in this population may explain this reduced ESBL-prevalence further. Indeed, the use of the veterinary antimicrobial enrofloxacin has been identified as selecting for faecal carriage of ESBL-producing *E. coli* (Moreno et al., 2008). This finding is explained by the presence of ESBL genes on plasmids

which carry multiple different resistance genes including those conferring low level resistance to fluoroquinolones (Strahilevitz et al., 2009; Jiang et al., 2008). As discussed previously, the use of fluoroquinolones in non-hospitalised animals is low, therefore, less selection pressure for ESBL-producing *E. coli* would be exerted than for vet visiting or hospitalised animals, where these drugs may be used more frequently (Huber et al., 2013; Johard et al., 2015; Tuerena et al., 2016; Wedley, 2012). Further causes of the reduced ESBL prevalence in this study compared to previous studies may be national variation in carriage rates and the diverse populations sampled in different studies. Furthermore, in this study statistical analysis allowed for clustering of data leading to a reduced true prevalence, whereas many other studies did not consider this.

Given the low numbers of ESBL-producing isolates identified phenotypically in this study, other mechanisms conferring resistance to extended-spectrum cephalosporins, such as AmpC enzymes, may also play an important role in this population. The predomination of AmpC over ESBL enzymes in both commensal and clinical isolates from dogs has previously been reported (Damborg et al., 2009; Gibson et al., 2010b; Sidjabat et al., 2006a; Wagner et al., 2014; Wedley et al., 2011). This is an interesting finding, given that the converse is true for human clinical isolates (Jacoby, 2009; Philippon et al., 2002; Wagner et al., 2014). This finding is likely due to differing selection pressures between canine and human populations. Increased use of amoxycylav in a human hospital has been linked with an increased prevalence of AmpC-producing isolates (Seiffert et al., 2013a); given this drug is the most commonly prescribed antimicrobial in UK small animal practice (Radford et al., 2011; Singleton et al., 2017), this may provide an explanation for the increased prevalence of AmpC-producing isolates reported in dogs, when compared to humans. Furthermore, cephalexin, a first generation cephalosporin, has been linked with selection for AmpC-mediated resistance (Damborg et al., 2011). This inexpensive antimicrobial is commonly prescribed in first opinion veterinary practice for the treatment of pyoderma in dogs (Beco et al., 2013; Summers et al., 2014), however its use has declined in human medicine due to its poor efficacy against Gram-negative bacteria (PHE, 2015), potentially reducing the selection pressure for AmpC-producing bacteria in humans versus dogs. The increased prevalence of potential AmpC-producing isolates in canine samples is concerning given not only their resistance to 3GCs but also beta-lactamase inhibitors such as clavulanic acid (Jacoby, 2009), leading to further reduced treatment options for isolates displaying this phenotype.

Within this study, the focus has been to investigate faecal carriage rates of AMR and ESBL-producing *E. coli* in kennelled dogs, therefore this study does not provide any insight into ESBL-producing clinical isolates or carriage rates by other companion animals in the UK. Monitoring of clinical veterinary isolates in the UK is undertaken by passive surveillance. This relies on submission of isolates to the Animal and Plant Health Agency (APHA) by private veterinary surgeons and further investigation of antimicrobial resistance in companion animal isolates is only pursued if there is a public health concern (VMD, 2015). Therefore, unlike in humans (ECDC, 2015; PHE, 2015) and food-producing animals (VMD, 2015), there is not a centralised system for monitoring resistant clinical isolates in companion animals and as such, it is difficult to assess the impact of AMR on small animal veterinary practice. Although some programs for clinical disease surveillance in companion animals, such as SAVSNET and VetCompass, have been initiated (O'Neill, 2015; Sánchez-Vizcaíno et al., 2015), these currently focus on disease syndromes and there are currently no outputs pertaining to antimicrobial resistance. The current absence of these data highlights the need for a more cohesive and synergistic approach to AMR surveillance in UK animals; requiring collaboration between multiple agencies, surveillance systems and veterinary laboratories to build a complete picture of the impact of antimicrobial resistance in the UK.

Within this study faecal samples were collected in excess of the numbers suggested by sample size calculations. While often sample collection would cease on reaching the required numbers, this study was linked to the project requiring the collection of both human and canine samples from the same premises, and as such collection of canine samples continued until the conclusion of human sampling. As described in Chapter Three, recruitment of human participants was challenging whereas, levels of compliance for canine sampling were at expected levels thus leading to an excess of canine samples overall. The increased number of samples collected does, however, ensure confidence in the estimated prevalence of ESBL-producing *E. coli*. It is, however, worth noting that faecal carriage of ESBL-producing *E. coli* is transient in dogs (Schmidt et al., 2015) and as such, shedding may be intermittent. Longitudinal carriage in this population should therefore be investigated, in order to fully assess carriage and spread of these resistant bacteria in the kennelled dog population.

Potential limitations within this study include selection bias due to the recruitment methods employed. Firstly, this study combined both human and animal faecal sampling and therefore recruitment of premises was more challenging. Of the kennel premises contacted only 17.5% (95% CI 12.1-23.9) agreed to participate which is markedly lower than in previous

studies recruiting animals where up to 50% of premises participated (Maddox et al., 2012; Wedley, 2012). The most common reason for lack of participation was the request for human faecal sampling alongside animal sampling; therefore despite random selection of premises, the staff at the premises recruited in this study may have had a higher level of interest in antimicrobial resistance or scientific research and may not be entirely representative of all kennel premises. Similarly, the reliance on kennel staff to recruit participants may mean that dog owners were not approached completely randomly. There is potential that the kennel staff approached regular clients more commonly and may not have approached owners during busy periods meaning that the sampled dogs were not truly representative of the study population. However, given the sampling frame for this study no other sampling strategies would have been appropriate. Despite these concerns, the signalment of the dogs recruited in this study equates to those reported in previous studies (Schmidt, 2014; Wedley, 2012) and as such appears representative of the UK dog population.

This study indicates a high prevalence of carriage of AMR *E. coli* in kennelled dogs. Resistance to first line antimicrobials was most prevalent while the prevalence of resistance to 3GCs was low. Carriage of ESBL-producing *E. coli* was lower than previously reported in dogs in the UK and other mechanisms of resistance to 3GCs predominated. Widespread carriage of AMR *E. coli* in kennelled dogs in the UK is concerning. The transient canine populations and high density of dogs present in these environments means that the potential exchange of resistant bacteria between large numbers of animals is high. Furthermore, frequent and close contact between people and pets may allow dissemination of resistance determinants between animals and humans in the community. Given the high level of AMR *E. coli* identified in this study, identification of risk factors associated with carriage is warranted. These risk factors are investigated in Chapter Five.

Chapter 5

Risk factors associated with faecal carriage of antimicrobial-resistant and extended-spectrum β -lactamase-producing *Escherichia coli* in kennelled dogs

5.1 Introduction

Antimicrobial resistance is recognised as an increasing problem in both human and veterinary medicine globally (Levy and Marshall, 2004; Wieler et al., 2011). Reduced efficacy of current treatment regimens against resistant clinical infections has led to treatment failures and associated increases in patient morbidity and mortality (Melzer and Petersen, 2007). While the economic and societal burden of antimicrobial resistance is strongly linked to the effects of resistant clinical infections (Cosgrove and Carmeli, 2003; Paladino et al., 2002), the role of commensal bacteria in the dissemination of antimicrobial resistance determinants should also be considered.

Escherichia coli (*E. coli*), a Gram-negative bacterium of the family *Enterobacteriaceae* is the predominant aerobe in the gastrointestinal tract of humans and dogs. Pathogenic strains of this bacterium are associated with both gastrointestinal and extra-intestinal disease (Johnson and Russo, 2002; Kaper et al., 2004; Nataro and Kaper, 1998); however commensal *E. coli* play a role in resistance against intestinal pathogens and are rarely associated with clinical infections (Hudault et al., 2001; van der Waaij et al., 1971). The gastrointestinal niche of commensal bacteria, including *E. coli*, is commonly exposed to antimicrobials used to treat clinical infections and this exposure selects for the development or acquisition of resistance determinants by the gastrointestinal commensal population. These bacteria may act as a reservoir of resistance genes and facilitate the dissemination of mobile genetic elements carrying these genes to pathogenic bacteria. Similarly, the frequent and close contact between people and their pets provides an environment for the potential zoonotic exchange of antimicrobial-resistant (AMR) bacteria.

Of particular concern, is production of extended-spectrum β -lactamase (ESBL) enzymes by members of the *Enterobacteriaceae*, including *E. coli*. These enzymes confer resistance to a wide range of β -lactam antimicrobials, including third and fourth generation cephalosporins. ESBL-producing isolates have been identified as a cause of both nosocomial and community acquired infections in human populations globally as well as a cause of clinical infections in veterinary species including dogs (Livermore and Hawkey, 2005; PHE, 2015; Timofte et al., 2011; Timofte et al., 2014a; Timofte et al., 2014b). In addition to ESBL-producing isolates, third generation cephalosporin (3GC) resistance in *E. coli* can be mediated by the production of AmpC enzymes. These enzymes additionally mediate resistance to β -lactamase inhibitors, such as clavulanic acid and therefore further reduce treatment options available (Jacoby, 2009). The faecal carriage of *E. coli* resistant to 3GCs has previously been reported in

numerous populations of dogs globally (Johard et al., 2015; Schaufler et al., 2015; Schmidt et al., 2015; Wedley et al., 2011), with a prevalence of up to 16% observed in healthy UK Labradors (Schmidt et al., 2015). While faecal carriage of ESBL-producing *E. coli* are reported in these populations, 3GC resistance is more commonly mediated by AmpC enzymes in healthy UK dogs (Schmidt et al., 2015; Wedley et al., 2011).

Previous studies have indicated numerous risk factors which may be associated with carriage of AMR and ESBL-producing *E. coli* in vet-visiting and healthy canine populations. Both hospitalisation and previous veterinary treatment have been identified as increasing the risk of faecal carriage of AMR *E. coli* in dogs (Damborg et al., 2011; Gibson et al., 2011a; Gibson et al., 2011b; Tuerena et al., 2016; Wedley, 2012). While some lifestyle related factors, including access to a raw food diet and contact with farm animals, have also been reported to increase the risk of carriage of AMR *E. coli* (Schmidt et al., 2015; Wedley, 2012). Interestingly, the attendance of dog dense environments including kennels has also been reported as increasing the risk of carriage of these bacteria (Belas et al., 2014; Procter et al., 2014); however dog and owner-related risk factors associated with this carriage in kennelled dogs have not been ascertained.

The aim of this study was to ascertain dog related risk factors associated with faecal carriage of AMR *E. coli* in kennelled dogs in the North of England

5.2 Materials and methods

5.2.1 Study population and data collection

Canine participants were recruited simultaneously from 33 boarding and rescue premises as previously described in Chapter Four. Briefly, owners of privately owned dogs were asked to give written informed consent for their pet to participate in the study, while consent for inclusion of dogs in rescue centres was given by the appropriate staff member in the centre. Following recruitment, the keeper of the animal was then asked to answer a two-part questionnaire detailing dog signalment, kennelling arrangements, history of ownership, animal contact and previous veterinary treatment together with a short section detailing the owner's contact with healthcare and animal dense environments. The kennel staff then collected a pea-sized fresh faecal sample on a swab for analysis. In this study, canine samples were selectively and non-selectively cultured for isolation of AMR and ESBL-producing *E. coli* and isolates underwent susceptibility testing against a panel of seven antimicrobials (ampicillin 10 µg, amoxycylav 30 µg, ciprofloxacin 1 µg, chloramphenicol 30µg, nalidixic acid

30µg tetracycline 30 µg and trimethoprim 2.5 µg). Further details of the methodology employed and the prevalence of AMR and ESBL-producing *E. coli* in dogs are detailed in Chapter Four.

5.2.2 Statistical analysis

All questionnaire and microbiological data were entered into a database (Microsoft Excel 2010, Microsoft Corporation) and the dataset was reviewed to ensure accuracy of input. Isolate antimicrobial susceptibility data for each outcome was coded as a binary variable using BSAC breakpoint values as either resistant (1) or non-resistant (0) (BSAC, 2015). Within this study, sample level data was considered the unit of interest, such that a sample with at least one resistant *E. coli* isolate was considered resistant. The antimicrobial resistance outcomes considered for analysis were antimicrobial resistance (resistance to any of the antimicrobials tested), independent resistance to each of the seven antimicrobials tested, 3GC resistance, multi-drug resistance (presence of an *E. coli* isolate resistant to at least three antimicrobial classes), and phenotypically confirmed ESBL-mediated resistance. Potential risk factors were identified from data provided in questionnaires and were examined for association with these defined resistance outcomes. Analysis of risk factors was undertaken in two parts; data from all dogs were included in the first set of analyses which examined dog related factors. The second set of analyses excluded rescue animals and focused solely on owned dogs in order that the effects of both animal and owner related factors on resistance outcomes could be assessed. All explanatory variables derived were binary or categorical in nature, with the exception of duration of kennelling, which was considered as a continuous variable. Data were exported into R (www.r-project.org) and statistical analyses were performed using the lme4 (Bates et al., 2014), and lmerTest (Hothorn et al., 2015) and mcgvc (Wood, 2016) statistical packages.

Initial univariable analysis was conducted on all explanatory variables using separate logistic regression models for each outcome. In order to account for clustering of data at the premises level all models included individual premises as a random effect. Variables which showed some association with each outcome, indicated by a likelihood ratio test statistic (LRTS) of $p < 0.25$ when compared to the null model were considered for inclusion into a multivariable model. Where response variable numbers were low, categorical variables were collapsed into a smaller number of categories when appropriate. In the case of low response numbers resulting in categories containing no responses, initial screening of associations was undertaken using Fisher's exact test. If a potential association between this variable and the

outcome of interest was present ($p < 0.25$), an artificial positive was placed in the category that would otherwise contain no data, allowing estimation of its association with the outcome of interest and as such, its inclusion in the multivariable model. This adjusted dataset was only used while this variable remained in the multivariable model.

In cases where nesting of variables identified in univariable analysis occurred (for example receipt of veterinary treatment and hospitalisation), a categorical variable incorporating both these variables was created to assess their association with the outcome. Only variables which remained significant following this analysis were included in the multivariable model. If any explanatory variables were found to be correlated following univariable analysis (correlation coefficient of ≥ 0.7), these were not deemed to be independent of each other and in order to avoid effects of collinearity only the variable with the smallest p-value was selected for inclusion in the multivariable model. Duration of kennelling was the only continuous variable considered during analysis; generalised additive model (GAM) plots were used to explore the relationship of this variable with respect to each outcome. If a significant non-linear relationship was identified, appropriate polynomial or piecewise fits of the relationship were explored for inclusion in the multivariable model.

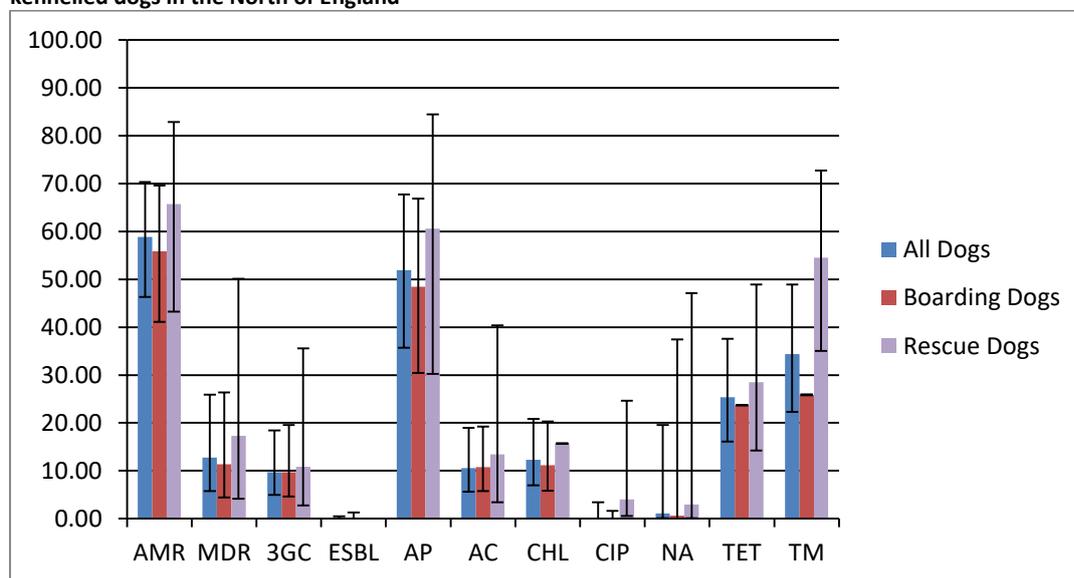
The final models were constructed by a manual backwards stepwise elimination. Variables were retained in the model if their exclusion resulted in a LRTS of $P < 0.05$, or if confounding, evidenced by change in the β coefficient of other explanatory variables of greater than 25%, was present. For variables retained in the model, the effect of random slopes and biologically plausible interaction terms were then tested and were retained if found to be significant (LRTS $p < 0.05$). All variables considered for initial inclusion were then added back into the model, in order to ensure no significant or confounding variables had been inadvertently excluded. For each of the final models, an intraclass correlation coefficient (ICC) was calculated using the latent variable approach (Goldstein et al., 2002), in order to assess the amount of variation remaining due to the premises versus sample level factors. Final models were checked for premises which may have had an increased influence by plotting premises level residuals against the overall mean. Where a significant difference was detected, data from these premises were checked for any errors.

5.3 Results

5.3.1 Prevalence of antimicrobial-resistant *E. coli*

Thirty-three boarding and rescue premises were recruited for this study and a total of 296 canine faecal samples (190 from boarding dogs and 106 from rescue dogs) were received, for which 292 questionnaires were returned. Further details on the population demographics including animal signalment, veterinary treatment and animal contact can be found in Chapter Four. The sample prevalence of carriage of AMR *E. coli* adjusted for clustering within premises is detailed in Figure 5.1.

Figure 5.1 The adjusted sample prevalence of AMR faecal *E. coli* within in a cross-sectional study of 296 kennelled dogs in the North of England



Key: AMR= antimicrobial resistance; MDR= multi-drug resistance; 3GC= third generation cephalosporin; ESBL= ESBL phenotype; AMP= ampicillin; AC= amoxyclav; CHL= chloramphenicol; CIP= ciprofloxacin; NA= nalidixic acid; TET= tetracycline; TM= trimethoprim

5.3.2 Risk factor analysis

5.3.2.1 All dogs

5.3.2.1.1 Univariable analysis

A total of 37 potential explanatory variables were identified from questionnaire responses and univariable analysis found that many of these variables were associated with the outcomes of interest (Table 5.1). GAM plots (Appendix III) indicated that duration of kennelling displayed a non-linear relationship ($p < 0.05$) with an ESBL phenotype and resistance to nalidixic acid and ciprofloxacin. Piecewise fits of this variable were constructed for each of these outcomes and while no association was identified between the transformed variable and nalidixic acid ($p = 0.27$), a piecewise fit showed some association with

ciprofloxacin resistance ($p=0.19$) and an ESBL phenotype ($p=0.22$). For these outcomes, three segment piecewise terms (<100 days, 100-449 days and ≥ 450 days and <100 days, 100-499 days and ≥ 500 days respectively) were found to be the best fit for the model.

When considering the size of dogs as an explanatory variable, collapse of the five categories into three was necessary due to low numbers of both toy and giant dogs sampled. Duration of kennelling and the boarding pattern of an animal were significantly correlated with each other when considering trimethoprim resistance, but no other outcomes. For this outcome, the boarding pattern of an animal was the best fit for the model and was selected for inclusion in multivariable analysis. Similarly, lifetime ownership of an animal was significantly correlated with the animal's origin for both multi-drug and trimethoprim resistance. In the case of trimethoprim resistance, lifetime ownership was more significant and was included in multivariable analysis, while the converse was true for multi-drug resistance. Contact with non-canine animals and wildlife were both identified as having a potential association with amoxycylav resistance. Construction of a polytomous variable including both these factors indicated that contact with wildlife alone ($p=0.142$) was the best fit for the model and this was therefore selected solely for inclusion in the associated multivariable model. Veterinary treatment, antimicrobial treatment, and hospitalisation within the last six months were identified as potential risk factors for antimicrobial resistance, 3GC, ampicillin, and amoxycylav resistance. In the case of antimicrobial ($p=0.009$), amoxycylav ($p<0.001$) and 3GC ($p=0.005$) resistance, veterinary treatment alone was identified as the variable with the best fit for the models, while recent hospitalisation ($p=0.006$) was the best fit for the ampicillin resistance model. These variables were therefore solely included in the corresponding multivariable model. Complete results of univariable analysis including polytomous variables created for nested variables are detailed in Appendix III.

Table 5.1 Variables considered for inclusion in the multivariable model for risk factors associated with faecal carriage of AMR *E. coli* in a cross-sectional survey of 292 kennelled dogs in the North of England following univariable analysis ($p < 0.25$)

Independent Variable	ANY	MDR	3GC	ESBL	AMP	AC	CHL	CIP	NA	TET	TM
Participant Demographics:											
Age category							Grey	Grey			Grey
Pure breed							Grey	Grey			
Size (when adult)	Grey	Black	Grey		Grey	Grey					Grey
Sex			Black	Grey				Grey			Grey
Neutering			Grey	Grey							
Same owner since pup		Grey					Grey				Grey
Origin of animal		Black			Black				Black	Black	Grey
Working dog			Grey			Grey	Grey				
Veterinary Treatment:											
Vet treatment within 6 months	Black		Black		Black	Black	Black			Grey	
Last vet treatment	Grey	Grey	Black		Grey	Black				Grey	
Antimicrobials within 6 months	Grey		Grey		Grey	Grey					Grey
Last antimicrobial treatment		Grey	Grey			Grey				Black	Grey
Hospitalisation within 6 months	Black		Black		Black	Black				Grey	Black
Home Environment:											
Fed complete dry food			Grey						Grey		
Fed cooked meat										Grey	
Fed dry mixer			Grey			Grey	Grey			Black	Black
Fed raw food		Black	Black		Grey		Grey		Black	Black	
Fed wet food				Black	Grey						
Housed indoors							Grey				Black
Housed in kennels											Grey
Housed outdoors		Grey					Grey	Grey	Grey	Black	
Multi-dog household			Grey								
Number of dogs in household				Grey	Grey						
In-contact dogs on antibiotics							Grey				
In-contact dogs hospitalised											Grey
Kennel Environment:											
Premises type						Grey					Grey
Boarder type					Grey		Grey				Black
Duration of kennelling (days)	Grey		Grey	Grey	Grey		Grey	Grey			Grey
Days Kennelled (categorical)									Grey		
Sharing kennel with another dog											
Animal contact:											
Contact with any other animals			Grey			Grey		Grey			
Cats											
Farm Animals	Black	Grey			Black		Grey			Black	
Horses	Grey				Grey						
Small Mammals	Grey				Grey				Black	Black	Grey
Wildlife	Grey				Grey	Grey	Black				

Key: AMR= antimicrobial resistance; MDR= multi-drug resistance; 3GC= third generation cephalosporin; ESBL= ESBL phenotype; AMP= ampicillin; AC= amoxyclav; CHL= chloramphenicol; CIP= ciprofloxacin; NA= nalidixic acid; TET= tetracycline; TM= trimethoprim

Grey=<0.25; Black=<0.05

Multiple explanatory variables were significantly associated ($p < 0.05$) with more than one of the outcomes of interest in this study. Receiving veterinary treatment within the previous six month period was associated increased risk of carriage of AMR *E. coli* ($p = 0.009$) and 3GC resistance ($p = 0.005$), as well as resistance to ampicillin ($p = 0.029$), amoxycylav ($p < 0.001$) and chloramphenicol ($p = 0.013$). Consumption of raw food was found to be associated with multidrug resistance ($p = 0.017$), 3GC resistance ($p = 0.025$), nalidixic acid ($p = 0.005$) and tetracycline ($p = 0.022$) resistance, increasing risk of carriage in all cases. A further four variables; origin of the dog, recent hospitalisation and contact with farm animals or small mammals were also significantly associated with multiple outcomes (Table 5.1). All variables identified as being significantly associated with at least one outcome during univariable analysis remained in multivariable models where constructed, with the exception of indoor housing which was found to be associated with trimethoprim solely on univariable analysis.

5.3.2.1.2 Multivariable analysis

Mixed effect logistic regression models were constructed for nine outcomes; the low carriage rate of both ESBL-producing and ciprofloxacin-resistant *E. coli* prevented further analysis of these outcomes. The models constructed indicated that the intraclass correlation coefficient for all outcomes was markedly higher than that reported in the humans sampled in the same premises. Values of between 0.25 and 0.58 were identified (Tables 5.2 and 5.3), with the exception of chloramphenicol (0.17) and nalidixic acid (0.81) resistance. In order to further check the role of individual premises on the models constructed, premises level residual plots were constructed for each outcome (Appendix III). These indicated that the random effects of some premises were significantly different from the overall mean for the final models. This was most noticeable in the case of nalidixic acid resistance where the prevalence of carriage was low. Examination of this plot revealed that the premises with a dog giving a sample positive for nalidixic acid resistance were those which were significantly different to the mean, explaining the high ICC for this outcome. For all outcomes, data from outlying premises was rechecked to ensure no errors were present, the data from these premises were correct so participants from these premises were retained in the model.

All models constructed contain multiple explanatory variables; Table 5.2 details the risk factors identified for multi-drug, ampicillin, nalidixic acid, tetracycline and trimethoprim resistance, while Table 5.3 describes the risk factors associated with amoxycylav, 3GC, chloramphenicol and antimicrobial resistance. The results indicate that no risk factors were associated with all outcomes investigated, however consumption of a raw food diet was a

risk factor for chloramphenicol, nalidixic acid, tetracycline and multi-drug resistance. Receiving veterinary treatment within the six months and the origin of dogs sampled were also both associated with four outcomes. Interestingly, recent antimicrobial treatment was only associated with carriage of tetracycline-resistant *E. coli*. Numerous further variables were associated with a single outcome; these included female sex which was identified solely as a risk factor for 3GC resistance and recent hospitalisation which was a risk factor for trimethoprim resistance. Being a working dog or having one owner since puppyhood were protective and contact with wildlife a risk factor for chloramphenicol resistance only, while contact with small mammals was exclusively associated with tetracycline resistance.

Random slope effects were investigated for all models and a random slope effect was identified ($p=0.037$) within the antimicrobial resistance model, suggesting that the effect of contact with farm animals differed between different premises. The inclusion of this random slope did not alter the nature, but did increase the magnitude of the effect caused by this contact.

Testing for biologically plausible interaction terms revealed an interaction between variables in two multivariable models. When considering tetracycline resistance, a significant multiplicative interaction was identified between consumption of raw food and consumption of dry mixer ($p=0.024$). Consumption of each of these diets alone increased risk of carriage by 64.4 and 5.1 times, respectively, however when fed together the level of risk was between these values ($OR=6.3$). An additional interaction term was detected between recent veterinary treatment and contact with farm animals when considering antimicrobial resistance. Exposure to these risk factors in isolation increased risk of carriage by 2.92 and 2.61×10^5 times, respectively, while exposure in conjunction increased the risk by 3.80×10^3 times.

Table 5.2 Multivariable, mixed effects logistic regression models of risk factors associated with any, amoxycylav, nalidixic acid, tetracycline and trimethoprim resistance in *E. coli* from faecal samples obtained from 292 kennelled dogs in the North of England

Variable	Category	Multi-drug resistance			Ampicillin			Nalidixic Acid			Tetracycline			Trimethoprim		
		OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Vet treatment within 6 months	No	-	-	-	(Ref)	-	-	-	-	-	-	-	-	-	-	-
	Yes	-	-	-	2.36	1.09-5.19	0.028	-	-	-	-	-	-	-	-	-
	>6 months	-	-	-	-	-	-	-	-	-	(Ref)	-	0.005	-	-	-
Last antimicrobial treatment	<1 month	-	-	-	-	-	-	-	-	-	0.43	0.09-2.19	0.313	-	-	-
	2-3 months	-	-	-	-	-	-	-	-	-	5.78	1.28-26.03	0.022	-	-	-
	4-6 months	-	-	-	-	-	-	-	-	-	8.24	1.43-47.52	0.018	-	-	-
Hospitalisation within 6 months	No	-	-	-	-	-	-	-	-	-	-	-	-	(Ref)	-	-
	Yes	-	-	-	-	-	-	-	-	-	-	-	-	6.16	1.25-30.48	0.017
Fed raw food	No	(Ref)	-	-	-	-	-	(Ref)	-	-	(Ref)	-	-	-	-	-
	Yes	18.91	2.66-134.37	0.003	-	-	-	53.08	2.91-969.4	0.005	64.42	6.73-616.19	<0.001	-	-	-
Fed dry mixer	No	-	-	-	-	-	-	-	-	-	(Ref)	-	-	-	-	-
	Yes	-	-	-	-	-	-	-	-	-	5.17	1.44-18.58	0.012	-	-	-
Boarder Type	Resident	-	-	-	13.56	1.31-140.5	0.029	-	-	-	-	-	-	(Ref)	-	0.046
	Overnight	-	-	-	7.72	0.92-65.05	0.060	-	-	-	-	-	-	0.30	0.10-0.88	0.028
	Day	-	-	-	(Ref)	-	0.029	-	-	-	-	-	-	0.30	0.05-1.69	0.173
	Rescue	-	-	-	43.67	2.54-750.6	0.009	-	-	-	-	-	-	1.51	0.41-5.57	0.540
	Rescue centre	(Ref)	-	0.003	(Ref)	-	0.007	(Ref)	-	0.005	(Ref)	-	<0.001	-	-	-
Origin of animals	Breeder	0.15	0.04-0.57	0.005	0.54	0.19-1.54	0.247	0.64	0.10-3.91	0.628	0.12	0.03-0.39	<0.001	-	-	-
	Home bred	0.03	0.00-0.52	0.015	0.407	0.06-2.71	0.353	0.05	0.00-2.12	0.115	0.01	0.00-0.13	0.001	-	-	-
	Previous owner	0.18	0.05-0.65	0.009	0.082	0.02-0.37	0.001	0.23	0.04-1.36	0.105	0.24	0.07-0.79	0.019	-	-	-
	Failed rehoming	0.11	0.02-0.64	0.014	0.087	0.01-0.68	0.020	0.07	0.01-0.62	0.016	0.26	0.05-1.31	0.103	-	-	-
Housed outdoors	No	(Ref)	-	-	-	-	-	-	-	-	(Ref)	-	-	-	-	-
	Yes	6.76	1.15-39.79	0.033	-	-	-	-	-	-	7.58	1.43-40.27	0.016	-	-	-
In contact with small mammals	No	-	-	-	-	-	-	-	-	-	(Ref)	-	-	-	-	-
	Yes	-	-	-	-	-	-	-	-	-	15.62	2.15-113.28	0.005	-	-	-
In contact with farm animals	No	-	-	-	(Ref)	-	-	-	-	-	-	-	-	-	-	-
	Yes	-	-	-	6.605	1.11-39.25	0.032	-	-	-	-	-	-	-	-	-
Interaction: Raw food x dry mixer*		-	-	-	-	-	-	-	-	-	0.02	0.00-0.78	0.024	-	-	-
n		253			247			267			249			262		
Premises variance [SE] and ICC		4.5 [0.13] 0.58			3.49 [0.12] 0.51			14.0 [0.23] 0.81			2.53 [0.10] 0.44			2.21 [0.09] 0.40		

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; SE=standard error

* Explanation of interaction terms: Dog fed raw food and no mixer, OR=64.43; Dog fed dry mixer and no raw food, OR=5.17; Dog is fed raw food and dry mixer, OR=6.33 (64.43 x 5.17 x 0.02).

Table 5.3 Multivariable, mixed effects logistic regression models of risk factors associated with ampicillin, third generation cephalosporin, chloramphenicol and multi-drug resistance in *E. coli* from faecal samples obtained from 292 kennelled dogs in the North of England

Variable	Category	Amoxyclav			3 rd Generation Cephalosporin			Chloramphenicol			Antimicrobial resistance†		
		OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Owned since pup	No	-	-	-	-	-	-	(Ref)	-	-	-	-	-
	Yes	-	-	-	-	-	-	0.30	0.11-0.79	0.012	-	-	-
Fed raw food	No	-	-	-	-	-	-	(Ref)	-	-	-	-	-
	Yes	-	-	-	-	-	-	4.65	1.21-17.97	0.032	-	-	-
Sex	Female	-	-	-	(Ref)	-	0.049	-	-	-	-	-	-
	Male	-	-	-	0.44	0.19-1.01	-	-	-	-	-	-	-
Size (when adult)	Small/Toy	(Ref)	-	0.028	(Ref)	-	0.033	-	-	-	-	-	-
	Medium	3.33	1.06-10.43	0.039	5.28	1.29-21.56	0.021	-	-	-	-	-	-
	Large/Giant	1.26	0.38-4.15	0.701	2.57	0.63-10.51	0.19	-	-	-	-	-	-
Vet treatment within 6 months	No	(Ref)	-	-	(Ref)	-	0.001	-	-	-	(Ref)	-	-
	Yes	5.72	2.16-15.14	<0.001	5.11	1.79-14.57	-	-	-	-	2.92	1.46-5.83	0.002
Housed outdoors	No	-	-	-	-	-	-	-	-	-	-	-	-
	Yes	-	-	-	-	-	-	-	-	-	-	-	-
Working dog	No	-	-	-	-	-	-	(Ref)	-	-	-	-	-
	Yes	-	-	-	-	-	-	0.07	0.00-0.99	0.016	-	-	-
Contact with farm animals	No	-	-	-	-	-	-	-	-	-	(Ref)	-	-
	Yes	-	-	-	-	-	-	-	-	-	2.61x10 ⁵	0.21-3.31x10 ¹¹	0.082
Contact with wildlife	No	-	-	-	-	-	-	(Ref)	-	-	-	-	-
	Yes	-	-	-	-	-	-	6.12	1.70-22.01	0.007	-	-	-
Interaction term													
Vet treatment x farm animals		-	-	-	-	-	-	-	-	-	0.005	0.00-27.52	0.030
n			261			260			262			259	
Premises variance [SE] and ICC			1.12 [0.07] 0.25			2.57[0.11] 0.44			0.70 [0.05] 0.18			1.38 [0.07] 0.30	

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; SE=standard error †Model includes farm animal random slope effect. * Explanation of interaction terms: Dog received veterinary treatment and had no contact with farm animals OR=2.92; Dog had contact with farm animals and no veterinary treatment OR=2.61x10⁵; Dog received veterinary treatment and had contact with farm animals OR=3.80x10³ (2.92x2.61x10⁵x0.005).

5.3.2.2 Owned dogs

In total, faecal samples and fully completed questionnaires were received back from 185 owned dogs from 25 boarding premises. Additional analysis of risk factors in this defined canine population allowed the inclusion of an additional 10 owner-related variables excluded from analysis for the total dog population.

5.3.2.2.1 Univariable analysis

Univariable analysis found many of the variables described when considering the complete dog population, were again significantly associated with the outcomes considered (Table 5.4). GAM plots showed that duration of kennelling displayed a significant non-linear relationship with ciprofloxacin, nalidixic acid and ESBL-mediated resistance (Appendix III). Appropriate piecewise fits were considered for inclusion of this variable during multivariable analysis for these outcomes. Piecewise fits of this variable were constructed for these outcomes; while no association was identified between the transformed variable and nalidixic acid ($p=0.884$) or ciprofloxacin ($p=0.93$) resistance, a piecewise fit showed some association with phenotypic ESBL mediated resistance ($p=0.082$). In this case, a two segment piecewise term (<525 days and ≥ 525 days) was found to be the best fit for the model.

Recent hospitalisation was a significant risk factor for five of the outcomes examined; resistance to 3GCs ($p=0.039$), ampicillin ($p=0.037$), amoxyclav ($p=0.003$), tetracycline ($p=0.019$) and trimethoprim ($p=0.019$). Similarly, consumption of a raw food diet was again identified as a risk factor for multiple outcomes (multidrug resistance, 3GC resistance, and resistance to chloramphenicol, tetracycline and nalidixic acid), mirroring the findings reported for the total dog population. There were however, some notable differences when considering the owned dogs alone. Firstly, having owners working with farm animals was a significant risk factor for a dog to carry AMR ($p=0.042$) or MDR *E. coli* ($p=0.002$) as well as resistance to amoxyclav ($p=0.044$), chloramphenicol ($p=0.002$), tetracycline ($p=0.002$) and trimethoprim ($p=0.033$). In keeping with this finding, dog contact with farm animals was also highlighted as having a significant association with four of the resistance outcomes investigated. Within the owned dog population, the size of the dog was also associated with an increased number of outcomes, when compared to the total dog population. Risk of carriage of AMR *E. coli* ($p=0.006$), ampicillin ($p=0.014$), amoxyclav ($p=0.010$), chloramphenicol ($p=0.019$) and trimethoprim ($p=0.023$) resistance were all increased in larger dogs. Conversely, while the origin from which an animal was obtained was significantly

associated with three outcomes for the total dog population, no associations were identified in the owned dog population. Complete results of univariable analysis are located in Appendix III.

Table 5.4 Variables considered for inclusion in the multivariable model for risk factors associated with faecal carriage of AMR *E. coli* in a cross-sectional survey of 185 boarding dogs in the North of England following univariable analysis ($p < 0.25$)

Independent Variable	AMR	MDR	3GC	ESBL	AMP	AC	CHL	CIP	NA	TET	TM
Participant details:											
Age category								Grey			
Pure breed		Grey								Black	
Size (when adult)	Black	Grey	Grey		Black	Black	Black	Black	Grey		Black
Sex	Grey	Grey		Grey	Black			Black		Grey	Black
Neutering				Grey							
Same owner since pup		Black			Grey		Grey			Grey	
Vet treatment within 6 months	Black		Grey		Grey	Black	Black	Grey			
Last vet treatment	Grey	Grey									
Last antimicrobial treatment	Grey										
Hospitalisation within 6 months	Grey	Grey	Black		Black	Black				Black	Black
Home environment:											
Fed complete dry food			Grey				Grey		Grey		Grey
Fed cooked meat											
Fed dry mixer			Grey						Grey		
Fed raw food		Black	Black	Grey	Grey		Black		Black	Black	Grey
Fed wet food		Grey		Black	Grey		Black				
Housed indoors							Grey				Grey
Housed in kennels				Grey							
Housed outdoors		Black					Grey	Grey	Grey	Black	Grey
Multi-dog household			Black			Grey					Grey
Number of dogs in household			Grey		Grey						
Working Dog		Grey					Grey				
Kennel environment:											
Boarder type	Grey			Grey	Black		Grey				Grey
Duration of kennelling (days)	Grey				Grey						Black
Sharing kennel with another dog											
Animal contact:											
Cats			Grey			Grey					
Farm Animals	Black		Grey		Black	Grey	Black			Black	
Horses	Grey							Grey			Grey
Small Mammals	Black								Black	Black	Grey
Wildlife	Grey		Wildlife		Grey	Black	Black				
Owner details:											
Works with animals	Grey	Grey			Grey		Grey				Grey
Works in farming	Black	Black	Grey		Grey	Black	Black		Grey	Black	Black
Works in human healthcare									Grey		Grey
Hospital healthcare			Grey								
General practice	Black				Black						Grey
Visited hospital in last month									Grey		
Hospital admission		Grey								Grey	Grey
Outpatient appointment	Grey										
Visiting patient		Black			Grey					Grey	

Key: AMR= antimicrobial resistance; MDR= multi-drug resistance; 3GC= third generation cephalosporin; ESBL= ESBL phenotype; AMP= ampicillin; AC= amoxycylav; CHL= chloramphenicol; CIP= ciprofloxacin; NA= nalidixic acid; TET= tetracycline; TM= trimethoprim
 Grey=<0.25; Black=<0.05

Numerous correlated variables were identified in this dataset including correlations between being a working dog and the owner working in farming, number of days kennelled and the boarding pattern of the dog, indoor versus outdoor housing and feeding of dry mixer versus complete dry diets. These correlations were examined for each of the affected outcomes and the variable that provided the best fit for the model was included. Nested explanatory variables were identified for eight of the eleven outcomes investigated during univariable analysis. The nested variables identified were working with animals and in the farming sector on five occasions, recent veterinary treatment and hospitalisation on five occasions, and working in human healthcare and in a GP surgery on one occasion. For most outcomes, construction of polytomous variables identified that inclusion of hospitalisation, working in farming or in a GP surgery alone was the best fit for the model and therefore these variables were solely included where appropriate. However, in the case of antimicrobial resistance, veterinary treatment alone ($p=0.016$) was found to be the best fit for the model and was subsequently selected for inclusion in the multivariable model for this outcome. Many variables identified as being statistically significant during univariable analysis were retained in the final models. However, receiving recent veterinary treatment, consumption of dry mixer or wet food diet, being housed outdoors, boarding pattern of the dog and duration of kennelling were found to be significantly associated with outcomes on univariable analysis only. Full results of univariable analyses including construction of polytomous variables can be found in Appendix III.

5.3.2.2.2 Multivariable analysis

Multivariable models were constructed for the same nine outcomes as reported for the total dog population. These models indicated that the proportion of variance occurring at the premises level varied between 0.0% for amoxyclav and 57.0% for nalidixic acid (Tables 5.5 and 5.6) indicating a variable influence of the premises depending on the outcome of interest. The proportion of overall variance attributed to the premises-level was greater in the total dog population for all outcomes, with the exception of trimethoprim resistance for which it was greater in the owned dog population. Plots of residual effects (Appendix III) indicated that there were fewer outlying premises when considering owned dogs than in the total dog population. Data from outlying premises had previously been checked during previous total dog analysis and was found to be correct and therefore data from these premises were retained in the final models.

Random slopes were examined for each of the models and were not significant, and no interaction terms were identified between variables remaining in the final models. When considering ampicillin resistance, low numbers of patients had been hospitalised within six months and as such there were no individuals who had been hospitalised and were negative for this outcome. In order that a model could be constructed, a false positive result was entered in the cell containing no data and this dataset was used. This variable remained in the final model and as such the estimates reported for this outcome should be viewed with caution. However, given the introduction of this false score, the odds ratio estimates are more conservative and should not therefore overestimate the effect of this variable.

The models constructed were grouped into two sets based on risk factors identified. Any antimicrobial, 3GC, ampicillin, amoxyclov and trimethoprim resistance had similar risk factors, which are located in Table 5.5. Risk factors for tetracycline, chloramphenicol, nalidixic acid and multi-drug resistance show less overlap and are detailed in Table 5.6. All models were multivariable in nature except for nalidixic acid resistance, in which only consumption of a raw diet ($p=0.004$) was retained.

No single risk factor was associated with all of the outcomes investigated and when compared to the risk factors identified for the total dog population ($n=15$), a slightly lower number of explanatory variables ($n=14$) are associated with the outcomes investigated in the owned dog population. Breed size was identified as a risk factor for antimicrobial, 3GC, ampicillin, amoxyclov and trimethoprim resistance. In all cases, small breed dogs displayed a lower risk of carriage of resistance than medium or larger breed dogs. Having been hospitalised within the last six months was identified as a risk factor in five outcomes; 3GC, ampicillin, amoxyclov, tetracycline and trimethoprim resistance. While dogs with owners working with farm animals were at increased risk of ampicillin, chloramphenicol, tetracycline and multi-drug resistance; consumption of a raw food diet was associated with an increased risk of 3GC, nalidixic acid and multidrug resistance. Additionally, an animal having multiple owners were at an increased risk of ampicillin and multidrug resistance, while contact with wildlife was identified as a risk factor for amoxyclov and chloramphenicol resistance. All other explanatory variables were associated with a single outcome and notably, having an owner who works in a human hospital was identified as a risk solely for carriage of *E. coli* resistant to 3GCs ($p=0.041$).

Table 5.5 Multivariable, mixed effects logistic regression models of risk factors associated with any, third generation cephalosporin, ampicillin, amoxycylav and trimethoprim-resistant *E. coli* in faecal samples obtained from 185 boarding dogs in the North of England

Variable	Category	Any resistance			3 rd Generation Cephalosporin			Ampicillin			Amoxycylav			Trimethoprim		
		OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Sex	Female	-	-	-	-	-	-	-	-	-	-	-	-	(Ref)	-	-
	Male	-	-	-	-	-	-	-	-	-	-	-	-	0.32	0.12-0.82	0.014
Size	Small/Toy	(Ref)	-	0.006	(Ref)	-	0.035	0.13	0.03-0.51	0.004	(Ref)	-	0.002	(Ref)	-	0.036
	Medium	1.20	0.41-3.50	0.736	17.28	1.10-272.1	0.043	0.21	0.06-0.71	0.012	28.06	1.86-423.0	0.016	3.60	0.89-14.60	0.073
	Large/Giant	4.33	1.42-13.13	0.009	11.28	0.75-170.7	0.080	(Ref)	-	0.002	8.31	0.58-119.2	0.119	5.46	1.34-22.23	0.018
Owned since pup	No	-	-	-	-	-	-	(Ref)	-	-	-	-	-	-	-	-
	Yes	-	-	-	-	-	-	0.36	0.13-1.03	0.048	-	-	-	-	-	-
Fed raw food	No	-	-	-	(Ref)	-	-	-	-	-	-	-	-	-	-	-
	Yes	-	-	-	6.89	1.44-32.91	0.024	-	-	-	-	-	-	-	-	-
Hospitalisation within 6 months	No	-	-	-	(Ref)	-	-	(Ref)	-	-	(Ref)	-	-	(Ref)	-	-
	Yes	-	-	-	17.89	1.13-284.8	0.040	15.82	1.06-236.7	0.019	51.31	4.42-595.4	<0.001	17.06	1.08-270.5	0.014
Contact with farm animals	No	(Ref)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Yes	25.74	2.54-261.2	<0.001	-	-	-	-	-	-	-	-	-	-	-	-
Contact with wildlife	No	-	-	-	-	-	-	-	-	-	(Ref)	-	-	-	-	-
	Yes	-	-	-	-	-	-	-	-	-	7.55	2.30-24.78	0.007	-	-	-
Owner works in farming	No	-	-	-	-	-	-	(Ref)	-	0.047	-	-	-	-	-	-
	Yes	-	-	-	-	-	-	25.19	1.04-608.8	-	-	-	-	-	-	-
Owner works in a hospital	No	-	-	-	(Ref)	-	0.041	-	-	-	-	-	-	-	-	-
	Yes	-	-	-	9.54	1.04-87.64	-	-	-	-	-	-	-	-	-	-
Owner works in a GP practice	No	-	-	-	-	-	-	(Ref)	-	0.014	-	-	-	-	-	-
	Yes	-	-	-	-	-	-	10.54	1.32-84.35	-	-	-	-	-	-	-
n		158			157			157			182			157		
Premises variance[SE] and ICC		1.20 [0.08] 0.27			1.60 [0.10] 0.33			3.16 [0.14] 0.49			4.00x10 ⁻¹⁴ [1.48x10 ⁻⁸] 1.22x10 ⁻¹⁴			2.84[0.13] 0.46		

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; SE=standard error

Table 5.6 Multivariable, mixed effects logistic regression models of risk factors associated with tetracycline, chloramphenicol, nalidixic acid and multi-drug resistant *E. coli* in faecal samples obtained from 185 boarding dogs in the North of England

Variable	Category	Tetracycline			Chloramphenicol			Nalidixic Acid			MDR		
		OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
Owned since pup	No	-	-	-	-	-	-	-	-	-	(Ref)	-	-
	Yes	-	-	-	-	-	-	-	-	-	0.25	0.08-0.72	0.008
Pure breed	No	(Ref)	-	-	-	-	-	-	-	-	-	-	-
	Yes	0.13	0.04-0.41	<0.001	-	-	-	-	-	-	-	-	-
Fed raw food	No	-	-	-	-	-	-	(Ref)	-	-	(Ref)	-	-
	Yes	-	-	-	-	-	-	24.70	3.46-176.38	0.004	6.68	1.65-27.02	0.008
Working dog	No	-	-	-	(Ref)	-	-	-	-	-	-	-	-
	Yes	-	-	-	0.04	0.00-0.81	0.006	-	-	-	-	-	-
Hospitalisation within 6 months	No	(Ref)	-	-	-	-	-	-	-	-	-	-	-
	Yes	35.70	3.21-397.31	<0.001	-	-	-	-	-	-	-	-	-
Contact with small mammals	No	(Ref)	-	-	-	-	-	-	-	-	-	-	-
	Yes	14.88	1.75-126.72	0.011	-	-	-	-	-	-	-	-	-
Contact with wildlife	No	-	-	-	(Ref)	-	-	-	-	-	-	-	-
	Yes	-	-	-	5.07	1.46-17.59	0.013	-	-	-	-	-	-
Owner works in farming	No	(Ref)	-	-	(Ref)	-	-	-	-	-	(Ref)	-	-
	Yes	61.30	4.59-817.99	0.002	26.18	2.65-258.98	0.002	-	-	-	32.57	2.83-375.13	0.004
Owner visited hospital patient	No	(Ref)	-	-	-	-	-	-	-	-	-	-	-
	Yes	0.10	0.01-0.88	0.015	-	-	-	-	-	-	-	-	-
n			157			158			161			160	
Premises variance [SE] and ICC			0.99 [0.08] 0.23			0.06 [0.02] 0.02			4.42 [0.17] 0.57			1.12 [0.08] 0.25	

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; SE=standard error

5.4 Discussion

Previous studies have identified potential risk factors for carriage of AMR *E. coli* in dogs; however, these have mainly focussed on dogs receiving veterinary treatment (Wedley, 2012), or those of specific breeds (Schmidt et al., 2015) and have not considered the kennelled dog population. This study has identified multiple animal and owner-related risk factors, many of which are associated with multiple resistance outcomes.

As previously reported in humans (Chapter Three), prior hospitalisation was a risk factor for multiple outcomes including carriage of 3GC-resistant *E. coli* in owned dogs. The relationship between prior hospitalisation and antimicrobial resistance in both dogs and other animal species has previously been described (Bryan et al., 2010; Gibson et al., 2011a; Gibson et al., 2011b; Maddox et al., 2011; Ogeer-Gyles et al., 2006). The increased risk of 3GC resistance in hospitalised dogs in particular is not unexpected; this population of animals will more commonly have suffered from complex disease syndromes including MDR infections that have not responded to previous treatments. Current guidelines suggest the use of 3GCs should be reserved for infections resistant to first line antimicrobials (BSAVA, 2016; OIE, 2015) and therefore the use of these drugs is likely higher in the hospitalised population. This increased exposure may therefore select for resistance, and the potential for nosocomial spread of resistance between hospitalised patients may drive the dissemination of resistance in this population. However, the absence of this variable as risk factor for 3GC resistance in the total dog population sampled is interesting, and may suggest differing antimicrobial use between owned dogs and rescue dogs. Indeed, cefovecin is the only 3GC authorised for use in dogs (NOAH, 2016), and the cost of this drug may limit its use in hospitalised rescue animals. Additionally, hospitalisation was identified as a risk factor for tetracycline resistance in owned dogs, but not when considering all dogs sampled. This may suggest differing use of this class of drugs in rescue and owned dog populations. Furthermore, animals that had been sourced from a rescue centre were more at risk of tetracycline resistance than dogs sourced elsewhere. Tetracyclines are commonly used as a treatment for canine respiratory infections, including infectious tracheobronchitis (Hughes et al., 2012) and outbreaks of this syndrome are common in rescue centres. This may potentially explain the difference in resistance to this antimicrobial class between owned and rescue animals. However, further investigation into differences in prescribing practices and circulating *E. coli* clones in these two populations is warranted.

When considering the total dogs sampled, veterinary treatment within the last six months emerged as an independent risk factor for antimicrobial, ampicillin, amoxycylav and 3GC resistance. Given animals treated in a veterinary practice are more likely to have been exposed to antimicrobials than those who have not, this finding is unsurprising and has been reported previously in horses (Maddox et al., 2011). The absence of veterinary treatment as a risk factor for any resistance outcomes when considering owned dogs alone may suggest a difference in reasons for veterinary treatment in rescue and non-rescue dogs; while non-rescue dogs may have received veterinary treatment for clinical problems, it is also likely they would need preventative treatments, such as vaccinations prior to staying in kennels. In contrast, animals in rescue centres have usually been surrendered by their owners and may have more chronic problems requiring medical treatment including antimicrobials. This potential difference between kennelled dog populations does, however, require further investigation.

Animals having received antimicrobials were found to be at increased risk of carriage of tetracycline-resistant *E. coli* when considering the total dog population, mirroring previous findings (Wedley, 2012). However, this finding was not mirrored when considering owned dogs alone. As previously discussed, the potential for increased use of tetracyclines for outbreaks of infectious tracheobronchitis within rescue populations may provide an explanation for this contrast. Interestingly, in contrast to many other studies, recent antimicrobial treatment was not found to be a significant risk factor for other outcomes in univariable or multivariable analyses (Belas et al., 2014; Gronvold et al., 2010; Lawrence et al., 2013; Wedley et al., 2017). The absence of this risk factor for other outcomes investigated may be explained by the increased dog density in a kennelled environment, which may allow for more effective dissemination of resistant organisms between animals, as previously reported by Harada et al. (2011). Indeed, attending a dog dense environment such as a doggy day care has also been identified as a risk factor for faecal carriage of AMR *E. coli* (Procter et al., 2014); suggesting transmission between animals plays an important role in dissemination of antimicrobial resistance in the canine population. Furthermore, Johnson et al. (2008b) report the exchange of faecal *E. coli* isolates between household members correlates with increased human and dog density.

Age was not found to be a significant risk factor for any resistance outcomes, mirroring the findings of previous studies (Schmidt et al., 2015; Wedley, 2012). However, breed size was associated with multiple outcomes in both the total and owned dog population and indicated

that large and giant breed dogs were at an increased risk of resistance. This finding has previously been reported (Procter et al., 2014), and may be explained by differences in lifestyle between large and small dog breeds; medium and large breed dogs have greater exercise requirements and are walked more frequently than small breed dogs (Degeling et al., 2012; Westgarth et al., 2008). Therefore, these dogs may be more likely to encounter other dogs as well as environmental sources of antimicrobial resistance. Female dogs were more likely to carry 3GC or trimethoprim-resistant *E. coli* in the total and owned dog populations respectively. This mirrors previous reports in both human and dog populations (Belas et al., 2014; Friedmann et al., 2009), yet the reasons for this are unclear and may represent differing behaviour or disease patterns between the two sexes or it may be that this variable is a proxy for other factors not investigated not in this study.

The diet of participants, in particular consumption of raw food, was a risk factor identified for multiple outcomes including multi-drug and 3GC resistance, mirroring findings reported in previous studies (Schmidt et al., 2015; Wedley et al., 2017). Both commercially produced raw diets and retail meat products, particularly poultry, have been shown to carry AMR *Enterobacteriaceae* (Finley et al., 2008; Johnson et al., 2007; Nilsson, 2015; Strohmeyer et al., 2006; Vincent et al., 2010), and feeding of these products has been shown to result in shedding of resistant bacteria in previously culture negative research animals (Finley et al., 2007). This suggests foodborne resistant isolates are able to colonise the gut of susceptible animals or alternatively transfer resistance determinants to previously susceptible gut commensals. In addition to raw food, feeding of a dry mixer was associated with tetracycline resistance when considering all dogs sampled. This finding has not been previously described, however dry diets have previously been reported to carry *E. coli* at a higher level than canned foods, which are sterilised in the canning process (Strohmeyer et al., 2006). An interaction was identified between feeding raw and dry mixer indicating an intermediate risk of tetracycline resistance when both foods are fed together (OR=6.33) compared to when either raw food (OR=64.43), or dry mixer (OR=5.17) was fed alone. The reason for this interaction, is likely explained by the fact that animals fed both diets will be eating less of the each individual diet. As such, they will have a reduced risk compared to those eating raw food alone, however, the addition of raw food to a dry mixer diet alone will expose dogs to an increased risk due to the larger odds ratio associated with raw food diets.

The potential role of farming on the dissemination of resistance was highlighted by both direct contact with farm animals and having an owner working in farming being identified as

risk factors for resistance outcomes. Contact with farm animals was a risk factor for antimicrobial resistance in the owned dog population, while having an owner working with farm animals was a risk factor for ampicillin, chloramphenicol, tetracycline and multi-drug resistance. Farm animals have previously been identified as faecal carriers of AMR bacteria including MDR isolates (Costa et al., 2009a; Karczmarczyk et al., 2011a; Miles et al., 2006; Varga et al., 2008) and this has been associated with antimicrobial use (Varga et al., 2009). Indeed, the prevalence of antimicrobial resistance is increased in intensively raised animal species, where the sale of antimicrobials is higher (de Jong et al., 2009; VMD, 2015). Given the nature of farming environments, it is therefore unsurprising that dog contact with these animals would lead to an increased prevalence of resistance, a finding which has been mirrored in other animal species (Maddox et al., 2011). On-farm transfer of resistant bacteria to humans working with farm animals has also previously been described (Dierikx et al., 2013; Hammerum et al., 2014; Jakobsen et al., 2010), and those working with farm animals have a higher prevalence of carriage of AMR *E. coli* than the general population (Huijbers et al., 2014). These individuals may therefore be more likely to transfer resistant isolates to their pets. Furthermore, dogs owned by farmers may also be more likely to be exposed to farm animal faeces in the environment, which may provide an additional reservoir of resistance.

An interaction term was identified between contact with farm animals and recent veterinary treatment when considering carriage of AMR *E. coli*, suggesting an intermediate risk of carriage of AMR *E. coli* in animals who had received recent veterinary treatment and had contact with farm animals ($OR=3.80 \times 10^3$), when compared to those who received veterinary treatment ($OR=2.92$) or had contact with farm animals alone ($OR=2.61 \times 10^5$). This interaction term is challenging to explain, but may suggest that animals in contact with farm animals receive different types of veterinary treatment to those which are not. In addition, animals receiving veterinary treatment may be less active than those which are not and therefore have less exposure to the farm environment, including animals and their faeces, which may lead to a reduced risk when compared to more active animals. This model also included a random slope indicating that the effect of contact with farm animals varied between premises. This effect may be explained by the varying husbandry systems and antimicrobial treatment received by animals in different farming sectors (Brunton et al., 2012; Stevens et al., 2007; VMD, 2016). Dogs on different premises may have had contact with different farm animal species and husbandry systems and therefore different levels and types of exposure.

Interestingly, all the risk factors associated with chloramphenicol resistance were environmental. This drug is not authorised for use in animals (NOAH, 2016) and is available only as a topical treatment in human medicine (DataPharm, 2016), and as such, it is likely resistance to this antimicrobial is due to co-selection for resistance to other antimicrobials such as ampicillin and tetracycline. Additionally, contact with wildlife was identified as a risk factor for carriage of resistance to chloramphenicol. Previous studies have identified wild rodents as a potential reservoir of resistance to antimicrobials including chloramphenicol (Williams et al., 2011) and this relationship warrants further investigation. Contact with small mammals in the home was also identified as a risk factor for tetracycline resistance, however the reasons for this association are not clear.

Additional associations between owner-related factors and resistance outcomes were identified; having an owner working in hospitals or a GP surgery were identified as risk factors for 3GC and ampicillin respectively. These risk factors appear to be plausible given the differing prescribing patterns of these drugs in primary and tertiary human healthcare facilities. Penicillins are the most commonly prescribed antimicrobial by general practitioners, while 3GC use is more predominant in hospitals (PHE, 2015) and this use will drive different selective pressures and therefore differing resistance profiles in community and hospitalised patients. Contact with these patients and their associated diagnostic samples may result in transfer of resistant *E. coli* or resistance determinants to members of staff, who could then transfer them to household pets (Stenske et al., 2009).

Within this population, the proportion of variance attributed to the premises largely ranged from 0.18 to 0.64. It is not unexpected that animals within the same premises would be more similar than those in other premises as these animals would receive more similar management. In many cases, the ICCs calculated here were higher than previously reported for horses housed on the same premises (Maddox et al., 2011). This may be explained by this increased dog density on premises when compared to horses, which may facilitate more contact and potential transmission of resistant clones between animals. In addition, horses on a yard would likely be cared for by their individual owners, whereas in a kennel environment all animals would be cared for by a small number of individuals, leading to more similarities in the management of these animals when compared to horses. Likewise, rescue centre dogs are more likely to be managed and treated as a single population, particularly during outbreaks of infectious disease. As such, the ICC for all models with the exception of trimethoprim resistance was higher in the total dog population when compared to owned

dogs alone. The ICCs for amoxyclav and chloramphenicol resistance were lower than that identified for other antimicrobials, indicating premises were of less importance for these outcomes. Chloramphenicol is not approved for use in animals and as such, prescribing practices of this drug will not differ between premises, which may explain the lack of variance at the premises level for resistance to this drug. Conversely, amoxyclav is the most frequently prescribed antimicrobial in small animal veterinary practice (Radford et al., 2011; Singleton et al., 2017), and as such, prescribing of this drug to animals may be very similar across premises.

Several potential limitations have been identified for this study; the recruitment methods employed are described Chapter Four, and are likely to have introduced some selection bias. Similarly, the recruitment of canine participants by kennel staff rather than random sampling may mean the sampled dogs were not truly representative of the study population. Recall bias may also have been introduced by participants when completing questionnaires. When writing the questionnaire most questions focussed on current exposures and very few asked for historical information which should minimise this effect, however validation of the information provided was not possible. The low prevalence of some outcomes precluded risk factor analysis; therefore, further studies with larger numbers of participants are required to assess risk factors for these outcomes. Similarly, many of the risk factors identified in this study have wide confidence intervals and thus, while there can be confidence in the effects reported, the magnitude of these effects is less certain. A larger sample size would have allowed increased confidence in the effects observed and may have allowed detection of risk factors with smaller effects, similarly an additional case-control study investigating carriage of ESBL-producing bacteria would be advisable. The population sampled in this study was restricted to kennelled dogs and as such, findings should not be extrapolated to the general dog population where exposures may be different.

Within this study, numerous risk factors for carriage of AMR *E. coli* in kennelled dogs have been identified. The importance of both human-animal contact and diet has been highlighted suggesting the potential for exchange of resistance determinants between humans, animal species and the environment. While this study does not provide information about risk factors for clinical infections, commensal *E. coli* can transmit resistance determinants to pathogenic isolates. Furthermore, knowledge of the risk factors for carriage of antimicrobial resistance in dogs housed in high densities may provide a clearer picture of the development and exchange of resistance between these animals.

Chapter 6

Molecular characterisation of *Escherichia coli* isolated from the faeces
of kennelled dogs and humans working with dogs

6.1 Introduction

First identified in 1885, *Escherichia coli* (*E. coli*) represent an important commensal of the gastrointestinal tract of humans and dogs (Berg, 1996; Escherich, 1989; Gordon and Cowling, 2003). In addition, this diverse bacterial species also includes pathogenic strains responsible for causing enteric and extra-intestinal disease (Johnson and Russo, 2002; Kaper et al., 2004; Nataro and Kaper, 1998), while commensal strains may also cause clinical disease in immunocompromised individuals (Packey and Sartor, 2009).

E. coli displays high levels of diversity, which can be attributed to both horizontal gene transfer and genetic recombination (Touchon et al., 2009). This diversity has led to the subdivision of the species into eight distinct phylogroups (A, B1, B2, C, D, E, F and *Escherichia* Clade I) based on the presence of housekeeping genes (Clermont et al., 2000; Clermont et al., 2013; Doumith et al., 2012). Some associations have been identified between these phylogroups and pathogenicity; phylogroups B2 and D, are most commonly associated with extra-intestinal disease, while phylogroups A, B1, and E are more frequently associated with enteric disease (Clermont et al., 2011; Johnson and Stell, 2000; Picard et al., 1999).

While commensal *E. coli* represent an important gastrointestinal barrier against pathogenic bacteria (Vollaard and Clasener, 1994), they are commonly exposed to antimicrobials used to treat disease. This exposure increases the selection pressure for bacterial acquisition of resistance genes, which may then be disseminated to pathogenic bacteria (Edlund et al., 1994; Jakobsson et al., 2010; Nyberg et al., 2007). The ecological niche of *E. coli*, alongside ease of culture means *E. coli* represents a good indicator bacteria for antimicrobial resistance determinants circulating in the gastrointestinal commensal population (De Graef et al., 2004; van den Bogaard and Stobberingh, 2000).

Of particular concern currently are the extended-spectrum β -lactamase (ESBL) enzymes. First described in 1983 (Knothe et al., 1983), these enzymes hydrolyse the β -lactam antimicrobials, conferring resistance to penicillins, oxyimino-cephalosporins and monobactams, while remaining susceptible to β -lactam inhibitors. ESBL-producing isolates have been increasingly associated with nosocomial and community-acquired infections within human medicine globally (ECDC, 2015; Fennell et al., 2012; Livermore et al., 2008) and have also been identified as a cause of clinical infections in dogs (Dierikx et al., 2012; O'Keefe et al., 2010; Timofte et al., 2011). The genes encoding these enzymes are located on plasmids, which commonly carry multiple resistance genes including those encoding

resistance to quinolone and aminoglycoside drugs. As such, ESBL-producing isolates frequently display a multi-drug resistant (MDR) phenotype, presenting a further clinical challenge (Paterson and Bonomo, 2005; Lavigne et al., 2006).

To date, four main classes of ESBL enzymes have been described; while parent TEM and SHV enzymes do not display intrinsic activity against oxyimino-cephalosporins, TEM and SHV variants gained extended activity via amino acid substitutions (Bush and Palzkill, 2015). Genes encoding these enzymes have been identified in *E. coli* isolates recovered from humans and animals globally (Briñas et al., 2003; Teshager et al., 2000; Tuerena et al., 2016) and predominated throughout the 1990's before being superseded by the CTX-M enzymes in the early 21st Century (Valentin et al., 2014; Livermore et al., 2007).

To date, over 160 CTX-M enzymes have been identified and are characterised into five clusters CTX-M1, CTX-M2, CTX-M8, CTX-M9 and CTX-M25 based on their amino acid sequences (McArthur et al., 2013). Within the human population, CTX-M-14 and CTX-M-15 belonging to clusters CTX-M9 and CTX-M1, respectively are most prevalent globally (Cantón et al., 2012). CTX-M-15 is of particular interest due to its global dissemination associated with the spread of the MDR pandemic *E. coli* sequence type (ST) 131 clone. Within the UK, CTX-M-15 is the most prevalent ESBL enzyme carried by human isolates (Livermore and Hawkey, 2005) and has also been identified in isolates of canine origin (Huber et al., 2013; Pomba et al., 2009; Timofte et al., 2011; Timofte et al., 2014b). In contrast to the picture in humans however, previous studies have indicated that CTX-M-1 predominates in clinical and non-clinical isolates of canine origin (Carattoli et al., 2005; Dierikx et al., 2012; Wedley et al., 2017).

The OXA enzymes are phylogenetically distinct from TEM, SHV and CTX-M enzymes. Classical OXA enzymes such as OXA-1 are not intrinsically resistant to third generation cephalosporins (3GC). However, variants with an extended spectrum activity have arisen through amino acid substitutions in a similar way to that previously described for TEM and SHV enzymes. While enzymes of this class have been identified in isolates of *E. coli*, they are most commonly carried by *Pseudomonas aeruginosa* (Bhattacharjee et al., 2007; Livermore, 1995).

The identification of numerous *bla*_{ESBL} genes of human importance in isolates of canine origin (Ewers et al., 2010; Ewers et al., 2014; Pomba et al., 2009; Timofte et al., 2014b), combined with the frequent and close contact between humans and dogs suggests that there is potential for transmission of resistance between these two species. Indeed, previous studies

have indicated household transmission of *E. coli* between humans and dogs globally (Carvalho et al., 2016; Damborg et al., 2009; Johnson et al., 2008a; Johnson et al., 2008b). However, no studies have investigated the potential for transmission of these isolates within the UK.

The aim of this study was to characterise *E. coli* isolates of canine and human origin within the North of England via identification of phylogroups, sequence types and the carriage of resistance genes by ESBL-producing and MDR isolates; and to assess the potential for transmission of *E. coli* between in-contact humans and dogs in defined premises.

6.2 Materials and methods

6.2.1 Isolation and genetic confirmation of *E. coli*

Faecal samples were collected from 296 dogs and 229 humans working with dogs in the North of England as previously described in Chapters Three and Four and subjected to selective and non-selective methods for the isolation of antimicrobial-resistant (AMR) *E. coli*. Briefly, 0.5ml of faecal homogenate was enriched in 4.5ml of buffered peptone water overnight at 37°C and streaked onto three methylene blue agar (EMBA) plates, one containing cefotaxime (1µg/ml), one containing ceftazidime (1µg/ml), and one containing no antimicrobials (Liebana et al., 2006). An additional EMBA plate was inoculated with the enriched faecal homogenate and four antimicrobial discs were directly applied (ampicillin 10µg, amoxycylav 30 µg, ciprofloxacin 1 µg, trimethoprim 2.5 µg), as previously described (Bartoloni et al., 1998). Following overnight incubation at 37°C colonies resembling *E. coli* were selected from the media as previously described. All isolates then underwent antimicrobial susceptibility testing against a panel of seven antimicrobials (ampicillin 10 µg, amoxycylav 30 µg, ciprofloxacin 1 µg, chloramphenicol 30µg, nalidixic acid 30µg tetracycline 30 µg and trimethoprim 2.5 µg), according to British Society of Antimicrobial Chemotherapy (BSAC) guidelines (BSAC, 2013). Additionally, all isolates selected from plates containing cefotaxime or ceftazidime were subjected to testing for ESBL production using the combination disc diffusion method (M'Zali et al., 2000). Isolates which were phenotypically resistant to 3GCs and amoxycylav were additionally tested for confirmation of AmpC production using the three disc diffusion method (Halstead et al., 2012).

DNA lysates were prepared for all isolates by transferring 1-3 colonies into 500µl sterile molecular grade water (Sigma-Aldrich, Dorset UK) in 1.5ml Eppendorf tubes heated at 100°C for 20 minutes. The lysate was then stored at 4°C until use. Isolates were then confirmed to

be *E. coli* by examination using PCR assays for the *E. coli*-specific *uidA* gene (McDaniels et al., 1996). Three isolates with a phenotypic appearance of *E. coli* on EMBA, which were negative for the *uidA* gene on PCR were forwarded to Dr Caroline Corless at the Royal Liverpool and Broadgreen University Hospitals NHS Trust for speciation using matrix-assisted laser desorption/ionization.

All media was sourced from Lab M Ltd Bury, UK, antimicrobial discs were obtained from MAST Group Ltd., Bootle, UK, while cefotaxime and ceftazidime powder was sourced from Sigma–Aldrich Company Ltd., Gillingham, UK.

6.2.2 Phylogenetic grouping and identification of *E. coli* ST131

Non-duplicate *E. coli* isolates of canine (n=508) and human (n=364) origin were assigned to seven recognised phylogroups (A, B1, B2, C, D, E and F) or other *Escherichia* clades using a series of multiplex PCR reactions as described by Clermont et al. (2013). Isolates designated as belonging to phylogroup B2 were then subjected to further PCR assays targeting the O25b *rfb* gene cluster and *pabB* gene described, to identify O25b-ST131 clonal isolates as previously (Clermont et al., 2008a; Clermont et al., 2009). Details of oligonucleotide sequences and conditions for PCR amplification are located in Table 6.1.

Table 6.1 Primers used for amplification of *E. coli* genes in this study including expected amplicon size and annealing temperatures

Target	Primer	Sequence 5'→3'	Amplicon size (bp)	Annealing (°C)	Reference
uidA	uidAF	CCAAAAGCCAGACAGAGT	623	58	McDaniels et al. (1996)
	uidAR	GCACAGCACATCAAAGAG			
chuA	chuA.1b	ATGGTACCGGACGAACCAAC	288		
	chuA.2	TGCCGCCAGTACCAAAGACA			
yjaA	yjaA.1b	CAAACGTGAAGTGTCCAGGAG	211	59	Clermont et al. (2013)
	yjaA.2b	AATGCGTTCCTCAACCTGTG			
TspE4.C2	TspE4C2.1b	CACTATTGTAAGGTCATCC	152	multiplex	
	TspE4C2.2b	AGTTTATCGCTGCGGGTCCG			
arpA	AceK.f	AACGCTATTCGCCAGCTTGC	400		
	ArpA1.r	TCTCCCCATACCGTACGCTA			
arpA	ArpAgpE.f	GATTCCATCTTGTCAAATATGCC	301	57	Clermont et al. (2013)
	ArpAgpE.r	GAAAAGAAAAAGAATTCCAAGAG			
trpA	trpAgpC.1	AGTTTTATGCCAGTGCAG	219	59	Clermont et al. (2013)
	trpAgpC.2	TCTGCGCCGTCACGCC			
trpA	trpBA.f	CGGCGATAAAGACATCTTCAC	489	57 or 59	Clermont et al. (2013)
	trpBA.r	GCAACGCGGCCTGGCGGAAG			
O25b rfb	rfb.1bis	ATACCGACGACGCCGATCTG	300	60	Clermont et al. (2008a)
	rfbO25b.r	TGCTATTATTATGCGCAGC			
ST131	trpA.F	GCTACGAATCTCTGTTTGCC	427	65	Clermont et al. (2009)
	trpA.R	GCAACGCGGCCTGGCGGAAG			
	O25pabBspe.F	TCCAGCAGGTGCTGGATCGT	347		
	O25pabBspe.R	GCGAAATTTTCGCCGTACTGT			

6.2.3 Characterisation of resistance genes

All *E. coli* isolates of canine and human origin demonstrating resistance to β -lactam antimicrobials during susceptibility testing were subjected to PCR in order to detect the presence of *bla*_{TEM} and *bla*_{SHV} genes as previously described (Dallenne et al., 2010). Isolates displaying an AmpC phenotype or resistance to clavulanic acid were additionally screened for carriage of plasmid-mediated *bla*_{AmpC} genes by multiplex PCR (Pérez-Pérez and Hanson, 2002). All isolates phenotypically resistant to at least one 3GC (cefepodoxime, ceftazidime, or cefotaxime) during combination disc diffusion testing were additionally tested for the presence of *bla*_{CTX-M} and *bla*_{OXA} genes (Boyd et al., 2004; Dallenne et al., 2010). All *bla*_{CTX-M} positive isolates were then characterised further using specific oligonucleotide primers for *bla*_{CTX-M} group 1, 2 and 9 genes (Batchelor et al., 2005; Carattoli et al., 2008; Hopkins et al., 2006a). All quinolone-resistant isolates were subjected to PCR targeting *qnrA*, *qnrB* and *qnrS* genes (Robicsek et al., 2006b), while those displaying resistance to ciprofloxacin were additionally tested for carriage of *aac(6')-Ib* (Park et al., 2006). The primer sequences and conditions for amplification of resistance genes are located in Table 6.2.

All PCR reactions were undertaken using 5x FIREPol® 12.5mMCl₂ Master Mix Ready to Load. Reactions testing for the presence of *uidA* and antimicrobial resistance genes were

undertaken in reaction volumes containing 4µl of master mix, 5pmol of each primer and 1µl of DNA lysate with the addition of sterile molecular grade water (Sigma-Aldrich, Dorset, UK) to make up a reaction volume of 25µl, with the exception of the detection of *bla*_{AmpC} genes where 5µl of DNA lysate was added. For the detection of *bla*_{AmpC} genes 5µl of DNA lysate was added. Reactions relating to phylogenetic grouping and detection of *E. coli* O25b:H4 ST131 were undertaken in reactions containing 4µl of master mix, 5pmol of each primer, 3µl of DNA lysate and sterile molecular grade water making up a reaction volume of 20µl. A bacterial isolate known to carry the gene of interest was included as a positive control and sterile molecular grade water was used as a negative control during each PCR assay. PCR amplification products were identified from their size by gel electrophoresis using peqGREEN-stained 2% agarose-Tris-acetate-EDTA gel, visualised by UV transillumination. A 100-bp ladder was used as a marker during visualisation of amplification products.

Table 6.2 Primers used for amplification of resistance genes in this study including expected amplicon size and annealing temperatures

Target	Primer	Sequence 5'→3'	Amplicon size (bp)	Annealing (°C)	Reference
bla_{TEM}	TSO-TF	CATTTCCGTGTCGCCCTTATTC	800	60 (multiplex)	Dallenne et al. (2010)
	TSO-TR	CGTTCATCCATAGTTGCCTGAC			
bla_{SHV}	TSO-SF	AGCCGCTTGAGCAAATTAAC	713	60 (multiplex)	Dallenne et al. (2010)
	TSO-SR	ATCCCGCAGATAAATCACCAC			
bla_{OXA}	TSO-OF	GGCACCAGATTCAACTTTCAAG	564	58	Boyd et al. (2004)
	TSO-OR	GACCCCAAGTTTCCTGTAAGTG			
bla_{CTX-M}	CTX-MU1	ATGTGCAGYACCAGTAARGTKATGGC	593	58	Boyd et al. (2004)
	CTX-MU2	TGGGTRAARTARGTSACCAGAAYCAGCGG			
bla_{CTX-M} Group 1	CTX-Mgp1F	CCCATGGTTAAAAAATCACTGC	876	55	Carattoli et al. (2008)
	CTX-Mgp1R	CAGCGCTTTTGCCGTCTAAG			
bla_{CTX-M} Group 2	CTX-Mgp2F	ATGATGACTCAGAGCATTTCGC	893	55	Hopkins et al. (2006a)
	CTX-Mgp2R	TCAGAAACCGTGGGTTACGAT			
bla_{CTX-M} Group 9	CTX-Mgp9F	ATGGTGACAAAGAGAGTGCAAC	876	55	Batchelor et al. (2005)
	CTX-Mgp9R	TTACAGCCCTTCGGCGATG			
bla_{AmpC}	CITMf	TGGCCAGAACTGACAGGCCAAA	462	64 (multiplex)	Pérez-Pérez and Hanson (2002)
	CITMr	TTTCTCCTGAACGTGGCTGGC			
	DHAMf	AACTTTCACAGGTGTGCTGGGT	405		
	DHAMr	CCGTACGCATACTGGCTTTGC			
	ACCMf	AACAGCCTCAGCAGCCGGTTA	346		
	ACCMr	TTCGCCGCAATCATCCCTAGC			
	EBCMf	TCGGTAAAGCCGATGTTGCGG	302		
	EBCMf	CTTCCACTGCGGCTGCCAGTT			
	FOXMF	AACATGGGGTATCAGGGAGATG	190		
	FOXMr	CAAAGCGCGTAACCGGATTGG			
MOXMF	GCTGCTCAAGGAGCACAGGAT	520			
MOXMR	CACATTGACATAGGTGTGGTGC				
qnrA	qnrAF	ATTTCTCACGCCAGGATTTG	516	53 (multiplex)	Robicsek et al. (2006b)
	qnrAR	GATCGGCAAAGGTTAGGTCA			
qnrB	qnrBF	GATCGTGAAAGCCAGAAAGG	469	53 (multiplex)	Robicsek et al. (2006b)
	qnrBR	ACGATGCCTGGTAGTTGTCC			
qnrS	qnrSF	ACGACATTTCGTCAACTGCAA	417	55	Park et al. (2006)
	qnrSR	TAAATTGGCACCCCTGTAGGC			
aac(6')-Ib	AAC(6)-Ib-F	TTGCGATGCTCTATGAGTGGCTA	420	55	Park et al. (2006)
	AAC(6)-Ib-r	CTCGAATGCCTGGCGTGT			

All primers were sourced from Eurofins MWG Operon, Ebersberg, Germany; PCR reagents, and 100-bp ladder were sourced from Solis Biodyne, Tartu, Estonia and peqGREEN was sourced from Peqlab, Fareham, UK.

6.2.4 Whole genome sequencing and multi-locus sequence typing

Isolates of canine and human origin displaying an ESBL phenotype, alongside a selection of isolates displaying an AmpC phenotype or resistance to at least four antimicrobial classes were selected for further examination using whole genome sequencing (WGS). In addition all isolates from two premises (one boarding and one rescue) with high levels of compliance were selected for WGS. Briefly, DNA was extracted from overnight cultures of selected isolates using QIAamp® DNA Mini Kit (Qiagen, Manchester, UK) according manufacturer's

instructions. DNA was then quantified and assessed for purity using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Cheshire, UK) before being forwarded to the University of Swansea and Dr Ben Pascoe for WGS. Sequence libraries were prepared using the Nextera XT v2 library preparation kit (Illumina, San Diego, USA) and sequenced on MiSeq desktop sequencers (Illumina, San Diego, USA) using MiSeq V3 sequencing cartridges. Genome assemblies on the 300 bp short read pair end data were undertaken using the *de novo* assembly algorithm SPAdes v3.3 (Bankevich et al., 2012). Overall, the average number of contiguous sequences (contigs) for the 132 genomes sequenced was 387, giving rise to an average total assembled genome size of 5,103,363 bp (average N₅₀: 56 kbp). Complete details of the number of contigs and assembled genome size for each of the isolates sequenced are available in Appendix IV. Assembled genomes were uploaded to the BIGSdb database and sequences were aligned to the K12 reference strain (accession number U00096) using MAFFT (Katoh and Standley, 2013) to produce an alignment of 5,451 bp on a gene by gene basis and concatenated into a contiguous sequence for each isolate genome including gaps (Sheppard et al., 2012).

On receipt of genome sequences, nucleotide sequences were uploaded in FASTA format to the online tools VirulenceFinder 1.5 (Joensen et al., 2014) and Resfinder 2.1 (Zankari et al., 2012) in order to ascertain carriage of virulence genes and plasmid mediated resistance genes. In each case a 90% threshold for identification and minimum query length of 60% were utilised. Nucleotide sequences were also uploaded to the Comprehensive Antibiotic Resistance Database (CARD) (<https://card.mcmaster.ca/>) in order to ascertain carriage of chromosomal quinolone resistance genes using the basic local alignment search tool (BLAST) Expect values (McArthur et al., 2013; Jia et al., 2017). Full details of the algorithms used by the CARD Resistance Gene Identifier (RGI) are not currently published, however a paper detailing these algorithms is in preparation and may provide further detail of how cut-offs are defined (Jia et al., 2017). Assessment for the presence of plasmid replicons was undertaken using the online PlasmidFinder 1.3 (Carattoli et al., 2014) with an identity threshold of 95%. Plasmid Multi-locus Sequence Typing (pMLST) of IncA/C, IncF, IncI1 and IncN replicons was then undertaken by comparing the sequence of replicon specific loci with allelic variants published in the PubMLST database (<https://pubmlst.org/plasmid/>) and allocation of an ST based on allelic combinations. *In silico* serotyping of isolates was undertaken using SerotypeFinder 1.1 (Joensen et al., 2015) in order to assess the O and H serogroups of each isolate using a 90% identity threshold and query length of 60%.

Sequences of seven housekeeping genes, *adhA*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA* (Wirth et al., 2006), were extracted from the complete sequence for each isolate and compared to those published within the *E. coli* MLST database (<http://enterobase.warwick.ac.uk/>). Allele numbers were then assigned for each loci and an isolate ST was then allocated based on these alleles. Where incomplete allele profiles were present, the MLST database was manually checked in order to ascertain in which ST the identified alleles were present. If only one ST matched the available data, the isolate was nominally allocated to this sequence type or clonal complex. In addition, MLST data from complete sequences were aligned using MUSCLE (Edgar, 2004), and a neighbour-joining phylogenetic tree of concatenated isolate sequences was constructed using MEGA version 7 (Kumar et al., 2016).

6.2.5 Conjugation experiments

Isolates resistant to 3GCs, displaying either an ESBL or MDR phenotype were selected for inclusion in conjugation experiments in order to determine their ability to transfer resistance determinants to a rifampin-resistant lactose-negative *E. coli* 26R793 recipient using the methods described by Karczmarczyk et al. (2011b). Briefly, donor and recipient isolates were cultured in nutrient broth overnight at 37°C. Equal volumes of donor and recipient cultures were then mixed and incubated at 37°C overnight without shaking. Selection for transconjugants was undertaken on MacConkey agar supplemented with a combination of 100 µg/ml rifampin and either 50 µg/ml nalidixic acid, 50 µg/ml trimethoprim, 2 µg/ml ceftazidime or 2 µg/ml of cefotaxime (Sigma–Aldrich, Gillingham, UK). Where present, three lactose-negative colonies were selected and sub-cultured on nutrient agar prior to antimicrobial susceptibility testing and PCR screening assays for appropriate ESBL or *bla*_{AmpC} genes. Where transfer of these genes was not confirmed by PCR, further PCR assays were undertaken to examine for the transfer of other *bla* genes

6.2.6 Statistical analysis

In order to examine the association between isolate-level antimicrobial resistance outcomes and *E. coli* phylogenetic groups in each of the species sampled, contingency tables were constructed and Chi-squared tests were undertaken. A Sidak-Bonferroni correction (Keppel and Wickens, 2004) was used to calculate an adjusted p-value of p<0.0006 based on 80 statistical comparisons undertaken for each species. Chi-squared tests were used to compare the association between *E. coli* phylogroups and species sampled. The corrected p-value for eight comparisons was p<0.006. In cases where expected cell values were less than five,

Fisher's exact method was used to calculate p values. Additionally, odds ratios and 95% confidence intervals were calculated from contingency tables used to undertake Chi-squared and Fisher's Exact tests. In order to examine the agreement between the presence of resistance genes identified by PCR assay and WGS of isolates, a Cohen's kappa statistic was calculated.

Multiple correspondence analysis plots were constructed in order to explore the association between host species, *E. coli* phylogenetic group and nine defined resistance outcomes (resistance to ampicillin (Amp), amoxyclav (AC), chloramphenicol (Chl), ciprofloxacin (Cip), nalidixic acid (N.A), tetracycline (Tet), trimethoprim (Tm), 3GCs (3GC) and multi-drug resistance (MDR)). Hierarchical clustering of the principle components of the MCA was then undertaken using Ward's method in order to group isolates based on their co-ordinates on these plots.

All statistical analyses were undertaken using R (R Core Team, 2015) and multiple correspondence analysis and hierarchical clustering were undertaken using the FactoMineR package (Lê et al., 2008).

6.3 Results

6.3.1 *E. coli* phylogenetic groups

In total, 364 human and 508 canine isolates of *E. coli* were assigned to phylogenetic groups (Table 6.3). In humans, most isolates belonged to phylogroup B2 followed by groups B1, A and D, with only a small number of isolates belonging to phylogroups C, E and F. In dogs, the most prevalent phylogroup identified was B1, followed by groups B2 and A with a low prevalence of groups C, D, E and F. Isolates belonging to phylogroup B1 were significantly more likely to be canine in origin (OR=1.89 95% CI 1.38-2.60, $p<0.0001$), while those belonging to groups B2 (OR=0.67 95% CI 0.50-0.90, $p=0.0051$) and D (OR=0.49 95% CI 0.23-0.65, $p<0.0001$) were significantly less likely to be isolated from dogs. No further differences were identified between host species and other phylogroups. In addition, 11 isolates (3.02% 95% CI 0.26-4.78) of human origin and two isolates (0.39% 95% CI 0.00-0.94) of canine origin belonging to group phylogroup B2 were found to belong to O25b-ST131 by PCR assays specific for this pandemic clone, however none of these isolates were resistant to 3GCs.

Table 6.3 The number and prevalence of faecal *E. coli* isolates assigned to phylogenetic groups A-F from 227 humans and 296 dogs in the North of England

Phylogroup	Human isolates (n=364)			Dog isolates (n=508)			P
	n	Prevalence	95% CI	n	Prevalence	95% CI	
A	62	17.0	13.2-20.9	96	18.9	15.5-22.3	0.41
B1	82	22.5	18.2-26.8	180	35.4	31.3-39.6	<0.0001
B2	142	39.0	34.0-44.0	152	29.9	25.9-33.9	0.0051
C	3	0.8	0.0-1.8	19	3.7	2.1-5.4	0.0068
D	46	12.6	9.2-16.1	27	5.3	3.4-7.3	<0.0001
E	6	1.6	0.3-3.0	8	1.6	0.5-2.7	0.93
F	17	4.7	2.5-6.8	9	1.8	0.6-2.9	0.013
Other Clades	6	1.6	0.3-3.0	17	3.3	1.8-4.9	0.12

Key: 95% CI = 95% confidence interval; P = P-value from Chi-squared test; Significance set at P < 0.006 (Sidak-Bonferroni correction)

6.3.2 Association between isolate phylogroups and antimicrobial resistance outcomes

6.3.2.1 Canine isolates

The prevalence of resistance to at least one antimicrobial in phylogroups A-F ranged from 25.0% (95% CI 0.0-55.0) in phylogroup E to 100% (95% CI 100.0) in phylogroup C. Isolates resistant to 3GCs originated from all phylogroups, however no ESBL-producing isolates were identified belonging to phylogroups B2 or D. Isolates belonging to phylogroup A displayed significantly higher levels of tetracycline (χ^2 p<0.0001) and trimethoprim (χ^2 p=0.00049) resistance when compared to other groups (Table 6.4). In contrast, isolates belonging to group B2 were associated with significantly lower levels of resistance to tetracycline (χ^2 p<0.0001) and nalidixic acid (χ^2 p=0.00028) as well as being less likely to display a MDR phenotype (χ^2 p<0.0001). Isolates belonging to group C were associated with increased levels of AMR (χ^2 p=0.00043), as well as specific resistance to amoxycylav (Fisher's Exact p=0.00016), nalidixic acid (Fisher's Exact p<0.0001) and trimethoprim (χ^2 p<0.0001). They were also significantly more likely to display a MDR phenotype (Fisher's Exact p<0.0001) than isolates belonging other groups (Table 6.4). No significant associations were identified for groups B1, D, E and F. Full details of the prevalence of each resistance outcome within each phylogroup can be found in Appendix IV.

Table 6.4 Significant associations between phylogenetic groups and the presence or absence of one of eleven antimicrobial resistance outcomes for 491 canine faecal *E. coli* isolates assigned to Phylogroups A-F

Resistance outcome	Phylogenetic Group								
	Phylogroup A (n=96)			Phylogroup B2 (n=152)			Phylogroup C (n=19)		
	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P
Any resistance							19	100.0 (100.0)	0.00043
MDR				4	2.6 (0.1-5.2)	<0.0001	13	68.4 (47.5-89.3)	<0.0001[†]
Amoxyclav							10	52.6 (30.2-75.1)	0.00016[†]
Nalidixic Acid				3	2.0 (0.0-4.2)	0.00028	9	47.4 (24.9-69.8)	<0.0001[†]
Tetracycline	59	56.3 (46.3-66.2)	<0.0001	19	12.5 (7.2-17.8)	<0.0001			
Trimethoprim	46	47.9 (37.9-57.9)	0.00049				16	84.2 (67.8-100.0)	<0.0001

Key: P=P value calculated using Chi-squared test; † P value calculated using Fisher's Exact method; 95% CI=95% confidence interval; MDR= multi-drug resistant phenotype; Significance set at $P < 0.00066$ (Sidak-Bonferroni correction).

6.3.2.2 Human isolates

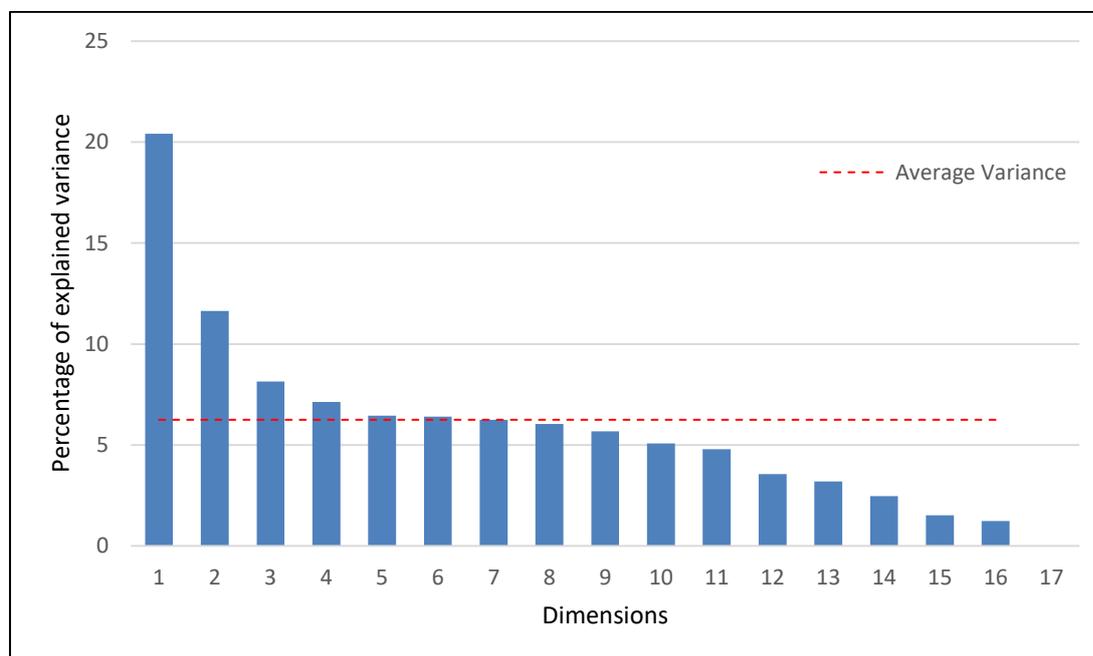
At least half of the isolates from all phylogroups displayed an AMR phenotype with the exception of phylogroup B1, in which 48.8% (95% CI 38.8-59.3) of isolates displayed a resistant phenotype. Of note were isolates from group C, which were all resistant to at least one antimicrobial. All phylogroups with the exception of group E contained isolates with 3GC resistance and the prevalence of phenotypic ESBL-producing isolates was highest in group A isolates (8.1% 95% CI 1.3-14.8), however this trend was not significant (Fisher's Exact $p=0.0089$). Resistance to ciprofloxacin was low in all phylogroups and was absent in isolates belonging to phylogroups C, E and F. Isolates belonging to phylogroup D were significantly more likely to be resistant to nalidixic acid ($\chi^2 p=0.00015$) and display a MDR phenotype ($\chi^2 p=0.00016$). No further significant differences in resistance outcomes were identified between phylogroups in isolates of human origin (Appendix IV).

6.3.3 Association between host species and resistance phenotypes

Multiple correspondence analysis and hierarchical clustering were utilised in order to assess the relationships between isolates, phylogenetic groups and the resistance outcomes defined above. The dataset comprised 849 isolates within 16 dimensions representing 11 variables, and these dimensions were examined in order to assess their contribution to the variation within the dataset (Figure 6.1). In total, six dimensions were found to contribute greater than average variance to the data and were therefore selected for inclusion in the

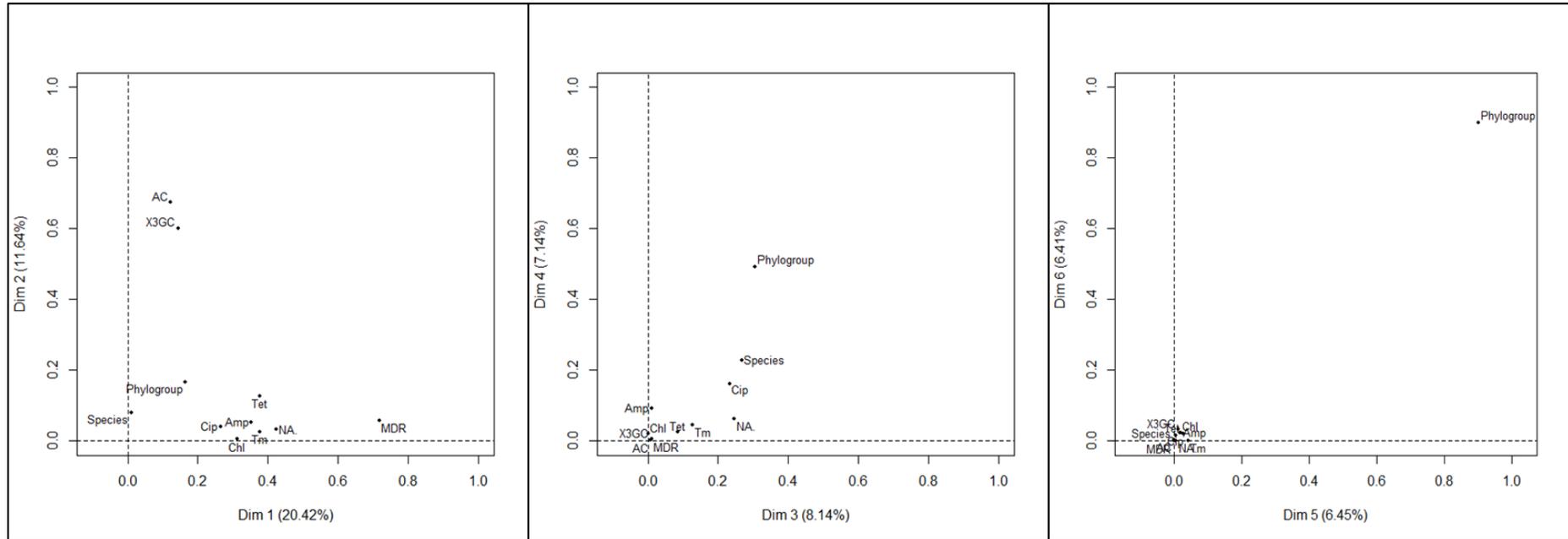
analysis. Interestingly however, these dimensions contributed a total of only 60.2% of the total variation.

Figure 6.1 Scree plot indicating variation in the dataset explained by each MCA dimension



Examination of variables contributing to the correspondence analysis (Figure 6.2) suggested the variables, multi-drug resistance ($\eta^2=0.72$) and nalidixic acid ($\eta^2=0.42$) were most correlated with the first dimension, while variables considering amoxyclav ($\eta^2=0.68$), and 3GC ($\eta^2=0.60$) resistance were most correlated to the second dimension. In contrast to dimensions one and two, most variables pertaining to resistance phenotypes showed little correlation with dimensions three to six and were clustered around the origin of the plot. *E. coli* phylogroup was the variable identified as having the highest correlation with these remaining dimensions (η^2 0.31-0.90). Full details of the correlation of all variables to each of the dimensions can be found in Appendix IV.

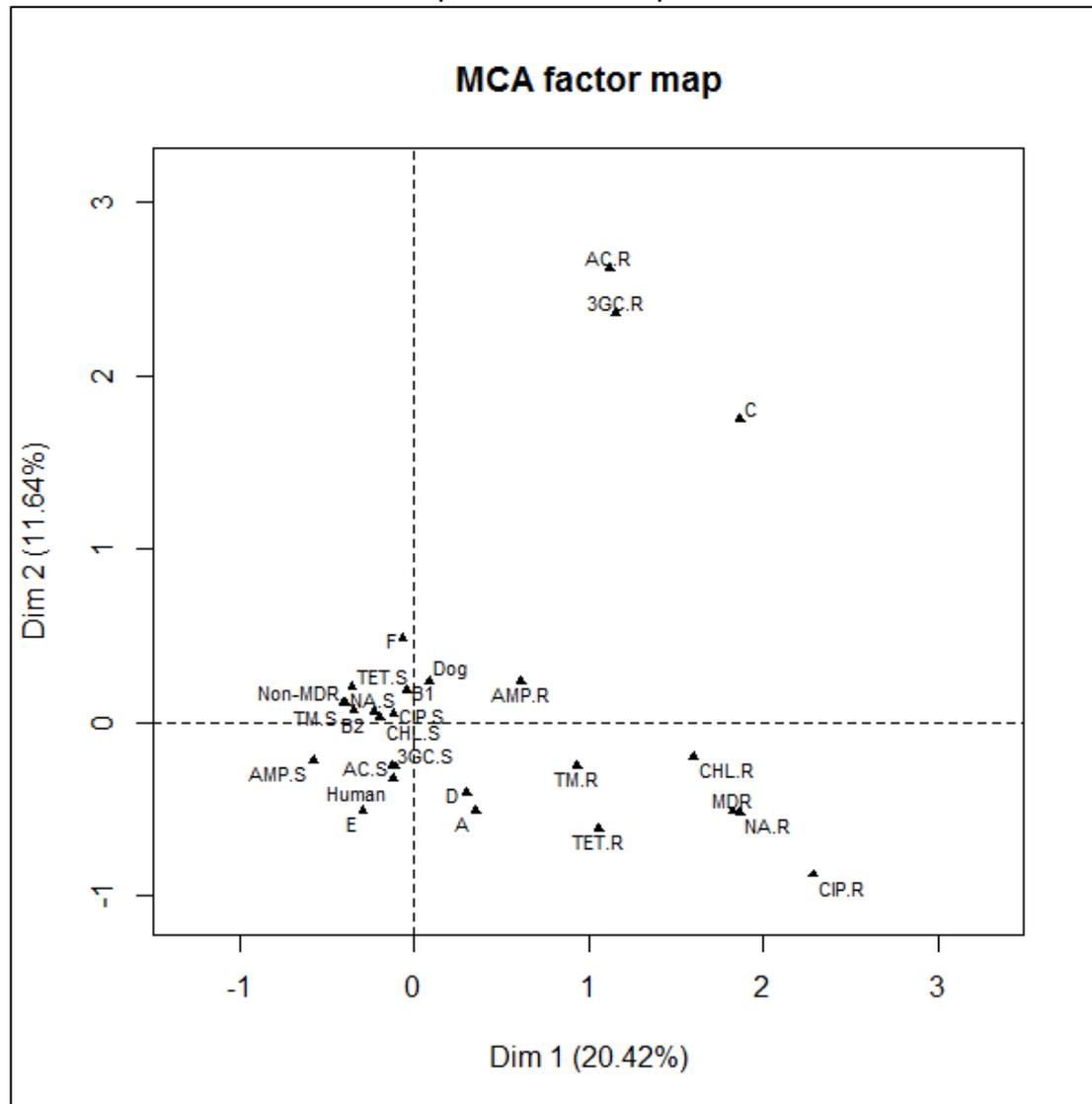
Figure 6.2 Correlation of variables with six dimensions included in correspondence analysis



Key: Amp=ampicillin; AC=amoxyclav; Chl=chloramphenicol; Cip=ciprofloxacin; MDR= multi-drug resistance; NA=nalidixic acid; Tet=tetracycline; Tm=trimethoprim; X3GC=third generation cephalosporin; Species=Host species; Phylogroup=*E. coli* phylogroups A-F

The 2D map (Figure 6.3), plotted from the MCA model of the first and second dimensions, indicates that these dimensions contributed 20.42% and 11.64% of the variance within the model, respectively. This model indicates that when considering dimensions one and two, sensitivity and resistance to each of the antimicrobials are opposed on both axes, with all resistant categories to the right of the origin of the plot. When considering variables pertaining to resistance outcomes, the bottom right quadrant indicates correlation between resistance to each of the quinolone antimicrobials tested, while nalidixic acid resistance also appears to be closely associated to multi-drug resistance. The position of these variables on the x-axis of the plot is mirrored by their contribution to dimension one with multi-drug and nalidixic acid resistance contributing most to the construction of this dimension. As previously reported for isolates of canine and human origin (Chapters Three and Four), resistance to tetracycline and trimethoprim appear to be correlated in dimension one. Examination of dimension two indicates high levels of contribution from variables relating to β -lactam resistance on the positive pole of the axis and fluoroquinolone resistance on the negative pole of the axis suggesting a potential negative correlation between resistance to these antimicrobial classes in this dataset. *E. coli* phylogroup B2 appears to be correlated with isolates displaying susceptible phenotypes and appears closely associated to nalidixic acid susceptibility and non-MDR phenotypes. In contrast, phylogroup C appears to be correlated with chloramphenicol, nalidixic acid and MDR phenotypes on dimension one and amoxycylav and 3GC resistance on dimension two. Contributions of all categories to each dimension are presented in Appendix IV.

Figure 6.3 Map showing the first two dimensions from a multiple correspondence analysis model of *E. coli* isolated from canine and human faecal samples received from 69 premises



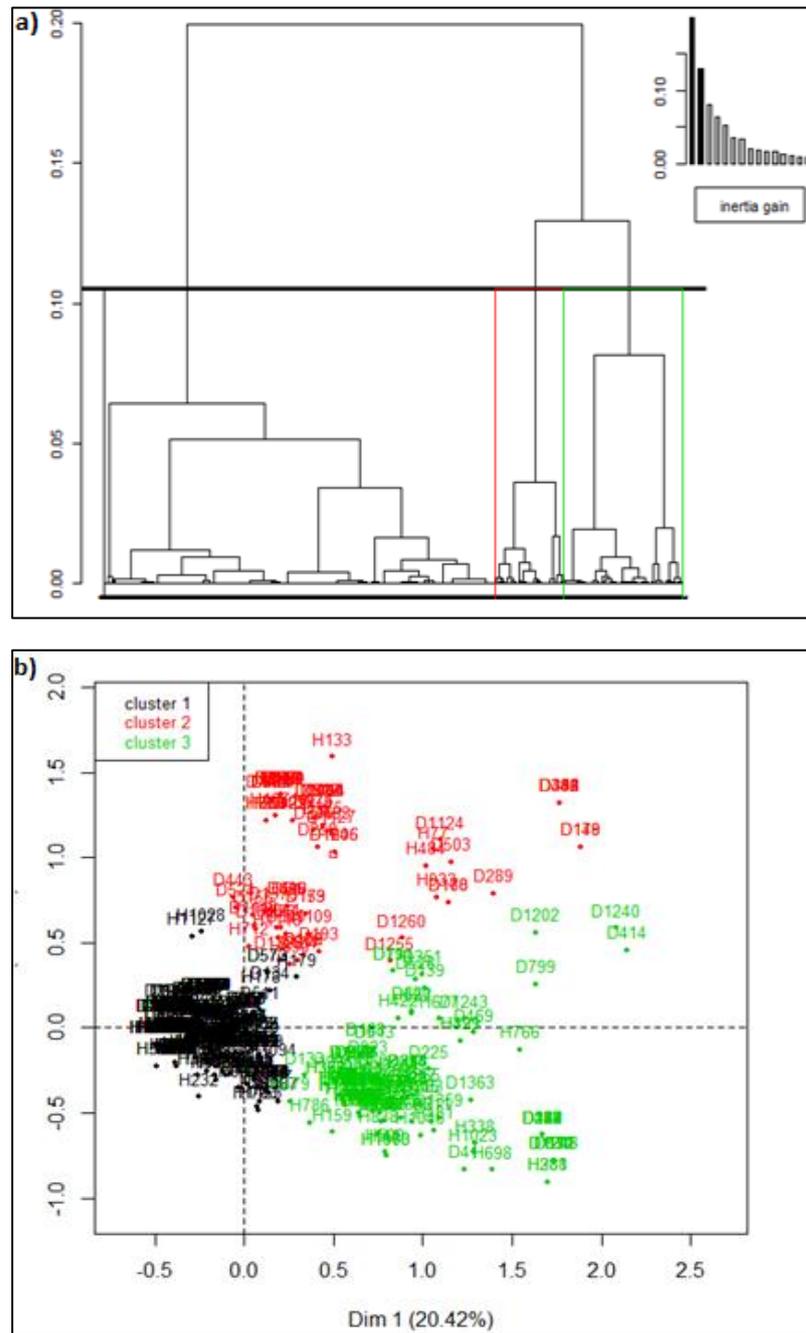
Key: AMP=ampicillin; AC=amoxyclav; CHL=chloramphenicol; CIP=ciprofloxacin; MDR= multi-drug resistance; NA=nalidixic acid; TET=tetracycline; TM=trimethoprim; 3GC=third generation cephalosporin; R=resistant; S=susceptible A-F=*E. coli* phylogroups A-F

Hierarchical cluster analysis performed on the MCA model identified three clusters (Figure 6.4). All clusters contained isolates of both human and canine origin, however Cluster Two was significantly associated with isolates of canine origin (χ^2 $p < 0.0001$). When considering antimicrobial resistance, the prevalence of multi-drug resistance as well as resistance to individual antimicrobial drugs varied between clusters (Table 6.5). Cluster One contained isolates with low levels of resistance to the majority of antimicrobial drugs tested while resistance to β -lactams was high in Clusters Two and Three. Indeed, isolates belonging to Cluster Two in particular displayed increased levels of resistance to amoxyclav and 3GCs. Isolates belonging to Cluster Three displayed high levels of resistance to both trimethoprim

and tetracycline and also demonstrated the highest prevalence of multi-drug resistance and resistance to quinolones.

All clusters contained at least one isolate from *E. coli* phylogroups A-F. However, phylogroup A was overrepresented in Cluster Three (χ^2 $p < 0.0001$), while group B2 was overrepresented in Cluster One (χ^2 $p < 0.0001$) and underrepresented in Cluster Three (χ^2 $p < 0.0001$). Additionally, phylogroup C was overrepresented in Cluster Two (Fisher's exact $p < 0.0001$) and underrepresented in Cluster One (Fisher's Exact $p < 0.0001$), while phylogroup D was overrepresented in Cluster 3 (χ^2 $p = 0.00053$). No significant associations with clusters were identified for phylogroups B1, E and F. Complete tables indicating the prevalence of each phylogroup and species of origin of isolates within each cluster can be located in Appendix IV.

Figure 6.4 Results of hierarchical clustering performed on outcome of multiple correspondence analysis of 849 isolates of *E. coli*



Key: a) Hierarchical tree indicating cluster formation and associated inertia gain; b) Factorial map indicating location of clusters and cluster membership of individuals.

Table 6.5 The prevalence of resistance to seven antimicrobials in 849 isolates of faecal *E. coli* of human and canine origin

Cluster	Dog isolates (n=491)	Human isolates (n=358)	Percentage of resistant isolates								
			Amp	AC	3GC	Tm	Tet	Chl	NA	Cip	MDR
1	348	272	32.3	0.0	0.3	18.2	13.2	3.1	1.6	0.0	0.0
2	69	16	100.0	82.4	81.2	35.3	16.5	21.2	14.1	3.5	21.2
3	74	70	88.9	9.0	2.1	78.5	81.9	38.2	48.6	26.4	91.7

Key: Amp=ampicillin; AC=amoxycylav; Chl=chloramphenicol; Cip=ciprofloxacin; MDR= multi-drug resistance; NA=nalidixic acid; Tet=tetracycline; Tm=trimethoprim; 3GC=third generation cephalosporin

 >25-50% of isolates >50-75% of isolates >75% of isolates

6.3.4 Characterisation of resistance genes

6.3.4.1 Canine isolates

6.3.4.1.1 Canine PCR results

In total, 312 *E. coli* isolates from 168 canine faecal samples were identified as being resistant to at least one antimicrobial tested in this study. All isolates displaying phenotypic resistance to β -lactam antimicrobials (n=257) were evaluated for the presence of *bla*_{TEM} and *bla*_{SHV} genes by PCR, while those resistant to clavulanic acid (n=63) were additionally examined for carriage of *bla*_{AmpC} genes. A total of 187 (72.7% 95% CI 67.3-78.2) isolates, including nine isolates phenotypically resistant to 3GCs, were identified as carrying *bla*_{TEM} genes. Carriage of *bla*_{SHV} genes was identified in eight (3.1% 95% CI 1.0-5.2) isolates, of which six were recovered from four dogs and were resistant to 3GCs. Of those isolates displaying phenotypic resistance to clavulanic acid, 29 (46.0% 95% CI 33.7-58.36) were identified as carrying *bla*_{AmpC} genes. The *bla*_{CIT} type genes were most prevalent (n=23), while both *bla*_{DHA} (n=4) and *bla*_{EBC} (n=2) were identified less frequently. A further 22 isolates were found to be phenotypic AmpC producers using the AmpC disc diffusion test, indicating chromosomally-mediated AmpC (cAmpC) production in these isolates. Twelve additional isolates tested negative on the AmpC disc diffusion test, eleven of which carried *bla*_{TEM} genes suggesting inhibitor-resistant TEM (IRT) production may be responsible for the amoxycylav-resistant phenotype of these isolates. Forty-seven isolates displayed resistance to quinolones and of these, nine (19.2% 95% CI 7.9-30.40) were found to carry *qnr* genes. The *qnrS* gene predominated (n=5), while *qnrA* (n=3) and *qnrB* (n=1) were less prevalent. Interestingly all *qnrS* genes were identified in ESBL-producing isolates, four of which were identified in four samples from dogs boarding at the same premises. A single ciprofloxacin-resistant isolate was identified as carrying an *aac(6')-Ib* gene. This isolate was not resistant to 3GCs, but did display resistance to five antimicrobial classes during susceptibility testing.

Sixty-three isolates from fifty samples displayed resistance to 3GCs. These isolates were initially examined using PCR to assess the carriage of *bla*_{CTX-M}, *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA} and *bla*_{AmpC} genes (Table 6.6). Five isolates, all of which displayed an ESBL phenotype were identified as carrying *bla*_{CTX-M} genes and further characterisation of these genes revealed them as belonging to CTX-M Group 1. The *bla*_{TEM} and *bla*_{SHV} genes were identified in nine and six isolates respectively, while *bla*_{OXA} genes were detected in three isolates. *bla*_{AmpC} genes were identified in 26 isolates, of which 22 belonged to the *bla*_{CIT} group. These results indicate that carriage of *bla*_{AmpC} genes, particularly those belonging to the *bla*_{CIT} group, are the most prevalent mechanism of resistance to 3GCs in the dogs sampled. Eight isolates carried more than one β -lactamase gene including one isolate which carried a *bla*_{TEM} and *bla*_{OXA} gene. For this isolate, it was therefore not possible to determine which *bla* gene conferred resistance to 3GCs from PCR results. Similarly, five isolates carried a *bla*_{CIT} gene concurrently with a *bla*_{TEM} (n=3) or *bla*_{OXA} (n=2) gene and as such further characterisation of the *bla*_{TEM} and *bla*_{OXA} genes was required in order to elucidate if the presence of a *bla*_{AmpC} gene was masking ESBL production. Sixteen isolates, which displayed phenotypic resistance to 3GCs during susceptibility testing, were not found to carry plasmid-mediated *bla* genes by PCR. Fourteen of these isolates displayed an AmpC phenotype using the AmpC disc diffusion test, indicating cAmpC production by these isolates. Further characterisation of 55 isolates displaying resistance to 3GCs was undertaken using WGS in order to identify the specific genes responsible for conferring this resistance. Isolates selected included all those displaying an ESBL phenotype or identified as carrying *bla*_{AmpC} genes on PCR alongside a selection of multi-drug and non-multidrug resistant isolates which displayed an AmpC phenotype in the absence of a *bla*_{AmpC} gene.

Table 6.6 Resistance genes detected by PCR in 63 canine faecal *E. coli* isolates phenotypically resistant to third generation cephalosporins

Isolate	Sample	Phylogroup	Resistance Phenotype	ESBL Phenotype	Gene Type Detected
29 [†]	7	A	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
93 [†]	13	B1	AMP AC	Non-ESBL	
139 [†]	37	A	AMP NA TM	ESBL	<i>bla</i> _{SHV} <i>qnrS</i>
148	38	B1	AMP AC	Non-ESBL	
149	38	C	AMP AC CIP NA TET	Non-ESBL	
151 [†]	38	A	AMP	ESBL	<i>bla</i> _{SHV}
159 [†]	39	F	AMP NA	ESBL	<i>bla</i> _{SHV} <i>qnrS</i>
173 [†]	41	F	AMP NA	ESBL	<i>bla</i> _{SHV} <i>qnrS</i>
178 [†]	42	C	AMP AC CIP NA TET	ESBL	
191 [†]	43	B1	AMP TET TM	ESBL	<i>bla</i> _{CTX-M Group 1} <i>bla</i> _{TEM}
193 [†]	38	A	AMP NA	ESBL	<i>bla</i> _{SHV} <i>qnrS</i>
194 [†]	39	A	AMP TET	ESBL	<i>bla</i> _{SHV}
216 [†]	47	B1	AMP AC	Non-ESBL	
258	56	B1	AMP AC	Non-ESBL	
289 [†]	63	D	AMP AC CHL TET TM	Non-ESBL	<i>bla</i> _{TEM} <i>bla</i> _{OXA}
306 [†]	64	B1	AMP AC	Non-ESBL	
319 [†]	66	B1	AMP AC TM	Non-ESBL	<i>bla</i> _{DHA}
330 [†]	68	B1	AMP AC	Non-ESBL	
341 [†]	70	C	AMP AC CHL NA TM	Non-ESBL	
345	71	B1	AMP AC	Non-ESBL	
346 [†]	71	B2	AMP AC TET	Non-ESBL	<i>bla</i> _{CIT}
348	71	C	AMP AC CHL NA TM	Non-ESBL	
355 [†]	72	C	AMP AC CHL NA TM	Non-ESBL	
361 [†]	73	B2	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
362 [†]	73	C	AMP AC CHL NA TM	Non-ESBL	
369 [†]	74	B1	AMP AC	Non-ESBL	
375 [†]	75	B1	AMP AC TET	Non-ESBL	<i>bla</i> _{CIT}
396 [†]	79	C	AMP AC CHL NA TM	Non-ESBL	
405 [†]	80	A	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
414 [†]	80	D	AMP AC CIP CHL NA TET TM	Non-ESBL	<i>bla</i> _{CIT}
424 [†]	82	B1	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
432 [†]	83	C	AMP AC CHL NA TM	Non-ESBL	
471 [†]	91	B2	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
483 [†]	93	D	AMP AC TM	Non-ESBL	<i>bla</i> _{CIT}
503 [†]	97	D	AMP AC CHL TM	Non-ESBL	<i>bla</i> _{OXA} <i>bla</i> _{CIT}
524 [†]	105	B2	AMP	Non-ESBL	
528 [†]	106	B1	AMP NA TET	ESBL	<i>bla</i> _{CTX-M Group 1} <i>qnrS</i>
540 [†]	109	B1	AMP TET	ESBL	<i>bla</i> _{CTX-M Group 1} <i>bla</i> _{TEM}
552 [†]	114	B2	AMP AC	Non-ESBL	
558 [†]	100	B2	AMP AC	Non-ESBL	
570 [†]	109	B1	AMP AC	Non-ESBL	
699 [†]	151	B2	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
714 [†]	145	B1	AMP TET TM	ESBL	<i>bla</i> _{CTX-M Group 1}

Isolate	Sample	Phylogroup	Resistance Phenotype	ESBL Phenotype	Gene Type Detected
754 [†]	163	B2	AMP AC CHL	Non-ESBL	<i>bla</i> _{CIT}
783 [†]	168	B2	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
977 [†]	213	B1	AMP AC TM	Non-ESBL	<i>bla</i> _{TEM}
1040 [†]	233	B1	AMP AC TM	Non-ESBL	<i>bla</i> _{TEM}
1045 [†]	234	A	AMP AC TET	Non-ESBL	<i>bla</i> _{CIT}
1109 ^{†*}	253	D	AMP CHL	Non-ESBL	<i>bla</i> _{CIT}
1110 ^{†*}	253	D	AMP	Non-ESBL	<i>bla</i> _{CIT}
1124	257	B1	AMP AC CHL TM	Non-ESBL	<i>bla</i> _{DHA}
1127 [†]	257	A	AMP AC TM	Non-ESBL	<i>bla</i> _{DHA}
1191	257	B1	AMP AC TM	Non-ESBL	<i>bla</i> _{DHA}
1201 [†]	259	B1	AMP AC	Non-ESBL	<i>bla</i> _{OXA} <i>bla</i> _{CIT}
1202 [†]	259	A	AMP AC CIP NA TET	Non-ESBL	<i>bla</i> _{CIT}
1206 [†]	260	A	AMP AC TET	Non-ESBL	<i>bla</i> _{TEM} <i>bla</i> _{CIT}
1224 [†]	265	A	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
1240 [†]	269	B1	AMP AC CIP CHL NA TET TM	Non-ESBL	<i>bla</i> _{TEM} <i>bla</i> _{CIT}
1259	267	B1	AMP AC	Non-ESBL	
1324 [†]	290	B1	AMP AC	Non-ESBL	
1328 [†]	284	E	AMP TET	ESBL	<i>bla</i> _{CTX-M Group 1}
1330 [†]	285	B1	AMP AC	Non-ESBL	<i>bla</i> _{TEM}
1404 [†]	298	B2	AMP AC	Non-ESBL	<i>bla</i> _{TEM} <i>bla</i> _{CIT}

Key: AM=ampicillin; AC=amoxyclov; CHL=chloramphenicol; CIP=ciprofloxacin; NA=nalidixic acid; TET=tetracycline; TM=trimethoprim; [†]isolates selected for WGS; *isolates harbouring *bla*_{CIT} classified as non-resistant to amoxyclov as zone diameter 15mm.

6.3.4.1.2 Canine WGS results

Of the 55 selected for sequencing (Table 6.6), 54 isolates from 48 faecal samples were successfully sequenced. Five isolates were identified as carrying a *bla*_{CTX-M} gene, all of which belonged to CTX-M group 1. The most prevalent *bla*_{CTX-M} gene was *bla*_{CTX-M-1} (n=3), with two isolates carrying *bla*_{CTX-M-15} and *bla*_{CTX-M-55} respectively. A *bla*_{SHV-12} gene was carried by four isolates, with an additional isolate carrying a variant showing 99.9% homology with this gene. Two amoxyclov-resistant isolates were identified as carrying potential inhibitor-resistant *bla*_{TEM-33} and *bla*_{TEM-78-like} genes respectively. Twenty-one isolates were identified as carrying *bla*_{CMY-2}, with two isolates identified as carrying *bla*_{DHA-1}, while a further amoxyclov-resistant isolate was identified as carrying a *bla*_{CMY-2-like} gene. No isolates carried extended-spectrum variants of the *bla*_{OXA} genes. In total, 38 isolates from 35 faecal samples were confirmed to carry plasmid-mediated β-lactamase genes although four of these isolates carried *bla* genes that did not confer 3GC resistance. Of the 17 isolates which did not carry plasmid-mediated β-lactamase genes, fifteen displayed an AmpC phenotype during AmpC phenotypic testing indicating cAmpC production by these isolates.

Examination of further resistance genes carried by the 38 isolates harbouring plasmid-mediated *bla* genes identified thirty-eight class-specific resistance genes including those conferring resistance to β -lactams, aminoglycosides, macrolides, phenicols, quinolones, tetracyclines, trimethoprim and sulphonamides (Table 6.7). The number of resistance genes carried by isolates ranged from one to twelve and aminoglycoside resistance genes were identified most frequently, with twenty isolates carrying these genes. Phenicol resistance genes were least prevalent, identified in only four isolates.

In silico plasmid replicon typing of the 38 isolates harbouring plasmid-mediated β -lactamase genes revealed plasmids belonging to seven incompatibility groups; IncF (n=20), IncI (n=33), IncQ (n=1), IncR (n=3), IncX, (n=8) and IncY (n=2), with all isolates carrying at least one replicon type. Five isolates additionally carried Col plasmids encoding bacteriocins. Some isolates (n=1) harboured up to four plasmid replicon types, however carriage of one (n=14) or two (n=20) Inc replicon types was most commonly identified. IncI1 plasmids were detected most frequently, with five known and two novel STs identified. IncI1 ST23 was identified alone or in combination in ten isolates, all of which carried *bla*_{CMY-2}, while ST43 was identified in only one isolate, which carried the inhibitor-resistant *bla*_{TEM-33} gene. IncX3 replicons were identified in five isolates, all of which carried *bla*_{SHV-12} and *qnrS1* genes.

Isolates sharing the same plasmid replicon and resistance genotype were identified in two premises (Table 6.7). Within Premises 9, two isolates (D346 and D361) identified as carrying *bla*_{CMY-2} associated with replicon types IncI1 ST23 and IncX1, while a further two isolates (D369 and D375) were identified as carrying *bla*_{CMY-2} and *tetB* associated with IncI1 ST23 and IncF RST F4*:A-:B- plasmid types. An additional single isolate within this premises carried *bla*_{CMY-2} associated with an IncI1 ST23 plasmid alone. This suggests that an IncI1 ST23 plasmid circulating within this premises may be responsible for transmission of *bla*_{CMY-2} and the addition of further plasmid types, such as IncF RST F4-like:A-:B- are responsible for the transfer of further resistance genes such as *tetB* between isolates. Within Premises 7, a boarding establishment, four isolates from three samples (D151, D159, D173 and D193), shared the same resistance genotype, all carrying *bla*_{SHV-12}, *aadA22*, *InuF* and *qnrS1* genes. Isolates D151, D159, and D173 harboured IncX3 and IncF RST F-:A-:B56-like replicon types, while D193 (isolated from the same sample as D151) harboured IncX3 alone suggesting an IncX3 replicon may be responsible for the transmission of *bla*_{SHV-12} associated multi-drug resistance in this premises. The isolation of bacteria with the same resistance genotype and plasmid replicon types from different animals on the same premises suggests the potential for transmission of 3GC-resistant bacteria between animals in the same environment.

6.3.4.2 Human isolates

6.3.4.2.1 Human PCR results

A total of 219 *E. coli* isolates originating from 154 human faecal samples displayed resistance to at least one antimicrobial drug during susceptibility testing. Of the 166 isolates displaying resistance to β -lactam antimicrobials, 129 (77.7% 95% CI 71.4-84.1) isolates carried *bla*_{TEM}

genes, including five displaying resistance to 3GCs. The number of isolates carrying *bla*_{SHV} genes was much lower, with only six (3.6% 95% CI 0.8-6.5) isolates possessing SHV-type β -lactamases. Of those isolates carrying a *bla*_{SHV} gene, three isolates were resistant to 3GCs. In total, 16 isolates were phenotypically resistant to amoxycylav, of which 43.8% (95% CI 19.4-68.1) were identified as carrying a plasmid-mediated *bla*_{AmpC} gene. Both *bla*_{CIT} and *bla*_{DHA}-type genes were carried by three isolates while a *bla*_{ACC} type gene was identified in a single isolate. An additional five isolates displayed an AmpC phenotype using AmpC phenotypic testing, suggesting cAmpC production. Forty-eight (21.9% 95% CI 16.4-27.4) isolates were resistant to quinolone antimicrobials and of these five isolates carried *qnr* genes. Three isolates possessed a *qnrA* gene, while two isolates carried a *qnrS* gene; in addition a single non-ESBL MDR isolate was identified as carrying an *aac(6')-Ib* gene variant. No isolates were identified as carrying *qnrB* genes.

Twenty isolates originating from 18 faecal samples, were phenotypically resistant to 3GCs and of these isolates, nine displayed an ESBL phenotype. Six isolates, all of which displayed an ESBL phenotype were identified as carrying *bla*_{CTX-M Group 1} genes. Five isolates were identified as carrying *bla*_{TEM} genes, while three isolates were found to possess *bla*_{SHV} genes. Five 3GC-resistant isolates were identified as carrying *bla*_{AmpC} genes comprising three *bla*_{CIT} and two *bla*_{DHA} genes. In addition five isolates confirmed as AmpC producers during phenotypic testing were found not to carry *bla*_{AmpC} genes, suggesting cAmpC production. A single amoxycylav-resistant isolate was identified as carrying a *bla*_{OXA} gene. Five isolates were identified as carrying multiple β -lactamase genes (Table 6.8). Of these isolates, two (H421 and H422) carried a *bla*_{TEM} and *bla*_{SHV} gene and as such it is not possible to elucidate which gene is responsible for conferring the ESBL phenotype from PCR results alone. Similarly, a single isolate carried both a *bla*_{AmpC} and a *bla*_{TEM} gene and therefore further gene characterisation is required in order to elucidate if the presence of an ESBL gene has been masked by AmpC production in this isolate. In order to identify the specific genes responsible for conferring both ESBL and AmpC-mediated resistance, all isolates displaying resistance to 3GCs were further characterised using WGS.

Table 6.8 Resistance genes detected by PCR in 20 human faecal *E. coli* isolates phenotypically resistant to third generation cephalosporins

Isolate	Sample	Resistance Phenotype	ESBL Phenotype	Gene Type Detected
77	17	AMP AC CHL TM	Non-ESBL	<i>bla</i> _{OXA}
93	20	AMP AC	Non-ESBL	
133	28	AMP AC	Non-ESBL	
399	88	AMP NA TET TM	ESBL	<i>bla</i> _{CTX-M Group 1} <i>bla</i> _{TEM}
421	95	AMP NA TET TM	ESBL	<i>bla</i> _{TEM} <i>bla</i> _{SHV} <i>qnrS</i>
422	95	AMP TET TM	ESBL	<i>bla</i> _{TEM} <i>bla</i> _{SHV}
484	112	AMP AC CHL TM	Non-ESBL	<i>bla</i> _{CIT} <i>bla</i> _{TEM}
669	147	AMP AC	Non-ESBL	<i>bla</i> _{CIT}
677	148	AMP NA TET TM	ESBL	<i>bla</i> _{CTX-M Group 1} <i>bla</i> _{TEM}
690	149	AMP TM	ESBL	<i>bla</i> _{CTX-M Group 1}
697	149	AMP CHL TET	ESBL	<i>bla</i> _{SHV}
712	152	AMP	ESBL	<i>bla</i> _{CTX-M Group 1}
766	163	AMP CIP NA TET TM	ESBL	<i>bla</i> _{CTX-M Group 1}
774	165	AMP AC	Non-ESBL	
933	192	AMP AC NA TET	Non-ESBL	<i>bla</i> _{CIT}
953	194	AMP AC	Non-ESBL	
971	200	AMP AC TM	Non-ESBL	<i>bla</i> _{DHA}
976	201	AMP AC TM	Non-ESBL	<i>bla</i> _{DHA}
1071	225	AMP AC	Non-ESBL	
1118	229	AMP TM	ESBL	<i>bla</i> _{CTX-M Group 1}

Key: AMP=ampicillin; AC=amoxyclov; CHL=chloramphenicol; NA=nalidixic acid; TET=tetracycline; TM=trimethoprim

6.3.4.2.2 Human WGS results

Of the 20 isolates resistant to 3GCs, all were sequenced successfully. Fifteen isolates from 13 human faecal samples were found to carry plasmid-mediated β -lactamases (Table 6.9). Six isolates were identified as carrying a *bla*_{CTX-M Group 1} gene, mirroring the findings of the CTX-M PCR assay. These genes were identified as *bla*_{CTX-M-15} (n=4) and *bla*_{CTX-M-1} (n=2). A *bla*_{SHV-12} gene was carried by three isolates, while a single isolate carried *bla*_{TEM-214}. Five isolates were confirmed as carrying *bla*_{AmpC} genes; two genes were identified as *bla*_{CMY-2}, while *bla*_{DHA-1}, *bla*_{DHA-1-like} and *bla*_{CMY-2-like} genes were each carried by a single isolate. Additionally, a single isolate was found to carry *bla*_{OXA-1} alone. No isolates carried genes conferring ESBL and AmpC-mediated resistance in combination mirroring the findings in canine isolates. Five additional isolates, all of which displayed an AmpC phenotype during double disc diffusion testing, did not carry any known plasmid-mediated *bla* genes.

The fifteen isolates found to harbour plasmid-mediated β -lactamase genes were found to carry up to ten additional resistance genes including those conferring resistance to aminoglycosides, macrolides, quinolones, tetracyclines, trimethoprim and sulphonamides

(Table 6.9). In contrast to canine isolates, a smaller number of class-specific resistance genes were identified in the human isolates (29 versus 39). However, a smaller number of isolates were examined and therefore this finding should be viewed with caution. Isolates most commonly carried genes conferring resistance to sulphonamides (n=12) and aminoglycosides (n=10), however phenicol resistance genes were only identified in a two isolates.

Plasmid typing of human isolates harbouring plasmid-mediated β -lactamase genes revealed an increased level of diversity when compared to canine isolates with nine plasmid incompatibility groups represented in the fifteen isolates (Table 6.9). The number of plasmids carried by isolates ranged from one (n=4) to four (n=2). Carriage of two replicon types was most commonly identified (n=6), with three isolates additionally carrying Col plasmids (Table 6.9). No plasmid could be identified from genomic data for a single isolate (H77). In contrast to isolates of canine origin, IncF plasmids were identified most frequently (n=13), with eleven RSTs identified. Two isolates (H971 and H976) originating from two individuals at the same premises were identified as carrying the same plasmid replicon type IncF RST F2:A-:B- and were found to harbour *bla*_{DHA-1}, *qnrB4*, *sul1* and *mphA* genes. In addition, isolate H971 carried a ColpVC plasmid and harboured a *dfrA17* gene, as did Isolate H1118. Two human and three canine isolates from five different premises harboured *bla*_{CTX-M-1} genes. Four of these isolates were confirmed to carry IncI1 ST3 replicons while the final isolate (H399) carried an IncI1 replicon, which could not be typed due to the absence of the *ardA* locus, all other loci alleles did however match ST3. Isolates carrying *bla*_{CTX-M-15} did not carry the same plasmid types.

Three isolates (H421, H422 and H697) originating from two individuals at different premises were identified as carrying an IncI1 ST26 replicon associated with carriage of *bla*_{SHV-12}, *aadA1* and *tetA* genes. Isolates H421 and H422 recovered from a single individual were identified as carrying the same plasmid replicon types and resistance genotype. These isolates did not however demonstrate the same resistance phenotype during antimicrobial susceptibility testing, with isolate H421 demonstrating resistance to nalidixic acid, while H422 remained susceptible.

6.3.4.3 Agreement between PCR and WGS data

The agreement between detection of carriage of plasmid-mediated *bla* genes by 3GC-resistant isolates using PCR and WGS methods was compared using the kappa (κ) statistic (Table 6.10). For all PCR assays there was perfect or almost perfect agreement between the two tests used indicated by a κ statistic of over 0.9 (Landis and Koch, 1977), indeed in the case of *bla*_{CTX-M}, *bla*_{OXA} and *bla*_{DHA} genes there was perfect agreement between the two tests. In the absence of perfect agreement, WGS identified an additional isolate carrying *bla*_{TEM}; however, PCR detected carriage of an additional *bla*_{SHV} gene by a single isolate. When comparing WGS and PCR for the carriage of *bla*_{CIT} genes, there was disagreement in two isolates, with one isolate PCR negative and WGS positive while the converse was true for the second isolate.

Table 6.10 Cross tabulation of the results of 75 third generation cephalosporin-resistant *E. coli* isolates tested for carriage of *bla* genes using both PCR and WGS methods

Gene		WGS Results			Kappa	p	
		Negative	Positive	Total			
<i>bla</i> _{TEM}	PCR Results	Negative	60	1	61	0.96	<0.0001
		Positive	0	14	14		
		Total	60	15	75		
<i>bla</i> _{SHV}	PCR Results	Negative	66	0	66	0.93	<0.0001
		Positive	1	8	9		
		Total	67	8	75		
<i>bla</i> _{OXA}	PCR Results	Negative	71	0	71	1	0.0018
		Positive	0	4	4		
		Total	71	4	75		
<i>bla</i> _{CTX-M}	PCR Results	Negative	64	0	64	1	<0.0001
		Positive	0	11	11		
		Total	64	11	75		
<i>bla</i> _{CIT}	PCR Results	Negative	49	1	50	0.94	<0.0001
		Positive	1	24	25		
		Total	50	25	75		
<i>bla</i> _{DHA}	PCR Results	Negative	71	0	71	1	0.0019
		Positive	0	4	4		
		Total	71	4	75		

6.3.4.4 Conjugation experiments

Thirty-four isolates, which were resistant to 3GCs and displayed an ESBL or MDR phenotype, were selected for examination of transfer of antimicrobial resistance genes to a rifampin-resistant recipient *E. coli* strain (*E. coli* 26R793). These isolates included twenty-nine isolates for which a plasmid-mediated β -lactamase gene was detected from whole genome sequences and five isolates that were found not to carry a plasmid-mediated β -lactamase gene during examination of whole genome sequences. All isolates bar one (H77) were found to carry plasmids during *in silico* examination of genome sequences. Of the isolates tested, 13 transferred resistance to the recipient strain successfully including transfer of *bla*_{CTX-M-1} (n=5), *bla*_{CTX-M-15} (n=2) *bla*_{CIT} (n=2) and *bla*_{TEM} (n=4) genes (Table 6.11). Interestingly, no isolates carrying the *bla*_{SHV} genes transferred this gene to the recipient strain suggesting reduced transmissibility of the plasmids harbouring the gene under lab conditions. Of those isolates that successfully conjugated, only four transconjugants displayed the same resistance phenotype and harboured the same *bla* genes as the donor strain. A single isolate (D714) carried only one plasmid and as such, carriage of *bla*_{CTX-M-1} by an IncI1 ST3 plasmid has been confirmed, however three additional donor isolates carried multiple plasmids and therefore it is not known if a single plasmid carrying all resistance genes was transferred or conjugation of multiple plasmids occurred. Interestingly, one isolate (D191) successfully transferred *bla*_{TEM}, but not the *bla*_{CTX-M-15} gene.

The MDR cAmpC producing isolates, did not transfer any resistance to the recipient strain indicating that while a resistance plasmid was present in these isolates it was not readily transferred.

Table 6.11 Details of conjugation results for thirty-four isolates of third generation cephalosporin-resistant *E. coli*

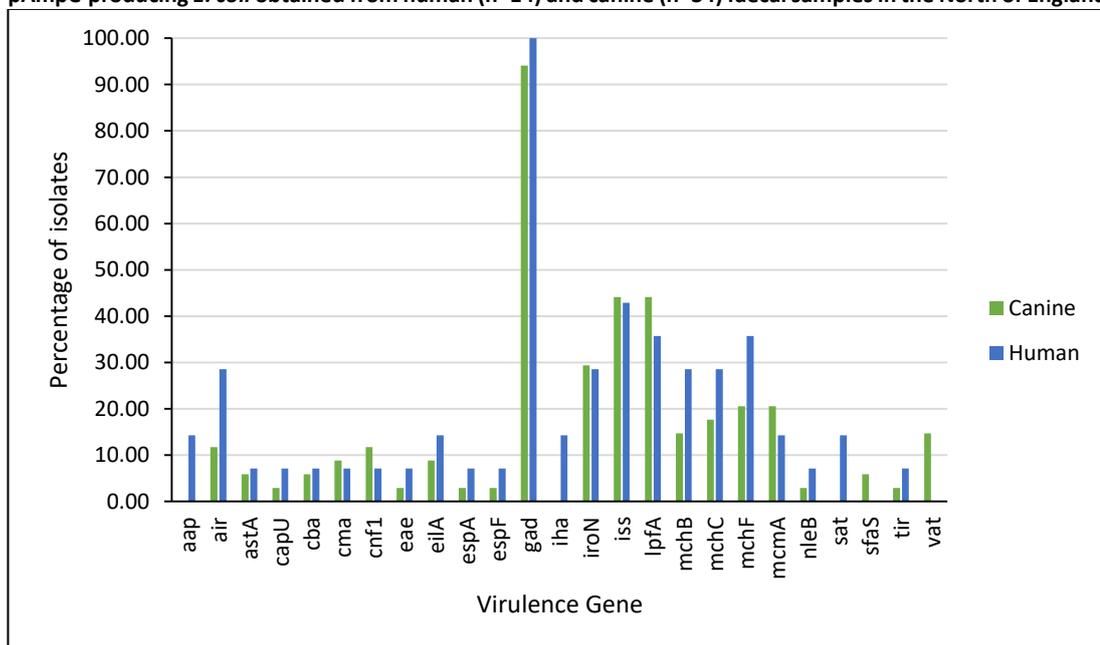
Isolate	Donor					Transconjugant		
	Resistance Phenotype	Plasmid Replicon Type				<i>bla</i> gene	Resistance Phenotype	<i>bla</i> gene type
		Inc F RST	Incl1 ST	IncX	Other			
H399	AMP NA TET TM	F52*:A-:B1	Unknown		IncQ1	<i>bla</i> _{CTX-M-1} <i>bla</i> _{TEM-1}	AMP TM	<i>bla</i> _{CTX-M}
H690	AMP TM	F40*:A-:B-	ST3		P0111	<i>bla</i> _{CTX-M-1}	AMP TM	<i>bla</i> _{CTX-M}
D540	AMP TET	F24:A-:B1	ST3			<i>bla</i> _{CTX-M-1} , <i>bla</i> _{TEM-1-like}	AMP TET	<i>bla</i> _{CTX-M}
D714	AMP TET TM		ST3			<i>bla</i> _{CTX-M-1}	AMP TET TM	<i>bla</i> _{CTX-M}
D1328	AMP TET	F24:A-:B1	ST3			<i>bla</i> _{CTX-M-1}	AMP TET	<i>bla</i> _{CTX-M}
H677	AMP NA TET TM	F29:A-:B10			IncB/O/K/Z, Col156	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1}	AMP TM	<i>bla</i> _{CTX-M}
H712	AMP	F84*:A-:B-		IncX4		<i>bla</i> _{CTX-M-15}		
H766	AMP CIP NA TET TM	F1:A1:B1			IncA/C ST7	<i>bla</i> _{CTX-M-15}		
H1118	AMP TM	F2:A-:B5			ColpVC	<i>bla</i> _{CTX-M-15}	AMP TM	<i>bla</i> _{CTX-M}
D191	AMP TET TM	F18:A-:B1			Col156	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{TEM-1-like}	AMP TET TM	<i>bla</i> _{TEM}
D528	AMP NA TET	F87*:A4:B1	Unknown			<i>bla</i> _{CTX-M-55}		
H421	AMP NA TET TM	C1*:A-:B12*	ST26		IncN ST1, IncY	<i>bla</i> _{SHV-12} , <i>bla</i> _{TEM-1}	AMP TET TM	<i>bla</i> _{TEM}
H422	AMP TET TM	C1*:A-:B12*	ST26		IncN ST1, IncY	<i>bla</i> _{SHV-12} , <i>bla</i> _{TEM-1}	AMP TET TM	<i>bla</i> _{TEM}
H697	AMP CHL TET	F-:A-: B25	ST26			<i>bla</i> _{SHV-12}		
D139	AMP NA TM			IncX3		<i>bla</i> _{SHV-12-like}		
D151	AMP	F-:A-:B56*		IncX3		<i>bla</i> _{SHV-12}		
D159	AMP NA	IncF RST F-:A-:B56*		IncX3		<i>bla</i> _{SHV-12}		
D173	AMP NA	IncF RST F-:A-:B56*		IncX3		<i>bla</i> _{SHV-12}		
D193	AMP NA			IncX3		<i>bla</i> _{SHV-12}		
D194	AMP TET			IncX3	IncR, IncY	<i>bla</i> _{SHV} (PCR only)		
D503	AMP AC CHL TM	F2-:A-:B-	ST2			<i>bla</i> _{CMY-2} , <i>bla</i> _{OXA-1}	AMP AC	<i>bla</i> _{CIT}
D1202	AMP AC CIP NA TET	F-:A-:B53	ST2			<i>bla</i> _{CMY-2}	AMP AC	<i>bla</i> _{CIT}
H484	AMP AC CHL TM	IncF RST F4*:A-:B-		IncX1		<i>bla</i> _{CMY-2-like} <i>bla</i> _{TEM-1}		
H933	AMP AC NA TET	F18:A-B20			IncB/O/K/Z	<i>bla</i> _{CMY-2}		
D414	AMP AC CIP CHL NA TET TM		ST23		IncI2, IncQ1, IncY	<i>bla</i> _{CMY-2}		
D1240	AMP AC CIP CHL NA TET TM		Unknown	IncX4	IncR	<i>bla</i> _{CMY-2} , <i>bla</i> _{TEM-78-like}	AMP CHL TET TM	<i>bla</i> _{TEM}
H77	AMP AC CHL TM	No plasmid replicons found				<i>bla</i> _{OXA-1}		
D289	AMP AC CHL TET TM	F67:A-: B3	Unknown		IncR, Col156	<i>bla</i> _{TEM-1} , <i>bla</i> _{OXA-1}		
D178	AMP AC CIP NA TET	F77:A:B16			IncQ1			
D341	AMP AC CHL NA TM	F67:A6:B38						
D355	AMP AC CHL NA TM	F67:A6:B38						
D362	AMP AC CHL NA TM	F67:A6:B38						
D396	AMP AC CHL NA TM	F67:A6:B38						
D432	AMP AC CHL NA TM	F67:A6:B38						

Key: AM=ampicillin; AC=amoxyclov; CHL=chloramphenicol; CIP= ciprofloxacin; NA=nalidixic acid; TET=tetracycline; TM=trimethoprim; *signifies allelic match of <100%

6.3.5 Virulence genes

A total of 42 different virulence genes were identified in the 48 ESBL and pAmpC-producing *E. coli*. Increased diversity in genes carried by human isolates was identified with 36 different virulence genes carried by 14 isolates, while 26 different virulence genes were carried by the 34 canine isolates. The most commonly identified virulence genes were *gad* (n=46), *iss* (n=21), *lfpA* (n=20), *iroN* (n=14) and *mchF* (n=12). With the exception of *mchF*, which was more prevalent in human isolates, carriage of these genes was equivocal in isolates of canine and human origin (Figure 6.5). Seventeen genes were identified in only a single isolate.

Figure 6.5 Virulence genes detected by *in silico* examination of whole genome sequences of 48 ESBL and pAmpC-producing *E. coli* obtained from human (n=14) and canine (n=34) faecal samples in the North of England



All isolates, with the exception of D139, were found to carry at least one virulence gene and carriage of one (n=10) or two (n=10) genes was most frequently identified. However two isolates H1118 and H399 were found to carry 15 and 17 virulence genes, respectively. Furthermore, the MDR isolate H399, harbouring *bla*_{CTX-M} was found to harbour *stx2*, *stx2A* variant d and *stx2B* variant d genes. Virulence genes were detected in isolates belonging to multiple phylogenetic groups, however some genes were more prevalent within some phylogroups (Table 6.12). Of particular note were *iss*, *iroN*, *mcmA* and *mch* genes, which were most prevalent in group B2 isolates, while *cnf1*, *sfaS* and *vat* genes were solely identified isolates belonging to this phylogroup. In contrast, *air* and *eilA* genes were most frequently carried by isolates belonging to group D and *lfpA* was most prevalent in B1 isolates. Statistical analysis of these relationships was not undertaken due to the small

number of isolates investigated. Full details of the prevalence of each of the virulence genes detected within each phylogroup can be located in Appendix IV.

Table 6.12 Virulence genes identified in more than one ESBL or pAmpC-producing isolate of *E. coli* obtained from kennelled dogs and in-contact humans in the North of England highlighting the presence of genes within phylogroups

Gene	Function	Phylogroup (%)					
		A (n=14)	B1 (n=12)	B2 (n=11)	D (n=6)	E (n=1)	F (n=4)
<i>air</i>	Enteroaggregative immunoglobulin repeat protein	0.0	0.0	9.1	83.3	0.0	50.0
<i>cnf1</i>	Cytotoxic necrotizing factor	0.0	0.0	45.5	0.0	0.0	0.0
<i>eilA</i>	<i>HilA</i> homolog (adherence)	0.0	0.0	0.0	66.7	0.0	25.0
<i>iroN</i>	Siderophore receptor (iron acquisition)	7.1	25.0	81.8	0.0	100.0	0.0
<i>iss</i>	Increased serum survival	28.6	50.0	90.9	0.0	100.0	0.0
<i>lpfA</i>	Long polar fimbriae colonisation	7.1	91.7	27.3	33.3	0.0	75.0
<i>mchB</i>	Microcin H47, part of Colicin H	7.1	8.3	63.6	0.0	0.0	0.0
<i>mchC</i>	MchC protein	7.1	8.3	72.7	0.0	0.0	0.0
<i>mchF</i>	ABC transporter protein MchF	7.1	8.3	90.9	0.0	0.0	0.0
<i>mcmA</i>	Microcin M, part of Colicin H	0.0	8.3	72.7	0.0	0.0	0.0
<i>sfaS</i>	S-fimbriae minor subunit	0.0	0.0	18.2	0.0	0.0	0.0
<i>vat</i>	Vacuolating autotransporter toxin	0.0	0.0	45.5	0.0	0.0	0.0

6.3.6 Serotype analysis

In silico serotyping of the 48 ESBL and pAmpC-producing *E. coli* allowed designation to O serogroups in 23 isolates, while 46 isolates were assigned an H serogroup. In total, 16 O antigens and 20 different H antigens were characterised (Table 6.13). The most frequently identified O antigens were O2 (n=4) and O8 (n=3). The most prevalent H antigens were H31 (n=6), H4 (n=5) and H18 (n=5). A single isolate (H677) harbouring *bla*_{CTX-M-15}, was found to belong to serotype O16:H5, associated with *E. coli* ST131. Of additional note, was a single EHEC O80:H2 isolate (H399) harbouring shiga toxin genes alongside genes located on the locus of enterocyte effacement (LEE) recovered from a human participant in this study.

Table 6.13 O and H serogroups and virulence genes carried by 48 isolates of ESBL and pAmpC-producing *E. coli*

Isolate	Phylogroup	O group	H group	Virulence Genes
H399	A	80	2	<i>eae espA espB espF gad iroN iss mchB mchC mchF nleA nleB nleC stx2 stx2A(d) stx2B(d) tir</i>
H421	A	-	25	<i>gad</i>
H422	A	-	25	<i>gad</i>
H484	B2	-	31	<i>cba cma gad iroN iss mchB mchC mchF mcmA</i>
H669	B2	-	31	<i>cnf1 gad iroN iss mchB mchC mchF mcmA</i>
H677	B2	16	5	<i>gad iha sat senB</i>
H690	A	154	4	<i>gad</i>
H697	A	133	4	<i>etpD gad iss</i>
H712	D	-	18	<i>aap air astA eilA gad lpfA</i>
H766	B1	-	21	<i>gad</i>
H933	B2	2	1	<i>air gad iroN iss lpfA mchF</i>
H971	F	-	6	<i>air gad lpfA</i>
H976	F	11	6	<i>air eilA gad lpfA</i>
H1118	B1	131	4	<i>aaic aap aatA aagR capU gad iha iss lpfA mchB mchC mchF ORF3 pic sat</i>
D29	A	31	38	<i>gad</i>
D139	A	9	4	
D151	A	-	48	<i>gad lpfA</i>
D159	F	-	26	<i>gad</i>
D173	F	-	26	<i>gad lpfA</i>
D191	B1	8	30	<i>capU gad iroN iss lpfA</i>
D193	A	-	38	<i>gad</i>
D319	B1	85	4	<i>cif eae espA espF espJ gad lpfA nleB sepA tccp tir</i>
D346	B2	-	31	<i>astA cnf1 gad iroN iss mchB mchC mchF mcmA sfaS vat</i>
D361	B2	75	31	<i>astA cnf1 gad iroN iss mchB mchC mchF mcmA sfaS vat</i>
D369	B1	-	7	<i>gad lpfA</i>
D375	B1	-	7	<i>gad lpfA</i>
D405	A	80	19	<i>gad</i>
D414	D	167	-	<i>eilA gad lpfA</i>
D424	B1	-	16	<i>gad lpfA</i>
D471	B2	-	28	<i>gad iroN iss mchB mchC mchF mcmA vat</i>
D483	D	-	18	<i>air gad</i>
D503	D	-	18	<i>air gad</i>
D528	B1	8	10	<i>gad iroN iss lpfA</i>
D540	B1	-	16	<i>cma gad iroN iss lpfA</i>
D699	B2	2	31	<i>cnf1 iroN iss mchB mchC mchF mcmA vat</i>
D714	B1	-	10	<i>gad iss lpfA mcmA</i>
D754	B2	-	-	<i>gad iroN iss lpfA mchC mchF mcmA</i>
D783	B2	2	31	<i>cnf1 gad iroN iss mchB mchC mchF mcmA vat</i>
D1109	D	2	18	<i>air cba cma eilA gad</i>
D1110	D	-	18	<i>air cba eilA gad</i>
D1127	A	26	32	<i>gad iss</i>
D1201	B1	-	21	<i>gad iss lpfA</i>
D1202	A	8	9	<i>gad iss</i>
D1206	A	141ab/ac	5	<i>gad</i>
D1224	A	-	19	<i>gad</i>
D1240	B1	-	28	<i>gad lpfA</i>
D1328	E	-	25	<i>cma gad iroN iss</i>
D1404	B2	93	28	<i>gad iss lpfA mchF</i>

6.3.7 Multi-locus sequence typing

Of the 132 AMR and non-AMR *E. coli* isolates, which underwent WGS, complete MLST data were available for 121 isolates. In addition, the incomplete allelic profiles of four isolates allowed nominal allocation to a ST. The ST of seven isolates could not be identified to this level due to incomplete sequence coverage of some loci. In total, 55 different MLST types were identified in isolates from both dogs and humans, with 29 sequence types occurring only once. An additional four novel STs were identified, all of which were represented by canine isolates. The most commonly identified STs were ST372 (n=10), ST10 (n=8), ST88 (n=6), ST95 (n=6) and ST162 (n=6), however these were not all equally distributed between canine and human isolates (Table 6.14).

Table 6.14 The nine most frequently identified MLST sequence types amongst 132 isolates of *E. coli* obtained from faecal samples of kennelled dogs and humans working with dogs in the North of England

Sequence Type	Total	Number of Isolates	
		Canine (n=88)	Human (n=44)
ST372	10	7	3
ST10	8	3	5
ST88	6	6	0
ST95	6	0	6
ST162	6	6	0
ST297	5	4	1
ST131	4	0	4
ST744	4	3	1
ST963	4	4	0

The 48 isolates confirmed as carrying *bla* genes encoding ESBL or pAmpC enzymes belonged to thirty-one different STs, of which 24 were identified in a single isolate. Few relationships were identified between STs and presence of *bla* resistance genes. Of note however, were isolates belonging to ST372 (n=7), which with the exception of one isolate which carried a *bla*_{CMY-2-like} gene, all carried *bla*_{CMY-2}. Five of these isolates were positive for carriage of an IncI1 ST23 plasmid, two of which originated from human and canine samples obtained from the same site (Premises 12) and displayed the same resistance phenotype (Table 6.15). In addition, isolates belonging to *E. coli* ST196 (n=2), ST457 (n=2) and ST3947 (n=2) were also recovered from samples from multiple individuals located at Premises 9, 7 and 61 respectively and in each case isolates belonging the same *E. coli* ST harboured the same plasmid replicon type. This may indicate potential transmission of ESBL and AmpC-producing *E. coli* between dogs kennelled at the same premises (Table 6.15).

Four isolates belonging ST963 were all associated with carriage of *bla*_{CMY-2}, these did not however have the same resistance genotype and phenotype. The five isolates carrying *bla*_{CTX-}

m-15 belonged to different STs, but did include a single human isolate belonging to O16:H5 ST131, which was not identified as ST131 during PCR screening.

In addition to isolates carrying plasmid-mediated *bla*_{ESBL} and *bla*_{AmpC} genes, isolates belonging to ST62, ST88 all showed MDR phenotypes. Five isolates belonging to ST62 were recovered from resident dogs in a single boarding premises and all carried IncF RST F18:A-B1 and IncQ1 plasmid replicon types. These isolates displayed the same resistance phenotype and harboured almost identical resistance genotypes (Table 6.16). Similar findings were observed when considering the isolates belonging to ST88, four of which were isolated from individual dogs on the same premises. These isolates were all positive for IncF RST F67:A6:B38 and had the same resistance phenotype and genotype. All isolates belonging to ST88 and ST4421 all displayed an AmpC phenotype during AmpC phenotypic testing in the absence of plasmid-mediated *bla*_{AmpC} genes suggesting cAmpC production.

Table 6.16 Details of *E. coli* isolates belonging to ST62, ST88 and ST4421, which do not carry a plasmid-mediated *bla*_{ESBL} or *bla*_{AmpC} gene

Isolate	Sequence		Host	Sample	Resistance Phenotype	Plasmid Replicon Type	Antimicrobial resistance gene															
	Type	Premises					A			B			C	D		E		F		G		H
							<i>tem-1</i>	<i>aadA1</i>	<i>aadA5</i>	<i>strA</i>	<i>strB</i>	<i>aph(3')-Ia</i>	<i>gyrA</i> variant	<i>ParC</i> variant	<i>tetA</i>	<i>tetB</i>	<i>dfrA1</i>	<i>dfrA5</i>	<i>dfrA17</i>	<i>sul1</i>	<i>sul2</i>	<i>catA1</i>
D142	62	7	Dog	37	AMP CHL CIP NA TET TM	IncF RST F18:A-B1, IncQ1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D157	62	7	Dog	39	AMP CHL CIP NA TET TM	IncF RST F18:A-B1, IncQ1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D164	62	7	Dog	40	AMP CHL CIP NA TET TM	IncF RST F18:A-B1, IncQ1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D182	62	7	Dog	42	AMP CHL CIP NA TET TM	IncF RST F18:A-B1, IncQ1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D189	62	7	Dog	43	AMP CHL CIP NA TET TM	IncF RST F18:A-B1, IncQ1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D1363	62	31	Dog	127	AMP CHL NA TET TM	IncF RST F18:A-B1, IncQ1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D178	88	7	Dog	42	AMP AC CIP NA TET	IncF RST F77:A:B16, IncQ1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D341	88	9	Dog	70	AMP AC CHL NA TM	IncF RST F67:A6:B38	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D355	88	9	Dog	72	AMP AC CHL NA TM	IncF RST F67:A6:B38	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D362	88	9	Dog	73	AMP AC CHL NA TM	IncF RST F67:A6:B38	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D396	88	9	Dog	79	AMP AC CHL NA TM	IncF RST F67:A6:B38, ColpVC	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D432	88	12	Dog	83	AMP AC CHL NA TM	IncF RST F67:A6:B38	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D977	4421	47	Dog	213	AMP AC TM	IncI1 ST3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
D1040	4421	50	Dog	233	AMP AC TM	IncI1 ST3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	

Key: Genes conferring resistance to: A= β -lactams; B= aminoglycosides; C= quinolones; D=trimethoprim; E=sulphonamides; F=phenicols G= macrolides; White squares indicate absence of a gene; black squares indicate a 100% homology to the reference sequence while grey squares indicate >98% homology; *signifies allelic match of <100%

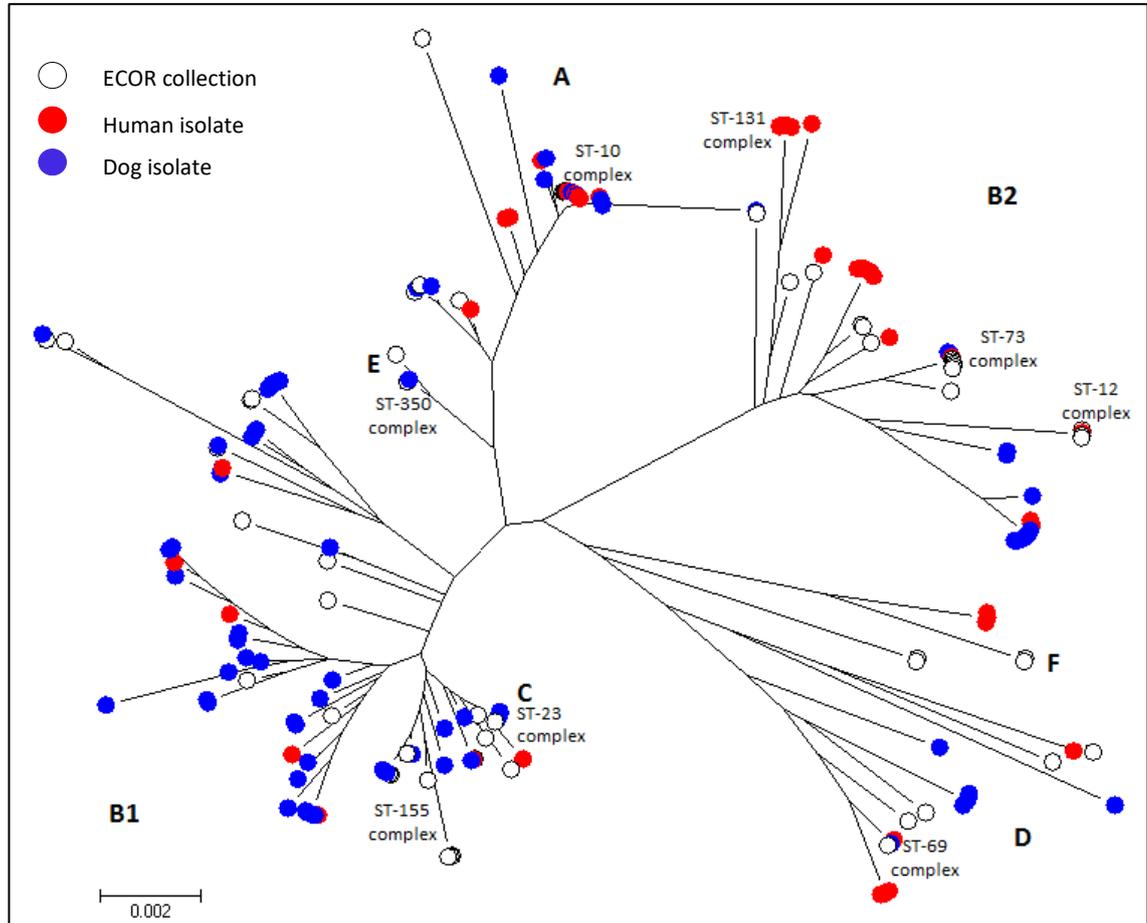
6.3.8 Phylogenetic analysis of MLST data

Phylogenetic analysis of MLST data from 132 isolates of canine (n=88) and human (n=44) origin alongside 67 reference strains from the ECOR Collection was undertaken using a neighbour-joining method. The constructed phylogenetic tree (Figure 6.6) indicates that isolates belonging to phylogroups B1 and B2 appear to be most numerous, while only small numbers of isolates belong to phylogroups E and F, supporting the PCR results previously reported. Both human and canine isolates are distributed throughout the tree indicating diversity in the *E. coli* population in these host species. Interestingly however, there is an increased density of canine isolates within phylogroup B1. Four isolates were identified as belonging to *E. coli* ST131, all of which were of human origin; however only one of these isolates was identified as carrying a *bla*_{CTX-M-15} gene and was identified as serotype O16:H5 rather than the O25b:H4 ST131 pandemic clone. ESBL-producing isolates were not clustered together and were located within multiple clusters on the tree, suggesting ESBL-producing isolates were not associated with a single clonal complex in either host species.

A further neighbour-joining phylogenetic tree was constructed using MLST data from all isolates obtained from Premises 12 and 30 in order to assess potential transfer of resistant and non-resistant *E. coli* between individuals in the same environment. Examination of the tree constructed (Figure 6.7) reveals some interesting patterns. Five isolates originating from humans working within the same premises (Premises 30) are located within the same cluster of the tree suggesting a high level of similarity of isolates in people working in this premises based on their STs (Cluster 1). Similarly, two clusters (Clusters 2 and 3) of canine isolates containing three and two isolates respectively from these premises are present on the tree. Investigation of the phenotypic resistance profiles revealed that in Cluster 1 two of the isolates of human origin shared the same resistance profile and carried the plasmid-mediated *bla*_{TEM-1} gene (Table 6.17). In Clusters 2 and 3, all of the canine isolates were susceptible to all antimicrobials tested and did not carry associated resistance genes. These findings suggest that in these premises, while there was no evidence of transmission between animals and staff, there is some evidence of transmission between dogs. When considering Premises 12, four small clusters of isolates were identified. One cluster contained two human, and one canine isolate (Cluster 4), with three further clusters each containing two canine isolates (Clusters 5-7). Investigation of the resistance profiles of these isolates revealed differing resistance phenotypes and genotypes between isolates located in Clusters 4 to 6 indicating that these were not clonal isolates spread between individuals. However,

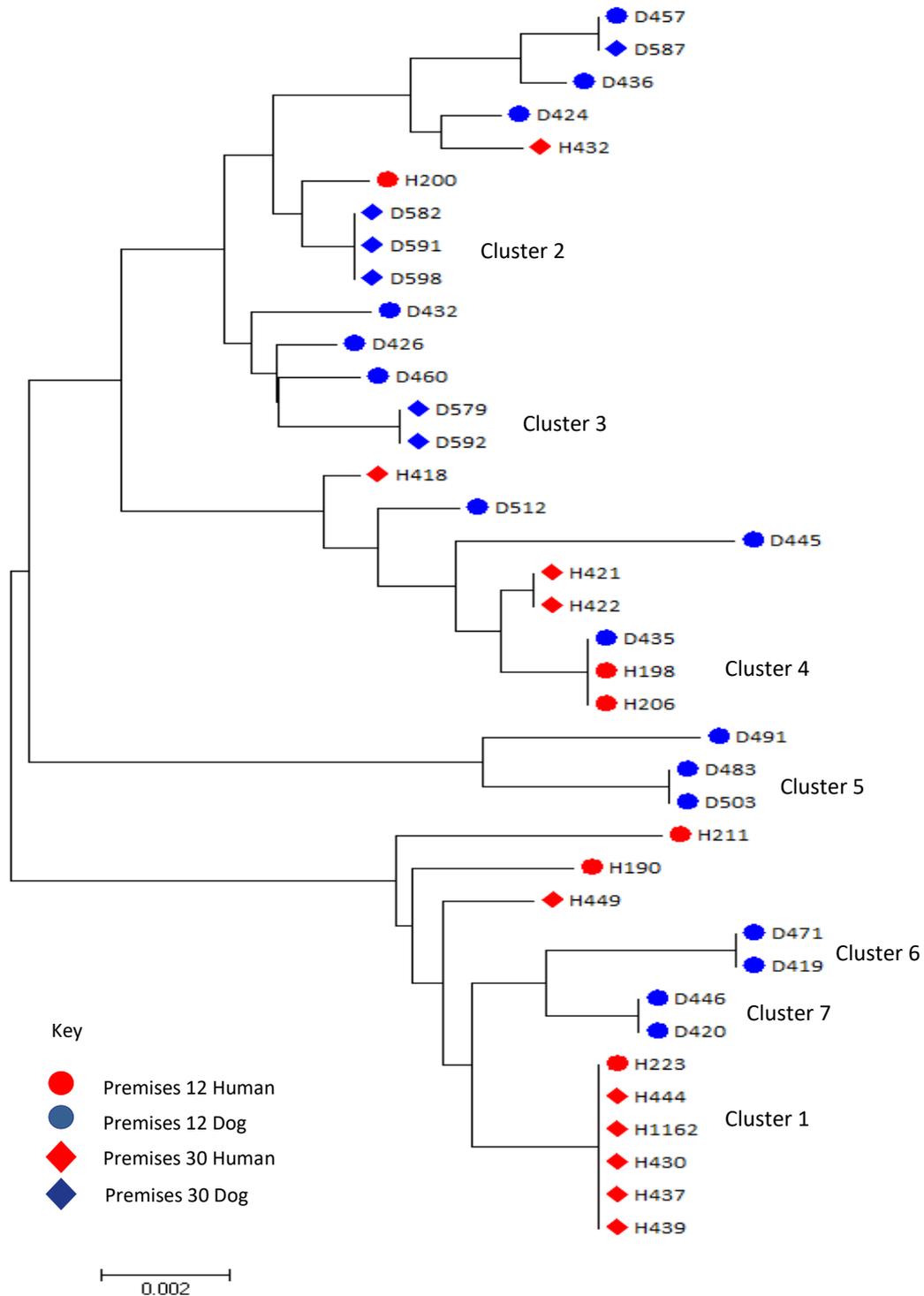
the two isolates in Cluster 7 shared the same resistance phenotype and genotype (Table 6.17).

Figure 6.6 Phylogenetic tree indicating relationships between 132 *E. coli* isolates recovered from humans and dogs in the North of England



Tree constructed using concatenated nucleotide sequences of seven *E. coli* housekeeping genes: *adh*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*.

Figure 6.7 Phylogenetic tree indicating relationships between 38 faecal *E. coli* isolates recovered from humans and dogs located at two kennel premises in the North of England



The tree was constructed from MLST data using a neighbour-joining method. Further details of isolates located in Clusters 1 to 7 can be located in Table 6.17 overleaf.

Table 6.17 Details of isolates located within Clusters 1 to 7 in Figure 6.7

Cluster	Premises	Premises		Host	Sample	Sequence		3GCR	Plasmids	Replicon Type	Resistance Genes	Virulence genes
		Type	Isolate			Type	3GCR					
1	12	Rescue	H223	Human	46	95	N	IncF RST F1:A1:B23, Col156, ColpVC	<i>Bla</i> _{TEM-1} , <i>tetB</i> , <i>dfrA1</i> , <i>sul1</i>	<i>gad</i> , <i>ireA</i> , <i>iss</i> , <i>senB</i> , <i>vat</i>		
			30	Boarding	H430	Human	97	95	N	IncB/O/K/Z, IncF RST F18:A-B8	<i>Bla</i> _{TEM-1}	<i>cba</i> , <i>cma</i> , <i>gad</i> , <i>iroN</i> , <i>iss</i> , <i>mchF</i>
		H437	Human		99	95	N	IncB/O/K/Z, IncF RST F18:A-B8	<i>Bla</i> _{TEM-1}	<i>cba</i> , <i>cma</i> , <i>gad</i> , <i>iroN</i> , <i>iss</i> , <i>mchF</i>		
		H439	Human		99	95	N	IncB/O/K/Z, IncF RST F18:A-B8	<i>Bla</i> _{TEM-1}	<i>cba</i> , <i>cma</i> , <i>gad</i> , <i>iroN</i> , <i>iss</i> , <i>mchF</i>		
		H444	Human	100	95	N	IncF RST F18:A-B8, Col156	<i>Bla</i> _{TEM-1} , <i>sul2</i>	<i>gad</i> , <i>ireA</i> , <i>iroN</i> , <i>iss</i> , <i>mchF</i>			
	H1162	Human	96	95	N	IncB/O/K/Z, IncF RSTF2:A-B1	<i>dfrA5</i>	<i>gad</i> , <i>ireA</i> , <i>iroN</i> , <i>iss</i> , <i>mchF</i> , <i>vat</i>				
2	30	Boarding	D582	Dog	118	1079	N	IncY		<i>gad</i> , <i>lpfA</i>		
			D591	Dog	120	1079	N	IncF RST F4*:A-B-, IncY		<i>gad</i> , <i>iss</i> , <i>lpfA</i>		
			D598	Dog	124	1079	N	IncY		<i>gad</i> , <i>iss</i> , <i>lpfA</i>		
3	30	Boarding	D579	Dog	117	2005	N	No plasmid replicons found		<i>gad</i> , <i>iss</i> , <i>lfpA</i>		
			D592	Dog	121	2005	N	No plasmid replicons found		<i>gad</i> , <i>iss</i> , <i>lfpA</i>		
4	12	Rescue	H198	Human	40	10	N	IncX1	<i>Bla</i> _{TEM-1} <i>qnrS</i>	<i>gad</i> , <i>iss</i>		
			H206	Human	43	10	N	Col156		<i>gad</i> , <i>iha</i> , <i>ireA</i> , <i>iss</i> , <i>sigA</i>		
			D435	Dog	84	10	N	IncI1 unknown ST, IncX1	<i>Bla</i> _{TEM-1} , <i>cmlA1-like</i> , <i>dfrA12</i> , <i>sul3</i> , <i>tetB</i>	<i>gad</i> , <i>iss</i> , <i>sfaS</i>		
5	12	Rescue	D483	Dog	93	963	Y	IncF RST F2:A-B-, IncI1 ST2	<i>Bla</i> _{CMY-2} , <i>sul2</i>	<i>air</i> , <i>gad</i>		
			D503	Dog	97	963	Y	IncF RST F2:A-B-, IncI1 ST2	<i>Bla</i> _{CMY-2} , <i>bla</i> _{OXA-1} , <i>sul1</i> , <i>sul2</i> , <i>floR-like</i>	<i>air</i> , <i>gad</i>		
6	12	Rescue	D419	Dog	81	372	N	IncI1 ST26, ColpVC, Col(MG828)	<i>Bla</i> _{TEM-33-like}	<i>gad</i> , <i>iroN</i> , <i>iss</i> , <i>mchB</i> , <i>mchC</i> , <i>mchF</i> , <i>mcmA</i> , <i>vat</i>		
			D471	Dog	91	372	Y	IncI1 ST43	<i>Bla</i> _{CMY-2}	<i>gad</i> , <i>iroN</i> , <i>iss</i> , <i>mchB</i> , <i>mchC</i> , <i>mchF</i> , <i>mcmA</i> , <i>vat</i>		
7	12	Rescue	D446	Dog	86	127	N	No plasmid replicons found	<i>Bla</i> _{TEM-1} , <i>tetB</i>	<i>cnf1</i> , <i>gad</i> , <i>iroN</i> , <i>iss</i> , <i>mcmA</i> , <i>sfaS</i> , <i>vat</i>		
			D420	Dog	82	127	N	No plasmid replicons found	<i>Bla</i> _{TEM-1} , <i>tetB</i>	<i>cnf1</i> , <i>gad</i> , <i>iroN</i> , <i>iss</i> , <i>mcmA</i> , <i>sfaS</i> , <i>vat</i>		

Key: 3GCR=third generation cephalosporin resistance; AM=ampicillin; AC=amoxyclov; CHL=chloramphenicol; CIP= ciprofloxacin; NA=nalidixic acid; TET=tetracycline; TM=trimethoprim; *signifies allelic match of <100%

6.4 Discussion

Within this study, the phylogenetic background and carriage of resistance genes by faecal *E. coli* originating from in-contact humans and dogs in the North of England have been investigated. The results of this study indicate a high level of diversity in the *E. coli* isolated from this population, both within and between host species, when considering both the phylogenetic background and carriage of resistance genes.

Within the canine population sampled, isolates belonging to phylogroup B1 were most prevalent and those isolates belonging to phylogroup B1 were significantly more likely to be canine than human in origin. The predominance of phylogroup B1 within canine populations is not unexpected and is supported by numerous previous studies (Damborg et al., 2009; Gordon and Cowling, 2003; Schmidt et al., 2015; Tenaillon et al., 2010). Indeed, Schmidt et al. (2015) postulated on the propensity of dogs to scavenge and explore environments such as soil and water courses, where B1 isolates are more likely to persist (Walk et al., 2007), as a potential predisposing factor for this predominance. In contrast to isolates of canine origin, human isolates were most frequently identified as belonging to phylogroup B2; reflecting findings reported by previous studies in temperate climates (Escobar-Páramo et al., 2004; Escobar-Páramo et al., 2006; Gordon et al., 2005; Watt et al., 2003; Zhang et al., 2002). These findings contrast to those in tropical climates where group A isolates predominate and suggest that multiple factors including climate, diet and levels of hygiene play an important role in determining commensal *E. coli* populations (Escobar-Páramo et al., 2004; Tenaillon et al., 2010). Isolates belonging to phylogroup D were significantly more likely to originate from humans while those belonging to phylogroup B2 also displayed this trend. This finding has been previously reported by Damborg et al. (2009) and may suggest a propensity for increased colonisation of the human gastrointestinal tract by ExPEC strains when compared to dogs. Indeed, the correlation between carriage of *E. coli* belonging to phylogroups B2 and D suggests the commensal niches of these phylogroups may be similar (Escobar-Páramo et al., 2004). Multiple host and environmental factors influence the commensal *E. coli* population and previous studies have indicated increased similarity of commensal *E. coli* between humans and in-contact animals when compared to control populations (Harada et al., 2012). This similarity may be due to both shared environments and inter-species transfer of *E. coli*. However, the current absence of data regarding commensal *E. coli* within the human population of the United Kingdom prevents comparison of this data with that of the

general population. Therefore, further studies investigating both dog owners and a control population with no canine contact are warranted in order to investigate this further.

Examination of the results of MCA indicated that there was a positive correlation between resistance to antimicrobials belonging to the same class. This cross-resistance is not unexpected given mechanisms of resistance commonly target a conserved element of these drugs. In the case of β -lactams, this target is the β -lactam ring present in all β -lactam drugs; hence, commonly isolates with resistance to amoxyclav or cefotaxime would additionally be resistant to ampicillin (Majiduddin et al., 2002). Similarly, resistance to quinolone drugs is commonly mediated through alterations to DNA gyrase-quinolone binding sites or quinolone efflux pumps both of which can have an effect on all drugs of this class (Drlica and Zhao, 1997; Hirai et al., 1986; Ruiz et al., 2012a). Interestingly however, a negative correlation was identified between resistance to quinolones and β -lactams, including 3GCs, indicated by their opposition along the second dimension of the MCA map. This finding was confirmed when examining the outcome of hierarchical clustering of MCA results, which indicated a decreasing percentage of isolates resistant to 3GCs in clusters with increased quinolone resistance. This finding may be surprising given previously reported MDR ESBL-producing isolates of *E. coli*, which commonly display resistance to additional drug classes including fluoroquinolones (Lavigne et al., 2006; Paterson and Bonomo, 2005). This finding is largely reported with co-localisation of *bla*_{CTX-M-15} and *qnr* genes (Lavigne et al., 2006; Liu et al., 2016), and is further linked to dissemination of the pandemic O25b:H4 ST131 clone harbouring the *aac(6')-Ib-cr* gene (Ewers et al., 2010; Pomba et al., 2009; Rogers et al., 2011). However, given the low prevalence of ESBL-producing versus AmpC-producing isolates identified in this study, alongside the propensity for high-level resistance to quinolone drugs to originate from chromosomal mutations (Martínez-Martínez et al., 2003; Ruiz, 2003; Ruiz et al., 2012a; Vila et al., 1994), selection for quinolone resistance may not be always be accompanied by co-selection for β -lactam resistance.

As previously described in Chapters Three and Four, correlation was identified between resistance to trimethoprim and tetracycline. A likely reason for this correlation is the presence of resistance genes against both antimicrobial classes on the same resistance plasmids, indeed previous studies have indicated the association of trimethoprim resistance to resistance against other antimicrobials in *E. coli* isolated from canine faeces (Schmidt et al., 2015; Wedley et al., 2011). Of additional interest was the apparent correlation between multi-drug resistance and nalidixic acid resistance supporting findings previously reported by

Platell et al. (2010). Given the low level of plasmid-mediated quinolone resistance genes carried by quinolone-resistant isolates in this study, this correlation cannot just be linked to localisation of quinolone resistance genes on plasmids carrying multiple resistance genes. It may however, indicate a propensity for chromosomal mutations encoding resistance to quinolones, such as GyrA Asp87Gly mutations, to reduce susceptibility to other antimicrobial classes (Webber et al., 2013). In addition, isolates displaying fluoroquinolone resistance due to chromosome-mediated resistance mechanisms may additionally harbour plasmids carrying multiple resistance genes or resistance may be due the presence of multi-drug efflux pumps conferring resistance to multiple drug classes (Piddock, 2006; Ruiz, 2003). Indeed, in this study, multiple are true; of the seventeen quinolone-resistant isolates, which underwent whole genome sequencing, thirteen displayed a multi-drug resistance phenotype. Examination of genes carried by these isolates indicated different mechanisms for quinolone resistance including plasmid-mediated *qnr* genes (n=4), mutations to *gyrA* or *parC* (n=5) and genes encoding multi-drug efflux pumps (n=4), in addition to acquired resistance mechanisms targeting other antimicrobial classes.

MCA indicated that resistance phenotypes contributed the largest amount of variation to the data, while host species sat closest to the origin of the plot and as such did not appear to be correlated strongly with resistance outcomes. This finding was not surprising since resistance against all antimicrobials tested was identified in isolates from both species, and resistance to ampicillin, tetracycline and trimethoprim were most prevalent in isolates from both humans and dogs. Indeed, many resistance genes of clinical importance in human medicine, including those conferring resistance to 3GCs and fluoroquinolones have also been identified in isolates of canine origin (Albrechtova et al., 2012; Timofte et al., 2011; Timofte et al., 2014a; Timofte et al., 2014b). However, hierarchical cluster analysis indicated that one cluster of isolates (Cluster 2) contained an increased prevalence of amoxycylav-resistant isolates and isolates of canine origin were significantly associated with this cluster. Given the increased use of amoxycylav in first opinion veterinary practice compared to human medicine (Mateus et al., 2011; PHE, 2015; PHE, 2016; Radford et al., 2011), it is not unexpected that the prevalence of resistance to this antimicrobial is higher in canine isolates than human isolates and as such, canine isolates would be more prevalent in this cluster.

The correlation of *E. coli* phylogroup with resistance outcomes appears to be more complex. The finding that this variable contributes less variation to the dataset than some resistance

outcomes is unsurprising given that resistance genes are commonly located on plasmids and can therefore be transferred between isolates of differing phylogeny (Livermore, 2003).

Our results do however seem to show some differences between *E. coli* phylogroups; MCA suggested that B2 isolates were correlated with more susceptible phenotypes while isolates belonging to group C were positively correlated with resistant phenotypes. Statistical analysis mirrored these findings indicating that in dogs, group B2 isolates were significantly less likely to display phenotypic resistance to tetracycline and nalidixic acid or have a MDR phenotype. This supports the findings of previous studies which have consistently found that canine isolates belonging to phylogroup B2 display reduced level of resistance to quinolones (Johnson et al., 2003b; Johnson et al., 2009a; Platell et al., 2010; Sato et al., 2014). Schmidt et al. (2015) additionally found that canine isolates belonging to phylogroup B2 were less likely to be MDR, a finding confirmed in this study. Given the correlation between quinolone resistance and an MDR phenotype identified in this study and previously (Platell et al., 2010), it is unsurprising that a coincident decrease in quinolone and multi-drug resistance was identified in B2 isolates. In contrast to the findings for canine isolates belonging to the B2 phylogroup, human isolates belonging to phylogroup D were significantly more likely to display quinolone and MDR phenotypes, mirroring the results of previous studies (Deschamps et al., 2009; Johnson et al., 2009a; Sato et al., 2014). This may suggest that group D isolates have a high prevalence of chromosomal resistance genes or an increased propensity for acceptance of plasmids carrying antimicrobial resistance genes. Additionally within this study, canine isolates belonging phylogroup C were identified as having an increased prevalence of multi-drug resistance and resistance to nalidixic acid, amoxycylav and trimethoprim. Massot et al. (2016) described a potential association between this phylogroup and antimicrobial resistance, however no further studies have reported this association or have used PCR incapable of identifying this phylogroup (Platell et al., 2010; Sato et al., 2014; Schmidt et al., 2015). As such, further investigation of the role of phylogroup C isolates in the dissemination of antimicrobial resistance is required.

Examination of isolates for carriage of *bla*_{ESBL} and *bla*_{AmpC} genes in this study was undertaken using two methods, PCR and WGS. In all cases there was very good agreement between the two methods for identification of *bla* genes. This finding supports the continued use of PCR as an initial screening tool in order to identify isolates carrying *bla* genes. Further examination of these isolates using sequencing methods can then be utilised in order to

characterise specific gene variants responsible for conferring resistance, alongside the genetic background of the isolates of interest.

Within the human population sampled, plasmid-mediated resistance to 3GCs was most frequently conferred by production of ESBL rather than pAmpC enzymes. These findings mirror those of previous reports investigating clinical and non-clinical *E. coli* isolates of human origin, which describe the predominance of ESBL-mediated resistance over production of pAmpC enzymes (Castillo et al., 2013; Hammerum et al., 2011; Potz et al., 2006; van Hoek et al., 2015). The presence of *bla*_{CTX-M} genes was identified in 67% of ESBL-producing isolates, with carriage of *bla*_{CTX-M-15} identified in four isolates, while *bla*_{CTX-M-1} was identified in two isolates. These findings echo those reported in previous studies investigating faecal carriage of ESBL-producing *Enterobacteriaceae* in healthy individuals and community patients in the UK and Northern Europe, which identify *bla*_{CTX-M-15} as the most prevalent CTX-M variant in these populations (Geser et al., 2012b; Reuland et al., 2013; Reuland et al., 2016; Valenza et al., 2014; Wickramasinghe et al., 2012). The ubiquitous nature of this enzyme globally has largely been linked to the dissemination of the pandemic *E. coli* ST131 clone, with which it is commonly associated (Coque et al., 2008; Lau et al., 2008; Woodford et al., 2011). Interestingly, only a single isolate carrying *bla*_{CTX-M-15} was identified as belonging to ST131 and furthermore this isolate was of serotype O16:H5 more commonly associated with *bla*_{CTX-M-14} (Dahbi et al., 2014; Zhong et al., 2015). The four isolates harbouring *bla*_{CTX-M-15} were isolated from individuals working at boarding, rescue and veterinary premises. These isolates belonged to different STs and were found to carry diverse plasmids. This indicates that while the mechanisms of ESBL resistance identified in this study mirror those prevalent in the wider population, dissemination is related to multiple resistance plasmids in different locations rather than transmission of the O25b:H4 ST131 pandemic clone in this population of individuals.

In addition to isolates harbouring ESBL genes, five isolates were identified as carrying *bla*_{AmpC} genes, with *bla*_{CMY-2} and *bla*_{DHA-1} genes characterised. There is currently a paucity of data regarding the carriage of plasmid-mediated *bla*_{AmpC} genes in the UK human population, making any comparison of this data with the general population challenging. However, a small number of studies have investigated carriage of *bla*_{AmpC} genes by healthy individuals in Europe. These studies identified *bla*_{CMY-2} and *bla*_{DHA-1} as the most prevalent *bla*_{AmpC} genes, supporting the findings presented here and highlighting the potential for healthy individuals

to act as a reservoir of these resistance determinants in community and hospital settings (Garrido et al., 2014; Reuland et al., 2015; Rodríguez-Baño et al., 2012; van Hoek et al., 2015).

In contrast to human isolates, the most common mechanism conferring resistance to 3GC resistance identified in dogs was production of AmpC enzymes. Carriage of these genes was identified in 64% of isolates harbouring a plasmid-mediated *bla* gene, with *bla*_{CMY-2} type genes most prevalent. Previous studies within the UK have identified the predominance of *bla*_{AmpC} carriage by isolates of faecal *E. coli* obtained from healthy (Schmidt et al., 2015; Wedley et al., 2011), as well as hospitalised and vet visiting dogs (Tuerena et al., 2016; Wedley et al., 2017). Furthermore, where characterised, CMY-2 production was identified as the sole mechanism of pAmpC-mediated resistance in all studies, supporting the findings reported here (Tuerena et al., 2016; Wedley et al., 2011; Wedley et al., 2017). The predominance of this mechanism of resistance in canine isolates when compared to those of humans is of interest. This suggests *bla*_{CMY-2} may be widespread within the canine population and highlights the potential for different drivers for development and dissemination of resistance between canine and humans populations.

The high prevalence of *bla*_{CMY-2} within *E. coli* isolates of canine origin may be due to the transmission of specific plasmids or bacterial clones within the canine population. Indeed, of the 22 isolates found to carry *bla*_{CMY-2} ten were associated with an IncI1 ST23 plasmid, IncI1 ST2 (n=5) and IncI1 ST43 plasmids (n=3) were also represented. Interestingly while IncI1 ST23 and IncI1 ST43 plasmids harbouring *bla*_{CMY-2} were identified in isolates from all premises types sampled, isolates harbouring IncI1 ST2 were recovered solely from animals within rescue centres. The predominance of IncI1 ST23 across the canine population sampled is of interest; historically *bla*_{CMY-2} has been associated with Inc A/C plasmids (Tagg et al., 2014), however CMY-2-producing *E. coli* isolates have more recently been associated with a diverse range of IncI1 plasmids, including ST2 and ST23 (Alonso et al., 2016; Bortolaia et al., 2014; Haenni et al., 2014; Weissman et al., 2013). The findings in this study may suggest that these plasmid types are responsible for the dissemination of *bla*_{CMY-2} in the kennelled dog population in the North of England. Furthermore, four CMY-2-producing isolates were identified as belonging to MLST ST372. Two isolates originating from dogs kennelled on the same premises carried IncI1 ST23 and IncX1 plasmids suggesting potential clonal dissemination of this strain between animals. The additional two isolates harboured *bla*_{CMY-2} associated with IncI1 ST23 alone, and were recovered from animals housed at separate premises. The recovery of isolates harbouring IncI1 ST2 solely from dogs within rescue

kennels may suggest circulation of an increased diversity of plasmids harbouring *bla*_{CMY-2} within this population when compared to boarding dogs, however this plasmid has been isolated from owned dogs in Europe suggesting it is not restricted to rescue populations (Haenni et al., 2014). Further analysis of the genome sequences from additional canine populations is required in order to assess the potential for IncI1 ST23 *E. coli* ST372-associated clonal dissemination of the *bla*_{CMY-2} gene alongside the role of IncI1 ST2 plasmids within the UK canine population.

A total of ten canine *E. coli* isolates were identified as ESBL-producers, 50% of these carried a *bla*_{CTX-M} gene, while the other 50% carried a *bla*_{SHV} gene. Of the CTX-M producers, *bla*_{CTX-M-1} associated with IncI1 ST3 was identified in three isolates, while *bla*_{CTX-M-15} and *bla*_{CTX-M-55} were each identified in a single isolate. These findings mirror the global picture relating to *bla*_{CTX-M} genes responsible for conferring resistance in healthy dogs where carriage of *bla*_{CTX-M-1} on IncI1 ST3 plasmids predominates (Dahmen et al., 2012; Grami et al., 2013; Haenni et al., 2014). One human isolate was additionally found to carry an IncI1 ST3 associated *bla*_{CTX-M-1} gene and was not associated with any animals carrying this gene within this study. This finding may indicate that while *bla*_{CTX-M-15} is most prevalent within human populations, suggesting largely separate dissemination of resistance mechanisms in the two species (Geser et al., 2012b; Reuland et al., 2016; Valenza et al., 2014), increased levels of animal contact may lead to transfer of animal associated plasmids into the wider human population (Madec et al., 2015)

In addition to the isolates carrying *bla*_{CTX-M} genes, four isolates recovered from three dogs located on the same premises were found to carry a *bla*_{SHV-12} gene alongside *qnrS1*, *aadA22* and *InuF* genes associated with an IncX3 plasmid, while three of the isolates were additionally associated with IncF RST F:-A-B56-like replicons. An additional isolate from a fourth dog harboured an IncX3 associated *bla*_{SHV-12-like} gene alongside *aadA1*, *dfrA1* and *InuF*. IncX3 plasmids have previously been associated with *bla*_{SHV-12} and *qnrS1* in isolates obtained from livestock and wastewater in Europe (Alonso et al., 2017; Dobiasova and Dolejska, 2016), both of which could act as a viable source of resistance determinants to kennelled dogs on a raw food diet. Two of the isolates, designated as ST3947 during MLST and carried the same resistance genotype associated with IncX3 and IncF replicon types, suggesting potential transmission of this strain between animals or acquisition from a shared source. The additional two isolates harbouring the same resistance genes were isolated from the same dog, and were identified as ST898 and ST206, respectively. Despite the divergent STs of these

isolates both carried IncX3 alone or in combination with IncF RST F-A-B56-like replicon, which may suggest horizontal transmission of an IncX3 plasmid harbouring *bla*_{SHV-12}, *aadA1*, *InuF* and *qnrS1* genes. However given failure of plasmids to transfer during conjugation experiments, carriage of *bla*_{SHV-12} on this premises may be associated with multiple IncX3 plasmids. Globally, IncX3 plasmids have additionally been associated with dissemination of *bla*_{NDM} genes alone or in combination with *bla*_{SHV-12} (Dobiasova and Dolejska, 2016; Feng et al., 2015; Huang et al., 2016); however, carbapenemase genes were not identified in this study.

Of further note is the presence of *bla*_{SHV-12} in three isolates recovered from two humans sampled at separate premises to the canine isolates carrying this gene. Plasmid typing revealed these isolates all carried IncI1 ST26 plasmids previously shown to harbour *bla*_{SHV-12} in isolates of human and meagreine origin (Accogli et al., 2013; Jones-Dias et al., 2016). The importance of the *bla*_{SHV-12} gene in conferring ESBL-mediated resistance in the canine population and in contact humans should not therefore be overstated based on current findings. The recovery of *bla*_{SHV-12} from animals on single premises may suggest this gene is not widespread and as such, is overrepresented in the canine population in this study. Additionally, although humans sampled in this study were also found to carry *bla*_{SHV-12}, the different plasmid replicon harbouring this gene suggests separate transmission of this gene within the two populations.

Of the 75 isolates displaying resistance to 3GCs which underwent WGS in this study, 26 of the 55 isolates displaying an AmpC phenotype were found not to carry a *bla*_{AmpC} gene. An additional eight isolates which were not sequenced were found to be AmpC producers during phenotypic testing, with an absence of *bla*_{AmpC} detection during PCR examination. These findings support those reported by Schmidt et al. (2015), who found an absence of *bla*_{AmpC} genes in nine of twelve phenotypic AmpC-producing *E. coli* isolates recovered from faeces of healthy UK dogs. In the absence of *bla*_{AmpC} genes, an AmpC phenotype in *E. coli* isolates is likely due to chromosomal mutation of the *ampD* promoter leading to hyper production of the AmpC enzyme (cAmpC) (Jacoby, 2009; Jaurin et al., 1981). Indeed, examination of clinical isolates of *E. coli* by Simner et al. (2011) found 44% of phenotypic AmpC-producing isolates contained mutations in the promoter and/or attenuator region of the *ampC* gene rather than carriage of a plasmid-mediated *bla*_{AmpC}. A single isolate displaying an ESBL phenotype alongside tetracycline resistance was found not to carry any *bla* genes. This isolate did

however, carry genes encoding multi-drug efflux pumps including AcrEF-TolC and MdtEF-TolC, which confer resistance to β -lactams, which may explain its resistance profile.

Of the 53 isolates found to carry *bla* genes, 44 isolates were found to carry additional class specific resistance genes. Isolates most commonly carried genes conferring resistance to aminoglycosides and sulphonamides, mirroring findings previously reported in both canine and human isolates (Card et al., 2015; Costa et al., 2008; Kirchner et al., 2014; Wedley et al., 2017). Indeed, the mechanisms of resistance identified in canine isolates echoed those in humans, indicating that mechanisms mediating antimicrobial resistance in canine *E. coli* isolates are largely consistent with those in isolates of human origin. Interestingly, an increased number of resistance genes were identified in isolates of canine origin, which could suggest greater diversity of resistance genes in this population. This finding may suggest increased exposure of dogs to a wider range of antimicrobials or increased contact with environmental sources of resistance genes including exposure to faeces from other animal species. However, an increased number of canine isolates were sequenced and as such, this increase in gene numbers may be a reflection of the greater number of isolates.

Examination of genotypes and resistance phenotypes of the isolates carrying *bla* genes identified some isolates that did not display phenotypic resistance to antimicrobials despite the presence of a resistance gene. Seven isolates harbouring *qnr* genes were not identified as displaying resistance to quinolone antimicrobials *in vitro*. The proteins encoded by these genes provide only low level resistance to quinolones (Martínez-Martínez et al., 1998), and as such, it is not unexpected that these isolates may not display a quinolone-resistant phenotype. In addition, one isolate carried genes encoding resistance to chloramphenicol and trimethoprim without displaying phenotypic resistance to these antimicrobials. The cause of this discrepancy is likely due to non-expression of the gene and can be explained by two potential mechanisms; the absence of promoter sequences (Podglajen et al., 1994) or silencing of the resistance gene (Enne et al., 2006). Alteration of selection pressure *in vivo*, for example administration of trimethoprim or chloramphenicol, may lead to upregulation of silenced genes and thus phenotypic resistance to these antimicrobials (Card et al., 2013).

Plasmid conjugation experiments revealed variability in the conjugative transfer of plasmids harbouring *bla* genes. Of the isolates carrying *bla*_{CTX-M} genes 64% transferred successfully including all isolates harbouring *bla*_{CTX-M-1}, while only 33% of isolates carrying *bla*_{CMY-2} conjugated. Furthermore, no isolates carrying *bla*_{SHV-12} successfully transferred plasmids harbouring this gene. This finding indicates variability in the ability for plasmids to transfer

under laboratory conditions mirroring findings reported by Hopkins et al. (2006b). Interestingly all IncI1 ST3 associated *bla*_{CTX-M-1} conjugated successfully suggesting why this plasmid is widespread among animal populations (Dahmen et al., 2012; Grami et al., 2013). The *bla*_{CMY-2} gene associated with IncI1 ST2 conjugated successfully, while isolates harbouring IncI1 ST23 did not. This finding was surprising given the predominance of this replicon type associated with *bla*_{CMY-2} carriage in this study and may further highlight the importance of *E. coli* ST372-associated clonal dissemination of this plasmid. Numerous donor isolates carried multiple *bla* genes; however the resultant transconjugant harboured only a single *bla* gene. These findings suggest that while ESBL and non-ESBL genes can be carried on the same plasmid (Boyd et al., 2004), isolates carried multiple plasmids only one of which was transferred to the recipient strain. One isolate harbouring *bla*_{CTX-M-15} and *bla*_{TEM-1} genes carried an IncF RST F18:A-B1 replicon alongside a Col156 replicon transferred only the *bla*_{TEM} gene to the recipient strain. This finding suggests either the presence of *bla* genes on the small Col156 plasmid or potential prophage inclusion of *bla*_{CTX-M-15} onto the bacterial chromosome (Guenther et al., 2017; Johnson et al., 2016). The variability in gene transfer among isolates is of interest and highlights that while commensal *E. coli* may act as a reservoir of resistance genes, knowledge of the plasmid harbouring resistance genes is important in predicting transmissibility. Additional typing of conjugative plasmids may provide additional information about the role of specific plasmids in the dissemination of resistance in canine and human populations and the ability of resistance genes to incorporate into the bacterial chromosome.

In total, 42 virulence-associated genes were identified in the 48 ESBL and pAmpC-producing isolates recovered in this study, with only a single isolate not carrying any virulence genes. An increased number of virulence genes were identified in isolates of human origin over those of canine origin (36 versus 26), suggesting greater diversity of *E. coli* in the human population sampled when compared to dogs sampled. This may be explained by the increased contact between dogs in the kennel environment as well a more similar lifestyle including food sources, all of which impact the composition of the intestinal microbiota (Gordon and Cowling, 2003; Penders et al., 2006) when compared to groups of humans working in a kennel environment. The *gad* gene encoding glutamate dehydrogenase was the most prevalent virulence gene identified. Induction of this enzyme occurs to maintain a physiological pH in acidic conditions such as those in the stomach (Capitani et al., 2003), and given the ecological niche of *E. coli* combined with a faeco-oral transmission route, the ubiquitous nature of this gene within isolates is expected.

The *iss* gene, linked to complement resistance and increased serum survival (Johnson et al., 2008c; Nolan et al., 2003), was identified alone or in combination with *iroN* in 21 isolates. Prevalence of these genes were increased in isolates belonging to phylogroup B2 confirming findings previously reported (Gordon et al., 2005; Massot et al., 2016). Indeed, *iss* is considered an important virulence determinant of avian pathogenic *E. coli* (APEC) (Johnson et al., 2008c; Nolan et al., 2003), while the siderophore encoded by *iroN* enables scavenging of iron from host tissues (Bäumler et al., 1998), and is associated with extraintestinal pathogenic *E. coli* (ExPEC) including APEC, neonatal meningitis-associated *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC) from multiple species (Bélanger et al., 2011; Negre et al.; Rodriguez-Siek et al., 2005; Ron, 2010).

The *lpfA* gene was identified in 20 isolates and showed a propensity to be carried by isolates belonging to phylogroup B1 mirroring reports by Balière et al. (2016) and Toma et al. (2006). The fimbrial protein encoded by this gene is associated with increased bacterial-epithelial adhesion and resulting diarrhoea caused by EHEC and EPEC (Afset et al., 2006; Botkin et al., 2012; Dogan et al., 2012). Microcin related determinants (*mchB*, *mchC*, *mchF* and *mcmA*) were identified in varying combinations in 12 isolates and predominated in B2 isolates. This finding has previously been reported in clinical and commensal isolates from dogs and humans respectively (Mickenková et al., 2016; Wagner et al., 2014). It has been postulated that this association is due to the predominance of human resident commensal isolates belonging to phylogroup B2 and bacteriocinogeny helping to promote and maintain stable colonisation within the intestinal niche (Mickenková et al., 2016). However, given the predominance of B1 isolates in canine samples, one may expect increased presence of *mch* determinants in canine isolates belonging to group B1, which was not observed. This suggests additional drivers for the production of microcin by isolates belonging to group B2 or alternate mechanisms for maintenance of resident B1 isolates within the canine intestine.

Of particular note was a single isolate harbouring seventeen virulence genes including genes encoding shiga toxin (*stx2*, *stx2A(d)*, *stx2B(d)*) and LEE-associated genes characteristic of EHEC isolates. Furthermore serotyping of this isolate revealed it to belong to the O80:H2 serotype (ST301), which has been associated with cases of haemolytic-uremic syndrome (HUS) (Mariani-Kurkdjian et al., 2014; Soysal et al., 2016; Wijnsma et al., 2017). This isolate harboured multiple resistance genes including *bla*_{CTX-M-1} and displayed a MDR phenotype. This is of particular interest given debate over the use of antimicrobials in patients with disease caused by EHEC. While antimicrobials are generally contraindicated in cases of

haemorrhagic colitis caused by EHEC due to enhanced release of toxins by dying bacteria, some studies suggest a benefit of antimicrobial treatment in established cases of HUS (Goldwater and Bettelheim, 2012; Menne et al., 2012; Rahal et al., 2015).

Examination of plasmid replicon types and virulence genes carried by isolates within this study revealed a highly diverse population and as such few associations between plasmid types and virulence determinants were observed. Isolates harbouring IncI1 ST3 plasmid types showed a propensity to harbour the *lpfA* gene, while isolates harbouring IncF RST F2-:A-:B- were shown to carry *air*. In addition, three isolates harbouring IncX3 plasmids were all shown to carry multiple virulence genes including *iroN*, *iss*, *mcmA*, *mchB*, *mchC* and *mchF*. These findings are of interest, while IncF plasmids have previously been identified as carrying virulence genes IncI1 and IncX3 plasmids are usually associated with resistance determinants rather than carriage of virulence determinants (Johnson and Nolan, 2009). While two of the isolates harbouring IncI1 ST3 also harboured an IncF plasmid replicon, three did not and in addition, two IncX1 plasmids were identified solely in combination with IncI1 ST23 replicons. These findings may suggest the possibility of carriage of virulence genes by IncI1 and IncX1 plasmids, however they may suggest a lack of sequencing coverage of additional plasmid replicons in these isolates.

The carriage of virulence genes by all but one of the ESBL and pAmpC-producing isolates in this study is of interest. All isolates were obtained from faecal samples from healthy individuals rather than clinical isolates and highlights the potential for commensal *E. coli* in humans and animals to act not only as a source of resistance genes, but also virulence genes. Furthermore, production of virulence factors by these commensal strains may lead to opportunistic infections, particularly in immunocompromised individuals (Packey and Sartor, 2009). Given the expression of some virulence genes, such as *mch* genes, may be related to commensalism as well as pathogenicity (Johnson et al., 2008a; Wold et al., 1992), longitudinal studies investigating virulence genes harboured by resident and transient *E. coli* in multiple species would be beneficial in order to fully assess the role commensal *E. coli* play in dissemination of virulence genes.

Within this study, a large number of *E. coli* STs were identified, indicating a high level of diversity in the commensal *E. coli* in the humans and dogs sampled. Interestingly, the most prevalent STs differed between species but some STs, including ST10 and ST372, were identified in both humans and dogs, suggesting that there does not appear to have been evolution of distinct canine-adapted subtypes within the population sampled. However,

given that the majority isolates selected for MLST were chosen due to their third generation or MDR phenotype, it may be some host-adapted strain were excluded from this analysis. Within the human population, ST95 and ST10 were most prevalent, while in dogs ST372, ST88 and ST162 were most commonly identified. Isolates belonging to ST95 and ST10 are well characterised and have previously been reported in clinical isolates of human and animal origin (Adams-Sapper et al., 2013; Stephens et al., 2015; Wagner et al., 2014). Interestingly in this study, all isolates belonging to ST95 were non-ESBL-producing isolates, which, with the exception of one isolate, carried *bla*_{TEM-1}. Five of these isolates originated on the same premises, and three (H430, H437 and H439) all harboured the same plasmid replicon types and virulence genes suggesting potential transmission of this isolate between individuals on the same premises. Isolates belonging to ST10 from both humans and dogs showed a diverse range of resistance phenotypes and no pattern of association with specific resistance genes, indicating that while ST10 was more prevalent than other sequence types this was not due to dissemination of a specific clone. In contrast, all six of the ST88 isolates identified in this study were MDR and displayed an AmpC-producing phenotype in the absence of pAmpC production suggesting cAmpC production in isolates belonging ST88. These findings mirror those previously reported in Europe suggesting cAmpC production in *E. coli* is commonly linked to isolates in the ST23 complex, including those belonging to ST88 in humans and animals (Crémet et al., 2010; Guillouzouic et al., 2009; Ortega et al., 2012). Four *E. coli* O8:H17 ST88 isolates (D341, D355, D362 and D396), harbouring the same IncF F67:A6:B8 plasmid replicon types and carrying identical resistance and virulence gene profile were recovered from four dogs within the same establishment, with a fifth O8:H7 ST88 isolate displaying the same profile recovered from an animal in a separate location. This finding indicates the presence of a MDR clone harbouring both chromosomal and plasmid mediated resistance mechanisms transmitted between dogs in close contact, which may also have been disseminated to the wider canine population.

Evidence of potential transmission of AMR *E. coli* between animals was identified in further premises with MDR *E. coli* O9:H9 ST62 isolates harbouring the same plasmids, resistance and virulence genotypes recovered from five dogs on the same premises. A further two dogs on an additional premises carried cAmpC-producing *E. coli* O91:H7 ST4421 with identical resistance and virulence genotypes associated with plasmid replicon IncI1ST3 plasmid carriage. These finding indicate the kennel environment may provide a suitable environment for the dissemination of AMR *E. coli* in the canine population

Of additional interest within this study, may be the potential dissemination of ST372 between dogs and humans within the same premises. In addition to the potential dissemination of ST372 between dogs discussed above, two further isolates, one human (H669) and one canine (D699), originating from a single boarding establishment were identified as ST372 and harboured an IncI1 ST23 plasmid replicon carrying the same resistance genes, as well as displaying the same resistance phenotype. There were however, some discrepancies between the two isolates; examination of serotypes indicated that the canine isolate was serotype O2:H31, while the human isolate was serotype O-:H31. Similarly while both isolates harboured *cnf1*, *iroN*, *iss*, *mcmA* and *mch* genes, the canine isolate additionally harboured *gad*, while, the human isolate harboured *vat*. These discrepancies may indicate these isolates were two distinct isolates, or they may indicate a lack of sequencing coverage of the *fliC* and *gad* genes in the human isolate and *vat* in the canine isolate. The finding of isolates belonging to the same H serogroup and ST, harbouring the same plasmid and carrying the same resistance genes does provide some evidence for the potential for transfer of a pAmpC-producing *E. coli* between dogs and humans on the same premises. This therefore highlights the potential for dogs to act as a reservoir of AMR isolates to in-contact humans. Data on transmission of *E. coli* between dogs and humans is limited but has previously been identified, with sharing of *E. coli* in up to 17% of households (Carvalho et al., 2016; Damborg et al., 2009; Harada et al., 2012; Johnson et al., 2008a; Ljungquist et al., 2016). This indicates the sharing of isolates within households may be higher than reported within kennel environments in this study. Indeed, despite the high density of dogs within kennel establishments, levels and type of contact between animals and staff may differ to those in households leading to reduced potential for inter-species bacterial transfer. An additional explanation for this reduced transmission may be increased levels of hand hygiene within the workplace. Previous studies have highlighted the potential role of hand hygiene in the prevention of inter-species transmission of *E. coli* (Damborg et al., 2009; Stenske et al., 2009) and individuals may be more conscious of hygiene protocols after handling animals in the workplace.

The sharing of isolates between dogs and humans identified in this study should be viewed with caution. Samples were taken from participants at a single time point and as such, temporal relationships or directionality of transfer cannot be examined. Furthermore, it is possible that the isolates were obtained from a shared source such as food, or the environment (Manges et al., 2007; Manges and Johnson, 2012; Randall et al., 2011; Randall et al., 2014), rather than transmitted between participants. With the exception of two

premises, only isolates showing resistance to 3GCs or MDR phenotypes were subjected to WGS. As such, this study may have underestimated the potential for transfer of *E. coli* between species by omitting non-MDR isolates. Additionally, no comment can be made on the complete picture of commensal *E. coli* carried by healthy dogs and humans or transfer of these isolates between individuals. Further longitudinal studies may help to provide additional data on the direction of transfer of *E. coli* between species as well as the role transient commensal strains may play in the dissemination of AMR *E. coli* between species (Tenailon et al., 2010). Additionally, environmental sampling may also provide information on possible shared sources of AMR *E. coli* within premises.

The results of this study indicate that commensal *E. coli* of canine and human origin share similar resistance phenotypes. Isolates belonging to all *E. coli* phylogroups were identified in both host species, however the most prevalent *E. coli* phylogroup in canine isolates was B1, while phylogroup B2 was most prevalent in humans. Within this population, carriage of ESBL or pAmpC-producing *E. coli* does not appear to be associated with widespread dissemination of specific resistant clones. However, the role of plasmid replicons such as IncI1 ST23, IncI1 ST3 and IncX3 in the dissemination of *bla*_{CMY-2}, *bla*_{CTX-M-1} and *bla*_{SHV-12}, respectively in the canine population does warrant further investigation. Similar mechanisms of resistance were identified in isolates of canine and human origin highlighting the potential for dogs to act as a potential reservoir of resistance genes. Furthermore, the potential inter-species transmission of pAmpC-producing *E. coli* is of concern and warrants further investigation.

Chapter 7

Concluding Discussion

7.1 Discussion

The global disease burden attributable to antimicrobial resistance is considered a significant public health concern (Woolhouse et al., 2016), impacting both human and veterinary medicine (Levy and Marshall, 2004; Wieler et al., 2011). Clinically, the appearance of isolates resistant to critically important antimicrobials, including third generation cephalosporins (3GC) and fluoroquinolones (OIE, 2015; WHO, 2017), has led to reduced treatment efficacy, with associated increases in patient morbidity and mortality (Melzer and Petersen, 2007). The impact of antimicrobial resistance, therefore, has multiple direct and indirect medical, economic and societal costs (Cosgrove and Carmeli, 2003; Cosgrove, 2006; Roberts et al., 2009; Taylor et al., 2014). The potential role of livestock, in the development and dissemination of antimicrobial resistance has previously been acknowledged (Khachatourians, 1998; Larsen et al., 2016; Marshall and Levy, 2011; Spoor et al., 2013). However, companion animals have also been highlighted as a potential source of antimicrobial resistance determinants (Schmidt et al., 2015; Tuerena et al., 2016; Wedley et al., 2011; Wedley et al., 2017). As such, resistance in both humans and animals should be considered in conjunction, in order to address the issue effectively (O'Neill, 2016).

While a considerable amount of research and surveillance has been previously undertaken in order to investigate the impact of antimicrobial-resistant (AMR) and extended-spectrum β -lactamase (ESBL)-producing *E. coli* in humans in the UK (Farrell et al., 2003; Lau et al., 2008; Livermore et al., 2008; PHE, 2015; Woodford et al., 2007), there is currently a paucity of information regarding faecal carriage of these bacteria in asymptomatic human populations. In contrast, more information is available regarding faecal carriage in UK dogs, where the reported prevalence of AMR *E. coli* ranges from 29.0-63.0% (Schmidt et al., 2015; Tuerena et al., 2016; Wedley et al., 2011; Wedley et al., 2017). However, to the author's knowledge, no UK studies have investigated carriage of AMR *E. coli* in dog dense environments such as kennels. In addition, given the reported sharing of *E. coli* isolates between humans and animals in close contact (Damborg et al., 2009; Johnson et al., 2008a; Johnson et al., 2008b), the potential for inter-species transmission of AMR *E. coli* and associated resistance determinants warranted further investigation.

The research undertaken in this study comprised three main objectives; to determine the prevalence of faecal carriage of AMR and ESBL-producing *E. coli* by kennelled dogs and humans working with dogs in clinical and non-clinical environments; to identify risk factors for this carriage in each population of interest; and finally, to characterise and subsequently

compare isolates of canine and human origin. In order to achieve these aims, two concurrent cross-sectional studies were undertaken, sampling kennelled dogs and humans within the same premises. This work involved recruitment of 229 human and 296 canine participants across 69 premises in the North of England, within a 75 mile radius of the University of Liverpool, Leahurst Campus.

Within the human population sampled, the prevalence of carriage of ESBL-producing *E. coli* was 3.1%, markedly lower than the 11.3% sample prevalence reported in a UK study by Wickramasinghe et al. (2012). However, the selection of medical patients as participants, alongside the ethnically diverse nature of the population sampled by Wickramasinghe et al precludes direct comparison with this study. Out with the UK, faecal carriage rates of 0.6-7.6% within the general human population have been identified (Leflon-Guibout et al., 2008; Meyer et al., 2012; Reuland et al., 2016; Rodrigues et al., 2016), mirroring the findings reported here. Additional studies investigating carriage rates in individuals working with animals in northern Europe have reported a largely increased prevalence of 2.5-19.1% (Dahms et al., 2015; Hammerum et al., 2014; Huijbers et al., 2014; Schmithausen et al., 2015). Participants in these studies all worked within the livestock sector, which does not offer direct comparison to those working with companion animals, but does provide evidence of the potential increased risk of carriage associated with high levels of animal contact. Given the current absence of UK data regarding faecal carriage of ESBL-producing *E. coli* in the general population it is difficult to comment on any potential increase in prevalence in the population sampled here. Ideally, a control population of individuals without canine contact would also have been recruited, however challenges of identification and recruitment of this population precluded its inclusion in the study.

Interestingly, the sample prevalence of 3GC-resistant *E. coli* was higher in kennelled dogs (9.7%) than humans (4.2%). Within the human population carriage of 3GC-resistant *E. coli* was lowest in humans working in clinical environments, when compared to those working in kennel premises. This finding may indicate that differing work environments and the nature of contact with animals and their faeces does impact on carriage rates. The prevalence of faecal carriage of ESBL-producing *E. coli* in kennelled dogs was however much lower than that reported for the human population sampled and indeed other studies investigating healthy dogs (Damborg et al., 2015; Wedley et al., 2011), indicating differences in the molecular epidemiology of resistance to 3GCs in the humans and dogs. This may therefore

suggest that, in the population sampled, dogs may not present a significant reservoir of ESBL-producing *E. coli* and as such the risk associated with canine contact may be low.

While a low prevalence of ESBL-producing *E. coli* was identified, carriage of AMR isolates was high in both human (68.7%) and canine (58.9%) populations. In both populations, resistance to ampicillin, trimethoprim or tetracycline was most frequently identified, as has previously been reported (Costa et al., 2008; De Graef et al., 2004; Nijsten et al., 1996; Schmidt et al., 2015; van den Bogaard et al., 2001; Wedley et al., 2011), indicating similar selection pressures and potential co-selection of resistance determinants in both host species. Ampicillin-resistant *E. coli* were present in samples from over half of the canine and human participants, which is not unexpected given that β -lactams are the most frequently prescribed antimicrobial class in both populations (PHE, 2015; Singleton et al., 2017). In addition, the previously reported finding that carriage of *bla*_{TEM-1}, offers minimal fitness cost to *E. coli* may offer an explanation as to its persistence within the intestinal niche (Karami et al., 2008), and the high prevalence of this gene in Gram-negative bacterial populations (Bradford, 2001). Of note however, was the increased prevalence of resistance to amoxycylav in dogs (10.6%) when compared to in-contact humans (3.1%). This finding highlights that while β -lactam use is prevalent in both populations, prescribing practices differ. Within small animal veterinary practice amoxycylav is the most frequently prescribed antimicrobial drug for use in dogs (Hughes et al., 2012; Radford et al., 2011; Singleton et al., 2017), while in human medicine this drug is not the first line antimicrobial of choice for primary care physicians and is reserved for emergent cases (PHE, 2015; PHE, 2016), leading to an increased selection for resistance to amoxycylav in canine populations over humans.

Statistical analyses undertaken in order to assess risk factors for carriage of antimicrobial resistance within the populations studied utilised mixed effects logistic regression models, with premises included as a random effect in order to account for clustering of individuals at this level. Due to the low prevalence of ESBL-producing *E. coli* in both dogs and humans, risk factors for this outcome could not be assessed. Similarly, the prevalence of ciprofloxacin resistance in dogs and amoxycylav resistance in humans precluded further analysis. For the outcomes analysed, results indicated the potentially complex interactions between host species and their environment, which may contribute to the carriage of resistant *E. coli*. Interestingly, the amount of variance attributable to the premises varied between the populations of interest. In most cases, the ICCs calculated were found to be higher in dogs than in the human population sampled. This finding may be due to multiple factors. Firstly,

the animals sampled were housed within the premises recruited in this study and would therefore be exposed to the same management factors, while humans in the study would only spend a defined period of their day within their place of work. These individuals would therefore have similar exposures in their work environment but may have differing contact and exposures in their home environment. Secondly, kennel environments are generally areas of high dog density which may facilitate increased contact and dissemination of *E. coli* between dogs, which is less likely to occur between smaller numbers of humans working together. Finally, dogs housed within a single environment such as a rescue centre may be treated as a single population especially during outbreaks of infectious disease, leading to similar selection pressures for the development of resistance in animals kennelled together.

While there was some variation in the risk factors associated with each outcome in different host species, some interesting patterns did emerge. Admittance to veterinary or human hospitals was a risk factor for multiple outcomes, including tetracycline and trimethoprim resistance in both species. This finding mirrors previous reports in multiple species (Luvsansharav et al., 2012; Maddox et al., 2011; Wedley, 2012), and can be attributed to nosocomial spread of resistant organisms and increased antimicrobial exposure in the hospital environment. In addition, while recent hospitalisation was a risk factor for carriage of resistance to 3GCs in dogs, so was being owned by someone who works in a human hospital. In addition, having received any kind of recent veterinary treatment was highlighted as a risk factor for numerous canine outcomes. These findings highlight the potential role of both human and veterinary facilities in the dissemination of AMR *E. coli* including hospital-acquired ESBL and AmpC-producing strains to multiple species in the wider community. Previous studies have identified the role of human healthcare facilities in the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in dogs (Boost et al., 2008; Soares Magalhães et al., 2010). The identification that owner contact with human healthcare facilities is a risk factor for canine carriage of 3GC-resistant *E. coli*, further emphasises that inter-species transmission of resistance is not unidirectional and humans may act as reservoirs of resistance to animal species.

The risk associated with recent antimicrobial treatment was variable, indeed for most outcomes investigated in this study exposure to antimicrobials was not significant. This finding further highlights that while antimicrobial treatment provides a direct selection pressure for the development of antimicrobial resistance (Blair et al., 2014; Jakobsson et al.,

2010), additional environmental factors are also important within the populations of interest.

The diet of dogs, and to a lesser extent humans, was identified as a significant risk factor for carriage of resistant *E. coli*. Raw food diets were associated with multiple resistance outcomes in dogs including multi-drug resistance as previously described (Schmidt et al., 2015; Wedley et al., 2017). Commercially produced raw diets and retail meats can harbour resistant *Enterobacteriaceae* (Finley et al., 2008; Johnson et al., 2007), which may then be able to colonise the gut of the consumer or transmit resistance determinants to resident microbial strains. In humans, regular consumption of specific meats, namely pork and poultry, were associated with carriage of resistance. The intensive production systems utilised by the pig and poultry industry have increased antimicrobial use per animal when compared to more extensive systems (VMD, 2016), and findings appear to indicate that contamination of meat products with resistant bacteria is occurring. These resistant isolates may survive the cooking process or result in cross contamination of surfaces in the kitchen and as such, acceptable microbial contamination levels of meat (FSA, 2016) may need to be revisited. Additionally, given the linkage between canine contact with farm animals or an owner working with farm animals and antimicrobial resistance identified in this study, further investigation into on-farm antimicrobial use in multiple livestock sectors should be undertaken.

Investigation of participant demographics was challenging when considering human participants in this study. Due to the anonymity afforded to participants, certain demographic data was not collected. There was, however, no association between age and carriage of AMR *E. coli* in humans or dogs. While this supports previous findings in dogs (Schmidt et al., 2015; Wedley et al., 2017), there is currently a lack of data on the impact of age on carriage of non-ESBL AMR *E. coli* in humans with which to compare. Previous studies have suggested increasing age is a risk factor for carriage of ESBL-producing *E. coli* in humans, with those aged over 65 at particular risk (Östholm-Balkhed et al., 2013; Toner et al., 2015). The sampling strategy used in this study selected for people of working age and, as such, a lack of participants over 65 may mean there was not sufficient statistical power to detect this effect. An additional demographic of interest was the type of premises participants were located on, with staff working at rescue kennels at most risk of carriage of ciprofloxacin-resistant *E. coli*. This finding was mirrored in the total canine population to some extent, as dogs originating from rescue centres were found to be at increased risk of carriage of

multiple resistance outcomes including quinolone resistance. Given many dogs within a rescue environment may have been surrendered by their owners with unknown histories, the proportion of dogs with chronic ongoing conditions including refractory infections may be increased in this population. As such, dissemination of multi-drug resistant (MDR) isolates including those demonstrating resistance to quinolones may be higher and zoonotic transfer may occur during handling. Further research into the dynamics of antimicrobial resistance within rescue centres is therefore warranted.

Characterisation of *E. coli* within this study was undertaken on multiple levels; all isolates underwent phylogenetic grouping, while resistant isolates additionally underwent PCR in order to assess carriage of plasmid-mediated β -lactamase and quinolone resistance genes. All isolates showing resistance to at least one 3GC or at least four antimicrobial classes, alongside all isolates from two premises with high levels of compliance were subjected to whole genome sequencing (WGS).

The commensal *E. coli* population is influenced by numerous factors including diet, climate and levels of hygiene (Escobar-Páramo et al., 2004; Tenaillon et al., 2010). In the canine population, isolates belonging phylogroup B1 predominated, while in humans isolates belonging to group B2 were most prevalent. This mirrors findings previously reported highlighting the propensity for colonisation of the human gastrointestinal tract by extraintestinal pathogenic *E. coli* (ExPEC) (Damborg et al., 2009; Escobar-Páramo et al., 2006; Gordon et al., 2005), while group B1 isolates are the most prevalent in dogs (Damborg et al., 2009; Tenaillon et al., 2010). In contrast to previous studies, which suggest a reduced prevalence of group B1 compared to group A isolates in human populations (Escobar-Páramo et al., 2006; Tenaillon et al., 2010), the findings of this study indicate the converse. Increased similarity in commensal *E. coli* between humans and in-contact animals has previously been reported (Harada et al., 2012), and may be due to both shared environmental sources and inter-species transmission. The current absence of data regarding commensal *E. coli* in the UK human population precludes any comparison with data collected during this study, and highlights need for further recruitment of a control population in order to investigate the findings further. Canine isolates belonging to phylogroup B2 were associated with a reduced prevalence of multi-drug, tetracycline and nalidixic acid resistance, conversely canine isolates belonging to phylogroup C and human isolates belonging to phylogroup D were more likely to display resistance to quinolones and have a MDR phenotype mirroring previous findings in human and canine studies (Johnson et al., 2009a; Massot et al., 2016; Schmidt et

al., 2015). This suggests the phylogenetic background of isolates may relate to the propensity of a bacterium to accept resistance plasmids and influence chromosomal mutation rates which negate resistance-associated fitness costs (Andersson and Hughes, 2010).

The commensal *E. coli* population identified within this study demonstrated a high level of diversity with a large number of STs identified, and while the most prevalent ST differed between species, many were identified in both humans and dogs. Examination of isolates harbouring β -lactamase genes identified more than 40 virulence-associated genes. The virulence genes identified varied between isolates of different phylogenetic groups, with those belonging to group B2 more likely to carry ExPEC associated genes including *iss* and *iroN* respectively linked with complement resistance and iron acquisition (Bäumler et al., 1998; Johnson et al., 2008c). In contrast *lpfA*, associated with increased epithelial adhesion, was more commonly identified in B1 isolates (Afset et al., 2006; Dogan et al., 2012). The evolution of these virulence genes in commensal strains has been attributed to other potential roles, including successful gut colonisation (Johnson et al., 2008a; Sheng et al., 2006; Wold et al., 1992). The carriage of these genes does, however, present the potential for commensal *E. coli* to cause opportunistic infections or act as a source of virulence determinants to other strains (Bingen et al., 1998; Packey and Sartor, 2009)

Characterisation of β -lactamase genes harboured by isolates highlighted the importance of *bla*_{TEM} genes in conferring resistance to broad spectrum β -lactams, including ampicillin (Bradford, 2001; Livermore, 2003). Indeed, of the 432 β -lactam resistant isolates recovered in this study 73.1% were found to harbour a *bla*_{TEM} gene during PCR examination. Of the 79 isolates which were resistant to amoxycylav, 45.5% harboured plasmid-mediated *bla*_{AmpC} genes, indicating the importance of additional β -lactamase inhibitor resistance mechanisms. Indeed, a further 27 isolates were identified as cAmpC producers. An additional 12 isolates harboured *bla*_{TEM} (n=11) or *bla*_{OXA} (n=1) genes, which may be responsible for this resistance phenotype. Furthermore, one of these isolates underwent WGS and was found to carry the inhibitor-resistant *bla*_{TEM-33} gene. Further sequencing of the *bla*_{TEM} genes from amoxycylav-resistant isolates will provide further information about the importance of inhibitor-resistant TEM (IRT) enzymes within the population studied.

Examination of the molecular epidemiology of isolates resistant to 3GCs identified an interesting contrast between the human and canine populations studied. In humans, the predominant mechanism for this resistance was the production of ESBL enzymes, while in dogs AmpC production predominated supporting previous studies of community human and

dog populations in the UK and Europe (Castillo et al., 2013; Hammerum et al., 2011; Schmidt et al., 2015; van Hoek et al., 2015; Wedley et al., 2011). The increased use of amoxycylav in canine populations when compared to humans may provide an explanation for this contrast (PHE, 2015; Singleton et al., 2017). Indeed, increased use of amoxycylav in human hospitals has been linked with an increased prevalence of AmpC-producing isolates within the patient population (Seiffert et al., 2013a).

Within the human population sampled, production of CTX-M enzymes was the most frequently identified mechanism of ESBL-mediated resistance, with *bla*_{CTX-M-15} most prevalent mirroring previous findings in healthy individuals and community patients in the UK and Europe (Geser et al., 2012b; Reuland et al., 2016; Wickramasinghe et al., 2012). The CTX-M-15-producing isolates were recovered from individuals working on all premises types sampled and while one isolate was identified as *E. coli* O16:H5 ST131, no isolates belonged to the pandemic O25b:H4 ST131 clone. Furthermore, the isolates belonged to four different *E. coli* STs and were found to harbour different plasmids suggesting the presence of different resistance plasmids in different locations rather than predominance of a single clone in this population.

In dogs, an equal number of ESBL-producing isolates harboured *bla*_{SHV-12} and *bla*_{CTX-M} genes. All of the *bla*_{SHV-12} genes were associated with IncX3 plasmids, which have previously been found to harbour *bla*_{SHV-12} in isolates originating from livestock and wastewater in Europe (Alonso et al., 2017; Dobiasova and Dolejska, 2016). Four of the five isolates harbouring *bla*_{SHV-12} were recovered from three animals on the same premises and two of the isolates belonged to *E. coli* ST3947 and displayed the same resistance phenotype and genotype, suggesting possible isolate transmission between individuals. The recovery of isolates harbouring IncX3-associated *bla*_{SHV-12} from multiple isolates on the same premises may additionally suggest horizontal transmission of an IncX3 plasmid or the presence of multiple IncX3 plasmids harbouring this gene, which warrants further investigation. The importance of *bla*_{SHV-12} in this population should not be overstated, given its predominance at a single premises. Of the CTX-M-producing isolates *bla*_{CTX-M-1} associated with Inc11 ST3 plasmids predominated, concurring with the global picture in dogs where this Inc11 ST3 plasmid-associated gene predominates (Dahmen et al., 2012; Grami et al., 2013; Haenni et al., 2014). Additionally, one human isolate was also found to carry *bla*_{CTX-M-1} associated with Inc11 ST3, and may suggest the potential for increased contact with animals to result in the transfer of animal associated plasmids into the human population (Madec et al., 2015).

The predominant plasmid-mediated AmpC (pAmpC) enzyme identified in isolates of canine and human origin in this study was CMY-2, previously reported as the principle pAmpC enzyme mediating resistance in canine and human populations (Garrido et al., 2014; Reuland et al., 2015; Tuerena et al., 2016; Wedley et al., 2011). The *bla*_{CMY-2} genes within this study were frequently associated with IncI1 ST23 plasmids in all premises types sampled, suggesting that horizontal transmission of this plasmid type may be largely responsible for the dissemination of *bla*_{CMY-2} within kennelled dogs in the North of England. In addition, four canine isolates belonging to *E. coli* ST372 were found to harbour IncI1 ST23-associated *bla*_{CMY-2}, two of which were isolated from dogs on the same premises and harboured the same resistance and virulence genotype. The additional two isolates were recovered from separate premises and harboured IncI1 ST23-associated CMY-2 alone. These findings suggest the potential circulation of multiple pAmpC-producing *E. coli* ST372 clones circulating within the kennelled dog population in the UK and further investigation of this may be necessary. Of particular additional concern, was the identification of potential transfer of a CMY-2-producing *E. coli* ST372 isolate between host species on the same premises. Previous studies have highlighted the transmission of *E. coli* between household members and pets with high levels of contact (Damborg et al., 2009; Harada et al., 2012; Johnson et al., 2008a). However, the identification of potential transmission of pAmpC-producing isolates between humans and dogs is of concern. Further examination of inter-species transmission of resistant *E. coli* is required, and should include longitudinal studies in order to assess the directionality of transfer.

Further evidence of transmission of non-ESBL or pAmpC-producing isolates was identified in this study including MDR clones belonging *E. coli* ST62 and ST88 which were isolated from multiple samples from dogs on Premises 7 and 9, respectively. These findings highlight not only the importance of ESBL and pAmpC enzymes as a cause of resistance in the canine population, but the potential roles of different AMR clones on different premises. In addition, potential transmission of non-MDR isolates was identified between staff working on the same premises. This finding suggests the potential for further dissemination of strains or resistance determinants to additional, in-contact individuals within the wider community, not sampled within this study.

Several limitations have been identified in this study. The selection of a limited geographical area for this study and the lack of control population mean that the findings cannot be generalised to the UK population of kennelled dogs and people working with dogs. Due to

the need for multiple visits to each premises in order to ensure human and canine samples were collected over the same time frame, it was not practical to extend the study over a larger area. Additionally, the lack of a human control population means no comparison can be made to individuals without canine contact in order to fully assess the risk of carriage of AMR and ESBL-producing *E. coli* associated with canine contact.

The sampling strategy employed, necessitated recruitment of canine and human participants within the same premises and may therefore have introduced selection bias. Despite random selection of premises, the aversion of many of the premises contacted to provide human faecal samples resulted in only 19.0% of those contacted agreeing to participate, which is much lower than previously reported for veterinary studies requesting animal faecal samples (Maddox et al., 2012; Wedley, 2012). This may mean that individuals within the participating premises had a higher level of interest in antimicrobial resistance or scientific research and may not be completely representative of the study population. Furthermore, 97 fewer human participants were recruited to the study than initial sample sizes suggested were required. The level of compliance associated with requests for human faecal samples is commonly poor (Hynam et al., 1995) and medical patients have identified embarrassment and hygiene issues as potential barriers to sample collection (Lecky et al., 2014). Multiple methods were employed to alleviate concerns related to these factors, including anonymous sampling and use of swabs for sampling, previously identified as the preferred method of sampling by Ellis et al. (2007). In total, 32.9% of individuals on participating premises were recruited to this study, an improvement to the 25.0% response rate reported by Reuland et al. (2016), which may be indicative of improved compliance due to the sampling methods utilised. The recruitment of a larger number of premises and subsequent reduced cluster sizes may, to some extent, have mitigated for the reduced sample size in the study, and as such, there can still be confidence that the prevalence of ESBL-producing *E. coli* in this population is low.

Within the canine study, the request for kennel staff to recruit dogs may not have resulted in a truly random selection of canine participants. While recruitment of kennelled dogs by the author may seem preferable in order to ensure random selection of participants, this would have proved logistically difficult. The comparison of signalment data from the dogs recruited in this study does, however, equate to those reported previously (Schmidt, 2014; Wedley, 2012) and as such appear representative of the UK canine population. Due to the

anonymity afforded to human participants, demographic data for this population could not be compared with the UK population.

The collection of only a single sample highlights another potential limitation of this study. Previous investigations into carriage of AMR and ESBL-producing *E. coli* have indicated that duration of carriage and faecal shedding is variable (Alsterlund et al., 2012; Schmidt, 2014; Tandé et al., 2010), as such a negative sample on a single occasion may not preclude the participant from carrying resistant *E. coli*. While repeated sampling of individuals would be preferable, this is not logistically possible in studies with a high number of participants.

The standardised microbiological methods used within this study have been previously validated and as such there can be confidence in recovery of resistant isolates when present. One area for further development may however be in methods used for DNA extraction and WGS. Despite using commercially available DNA extraction and sequencing kits, there was a potential lack of sequencing coverage of some virulence and serotyping genes noted for some isolates. A Nanodrop spectrophotometer (Thermo Fisher Scientific, Cheshire, UK) was used to quantify and assess the purity of the DNA extracted, however additional methods including utilisation of a Qubit fluorometer (Thermo Fisher Scientific, Cheshire, UK) and PCR in order to ensure DNA integrity, may have been of additional use prior to sequencing (Kapp et al., 2015; Simbolo et al., 2013). Due to the agreement of PCR and WGS methods in the detection of *bla* genes, there can however be confidence in the detection of these resistance genes.

7.2 Further work

This study has provided novel information regarding the prevalence of AMR *E. coli* including ESBL-producing isolates in kennels dogs and people working with dogs. However, there is still a considerable amount of evidence which could be provided by additional research. The current absence of data regarding faecal carriage of ESBL-producing *E. coli* by the UK general population made comparisons with this study challenging. An additional cross-sectional study in the UK is therefore warranted.

Risk factors were identified for numerous outcomes but the low prevalence of faecal carriage of ESBL-producing *E. coli* in both species alongside ciprofloxacin and amoxycylav-resistant isolates in dogs and humans, respectively, precluded analysis of these outcomes. Given the importance of 3GCs and fluoroquinolones in global medicine (WHO, 2017), further investigation of these outcomes involving a larger cross-sectional or case-control study

should be considered. In addition, given the importance of both farm animals and diet in carriage of AMR *E. coli* highlighted in this study, further investigation of the role of food in transmission of resistant isolates may be needed. Longitudinal studies sampling at multiple stages throughout the food chain may provide further information and highlight potential areas of transmission or contamination which can be addressed.

Additional molecular characterisation of isolates in this study would help to provide further information regarding the role of specific plasmids in the dissemination of resistance. While WGS identified plasmid replicons and plasmid sequence types carried by isolates, multiple replicons were identified for a large number of isolates. Replicon typing and pMLST of conjugative plasmids would therefore allow further identification of specific plasmids associated with horizontal transfer of *bla*_{ESBL} and *bla*_{AmpC} genes. Further assessment of some resistance genes may also be justified. Although all isolates resistant to 3GCs underwent WGS, the mechanism responsible for amoxyclav resistance in more than half of isolates was not elucidated. Sequencing of *bla*_{TEM} genes carried by amoxyclav-resistant isolates will provide further information on the molecular epidemiology of resistance to this drug.

While potential transmission of *E. coli* between humans and dogs was identified in this study, it may have been underestimated due to the sequencing of all isolates from two premises only, alongside 3GC and MDR isolates. Sequencing of further isolates from multiple premises may, therefore provide additional information about circulating strains in dogs and humans and the potential for transmission of isolates between host species. Within this study samples were collected at only a single time point, and no information can be provided about directionality of transfer or the potential role of shared environmental sources of *E. coli*. Hence, further longitudinal studies are warranted, which sample animals, humans and their environments, in order to gain a more in depth picture of *E. coli* transmission dynamics. Inclusion of animals and humans in the home environment, where frequency and levels of contact are likely increased would help to ensure this transmission is not underestimated.

7.3 Conclusion

The findings presented in this study indicate a high prevalence of faecal carriage of AMR *E. coli* in both humans and dogs. However, carriage of resistance to 3GCs was low in both populations. While similar mechanisms of resistance were largely identified in both species, the predominant mechanisms responsible for 3GC resistance differ, with *bla*_{ESBL} genes predominating in humans, while *bla*_{AmpC} genes are most prevalent in dogs. Carriage of these

genes in the population studied does not appear to be associated with widespread transmission of specific resistant clones. However, the identification of possible transmission of pAmpC-producing *E. coli* between dogs and in-contact humans highlights the potential for dogs to act as a reservoir of resistance genes. The identification of numerous healthcare, animal and environment-associated risk factors emphasises the complex epidemiology of antimicrobial resistance. Indeed, this study highlights the need for a multifaceted, cohesive approach to this global issue, to which all stakeholders must acquiesce.

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Appendices

Appendix I

Material pertaining to Chapter Three



Participant Information Sheet

Human contact with companion animals and the risk of acquiring antibiotic-resistant bacteria.

You are being invited to participate in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve.

Please read the following information carefully and feel free to ask us if there is anything you do not understand.

What is the purpose of the study?

Antibiotic resistance has become an increasing problem in human medicine, and these resistant bacteria have been highlighted in recent years as having an increasing effect on hospital-acquired infections and in the community worldwide. Infections involving these bacteria are commonly multidrug resistant, leading to reduced treatment options and increased mortality.

People have frequent and close contact with companion animals and, given that antibiotic-resistant bacteria have already been identified in both hospitalised and non-hospitalised animals, they may act as an important link in the transfer of antibiotic resistance. Currently there is little data available on the risk of carriage of antibiotic-resistant bacteria in humans in contact with companion animals, therefore the objectives of this study are to determine the carriage rates in these individuals and to assess factors which affect this carriage. This information will allow us to identify potential measures which may reduce the potential for spread of antibiotic resistance.

Why have I been chosen to take part?

You have been contacted as your working environment involves regular contact with dogs.

Do I have to take part?

Participation in this study is voluntary and participants are free to withdraw from the study at any time.

What will happen if I take part?

If you agree to take part in the study we will ask you to provide us with a faecal sample and fill in a short questionnaire. All samples and the questionnaire will be anonymised so that you cannot be identified. **By providing a faecal sample and filling in a questionnaire you are providing informed consent for your data to be included in the study.**

Faecal samples will undergo microbiological testing to identify any antibiotic-resistant bacteria present in the sample, followed by further testing to identify the genes responsible for this resistance. Data from questionnaires will be entered into a secure database and analysed to identify any risk factors for carriage of resistant bacteria.

Are there any risks or benefits in taking part?

No personal risks or benefits will be experienced by participants taking part in this study. Benefits of the study are provision of increased information about the potential role animals play in antibiotic resistance and the risk factors for carriage of resistant bacteria. This will further understanding about the spread of antibiotic resistance and therefore help in the development of potential prevention strategies.

Will my participation be kept confidential?

Participation in the study is confidential. All data will be anonymised and entered into a database which will be securely stored and password protected on university computers for up to 5 years. Access to data from this study will be available only to investigators involved in the project.

What will happen to the results of the study?

We aim to publish the results of this study in an appropriate scientific journal. Within any published articles no participants will be identifiable from the results.

As samples are anonymised we are unable to provide participants with their individual results. We will however, provide each premise with a copy of the report at the end of the study.

What will happen if I want to stop taking part?

Participants are free to withdraw from the study at any time. If you wish to withdraw from the study please contact Mrs Emma Ormandy quoting your unique reference number. This will allow us to remove your sample and any data provided from the study.

What should I do if there is a problem?

If you are unhappy, or if there is a problem, please feel free to let us know by contacting Dr Nicola Williams on 0151 795 6051 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 at 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 researchers involved and the details of the complaint you wish to make.

Who can I contact if I have further questions?

Mrs Emma Ormandy
School of Veterinary Science
University of Liverpool
Neston
CH64 7TE
0151 795 6040
E.E.Ormandy@liverpool.ac.uk

Thank you again for your time.



Human Contact with Companion Animals and the Risk of Acquiring Antibiotic-resistant Bacteria Questionnaire

As part of the research into the potential role companion animals' play in the spread of antibiotic resistance we are asking people who work with dogs to provide us with a faecal sample and fill in this questionnaire.

Your participation in this study will help us to better understand the role companion animals play in the spread of antibiotic resistance.

Completion and return of this questionnaire alongside provision of a faecal sample will be taken as informed consent for the use of the data in our study as described in the accompanying letter.

Participation in this study is entirely voluntary and all responses will remain anonymous and completely confidential.

All information from this questionnaire is strictly confidential and will be available only to the investigators. All data is anonymised and no individuals will be identifiable from published data.

Answering the questions

This questionnaire consists of the three short sections

Section one: Information about you

Section two: Information about your work

Section three: Information about your home environment

Please answer all questions by marking an in the box or by writing in the boxes provided using BLOCK CAPITAL letters.

E.g. Have you received antibiotic treatment within the last 6 months?

Yes No Don't know

If you make a mistake or change your answer please fill in the first box completely and put a clear cross in the correct box.

Once completed please return the questionnaire alongside your faecal sample using the pre-paid envelope provided.

If you have any questions or queries please contact

Mrs Emma Ormandy
School of Veterinary Science
University of Liverpool
Neston
CH64 7TE
0151 795 6040
E.E.Ormandy@liverpool.ac.uk

ABOUT YOU

1. How old are you?

- 16-25 years 26-35 years
 36-45 years 46-55 years
 56-65 years Over 65 years

2. Have you received any antibiotic treatment within the last 6 months?

- Yes No Don't know

If yes, when was the most recent treatment course?

- Within last month
 2-3 months ago
 Over 3 months ago

Please indicate antibiotic treatment (e.g. penicillin) if known:-

3. Have you been hospitalised within the last 6 months?

- Yes No Don't know

If yes, how long ago?

- Within last month
 2-3 months ago
 Over 3 months ago

4. When did you last travel outside the UK?

- Within last 3 months 10-12 months ago
 4-6 months ago Over 12 months ago
 7-9 months ago

Please indicate where you travelled to on this occasion?

ABOUT YOUR WORK

5. How long have you worked here?

Years Months

6. What is your role?

Staff Volunteer

7. Do you work here full-time?

Yes No prefer not to say

8. Which animals do you come into direct contact with at work on a daily basis?
(Tick any boxes which apply)

Dogs Horses
 Cats Farm animals (please specify) _____
 Small mammals(e.g. hamster) Other (please specify) _____

9. Please specify the percentage of your time at work spent on the following:

Direct contact with dogs	<input type="text"/>	Office work	<input type="text"/>
Direct contact with other animals	<input type="text"/>	Cleaning dog kennels	<input type="text"/>
Reception work	<input type="text"/>		

Other (please specify)

YOUR HOME ENVIRONMENT

10. Has any member of your household been hospitalised within the last 6 months?

Yes No Don't know

11. Do you have direct contact with companion animals at home?

Yes No

If yes, which animals do you come into contact with?

(Tick any boxes which apply)

Dogs Horses
 Cats Other (please specify) _____
 Small mammals (e.g. hamster)

12. Do you have direct contact with farm animals at home?

Yes No

If yes, which animals do you come into contact with?

(Tick any boxes which apply)

Dairy cattle Pigs
 Beef cattle Poultry
 Sheep Other (please specify) _____

13. Do you eat meat?

Yes No

If yes, please indicate which meat you eat three or more times weekly

(Tick any boxes which apply)

beef poultry
 lamb fish
 pork other (please specify) _____

14. Do you eat salad leaves three or more times weekly?

yes no

We welcome any feedback from our participants.

Please indicate any further comments or concerns regarding this study:

If you would like to discuss your comments further please do not hesitate to email the study team at e.e.ormandy@liverpool.ac.uk

Many thanks for your participation

Results of univariable analysis

Complete results of univariable analysis for all outcomes of interest are presented on pages 241 to 258

Table I-a Results of univariable analysis for risk factors associated with antimicrobial and multi-drug resistance in faecal samples obtained from 220 people working with dogs in the North of England

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age Adjusted	16-35	(Ref)	-	-	-	-	2.221	0.329	-	(Ref)	-	-	-	-	0.008	0.996	-
	36-55	0.473	0.324	1.604	0.850	3.028	-	-	-	0.006	0.357	1.006	0.499	2.028	-	-	-
	55+	0.369	0.455	1.446	0.593	3.527	-	-	-	-0.038	0.502	0.963	0.360	2.577	-	-	-
Eat Meat		0.133	0.514	1.142	0.417	3.130	0.066	0.797	-	0.541	0.678	1.717	0.454	6.487	0.687	0.407	-
Beef		-0.045	0.303	0.956	0.527	1.732	0.022	0.881	-	-0.207	0.351	0.813	0.408	1.617	0.354	0.552	-
Lamb		0.341	0.415	1.406	0.624	3.170	0.690	0.406	-	-0.598	0.506	0.550	0.204	1.483	1.517	0.218	-
Pork		0.396	0.350	1.486	0.748	2.952	1.315	0.252	-	-0.151	0.391	0.859	0.399	1.849	0.152	0.696	-
Poultry		0.422	0.327	1.525	0.804	2.892	1.633	0.201	0.099	0.640	0.419	1.897	0.835	4.311	2.522	0.112	-
Fish		0.385	0.337	1.470	0.760	2.843	1.338	0.247	-	-0.217	0.380	0.805	0.382	1.695	0.331	0.565	-
Game		1.166	1.119	3.209	0.358	28.788	1.358	0.244	-	-0.571	1.146	0.565	0.060	5.342	0.273	0.601	0.205
Salad		0.273	0.316	1.313	0.707	2.440	0.747	0.387	-	0.296	0.369	1.345	0.653	2.772	0.662	0.416	-
International Travel within 3 months		-0.171	0.343	0.843	0.430	1.650	0.249	0.618	-	0.305	0.367	1.357	0.661	2.786	0.681	0.409	-
International Travel within 6 months		-0.171	0.310	0.843	0.459	1.546	0.307	0.580	-	0.032	0.338	1.032	0.533	2.001	0.009	0.925	-
International Travel within 9 months		-0.410	0.308	0.663	0.363	1.214	1.808	0.179	-	-0.067	0.334	0.936	0.487	1.799	0.040	0.842	-
International Travel within 12 months		-0.285	0.306	0.752	0.412	1.370	0.875	0.350	-	-0.043	0.336	0.958	0.496	1.849	0.016	0.899	-
Last international travel	None	(Ref)	-	-	-	-	3.826	0.575	-	(Ref)	-	-	-	-	2.115	0.833	-
	<3m	-0.415	0.668	0.661	0.178	2.448	-	-	-	0.548	0.732	1.730	0.412	7.270	-	-	-
	3m-<6m	-0.349	0.725	0.705	0.170	2.918	-	-	-	-0.117	0.830	0.890	0.175	4.524	-	-	-
	6m-<9m	-1.221	0.838	0.295	0.057	1.523	-	-	-	-0.161	1.033	0.852	0.112	6.448	-	-	-
	9m-12m	0.172	0.878	1.188	0.213	6.632	-	-	-	0.408	0.899	1.504	0.258	8.757	-	-	-
	>12m	-0.164	0.654	0.848	0.235	3.060	-	-	-	0.410	0.710	1.507	0.375	6.059	-	-	-
Antimicrobial Treatment within 1		0.223	0.722	1.250	0.304	5.145	0.098	0.754	-	0.585	0.685	1.794	0.468	6.874	0.681	0.409	-
Antimicrobial Treatment within 3		0.778	0.491	2.178	0.832	5.701	2.768	0.096	-	-0.127	0.488	0.881	0.338	2.295	0.068	0.794	-
Antimicrobial Treatment within 6		0.596	0.392	1.815	0.842	3.913	2.437	0.118	-	-0.100	0.410	0.905	0.405	2.022	0.060	0.806	-
Last antimicrobial	>3m	(Ref)	-	-	-	-	3.457	0.178	-	-	-	-	-	-	1.567	0.457	-
	<1m	0.306	0.719	1.358	0.332	5.562	-	-	-	0.533	0.690	1.703	0.441	6.583	-	-	-
	2-3m	1.098	0.657	2.997	0.827	10.854	-	-	-	-0.595	0.666	0.551	0.150	2.033	-	-	-
Hospitalisation with 1 month		0.586	1.163	1.796	0.184	17.557	0.276	0.599	-	2.644	1.154	14.063	1.464	135.065	6.808	0.009	-
Hospitalisation within 3 months		1.375	1.086	3.955	0.471	33.240	2.145	0.143	-	1.539	0.740	4.660	1.094	19.857	4.361	0.037	-
Hospitalisation within 6 months		0.482	0.699	1.620	0.412	6.370	0.505	0.477	-	0.754	0.626	2.126	0.623	7.257	1.367	0.242	-
Last Hospitalisation	>3m	-	-	-	-	-	-	-	0.537	(Ref)	-	-	-	-	6.838	0.033	-
	<1m	-	-	-	-	-	-	-	-	2.649	1.156	14.134	1.465	136.356	-	-	-
	2-3m	-	-	-	-	-	-	-	-	0.213	1.206	1.237	0.116	13.151	-	-	-
Household member hospitalised		0.910	0.666	2.483	0.673	9.168	2.119	0.146	-	-1.090	0.003	0.336	0.334	0.339	2.250	0.134	-

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Animal contact at home:																	
Dog		0.144	0.346	1.155	0.586	2.275	0.172	0.678		-0.027	0.385	0.974	0.457	2.072	0.005	0.945	-
Cat		0.003	0.308	1.003	0.548	1.833	0.000	0.993		0.014	0.334	1.014	0.527	1.949	0.002	0.968	-
Small Mammals		0.000	0.373	1.000	0.481	2.079	0.000	0.999		-0.012	0.412	0.989	0.441	2.216	0.001	0.978	-
Horse		0.408	0.420	1.504	0.661	3.424	0.982	0.322		0.147	0.432	1.159	0.497	2.703	0.114	0.735	-
Fish		-0.801	1.066	0.449	0.056	3.630	0.557	0.456		-	-	-	-	-	-	-	0.575
Reptiles		0.874	0.826	2.397	0.475	12.100	1.288	0.256		-1.300	1.091	0.273	0.032	2.315	1.900	0.168	0.071
Birds		0.762	0.838	2.142	0.414	11.069	0.925	0.336		-0.108	0.851	0.897	0.169	4.757	0.016	0.899	-
Farm Animal		-0.463	0.375	0.629	0.302	1.312	1.511	0.219	-	-0.026	0.428	0.975	0.421	2.255	0.004	0.952	-
Cattle		-0.683	0.617	0.505	0.151	1.694	1.214	0.271	-	0.311	0.668	1.365	0.368	5.057	0.206	0.650	-
Dairy		-2.063	1.237	0.127	0.011	1.435	3.384	0.066	-	-	-	-	-	-	-	-	0.575
Beef		-0.839	0.638	0.432	0.124	1.510	1.724	0.189	-	0.484	0.673	1.623	0.434	6.072	0.489	0.484	-
Small Ruminants		-0.658	0.570	0.518	0.169	1.584	1.299	0.254	-	0.547	0.614	1.729	0.519	5.757	0.761	0.383	-
Poultry		-0.144	0.412	0.866	0.386	1.940	0.121	0.728	-	-1.217	0.207	0.296	0.197	0.444	0.005	0.946	-
Pigs		-0.143	1.315	0.867	0.066	11.407	0.012	0.914	-	0.137	1.412	1.147	0.072	18.246	0.009	0.924	-
Polytomous Variables																	
Farm, dairy and beef	No farm	(Ref)	-	-	-	-	8.1968	0.085	-	-	-	-	-	-	-	-	-
	No cattle	-0.343	0.432	0.709	0.304	1.654	-	-	-	-	-	-	-	-	-	-	-
	Dairy	16.036	5121.8	9.21E+06	0	Inf	-	-	-	-	-	-	-	-	-	-	-
	Beef	-0.119	0.756	0.887	0.202	3.903	-	-	-	-	-	-	-	-	-	-	-
	Both	-	5125.7	0	0	Inf	-	-	-	-	-	-	-	-	-	-	-
Farm and dairy	No farm	(Ref)	-	-	-	-	3.9062	0.142	-	-	-	-	-	-	-	-	-
	No dairy	-0.291	0.399	0.748	0.342	1.634	-	-	-	-	-	-	-	-	-	-	-
	Dairy	-2.107	1.237	0.122	0.011	1.373	-	-	-	-	-	-	-	-	-	-	-
Farm and beef	No farm	(Ref)	-	-	-	-	2.1578	0.340	-	-	-	-	-	-	-	-	-
	No beef	-0.29	0.436	0.748	0.318	1.76	-	-	-	-	-	-	-	-	-	-	-
	Beef	-0.88	0.641	0.415	0.118	1.457	-	-	-	-	-	-	-	-	-	-	-
Contact with dairy cattle alone is most significant so included in the multivariable model																	
Premises Type	Boarding	(Ref)	-	-	-	-	7.318	0.026	-	(Ref)	-	-	-	-	0.401	0.818	-
	Rescue	-0.590	0.560	0.554	0.185	1.661	-	-	-	0.340	0.639	1.404	0.401	4.916	-	-	-
	Vet	-0.915	0.349	0.401	0.202	0.793	-	-	-	-0.053	0.388	0.948	0.444	2.026	-	-	-
Staff		-0.272	0.888	0.762	0.134	4.340	0.096	0.757	-	-0.316	0.004	0.729	0.724	0.734	0.107	0.744	-
Full time work		0.300	0.349	1.350	0.681	2.677	0.741	0.389	-	0.168	0.391	1.183	0.549	2.547	0.186	0.666	-

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Duration of employment	0-24m	(Ref)	-	-	-	-	4.200	0.241	-	(Ref)	-	-	-	-	4.130	0.248	-
	25-60m	0.778	0.447	2.176	0.907	5.223	-	-	-	0.553	0.477	1.739	0.683	4.429	-	-	-
	61-120m	0.076	0.439	1.079	0.456	2.552	-	-	-	-0.536	0.586	0.585	0.185	1.846	-	-	-
	>120m	0.526	0.384	1.693	0.798	3.591	-	-	-	0.247	0.444	1.281	0.536	3.057	-	-	-
Workplace animal contact:																	
	Dog	-0.309	0.470	0.734	0.292	1.842	0.443	0.506	-	0.094	1.243	1.099	0.096	12.551	0.006	0.939	-
	Cat	-0.619	0.316	0.539	0.290	1.000	3.890	0.049	-	0.054	0.498	1.055	0.397	2.802	0.012	0.914	-
	Small Mammal	0.361	0.455	1.435	0.588	3.500	0.652	0.419	-	0.229	0.355	1.257	0.627	2.522	0.423	0.515	-
	Horse	0.277	0.427	1.320	0.572	3.045	0.433	0.510	-	0.394	0.461	1.482	0.601	3.658	0.719	0.397	-
	Farm Animal	-0.108	0.773	0.898	0.197	4.087	0.019	0.890	-	0.108	0.449	1.114	0.462	2.684	0.057	0.812	-
	Cattle	0.033	0.751	1.034	0.237	4.507	0.002	0.964	-	-0.216	0.900	0.805	0.138	4.703	0.060	0.807	-
	Small Ruminant								1.000	0.287	0.763	1.333	0.299	5.944	0.136	0.712	-
	Poultry	0.415	0.520	1.515	0.547	4.197	0.667	0.414	-	-0.201	0.557	0.818	0.274	2.439	0.132	0.716	-
	Reptile	-0.344	0.831	0.709	0.139	3.610	0.170	0.680	-	-0.821	1.135	0.440	0.048	4.074	0.601	0.438	-
	Birds	0.030	0.652	1.030	0.287	3.701	0.002	0.963	-	-0.488	0.817	0.614	0.124	3.042	0.382	0.537	-
	Wildlife	-0.644	1.497	0.525	0.028	9.867	0.182	0.670	-	1.534	1.575	4.635	0.212	101.480	0.925	0.336	-
Job Role:																	
	Handling dogs	-0.910	0.808	0.403	0.083	1.964	1.465	0.226	-	-0.054	0.721	0.947	0.231	3.889	0.006	0.940	-
	Handling other animals	-0.653	0.366	0.520	0.254	1.067	3.289	0.070	-	-0.269	0.368	0.764	0.372	1.573	0.518	0.472	-
	Reception work	0.136	0.314	1.146	0.619	2.121	0.189	0.664	-	-0.002	0.337	0.998	0.515	1.934	0.000	0.996	-
	Office work	-0.003	0.306	0.997	0.548	1.816	0.000	0.993	-	0.026	0.338	1.026	0.529	1.989	0.006	0.940	-
	Cleaning kennels	0.086	0.316	1.090	0.586	2.026	0.074	0.786	-	0.084	0.350	1.087	0.548	2.158	0.057	0.811	-

P values quoted are from the likelihood ration test statistic; OR=Odds Ratio; 95% CI= 95% Confidence Interval

Table I-b Results of univariable analysis for risk factors associated with third generation cephalosporin and ESBL-mediated resistance in faecal samples obtained from 220 people working with dogs in the North of England

Variable	Category	Third Generation Cephalosporin Resistance							ESBL Phenotype								
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age Adjusted	16-35	(Ref)	-	-	-	-	4.447	0.108	-	(Ref)	-	-	-	-	-6.570	1	-
	36-55	-0.843	0.677	0.431	0.114	1.624			-	-0.087	1.422	0.917	0.056	14.878			-
	55+	0.854	0.794	2.349	0.495	11.145			-	2.462	1.138	11.733	1.261	109.137			-
Eat Meat		-1.590	1.160	0.204	0.021	1.979	1.769	0.183	-	-	-	-	-	-	-	-	1
Beef		0.006	0.784	1.006	0.216	4.671	0.000	0.994	-	-	5.726	0.000	0.000	0.668	3.515	0.061	-
Lamb		1.413	0.718	4.108	1.006	16.769	2.363	0.124	-	3.053	1.134	21.176	2.296	195.326	9.140	0.003	-
Pork		-1.618	1.391	0.198	0.013	3.030	1.831	0.176	-	-1.110	3.180	0.329	0.001	167.905	0.138	0.710	-
Poultry		-0.705	0.002	0.494	0.492	0.496	0.706	0.401	-	-	-	-	-	-	-	-	0.3254
Fish		0.490	0.653	1.632	0.454	5.863	0.935	0.334	-	2.463	3.046	11.740	0.030	4593.253	0.887	0.346	-
Game		2.073	1.274	7.948	0.655	96.474	1.026	0.311	-	-	6.378	0.000	0.000	4.339	4.065	0.044	-
Salad		1.380	1.099	3.975	0.461	34.260	1.939	0.164	-	0.530	4.116	1.699	0.001	5417.000	0.018	0.892	-
Polytomous Variables																	
Meat, lamb and pork	Vegetarian	(Ref)	-	-	-	-	8.395	0.078	-	-	-	-	-	-	-	-	-
	Eat meat	-1.894	1.272	0.151	0.012	1.823	-	-	-	-	-	-	-	-	-	-	-
	Pork >3x	-4.243	2.737	0.014	0.000	3.068	-	-	-	-	-	-	-	-	-	-	-
	Lamb >3x	1.141	1.732	3.129	0.105	93.215	-	-	-	-	-	-	-	-	-	-	-
	Both >3x	-2.095	2.212	0.123	0.002	9.403	-	-	-	-	-	-	-	-	-	-	-
Meat and pork	Vegetarian	(Ref)	-	-	-	-	3.296	0.193	-	-	-	-	-	-	-	-	-
	Eat meat	-1.472	1.187	0.230	0.022	2.352	-	-	-	-	-	-	-	-	-	-	-
	Pork >3x	-3.021	1.846	0.049	0.001	1.817	-	-	-	-	-	-	-	-	-	-	-
Meat and Lamb	Vegetarian	(Ref)	-	-	-	-	4.578	0.101	-	-	-	-	-	-	-	-	-
	Eat meat	-1.931	1.221	0.145	0.013	1.587	-	-	-	-	-	-	-	-	-	-	-
	Lamb >3x	-0.226	1.348	0.797	0.057	11.188	-	-	-	-	-	-	-	-	-	-	-
Eating meat and regular consumption of both pork and lamb most significant so included in multivariable model																	
International Travel within 3 months		0.228	0.632	1.256	0.364	4.333	0.128	0.721	-	-1.190	3.317	0.304	0.000	202.690	0.170	0.680	-
International Travel within 6 months		0.282	0.565	1.326	0.438	4.013	0.245	0.621	-	0.904	1.688	2.470	0.090	67.514	0.292	0.589	-
International Travel within 9 months		-0.006	0.567	0.994	0.327	3.020	0.000	0.991	-	0.067	1.432	1.070	0.065	17.713	0.002	0.966	-
International Travel within 12 months		-0.171	0.603	0.842	0.258	2.747	0.082	0.774	-	-1.286	1.766	0.276	0.009	8.807	0.506	0.477	-

Variable	Category	Third Generation Cephalosporin Resistance							ESBL Phenotype								
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Premises Type	Boarding	(Ref)	-	-	-	-	0.307	0.858		(Ref)	-	-	-	-	0.064	0.969	-
	Rescue	0.636	1.203	1.868	0.179	19.949	-	-		1.018	3.358	2.767	0.004	1996	-	-	-
	Vet	0.250	0.751	1.284	0.295	5.595	-	-		0.013	0.011	1.013	0.992	1.035	-	-	-
Staff		-0.903	1.459	0.405	0.023	7.075	0.368	0.544		-1.777	1.161	0.169	0.017	1.645	-13.216	1	-
Full time work		0.663	0.728	1.940	0.466	8.081	0.922	0.337		-0.282	1.604	0.755	0.033	17.489	0.031	0.861	-
Duration of employment	0-24m	(Ref)	-	-	-	-	3.163	0.367		-	-	-	-	-	-	-	0.702
	25-60m	-0.712	0.806	0.490	0.101	2.381	-	-		-	-	-	-	-	-	-	-
	61-120m	-1.710	1.145	0.181	0.019	1.706	-	-		-	-	-	-	-	-	-	-
	>120m	-0.337	0.669	0.714	0.193	2.649	-	-		-	-	-	-	-	-	-	-
Workplace animal contact:																	
Dog		-	-	-	-	-	-	-	1.000	-	-	-	-	-	-	-	1.000
Cat		-0.387	0.795	0.679	0.143	3.229	0.229	0.632		-2.598	0.013	0.074	0.072	0.076	1.632	0.201	-
Small Mammal		0.448	0.631	1.565	0.454	5.390	0.530	0.466		0.528	1.561	1.696	0.079	36.178	0.117	0.733	-
Horse		-1.012	1.136	0.364	0.039	3.370	0.889	0.346		-	-	-	-	-	-	-	0.594
Farm Animal		2.993	1.436	19.94	1.196	332.513	5.123	0.024		3.156	2.418	23.478	0.205	2685.308	2.312	0.128	-
Cattle		0.443	1.367	1.558	0.107	22.709	0.099	0.753		-	-	-	-	-	-	-	1.000
Small Ruminant		4.161	2.067	64.153	1.116	3687.905	4.841	0.003		-	-	-	-	-	-	-	1.000
Poultry		0.760	0.818	2.139	0.431	10.623	0.821	0.365		-0.792	3.466	0.453	0.001	404	0.062	0.804	-
Polytomous variable																	
Contact with farm animals incl. sheep	No contact	(Ref)	-	-	-	-	6.559	0.038		-	-	-	-	-	-	-	-
	Non-sheep	1.932	1.529	6.904	0.345	138.201	-	-		-	-	-	-	-	-	-	-
	Sheep	4.460	2.086	86.483	1.449	5160.893	-	-		-	-	-	-	-	-	-	-
Contact with sheep alone most significant so included in multivariable model																	
Reptile		0.609	1.42	1.839	0.122	30.256	0.168	0.682		-	-	-	-	-	-	-	1.000
Birds		0.935	0.98	2.547	0.369	17.560	0.789	0.374		-1.554	3.432	0.211	0.000	176	0.268	0.605	-
Wildlife		-	-	-	-	-	-	-	1.000	-	-	-	-	-	-	-	1.000
Job Role:		-	-	-	-	-	-	-		-	-	-	-	-	-	-	-
Handling dogs		-	-	-	-	-	-	-	0.606	-	-	-	-	-	-	-	1.000
Handling other animals		-0.417	0.60	0.659	0.201	2.161	0.454	0.501		8.020	0.014	3040	2958	3125	3.400	0.065	-
Reception work		-0.457	0.56	0.633	0.210	1.912	0.650	0.420		-1.033	0.877	0.356	0.064	1.986	-13.403	1	-
Office work		0.670	0.00	1.954	1.945	1.964	1.322	0.250		1.234	1.257	3.434	0.292	40.375	0.529	0.467	-
Cleaning kennels		0.641	0.68	1.899	0.501	7.202	1.006	0.316		9.765	5.168	17418	0.694	4.4E+08	5.322	0.021	-

P values quoted are from the likelihood ration test statistic; OR=Odds Ratio; 95% CI= 95% Confidence Interval

Table I-c Results of univariable analysis for risk factors associated with ampicillin and amoxycylav resistance in faecal samples obtained from 220 people working with dogs in the North of England

Variable	Category	Ampicillin Resistance							Amoxycylav Resistance								
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age Adjusted	16-35	(Ref)	-	-	-	-	0.948	0.623	-	-	-	-	-	-	1.304	0.521	-
	36-55	0.267	0.306	1.306	0.717	2.379	-	-	-	-0.752	0.665	0.472	0.128	1.738	-	-	-
	55+	-0.030	0.424	0.971	0.423	2.227	-	-	-	-0.283	0.905	0.753	0.128	4.441	-	-	-
Eat Meat		0.009	0.490	1.009	0.387	2.636	0.000	0.985	-	-1.639	0.974	0.194	0.029	1.310	3.038	0.081	-
Beef		-0.166	0.285	0.847	0.484	1.482	0.339	0.560	-	-0.244	0.634	0.784	0.226	2.714	0.144	0.705	-
Lamb		0.805	0.397	2.237	1.028	4.868	4.366	0.037	-	-0.045	0.871	0.956	0.173	5.267	0.003	0.958	-
Pork		0.747	0.330	2.110	1.105	4.030	5.355	0.021	-	-1.762	1.123	0.172	0.019	1.552	3.799	0.051	-
Poultry		0.364	0.312	1.439	0.780	2.653	1.350	0.245	-	-0.902	0.680	0.406	0.107	1.539	1.859	0.173	-
Fish		0.627	0.320	1.871	1.000	3.502	4.021	0.045	-	0.154	0.003	1.166	1.160	1.173	0.057	0.812	-
Game		1.680	1.114	5.366	0.605	47.630	3.105	0.078	0.019	1.384	1.406	3.990	0.254	62.711	0.895	0.344	-
Salad		0.462	0.299	1.587	0.884	2.850	2.439	0.118	-	0.934	0.792	2.545	0.538	12.026	1.693	0.193	-
Polytomous Variables																	
Meat, pork and poultry	Vegetarian	-	-	-	-	-	-	-	-	(Ref)	-	-	-	-	6.157	0.188	-
	Eat meat	-	-	-	-	-	-	-	-	-1.125	1.137	0.325	0.035	3.015	-	-	-
	Pork>3x	-	-	-	-	-	-	-	-	-135.8	2.54E077	0.000	0.000	Inf	-	-	-
	Poultry>3x	-	-	-	-	-	-	-	-	-1.461	0.981	0.232	0.034	1.586	-	-	-
	Both>3x	-	-	-	-	-	-	-	-	-2.998	1.606	0.050	0.002	1.163	-	-	-
Meat and pork	Vegetarian	-	-	-	-	-	-	-	-	(Ref)	-	-	-	-	3.370	0.186	-
	Eat meat	-	-	-	-	-	-	-	-	-1.306	1.110	0.271	0.031	2.386	-	-	-
	Pork>3x	-	-	-	-	-	-	-	-	-1.788	1.040	0.167	0.022	1.285	-	-	-
Meat and poultry	Vegetarian	-	-	-	-	-	-	-	-	-2.442	1.277	0.087	0.007	1.062	5.882	0.053	-
	Eat meat	-	-	-	-	-	-	-	-	(Ref)	-	-	-	-	-	-	-
	Poultry>3x	-	-	-	-	-	-	-	-	-3.097	1.684	0.045	0.002	1.226	-	-	-
Eating pork alone most significant so included in multivariable model																	
International Travel within 3 months		0.156	0.322	1.169	0.622	2.197	0.236	0.627	-	0.450	0.645	1.568	0.443	5.547	0.459	0.498	-
International Travel within 6 months		-0.019	0.287	0.981	0.559	1.722	0.005	0.946	-	0.666	0.608	1.946	0.591	6.410	1.125	0.289	-
International Travel within 9 months		-0.101	0.282	0.904	0.520	1.571	0.129	0.720	-	0.389	0.617	1.476	0.441	4.943	0.383	0.536	-
International Travel within 12 months		-0.008	0.286	0.992	0.567	1.736	0.001	0.977	-	0.384	0.646	1.468	0.414	5.212	0.340	0.560	-

Variable	Category	Ampicillin Resistance								Amoxycylav Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Premises Type	Boarding	(Ref)	-	-	-	-	2.855	0.240	-	(Ref)	-	-	-	-	0.182	0.913	-
	Rescue	0.050	0.555	1.051	0.354	3.120	-	-	-	-0.203	1.396	0.816	0.053	12.602	-	-	-
	Vet	-0.496	0.323	0.609	0.323	1.148	-	-	-	0.253	0.772	1.288	0.284	5.842	-	-	-
Staff		0.283	0.770	1.327	0.294	6.000	0.135	0.713	-	-	-	-	-	-	-	-	1.000
Full time work		0.300	0.328	1.350	0.710	2.569	0.845	0.358	-	1.614	0.003	5.025	4.997	5.053	3.256	0.071	-
Duration of employment	0-24m	(Ref)	-	-	-	-	2.454	0.4842	-	(Ref)	-	-	-	-	1.237	0.744	-
	25-60m	0.435	0.404	1.545	0.700	3.410	-	-	-	0.116	0.800	1.123	0.234	5.390	-	-	-
	61-120m	0.139	0.426	1.150	0.499	2.647	-	-	-	-0.559	0.935	0.572	0.091	3.575	-	-	-
	>120m	0.518	0.362	1.679	0.825	3.416	-	-	-	-0.666	0.822	0.514	0.103	2.575	-	-	-
Workplace animal contact:																	
Dog		-0.088	1.194	0.415	0.040	4.308	0.603	0.438	0.132	-	-	-	-	-	-	-	1.000
Cat		-0.167	0.425	0.846	0.368	1.945	0.154	0.695	-	0.822	0.003	2.274	2.262	2.287	0.594	0.441	-
Small Mammal		-0.324	2891	0.410	1.274		1.246	0.264	-	1.134	0.735	3.109	0.737	13.120	2.699	0.100	-
Horse		0.284	0.407	1.328	0.598	2.950	0.488	0.485	-	-0.705	1.168	0.494	0.050	4.876	0.386	0.535	-
Farm Animal		0.261	0.389	1.298	0.605	2.784	0.4537	0.5006	-	0.759	0.898	2.136	0.368	12.404	0.751	0.386	-
Cattle		0.422	0.756	1.526	0.347	6.707	0.318	0.573	-	0.720	1.350	2.054	0.146	28.960	0.253	0.615	-
Small Ruminant		0.594	0.732	1.811	0.432	7.598	0.683	0.409	-	3.544	2.359	34.606	0.340	3526.082	3.934	0.047	-
Poultry		0.111	0.458	1.118	0.453	2.744	0.059	0.808	-	0.522	0.938	1.685	0.268	10.597	0.297	0.586	-
Reptile		-0.276	0.778	0.759	0.165	3.489	0.126	0.723	-	0.865	1.406	2.374	0.151	37.372	0.333	0.564	-
Birds		-0.120	0.601	0.887	0.273	2.881	0.040	0.841	-	1.348	0.003	3.850	3.830	3.870	1.555	0.212	-
Wildlife		-0.139	1.480	0.870	0.048	15.824	0.009	0.925	-	-	-	-	-	-	-	-	1.000
Job Role:																	
Handling dogs		-0.441	0.650	0.644	0.180	2.303	0.470	0.493	-	-	-	-	-	-	-	-	1.000
Handling other animals		-0.129	0.325	0.879	0.465	1.662	0.157	0.692	-	0.326	0.003	1.386	1.378	1.393	0.205	0.651	-
Reception work		-0.069	0.287	0.933	0.531	1.640	0.058	0.811	-	-0.357	0.606	0.700	0.213	2.295	0.347	0.556	-
Office work		0.024	0.285	1.024	0.586	1.790	0.007	0.933	-	0.682	0.637	1.978	0.567	6.898	1.183	0.277	-
Cleaning kennels		0.188	0.299	1.206	0.671	2.168	0.399	0.528	-	0.583	0.685	1.792	0.468	6.862	0.780	0.377	-

P values quoted are from the likelihood ration test statistic; OR=Odds Ratio; 95% CI= 95% Confidence Interval

Table I-d Results of univariable analysis for risk factors associated with chloramphenicol and ciprofloxacin resistance in faecal samples obtained from 220 people working with dogs in the North of England

Variable	Category	Chloramphenicol Resistance								Ciprofloxacin Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age Adjusted	16-35	(Ref)	-	-	-	-	2.154	0.341	-	(Ref)	-	-	-	-	1.262	0.532	-
	36-55	0.734	0.512	2.083	0.763	5.686	-	-	-	-0.353	0.621	0.703	0.208	2.371	-	-	-
	55+	0.364	0.744	1.439	0.335	6.190	-	-	-	0.513	0.750	1.671	0.384	7.264	-	-	-
Eat Meat		0.235	0.854	1.265	0.237	6.752	0.078	0.779	-	-0.114	0.869	0.892	0.162	4.903	0.016	0.898	-
Beef		-0.298	0.490	0.743	0.284	1.939	0.380	0.538	-	0.164	0.552	1.178	0.399	3.472	0.087	0.768	-
Lamb		-0.670	0.726	0.512	0.123	2.125	0.954	0.329	-	0.387	0.667	1.473	0.398	5.449	0.309	0.578	-
Pork		-0.860	0.622	0.423	0.125	1.430	2.162	0.141	-	0.490	0.589	1.632	0.515	5.177	0.679	0.410	-
Poultry		-0.242	0.522	0.785	0.282	2.186	0.214	0.644	-	0.366	0.639	1.441	0.412	5.040	0.344	0.558	-
Fish		-0.233	0.519	0.792	0.286	2.191	0.205	0.651	-	0.307	0.584	1.359	0.433	4.271	0.274	0.601	-
Game		-	-	-	-	-	-	-	0.601	0.619	1.279	1.858	0.152	22.770	0.210	0.647	-
Salad		-0.122	0.483	0.886	0.343	2.283	0.063	0.802	-	1.073	0.692	2.925	0.754	11.350	2.864	0.091	-
International Travel within 3 months		-0.302	0.550	0.739	0.252	2.170	0.312	0.576	-	-0.637	0.709	0.529	0.132	2.122	0.901	0.343	-
International Travel within 6 months		0.190	0.466	1.210	0.486	3.014	0.166	0.683	-	-0.204	0.563	0.816	0.271	2.458	0.132	0.717	-
International Travel within 9 months		-0.112	0.464	0.894	0.360	2.219	0.059	0.809	-	-0.157	0.551	0.855	0.290	2.514	0.081	0.776	-
International Travel within 12 months		-0.320	0.473	0.726	0.287	1.834	0.464	0.496	-	-0.263	0.544	0.769	0.265	2.233	0.233	0.629	-
Last international travel	None	-	-	-	-	-	-	-	0.572	(Ref)	-	-	-	-	1.628	0.898	-
	<3m	-	-	-	-	-	-	-	-	-0.267	1.300	0.766	0.060	9.780	-	-	-
	4-6m	-	-	-	-	-	-	-	-	0.764	1.352	2.147	0.152	30.418	-	-	-
	7-9m	-	-	-	-	-	-	-	-	0.561	1.651	1.752	0.069	44.524	-	-	-
	10-12m	-	-	-	-	-	-	-	-	-0.285	1.670	0.752	0.028	19.844	-	-	-
	>12m	-	-	-	-	-	-	-	-	0.477	1.219	1.612	0.148	17.590	-	-	-
Antimicrobial Treatment within 1 month		-	-	-	-	-	-	-	0.367	-	-	-	-	-	-	-	1.00
Antimicrobial Treatment within 3		0.165	0.646	1.180	0.332	4.188	0.064	0.800	-	0.370	0.722	1.448	0.352	5.959	0.252	0.615	-
Antimicrobial Treatment within 6 month		0.432	0.518	1.541	0.558	4.257	0.670	0.413	-	0.462	0.604	1.587	0.485	5.186	0.558	0.455	-
Last antimicrobial treatment	None	-	-	-	-	-	-	-	0.332	-	-	-	-	-	-	-	0.364
	<1m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2-3m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hospitalisation with 1 month		0.290	1.343	1.337	0.096	18.575	0.045	0.832	-	-	-	-	-	-	-	-	1.000
Hospitalisation within 3 months		0.646	0.994	1.907	0.272	13.393	0.390	0.532	-	-	-	-	-	-	-	-	1.000
Hospitalisation within 6 months		-0.203	1.021	0.816	0.110	6.036	0.041	0.840	-	0.036	1.149	1.037	0.109	9.858	0.001	0.975	-
Last Hospitalisation	>3m	-	-	-	-	-	-	-	0.491	-	-	-	-	-	-	-	0.508
	<1m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2-3m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Household member hospitalised		-0.547	1.012	0.579	0.080	4.205	0.327	0.568	-	0.910	1.039	2.485	0.325	19.028	0.777	0.378	-

Variable	Category	Chloramphenicol Resistance								Ciprofloxacin Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Animal contact at home:																	
Dog		0.384	0.579	1.468	0.472	4.568	0.454	0.500	-	-0.267	0.608	0.766	0.233	2.520	0.187	0.665	-
Cat		0.311	0.498	1.360	0.514	3.626	0.402	0.526	-	-1.126	0.632	0.324	0.094	1.119	3.680	0.055	-
Small Mammals		0.016	0.568	1.016	0.334	3.095	0.001	0.998	-	-0.311	0.719	0.733	0.179	2.997	0.196	0.658	-
Horse		-0.794	0.784	0.452	0.097	2.104	1.192	0.275	-	-0.478	0.824	0.620	0.123	3.117	0.362	0.548	-
Fish		-	-	-	-	-	-	-	1.000	-	-	-	-	-	-	-	1.000
Reptiles		-0.612	1.206	0.542	0.051	5.765	0.287	0.592	-	0.128	1.169	1.137	0.115	11.251	0.117	0.914	-
Birds		1.245	1.049	3.473	0.444	27.167	0.133	0.248	-	0.512	1.219	1.668	0.153	18.173	0.164	0.685	-
Farm Animal		0.359	0.574	1.432	0.465	4.411	0.371	0.543	-	-0.472	0.828	0.624	0.123	3.158	0.343	0.557	-
Cattle		-0.398	1.120	0.672	0.075	6.033	0.136	0.712	-	0.317	1.130	1.373	0.150	12.568	0.065	0.799	-
Dairy		-	-	-	-	-	-	-	1.000	-	-	-	-	-	-	-	1.000
Beef		-0.093	1.083	0.911	0.109	7.615	0.008	0.931	-	-0.093	1.083	0.911	0.109	7.615	0.115	0.735	-
Small Ruminants		0.464	0.851	1.590	0.300	8.426	0.278	0.598	-	-0.004	1.166	0.996	0.101	9.793	0.000	0.994	-
Poultry		0.194	0.630	1.214	0.353	4.169	0.092	0.762	-	-0.229	0.835	0.795	0.155	4.091	0.077	0.782	-
Pigs		0.371	1.724	1.448	0.049	42.506	0.044	0.833	-	-	-	-	-	-	-	-	1.000
Premises Type	Boarding	(Ref)	-	-	-	-	0.099	0.952	-	(Ref)	-	-	-	-	12.991	0.002	-
	Rescue	-0.252	1.054	0.777	0.098	6.134	-	-	-	3.639	1.407	38.045	2.416	599.202	-	-	-
	Vet	-0.164	0.297	0.849	0.264	2.734	-	-	-	2.317	1.127	10.140	1.114	92.308	-	-	-
Staff		0.014	1.349	1.014	0.072	14.266	0.000	0.992	-	-	-	-	-	-	-	-	-
Full time work		1.334	0.770	3.796	0.839	17.181	4.035	0.045	-	0.970	0.801	2.638	0.549	12.674	1.758	0.185	-
Duration of employment	0-24m	(Ref)	-	-	-	-	4.110	0.250	-	(Ref)	-	-	-	-	0.890	0.828	-
	25-60m	0.681	0.674	1.976	0.527	7.402	-	-	-	0.054	0.784	1.055	0.227	4.903	-	-	-
	61-120m	-0.942	0.886	0.390	0.069	2.216	-	-	-	0.335	0.791	1.398	0.296	6.595	-	-	-
	>120m	-0.041	0.651	0.960	0.268	3.440	-	-	-	-0.405	0.764	0.667	0.149	2.981	-	-	-
Workplace animal contact:																	
Dog		-0.570	1.438	0.566	0.034	9.466	0.148	0.700	-	-	-	-	-	-	-	-	1.000
Cat		-0.121	0.695	0.886	0.227	3.456	0.030	0.863	-	-0.827	0.721	0.438	0.106	1.798	1.253	0.263	-
Small Mammal		0.066	0.515	1.069	0.340	2.930	0.017	0.897	-	-0.330	0.618	0.719	0.214	2.417	0.297	0.586	-
Horse		-0.585	0.805	0.557	0.115	2.700	0.575	0.448	-	1.109	1.098	0.330	0.038	2.834	1.248	0.264	-
Farm Animal		0.342	0.602	1.403	0.433	4.582	0.310	0.578	-	-0.363	0.828	0.695	0.137	3.522	0.203	0.653	-
Cattle		0.278	1.145	1.321	0.140	12.467	0.056	0.813	-	-	-	-	-	-	-	-	1.000
Small Ruminant		0.907	0.918	2.478	0.410	14.977	0.874	0.350	-	-	-	-	-	-	-	-	1.000
Poultry		0.410	0.701	1.507	0.382	53949	0.329	0.566	-	-0.653	1.108	0.520	0.059	4.568	0.395	0.530	-
Reptile		0.992	1.130	2.698	0.294	24.722	0.729	0.393	-	0.560	1.100	1.750	0.203	15.120	0.282	0.595	-
Birds		0.505	0.941	1.656	0.262	10.483	0.273	0.601	-	-0.347	1.231	0.707	0.630	7.895	0.086	0.769	-

Variable	Category	Chloramphenicol Resistance								Ciprofloxacin Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Wildlife		2.536	1.813	12.629	0.361	441.467	1.929	0.165	-	-	-	-	-	-	-	-	1.000
Job Role:																	
Handling dogs		0.420	1.166	1.522	0.155	14.975	0.140	0.709	-	-0.296	1.179	0.744	0.074	7.507	0.060	0.807	-
Handling other animals		-0.503	0.500	0.605	0.227	1.612	0.966	0.326	-	0.729	0.560	0.483	0.161	1.446	1.570	0.210	-
Reception work		0.069	0.470	1.071	0.426	2.692	0.021	0.884	-	-0.112	0.572	0.894	0.292	2.741	0.038	0.846	-
Office work		0.010	0.472	1.010	0.401	2.547	0.000	0.982	-	0.704	0.579	2.022	0.650	6.295	1.555	0.212	-
Cleaning kennels		0.446	0.506	1.562	0.579	4.213	0.806	0.369	-	-0.150	0.561	0.861	0.287	2.584	0.070	0.791	-

P values quoted are from the likelihood ration test statistic; OR=Odds Ratio; 95% CI= 95% Confidence Interval

Table I-e Results of univariable analysis for risk factors associated nalidixic acid and tetracycline resistance in faecal samples obtained from 220 people working with dogs in the North of England

Variable	Category	Nalidixic Acid Resistance								Tetracycline Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age Adjusted	16-35	(Ref)	-	-	-	-	0.183	0.912	-	(Ref)	-	-	-	-	0.520	0.771	-
	36-55	-0.139	0.414	0.870	0.386	1.960	-	-	-	-0.138	0.336	0.871	0.451	1.685	-	-	-
	55+	-0.220	0.595	0.803	0.250	2.578	-	-	-	0.185	0.459	1.203	0.489	2.958	-	-	-
Eat Meat		-0.062	0.662	0.940	0.257	3.437	0.009	0.925	-	0.368	0.580	1.444	0.463	4.501	0.412	0.521	-
Beef		-0.090	0.404	0.914	0.413	2.019	0.050	0.823	-	0.198	0.319	1.219	0.652	2.276	0.383	0.536	-
Lamb		-0.202	0.552	0.817	0.277	2.411	0.137	0.711	-	0.104	0.415	1.110	0.492	2.502	0.062	0.803	-
Pork		-0.171	0.454	0.843	0.346	2.054	0.143	0.705	-	0.159	0.350	1.172	0.590	2.329	0.202	0.653	-
Poultry		0.212	0.449	1.237	0.513	2.983	0.227	0.634	-	0.925	0.392	2.522	1.170	5.435	6.062	0.014	-
Fish		0.385	0.430	1.469	0.632	3.414	0.804	0.370	-	-0.152	0.345	0.859	0.436	1.689	0.195	0.659	-
Game		-0.344	1.241	0.709	0.062	8.079	0.081	0.776	-	-0.069	0.929	0.934	0.151	5.766	0.005	0.941	-
Salad		0.697	0.446	2.008	0.838	4.810	2.614	0.106	-	0.109	0.341	1.115	0.572	2.173	0.102	0.749	-
International Travel within 3 months		0.161	0.424	1.175	0.511	2.698	0.142	0.706	-	0.018	0.354	1.018	0.509	2.036	0.003	0.960	-
International Travel within 6 months		0.064	0.386	1.066	0.500	2.273	0.027	0.869	-	0.063	0.317	1.065	0.572	1.983	0.040	0.842	-
International Travel within 9 months		-0.087	0.381	0.917	0.434	1.936	0.052	0.820	-	-0.032	0.312	0.969	0.525	1.787	0.010	0.920	-
International Travel within 12 months		0.052	0.386	1.053	0.494	2.245	0.018	0.893	-	0.151	0.320	1.163	0.621	2.176	0.223	0.637	-
Last international travel	None	(Ref)	-	-	-	-	1.985	0.851	-	(Ref)	-	-	-	-	1.962	0.854	-
	<3m	-0.275	0.733	0.759	0.180	3.196	-	-	-	0.426	0.689	1.531	0.397	5.906	-	-	-
	4-6m	-0.521	0.823	0.594	0.118	2.983	-	-	-	0.494	0.745	1.639	0.381	7.052	-	-	-
	7-9m	-1.309	1.255	0.270	0.023	3.162	-	-	-	-0.023	0.927	0.977	0.159	6.017	-	-	-
	10-12m	0.036	0.913	1.036	0.173	6.209	-	-	-	0.991	0.840	2.694	0.519	13.987	-	-	-
	>12m	-0.523	0.712	0.593	0.147	2.392	-	-	-	0.377	0.665	1.458	0.396	5.373	-	-	-
Antimicrobial Treatment within 1 month		-	-	-	-	-	-	-	0.224	1.363	0.692	3.909	1.008	15.159	4.005	0.045	-
Antimicrobial Treatment within 3 months		-0.679	0.627	0.507	0.148	1.735	1.312	0.252	-	-0.040	0.446	0.961	0.401	2.305	0.008	0.929	-
Antimicrobial Treatment within 6 month		-0.775	0.543	0.461	0.159	1.334	2.323	0.127	-	-0.224	0.386	0.799	0.375	1.703	0.341	0.559	-
Last antimicrobial	None	-	-	-	-	-	-	-	0.3452	(Ref)	-	-	-	-	6.747	0.034	-
	<1m	-	-	-	-	-	-	-	-	1.288	0.692	3.626	0.934	14.088	-	-	-
	2-3m	-	-	-	-	-	-	-	-	-1.020	0.667	0.361	0.098	1.334	-	-	-
Hospitalisation with 1 month		0.837	1.054	2.308	0.293	18.206	0.595	0.440	-	2.103	1.184	8.187	0.804	83.364	3.940	0.047	-
Hospitalisation within 3 months		0.732	0.817	2.079	0.419	10.312	0.755	0.385	-	0.984	0.743	2.676	0.623	11.489	1.752	0.186	-
Hospitalisation within 6 months		0.538	0.713	1.712	0.423	6.933	0.536	0.464	-	0.161	0.645	1.175	0.332	4.159	0.061	0.805	-
Last Hospitalisation	>3m	(Ref)	-	-	-	-	0.783	0.854	-	(Ref)	-	-	-	-	3.991	-	-
	<1m	0.845	1.060	2.329	0.299	18.595	-	-	-	-0.790	0.175	0.454	0.322	0.640	-	-	-
	2-3m	0.574	1.291	1.775	0.141	22.310	-	-	-	-0.273	1.221	0.761	0.069	8.340	-	-	-
Household member hospitalised		0.328	0.681	1.389	0.366	5.274	0.227	0.634	-	-0.362	0.613	0.696	0.210	2.312	0.363	0.547	-

Variable	Category	Nalidixic Acid Resistance								Tetracycline Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Animal contact at home:																	
Dog		-0.372	0.436	0.689	0.293	1.621	0.722	0.395	-	0.036	0.365	1.037	0.507	2.119	0.010	0.921	-
Cat		-0.812	0.412	0.444	0.198	0.995	4.176	0.041	-	-0.642	0.316	0.526	0.283	0.978	4.144	0.042	-
Small Mammals		-0.724	0.551	0.485	0.165	1.426	1.941	0.164	-	0.173	0.384	1.189	0.560	2.522	0.201	0.654	-
Horse		0.035	0.521	1.036	0.373	2.876	0.005	0.946	-	0.176	0.411	1.193	0.533	2.670	0.180	0.671	-
Fish		-	-	-	-	-	-	-	1.000	-0.590	1.274	0.554	0.046	6.733	0.229	0.632	-
Reptiles		-1.268	1.165	0.281	0.029	2.761	1.507	0.220	-	-1.727	1.094	0.178	0.021	1.518	3.556	0.059	-
Birds		0.365	0.923	1.441	0.236	8.803	0.151	0.697	-	-0.554	0.852	0.575	0.108	3.051	0.448	0.503	-
Farm Animal		-1.006	0.628	0.366	0.107	1.253	3.065	0.080	-	-0.141	0.409	0.869	0.390	1.936	0.119	0.730	-
Cattle		0.186	0.795	1.205	0.254	5.721	0.054	0.817	-	-0.177	0.685	0.837	0.219	3.207	0.068	0.794	-
Dairy		-	-	-	-	-	-	-	1.000	-0.509	1.245	0.601	0.052	6.896	0.176	0.675	-
Beef		0.399	0.807	1.491	0.306	7.253	0.234	0.628	-	-0.534	0.762	0.586	0.132	2.609	0.522	0.470	-
Small Ruminants		0.050	0.779	1.051	0.228	4.837	0.041	0.949	-	-0.332	0.665	0.717	0.195	2.640	0.257	0.612	-
Poultry		-1.517	0.790	0.219	0.047	1.033	5.049	0.025	-	-0.264	0.451	0.768	0.317	1.858	0.351	0.553	-
Pigs		0.101	1.531	1.106	0.055	22.220	0.004	0.948	-	-0.473	1.406	0.623	0.040	9.802	0.117	0.732	-
Polytomous variable																	
Contact with farm animals incl. poultry	No contact	(Ref)	-	-	-	-	5.061	0.080	-	-	-	-	-	-	-	-	-
	Non-	0.109	0.988	1.115	0.161	7.724	-	-	-	-	-	-	-	-	-	-	-
	Poultry	-1.509	0.792	0.221	0.047	1.045	-	-	-	-	-	-	-	-	-	-	-
Contact with poultry alone most significant so included in multivariable model																	
Premises Type	Boarding	(Ref)	-	-	-	-	1.432	0.488	-	(Ref)	-	-	-	-	0.881	0.644	-
	Rescue	0.843	0.816	2.323	0.469	11.499	-	-	-	-0.094	0.638	0.910	0.261	3.180	-	-	-
	Vet	0.450	0.505	1.569	0.583	4.224	-	-	-	-0.348	0.378	0.706	0.337	1.482	-	-	-
Staff		0.239	1.229	1.270	0.114	14.111	0.039	0.844	-	-0.604	0.894	0.547	0.095	3.150	0.454	0.500	-
Full time work		0.259	0.456	1.293	0.530	3.168	0.333	0.564	-	-0.023	0.358	0.977	0.485	1.970	0.004	0.949	-
Duration of employment	0-24m	(Ref)	-	-	-	-	2.128	0.546	-	(Ref)	-	-	-	-	0.818	0.845	-
	25-60m	0.642	0.556	1.899	0.639	5.648	-	-	-	0.369	0.449	1.446	0.600	3.488	-	-	-
	61-120m	-0.123	0.617	0.884	0.264	2.964	-	-	-	0.003	0.478	1.003	0.393	2.557	-	-	-
	>120m	0.035	0.531	1.035	0.366	2.931	-	-	-	0.101	0.411	1.107	0.495	2.475	-	-	-
Workplace animal contact:																	
Dog		0.042	1.344	1.043	0.075	14.533	0.001	0.975	-	-0.770	1.101	0.463	0.054	4.008	0.483	0.487	-
Cat		-0.170	0.572	0.844	0.275	2.590	0.087	0.768	-	-0.679	0.467	0.507	0.203	1.265	2.135	0.144	-
Small Mammal		-0.195	0.411	0.823	0.368	1.842	0.227	0.634	-	-0.264	0.327	0.768	0.404	1.458	0.647	0.421	-
Horse		-0.169	0.591	0.844	0.265	2.689	0.084	0.773	-	0.458	0.444	1.582	0.662	3.779	1.037	0.309	-

Variable	Category	Nalidixic Acid Resistance								Tetracycline Resistance							
		β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Farm Animal		-0.165	0.549	0.848	0.289	2.487	0.092	0.762	-	0.028	0.430	1.029	0.442	2.392	0.004	0.948	-
Cattle		-0.055	1.018	0.947	0.129	6.967	0.003	0.957	-	-0.854	0.948	0.426	0.066	2.727	0.910	0.340	-
Small Ruminant		-0.214	0.997	0.808	0.114	5.698	0.048	0.827	-	0.314	0.745	1.369	0.318	5.895	0.173	0.677	-
Poultry		-1.087	0.823	0.337	0.067	1.694	2.134	0.144	-	-0.052	0.513	0.950	0.347	2.597	0.010	0.920	-
Reptile		-0.071	1.058	0.932	0.117	7.414	0.005	0.947	-	-1.335	1.154	0.263	0.027	2.526	1.642	0.200	-
Birds		-0.290	0.865	0.748	0.137	4.079	0.117	0.732	-	-0.492	0.719	0.611	0.149	2.499	0.492	0.483	-
Wildlife		1.634	1.648	5.122	0.202	129.599	0.956	0.328	-	1.241	1.581	3.458	0.156	76.721	0.605	0.437	-
Job Role:																	
Handling dogs		-0.970	0.736	0.379	0.090	1.602	1.651	0.199	-	-0.091	0.681	0.913	0.240	3.466	0.018	0.894	-
Handling other animals		-0.685	0.423	0.504	0.220	1.156	2.620	0.106	-	-0.500	0.354	0.607	0.303	1.215	1.983	0.159	-
Reception work		0.248	0.398	1.282	0.587	2.798	0.396	0.529	-	0.225	0.323	1.252	0.664	2.359	0.486	0.486	-
Office work		0.124	0.389	1.132	0.528	2.425	0.101	0.750	-	-0.058	0.319	0.943	0.505	1.763	0.033	0.855	-
Cleaning kennels		-0.204	0.390	0.815	0.380	1.751	0.272	0.602	-	0.095	0.328	1.099	0.578	2.090	0.084	0.772	-

P values quoted are from the likelihood ration test statistic; OR=Odds Ratio; 95% CI= 95% Confidence Interval

Table I-f Results of univariable analysis for risk factors associated trimethoprim resistance in faecal samples obtained from 220 people working with dogs in the North of England

		Trimethoprim Resistance							
Variable	Category	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age Adjusted	16-35	(Ref)	-	-	-	-	3.717	0.156	-
	36-55	0.193	0.330	1.213	0.636	2.313	-	-	-
	55+	0.837	0.435	2.309	0.984	5.419	-	-	-
Eat Meat		1.550	0.769	4.713	1.044	21.285	5.550	0.018	-
Beef		0.217	0.304	1.242	0.684	2.255	0.507	0.476	-
Lamb		-0.244	0.415	0.784	0.347	1.770	0.352	0.553	-
Pork		-0.062	0.341	0.940	0.481	1.835	0.033	0.856	-
Poultry		0.887	0.370	2.429	1.175	5.020	6.144	0.013	-
Fish		0.007	0.329	1.007	0.529	1.920	0.000	0.982	-
Game		-0.219	0.891	0.803	0.140	4.610	0.062	0.804	-
Polytomous variable									
Eat meat and poultry	Vegetarian	(Ref)	-	-	-	-	7.972	0.019	-
	Eat meat	1.056	0.838	2.874	0.556	14.856	-	-	-
	Poultry> 3x weekly	1.672	0.771	5.322	1.174	24.124	-	-	-
Regular consumption of poultry most significant so included in multivariable model									
Salad		0.055	0.319	1.057	0.566	1.973	0.030	0.863	-
International Travel within 3 months		0.193	0.335	1.213	0.629	2.339	0.326	0.568	-
International Travel within 6 months		-0.212	0.315	0.809	0.436	1.499	0.461	0.497	-
International Travel within 9 months		-0.300	0.307	0.741	0.405	1.353	0.972	0.324	-
International Travel within 12 months		-0.336	0.312	0.715	0.388	1.317	1.188	0.276	-
Last international travel	None	(Ref)	-	-	-	-	4.042	0.543	-
	<3m	-0.059	0.615	0.942	0.282	3.145	-	-	-
	4-6m	-0.936	0.720	0.392	0.096	1.608	-	-	-
	7-9m	-0.640	0.871	0.527	0.096	2.908	-	-	-
	10-12m	-0.307	0.788	0.736	0.157	3.446	-	-	-
	>12m	-0.028	0.596	0.973	0.302	3.128	-	-	-
Antimicrobial Treatment within 1 month		0.120	0.673	1.127	0.301	4.218	0.031	0.859	-
Antimicrobial Treatment within 3 months		0.131	0.421	1.140	0.499	2.605	0.096	0.757	-
Antimicrobial Treatment within 6 month		0.138	0.358	1.148	0.569	2.314	0.147	0.702	-
Last antimicrobial treatment	None	(Ref)	-	-	-	-	0.096	0.953	-
	<1m	0.132	0.675	1.141	0.304	4.284	-	-	-
	2-3m	0.131	0.512	1.140	0.418	3.108	-	-	-

		Trimethoprim Resistance							
Variable	Category	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Hospitalisation with 1 month		2.197	1.161	8.999	0.924	87.667	4.661	0.031	-
Hospitalisation within 3 months		1.572	0.767	4.817	1.072	21.646	4.633	0.031	-
Hospitalisation within 6 months		0.951	0.602	2.588	0.795	8.426	2.479	0.115	-
Last Hospitalisation	>3m	(Ref)	-	-	-	-	5.374	0.068	-
	<1m	2.221	1.167	9.218	0.936	90.748	-	-	-
	2-3m	0.909	1.070	2.481	0.305	20.210	-	-	-
Household member hospitalised		0.240	0.537	1.271	0.444	3.641	0.195	0.659	-
Animal contact at home:									
Dog		-0.148	0.348	0.862	0.436	1.705	0.181	0.670	-
Cat		-0.231	0.300	0.794	0.411	1.430	0.585	0.444	-
Small Mammals		-0.265	0.380	0.767	0.364	1.616	0.494	0.482	-
Horse		0.455	0.384	1.577	0.743	3.344	1.391	0.238	-
Fish		0.843	1.069	2.330	0.287	18.942	0.619	0.432	-
Reptiles		-1.692	1.079	0.184	0.022	1.527	3.618	0.057	-
Birds		0.448	0.710	1.565	0.389	6.299	0.392	0.531	-
Farm Animal		0.344	0.376	1.41	0.675	2.946	0.824	0.364	-
Cattle		0.940	0.604	2.560	0.787	8.324	2.410	0.121	-
Dairy		0.351	1.201	0.704	0.067	7.409	0.089	0.765	-
Beef		0.762	0.625	2.143	0.630	7.292	1.453	0.228	-
Polytomous variable									
Contact with all cattle vs beef cattle	No contact	(Ref)	-	-	-	-	3.715	0.156	-
	Non-beef	17.666	4661	4.70E+07	0.000	Inf	-	-	-
	Beef cattle	0.777	0.614	2.174	0.653	7.239	-	-	-
Contact with all cattle most significant so included in multivariable model									
Small Ruminants		0.240	0.578	1.383	0.445	4.297	0.307	0.580	-
Poultry		0.292	0.405	1.339	0.606	2.960	0.514	0.474	-
Pigs		1.312	1.305	3.714	0.288	47.920	1.037	0.309	-
Premises Type	Boarding	(Ref)	-	-	-	-	1.617	0.446	-
	Rescue	-0.173	0.560	0.841	0.281	2.523	-	-	-
	Vet	-0.424	0.330	0.654	0.343	1.249	-	-	-
Staff		-0.254	0.800	0.776	0.162	3.724	0.092	0.753	-
Full time work		0.166	0.356	1.181	0.588	2.373	0.222	0.637	-

Trimethoprim Resistance									
Variable	Category	β	SE	OR	L95%CI	U95%CI	LRT statistic	P	FE
Duration of employment	0-24m	-0.596	0.289	0.551	0.313	0.971	1.354	0.716	-
	25-60m	-0.389	0.447	0.678	0.282	1.629	-	-	-
	61-120m	-0.287	0.462	0.750	0.304	1.855	-	-	-
	>120m	0.035	0.388	1.035	0.484	2.215	-	-	-
Workplace animal contact:									
Dog		0.605	1.240	1.831	0.161	20.813	0.257	0.612	-
Cat		0.609	0.494	1.838	0.698	4.842	0.200	0.200	-
Small Mammal		-0.288	0.305	0.750	0.412	1.365	0.871	0.351	-
Horse		0.597	0.410	1.816	0.813	4.055	2.086	0.149	-
Farm Animal		0.624	0.393	1.866	0.863	4.032	2.489	0.115	-
Cattle		0.961	0.719	2.613	0.638	10.701	1.729	0.189	-
Small Ruminant		0.728	0.684	2.071	0.542	7.912	1.090	0.296	-
Poultry		0.626	0.458	1.871	0.762	4.595	1.820	0.177	-
Reptile		-0.361	0.884	0.697	0.123	3.944	0.172	0.678	-
Birds		0.341	0.628	1.406	0.411	4.811	0.291	0.589	-
Wildlife		0.995	1.525	2.704	0.136	53.763	0.420	0.517	-
Polytomous variable									
Contact with farm animals, cattle and poultry	No contact	(Ref)	-	-	-	-	4.055	0.399	-
	Non-cattle/poultry	0.654	0.834	1.923	0.375	9.867	-	-	-
	Cattle	0.386	0.972	1.471	0.219	9.881	-	-	-
	Poultry	0.431	0.504	1.539	0.572	4.136	-	-	-
	Cattle and poultry	1.901	1.201	6.690	0.635	70.453	-	-	-
Farm animals and cattle	No contact	(Ref)	-	-	-	-	2.912	0.233	-
	Non-cattle	0.485	0.445	1.625	0.679	3.885	-	-	-
	Cattle	1.023	0.726	2.782	0.670	11.555	-	-	-
Farm animals and Poultry	No contact	(Ref)	-	-	-	-	2.518	0.284	-
	Non-poultry	0.538	0.638	1.713	0.491	5.983	-	-	-
	Poultry	0.665	0.463	1.945	0.785	4.819	-	-	-
Contact with farm animals most significant so included in multivariable model									
Job Role:									
Handling dogs		0.449	0.714	1.567	0.387	6.347	0.419	0.518	-
Handling other animals		0.139	0.349	1.149	0.580	2.276	0.159	0.690	-
Reception work		0.312	0.304	0.732	0.403	1.329	1.052	0.305	-
Office work		-0.063	0.303	0.939	0.519	1.700	0.043	0.836	-
Cleaning kennels		0.279	0.317	1.322	0.710	2.463	0.788	0.375	-

P values quoted are from the likelihood ration test statistic; OR=Odds Ratio; 95% CI= 95% Confidence Interval

Residual plots

Residual plots were utilised to assess if any premises were exerting an increased influence on the mixed effect models constructed for each outcome. Where a significant difference was detected, data from these premises were checked for any error

Figure I-a Premises level residuals plotted against the overall mean for each mixed effect model (n=66 premises).

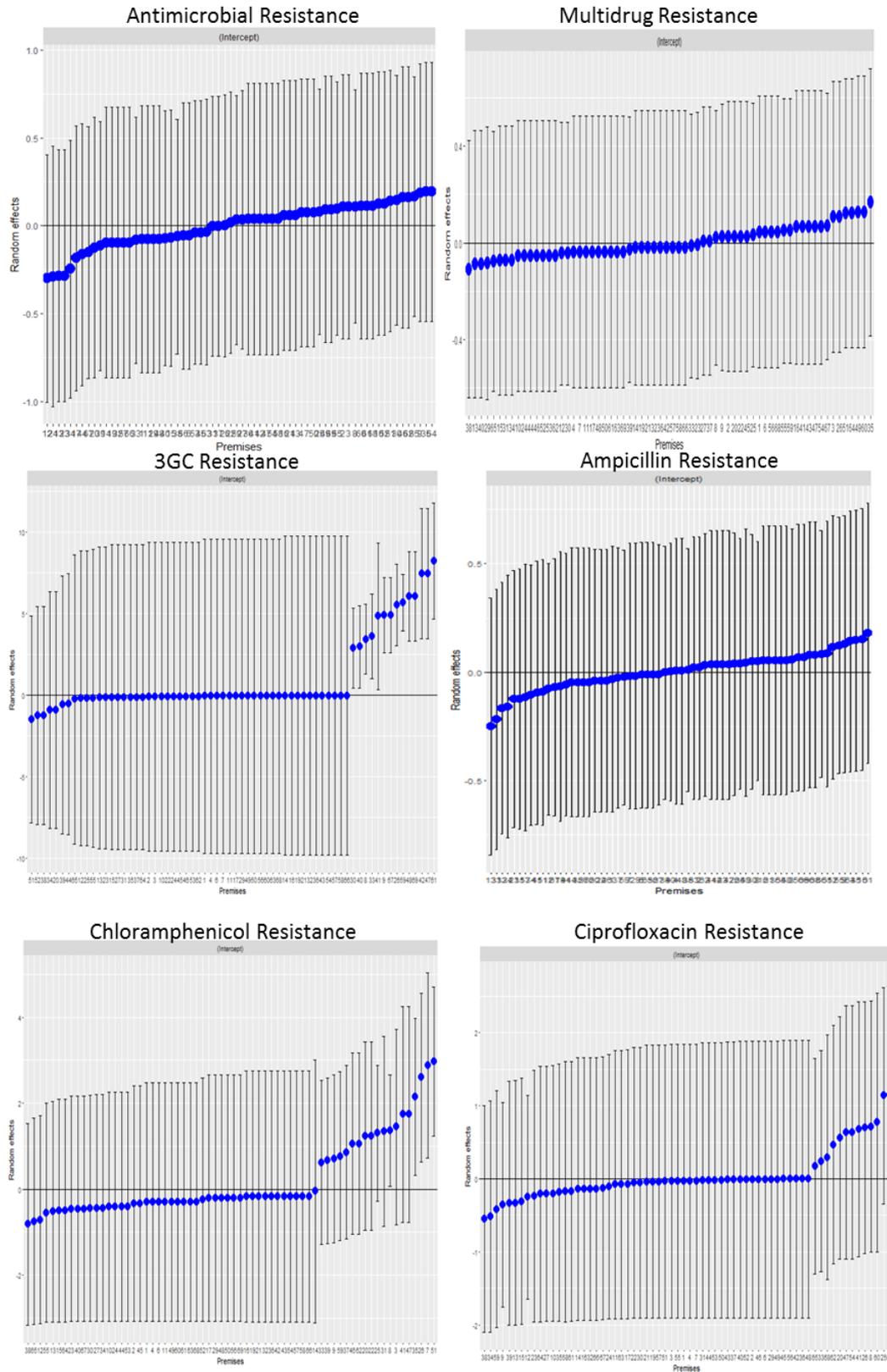
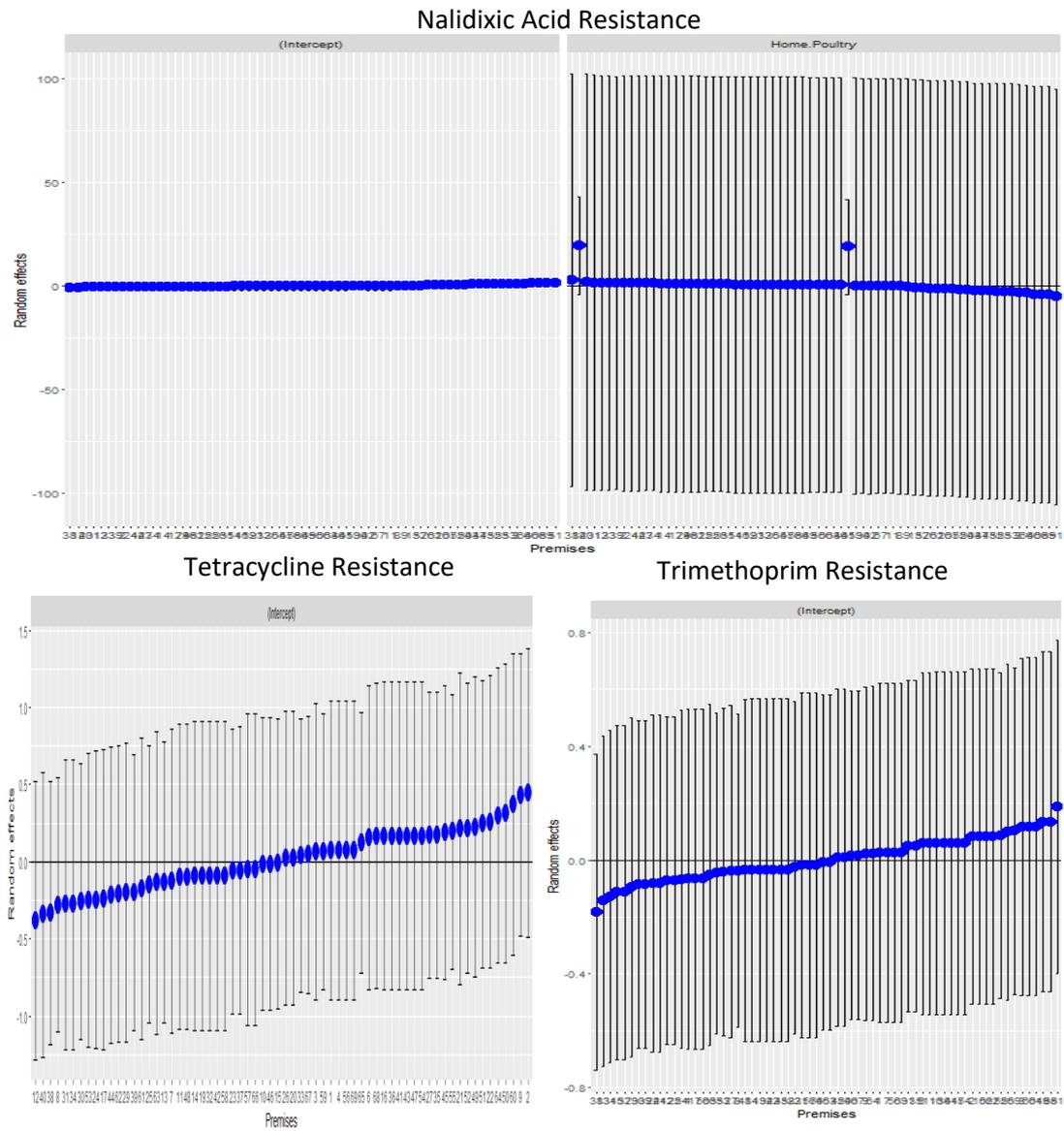


Fig I-a cont.



Appendix II

Material pertaining to Chapter Four



Participant Information Sheet

Antibiotic-resistant Bacteria in Kennelled Dogs.

You are being invited to participate in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please read the following information carefully and feel free to ask us if there is anything you do not understand.

What is the purpose of the study?

Antibiotic resistance has become an increasing problem in veterinary medicine, and these resistant bacteria have been highlighted having an increasing effect on animal patients. Infections involving these bacteria are commonly multidrug resistant, leading to reduced treatment options and increased mortality.

Antibiotic-resistant bacteria have already been identified in both hospitalised and non-hospitalised animals, and therefore they may act as an important link in the transfer of antibiotic resistance. Currently there is little data available on the risk of carriage of antibiotic-resistant bacteria in kennelled dogs; therefore the objective of this study is to determine the carriage rates in these dogs and investigate factors which may affect this carriage. This information will allow us to identify potential measures which may reduce the potential for spread of antibiotic resistance.

Why have I been chosen to take part?

You are being asked to take part as your dog is being boarded at a participating kennels.

Do I have to take part?

Participation in this study is voluntary and participants are free to withdraw from the study at any time.

What will happen if I take part?

If you agree to take part in the study we will ask you to sign a consent form to confirm your participation in the study. We will then ask you to provide us with a few details about your pet and fill in a short questionnaire. All samples and the questionnaire will be anonymised so that you and your pet cannot be identified.

A sample of your pet's faeces will be taken during their stay which will undergo microbiological testing to identify any antibiotic-resistant bacteria present in the sample, followed by further testing to identify the genes responsible for this resistance. Data from questionnaires will be entered into a secure database and analysed to identify any risk factors for carriage of resistant bacteria.

Are there any risks or benefits in taking part?

No personal risks or benefits will be experienced by participants taking part in this study. Benefits of the study are provision of increased information about carriage rates and the risk factors for carriage of resistant bacteria. This will further understanding about the spread of antibiotic resistance and therefore help in the development of potential prevention strategies.

Will my participation be kept confidential?

Participation in the study is confidential. All data will be anonymised and entered into a database which will be securely stored and password protected on university computers for up to 5 years. Access to data from this study will be available only to investigators involved in the project.

What will happen to the results of the study?

We aim to publish the results of this study in an appropriate scientific journal. Within any published articles no participants will be identifiable from the results.

As samples are anonymised we are unable to provide participants with their individual results. We will however, provide each of the premises with a copy of the report at the end of the study.

What will happen if I want to stop taking part?

Participants are free to withdraw from the study at any time. If you wish to withdraw from the study please contact Mrs Emma Ormandy quoting your unique reference number. This will allow us to remove your sample and any data provided from the study.

What should I do if there is a problem?

If you are unhappy, or if there is a problem, please feel free to let us know by contacting Dr Nicola Williams on 0151 795 6051 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 at 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 researchers involved and the details of the complaint you wish to make.

Who can I contact if I have further questions?

Mrs Emma Ormandy
School of Veterinary Science
University of Liverpool
Neston
CH64 7TE
0151 795 6041
E.E.Ormandy@liverpool.ac.uk

Thank you again for your time.



UNIVERSITY OF
LIVERPOOL

Committee on Research Ethics

PARTICIPANT CONSENT FORM

Title of Research Project: Antibiotic-resistant Bacteria in Kennelled Dogs

Researcher(s): Dr Nicola Williams
Dr Gina Pinchbeck
Prof. Sarah O'Brien
Prof. Susan Dawson
Mrs Emma Ormandy

Please
initial or
cross the
box

1. I confirm that I have read and have understood the information sheet dated March 2014 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 without giving any reason, without my rights being affected. In addition, should I not wish to answer any particular question or questions, I am free to decline.
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.
4. I agree to take part in the above study.
5. I understand that confidentiality and anonymity will be maintained and it will not be possible to identify me in any publications.
6. I agree for the data collected from me to be used in relevant future research

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

Principal Investigator:
Dr Nicola Williams
School of Veterinary Science
University of Liverpool
Neston
CH64 7TE
0151 795 6051
njwillms@liverpool.ac.uk

Student Researcher:
Mrs Emma Ormandy
School of Veterinary Science
University of Liverpool
Neston
CH64 7TE
0151 795 6040



Antibiotic-resistant Bacteria in Kennelled Dogs Questionnaire

As part of the research into antibiotic resistance we are asking owners of dogs staying at boarding kennels to fill in this questionnaire and allow a sample of their pet's faeces to be collected.

Thank you for agreeing to take part in this study, your participation will help us to better understand antibiotic resistance in dogs and how this resistance may spread.

How to complete the form:

Please answer all questions by marking an X in the box or by writing in the boxes provided using BLOCK CAPITAL letters.

If you make a mistake or change your answer please fill in the first box completely and put a clear cross in the correct box.

Once completed please return the questionnaire to the kennel staff.

Participation in this study is entirely voluntary and all information from this questionnaire is strictly confidential and will be available only to the investigators. All data is anonymised and no individuals will be identifiable from published data.

1. Approximately how old is your dog?

Years
 Months
 Don't know

2. What is the sex of your dog?

Male
 Female

3. Is your dog neutered?

Yes
 No
 Don't know

4. What breed is your dog?

Purebred
 Crossbreed
 Don't know

If known please state breed

5. What is the approximate size (when adult) of your dog?

Toy
 Small (terrier)
 Medium (collie)
 Large (Labrador)
 Giant (Great Dane)

6. What is your dog's date of arrival at the kennels (dd/mm/yy)?

/ /

7. How long is your dog staying in kennels?

--	--

 Nights

8. Is your dog sharing a kennel with other dogs?

Yes No Don't know

If yes how many?

--	--

Thank you for answering those questions. We would be very grateful if we could leave you with a further questionnaire about your dog's home environment and health for you to fill in, in your own time, and return in the pre-paid envelope provided.

We welcome any feedback from our participants.

Please indicate any further comments or concerns regarding this study:

--

If you would like to discuss your comments further please do not hesitate to email the study team at E.E.Ormandy@liverpool.ac.uk

Many thanks for your participation

Date of Sampling	<table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table> / <table border="1"><tr><td></td><td></td></tr></table>						



Antibiotic-resistant Bacteria in Kennelled Dogs Questionnaire

As part of our research into antibiotic resistance we are investigating carriage rates of antibiotic-resistant bacteria in kennelled dogs and factors which affect it. In order to do this we are asking dog owners to fill in this questionnaire and allow a sample of their pet's faeces to be collected.

Your participation in this study will help us to better understand antibiotic resistance in dogs and how this resistance is spread.

Participation in this study is entirely voluntary and all responses will remain anonymous and completely confidential.

All information from this questionnaire is strictly confidential and will be available only to the investigators.

Answering the questions

This questionnaire consists of the four short sections

Section 1: Information about your dog

Section 2: Information about your dog's veterinary history

Section 3: Information about your dog's home environment

Section 4: Information about you

Please answer all questions by marking an X in the box or by writing in the boxes provided using BLOCK CAPITAL letters.

E.g. Have you owned your dog all its life (since being 12 week old puppy)?

Yes
 No
 Don't know

If you make a mistake or change your answer please fill in the first box completely and put a clear cross in the correct box.

Once completed please return the questionnaire to the kennel staff.

If you have any questions or queries please contact

Mrs Emma Ormandy
 School of Veterinary Science
 University of Liverpool
 Neston
 CH64 7TE
 0151 795 6040
 E.E.Ormandy@liverpool.ac.uk

We welcome any feedback from our participants.

Section 1: About your dog

1. Have you owned your dog all its life (since being 12 week old pup)?

Yes No Don't know

2. Where did you get your dog from (e.g. breeder, pet shop, rescue centre)?

3. Is your dog a working dog?

Yes No Don't know

If yes, please specify (e.g. guard dog, farm dog).

Section 2: About your dog's veterinary history

4. Has your dog received any veterinary treatment within the last 6 months?

Yes No Don't know

- 4a. If yes, when was the most recent visit?

Within the last month

2-3 months ago

3-6 months ago

- 4b. Please indicate your dog's symptoms.

- 4c. Was any antibiotic treatment given?

Yes No Don't know

- 4d. Please indicate the name of the antibiotic treatment if known:-

5. Has your dog been hospitalised at the vets within the last 6 months?

Yes No Don't know

5a. If yes, how long ago was this?

Within last month

2-3 months ago

3-6 months ago

5b. What was the reason for the hospitalisation?

6. Is your dog currently receiving any treatment for an on-going condition (excluding antibiotics)?

Yes

No

Don't know

6a. If yes, what condition is being treated?

6b. Please indicate the name of your pet's current treatment if known:-

Section 3: ABOUT YOUR DOG'S HOME ENVIRONMENT

7. Which of the following food types does your dog eat on a regular basis (at least 3 times a week)?

(Tick any boxes which apply)

Wet food (tins/pouches)

Raw meat and bones

Dry mixer

Cooked meat

Complete dry food

Other (please specify) _____

8. Where is your dog housed when at home?

Indoors Outdoors

Kennels Other (please specify) _____

9. Does your dog come into direct contact with other dogs at home?

- Yes No Don't know

9a. If yes how many other dogs are present?

9b. Have any of these other dogs recently (within the last month) had any veterinary treatment with antibiotics?

- Yes No Don't know

9b. Have any of these dogs been hospitalised within the last month?

- Yes No Don't know

10. Does your dog come into regular contact with other animals (not dogs)?

- Yes No Don't know

If yes please specify (Tick all that apply)

- Cats Small mammals (e.g. hamster)
 Horses Wildlife
 Farm animals Other (please specify) _____

11. How many times has your dog stayed at kennels within the last 12 months (including this stay)?

Section 4: About you

12. Do you, or anyone in your household, work in the health-care or veterinary/farming industry?

- Yes No Don't know

If yes, in what setting?

- Hospital Nursing home
 GP surgery Farming
 Veterinary practice Other (please specify) _____

13. Have you, or anyone else in your household, been into a hospital within the last month (including visiting)?

- Yes No Don't know

If yes, what was the reason for this?

- Hospital admission
 Visiting
 Outpatient appointment
 Other (please specify) _____

Thank you for filling in this questionnaire.

Please indicate any further comments or concerns regarding this study:

If you would like to discuss your comments further please do not hesitate to email the study team at E.E.Ormandy@liverpool.ac.uk

Many thanks for your participation

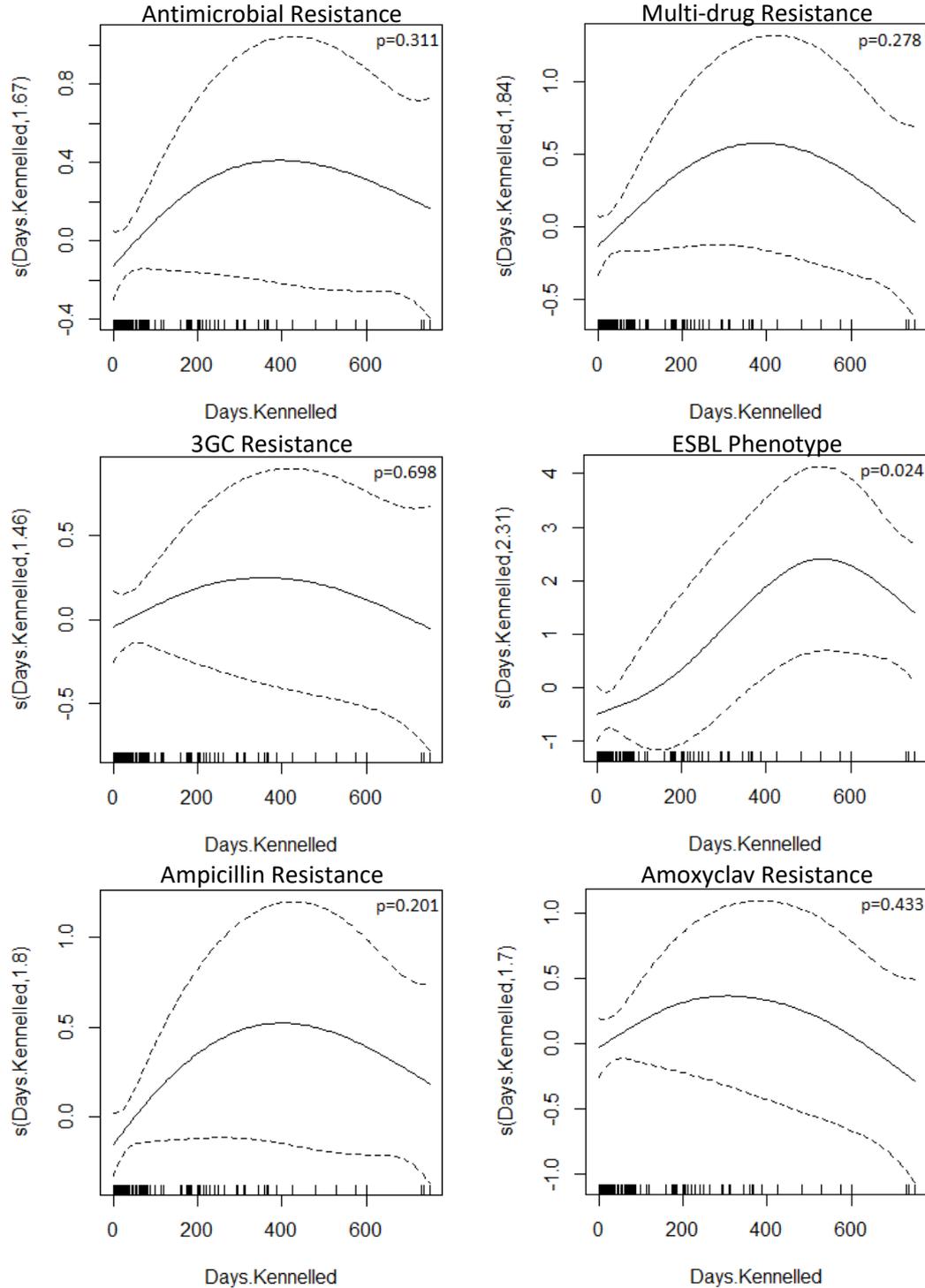
Appendix III

Material pertaining to Chapter Five

Total Dog Population

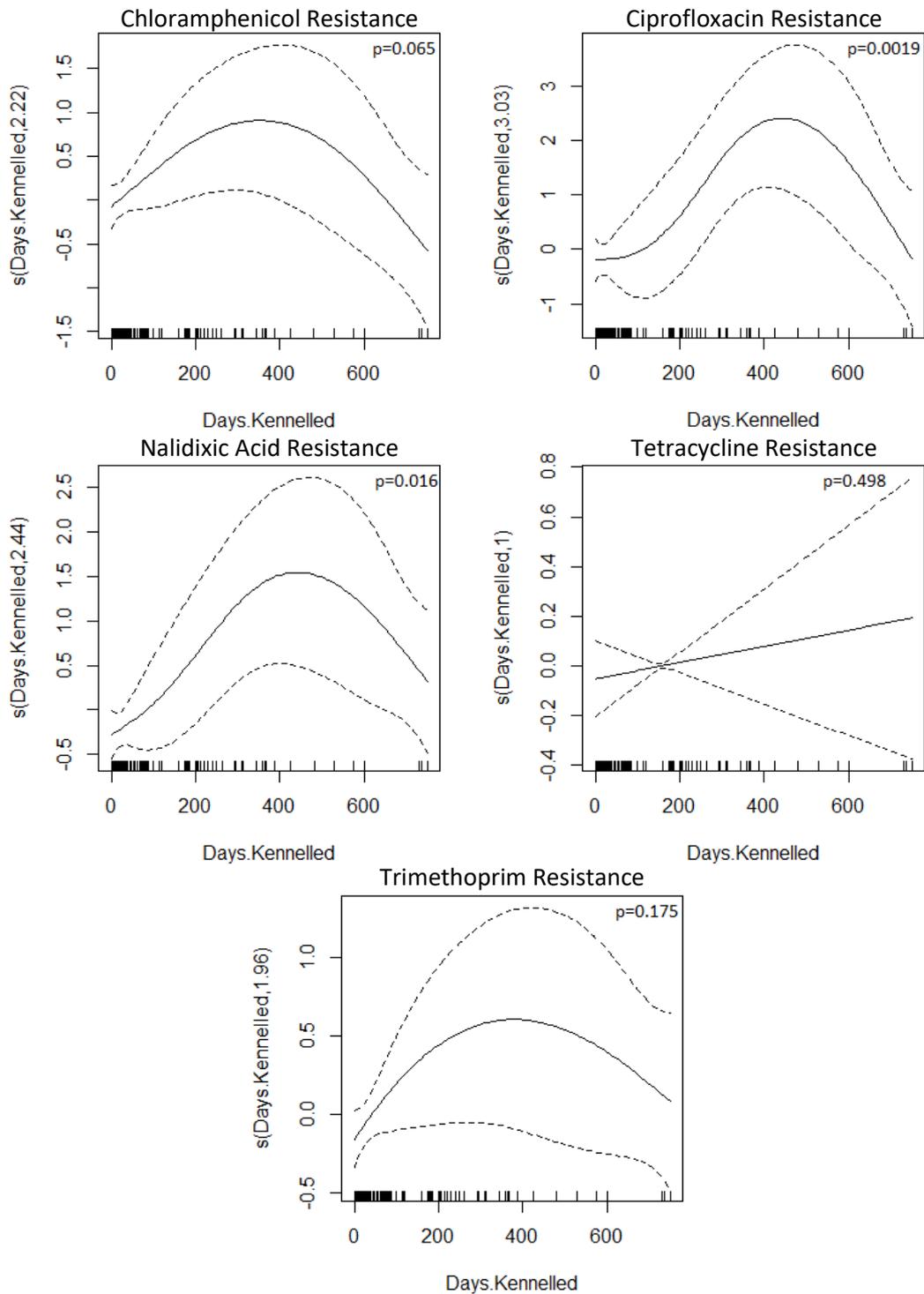
Generalised Additive Model (GAM) plots for duration of kennelling

Figure III-a Plots indicating the functional form of duration of kennelling modelled in generalised additive models for each outcome of interest utilising spline smoothers. The plots provide an estimate of the shape of the relationship between the explanatory variable on the x axis and the modelled outcome variable plotted on a centred log scale on the y axis



Key: Dashed lines indicate 95% confidence intervals; Rug plots on x-axis indicate data points; P values indicate the significance of smooth terms calculated using Chi squared test.

Figure III-a cont.



Key: Dashed lines indicate 95% confidence intervals; Rug plots on x-axis indicate data points; P values indicate the significance of smooth terms calculated using Chi squared test

Results of univariable analysis

Complete results of univariable analysis for all outcomes of interest are presented on pages 276 to 292

Table III-a Results of univariable analysis for risk factors associated with antimicrobial and multi-drug resistance in faecal samples obtained from 292 kennelled dogs in the North of England

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Premises type	Boarding	(Ref)	-	-	-	-	0.584	0.445	-	(Ref)	-	-	-	-	0.218	0.641	-
	Rescue centre	0.432	0.558	1.540	0.516	4.593	-	-	-	0.410	0.871	1.506	0.273	8.299	-	-	-
Age	<1	(Ref)	-	-	-	-	0.209	0.901	-	(Ref)	-	-	-	-	0.322	0.851	-
	1-6years	0.037	0.593	1.037	0.324	3.317	-	-	-	0.065	0.750	1.068	0.246	4.642	-	-	-
	7+ years	-0.101	0.604	0.904	0.276	2.956	-	-	-	-0.151	0.757	0.860	0.195	3.788	-	-	-
Sex	Female	(Ref)	-	-	-	-	0.384	0.535	-	(Ref)	-	-	-	-	0.965	0.326	-
	Male	-0.171	0.273	0.843	0.493	1.441	-	-	-	-0.346	0.353	0.707	0.354	1.414	-	-	-
Neutered		0.231	0.310	1.260	0.687	2.312	0.549	0.459	-	0.279	0.451	1.322	0.546	3.200	0.388	0.533	-
Sex	Male entire	(Ref)	-	-	-	-	1.760	0.624	-	(Ref)	-	-	-	-	1.139	0.768	-
	Male neutered	0.500	0.407	1.649	0.743	3.660	-	-	-	0.472	0.574	1.603	0.520	4.939	-	-	-
	Female entire	0.481	0.469	1.617	0.646	4.052	-	-	-	0.568	0.661	1.764	0.483	6.442	-	-	-
	Female	0.354	0.400	1.425	0.651	3.120	-	-	-	0.530	0.570	1.699	0.556	5.191	-	-	-
Pure breed		0.257	0.317	1.292	0.694	2.406	0.648	0.421	-	0.262	0.407	1.300	0.586	2.884	0.422	0.516	-
Size	Small/Toy	(Ref)	-	-	-	-	5.549	0.062	-	(Ref)	-	-	-	-	5.997	0.050	-
	Medium	0.399	0.377	1.490	0.712	3.117	-	-	-	0.948	0.556	2.581	0.868	7.672	-	-	-
	Giant	0.879	0.385	2.408	1.133	5.116	-	-	-	1.297	0.559	3.658	1.224	10.936	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	2.985	0.394	-	(Ref)	-	-	-	-	0.455	0.929	-
	Overnight	-0.461	0.457	0.630	0.257	1.543	-	-	-	-0.348	0.660	0.706	0.194	2.575	-	-	-
	Day	-1.048	0.781	0.350	0.076	1.618	-	-	-	-0.645	1.076	0.525	0.064	4.321	-	-	-
	Rescue	0.190	0.567	1.209	0.398	3.674	-	-	-	-0.056	0.780	0.946	0.205	4.366	-	-	-
Duration of kennelling	(days)	0.001	0.001	1.001	1.000	1.002	1.495	0.221	-	0.000	0.001	1.000	0.999	1.002	0.022	0.882	-
Duration of kennelling (Categorical)	Up to 2 weeks	(Ref)	-	-	-	-	3.246	0.517	-	(Ref)	-	-	-	-	1.124	0.891	-
	2 w-1m	-0.340	0.589	0.712	0.225	2.257	-	-	-	-0.312	0.706	0.732	0.184	2.920	-	-	-
	1-3m	0.278	0.523	1.321	0.474	3.683	-	-	-	-0.683	0.710	0.505	0.126	2.031	-	-	-
	4-6m	0.823	0.747	2.278	0.526	9.859	-	-	-	-0.013	0.906	0.987	0.167	5.830	-	-	-
	>6 m	0.498	0.387	1.645	0.771	3.509	-	-	-	-0.151	0.546	0.860	0.295	2.508	-	-	-
Sharing kennel		0.229	0.378	1.258	0.599	2.641	0.364	0.546	-	0.136	0.508	1.146	0.423	3.102	0.071	0.790	-
Working Dog		0.044	0.659	1.045	0.287	3.806	0.004	0.947	-	-0.412	0.990	0.663	0.095	4.615	0.178	0.674	-
Life Owner		-0.402	0.346	0.669	0.340	1.319	1.323	0.250	-	-1.574	0.491	0.207	0.079	0.543	3.574	0.057	-

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Origin	Rescue	(Ref)	-	-	-	-	4.924	0.295	-	(Ref)	-	-	-	-	11.126	0.025	-
	Breeder	-0.496	0.435	0.609	0.260	1.428	-	-	-	-0.991	0.576	0.371	0.120	1.148	-	-	-
	Home bred	-0.964	0.757	0.381	0.086	1.681	-	-	-	-1.230	1.076	0.292	0.035	2.409	-	-	-
	Previous owner	-1.028	0.511	0.358	0.131	0.974	-	-	-	-1.697	0.672	0.183	0.049	0.684	-	-	-
	Other	-1.085	0.797	0.338	0.071	1.613	-	-	-	-2.445	0.897	0.087	0.015	0.503	-	-	-
Veterinary treatment within 6 months		0.848	0.323	2.336	1.241	4.396	6.913	0.009	-	0.364	0.428	1.439	0.623	3.327	0.723	0.395	-
Last vet treatment	(Ref)	-	-	-	-	-	6.196	0.102	-	(Ref)	-	-	-	-	5.244	0.155	-
		0.706	0.373	2.026	0.975	4.206	-	-	-	-0.087	0.487	0.916	0.353	2.378	-	-	-
		1.000	0.475	2.720	1.071	6.905	-	-	-	-0.507	0.651	0.602	0.168	2.157	-	-	-
		0.570	0.569	1.769	0.580	5.391	-	-	-	1.200	0.637	3.321	0.953	11.572	-	-	-
Antimicrobials within 6 months		0.482	0.384	1.619	0.762	3.437	1.571	0.210	-	0.347	0.448	1.414	0.588	3.401	0.592	0.442	-
Last antimicrobial treatment	(Ref)	-	-	-	-	-	3.219	0.359	-	(Ref)	-	-	-	-	4.273	0.234	-
		0.969	0.560	2.635	0.880	7.895	-	-	-	0.506	0.602	1.658	0.510	5.394	-	-	-
		0.002	0.649	1.002	0.281	3.575	-	-	-	-0.877	0.897	0.416	0.072	2.413	-	-	-
Hospitalisation within 6 months		0.125	0.760	1.133	0.256	5.024	-	-	-	1.308	0.860	3.700	0.685	19.985	-	-	-
	Polytomous variable	1.646	0.877	5.185	0.929	28.941	4.221	0.040	-	0.899	0.807	2.457	0.506	11.939	1.211	0.271	-
Vet Treatment, antimicrobials and hospitalisation	No Treatment	(Ref)	-	-	-	-	10.628	0.031	-	-	-	-	-	-	-	-	-
	No Ab Tx or	0.720	0.356	2.055	1.023	4.130	-	-	-	-	-	-	-	-	-	-	-
	Ab Tx	0.766	0.450	2.152	0.890	5.202	-	-	-	-	-	-	-	-	-	-	-
	Hosp	16.451	1688	1.39E+07	0.000	Inf	-	-	-	-	-	-	-	-	-	-	-
	Ab Tx and Hosp	1.326	0.930	3.765	0.608	23.323	-	-	-	-	-	-	-	-	-	-	-
Vet treatment and antimicrobials	No treatment	(Ref)	-	-	-	-	6.929	0.031	-	-	-	-	-	-	-	-	-
	No Ab Tx	0.828	0.360	2.289	1.131	4.631	-	-	-	-	-	-	-	-	-	-	-
	Ab Tx	0.884	0.422	2.420	1.057	5.538	-	-	-	-	-	-	-	-	-	-	-
Vet treatment and hospitalisation	No treatment	(Ref)	-	-	-	-	8.824	0.012	-	-	-	-	-	-	-	-	-
	No Hosp	0.716	0.334	2.046	1.063	3.937	-	-	-	-	-	-	-	-	-	-	-
	Hospitalisation	1.903	0.877	6.704	1.201	37.416	-	-	-	-	-	-	-	-	-	-	-
Veterinary treatment alone most significant so included in multivariable model																	
Fed wet food		-0.150	0.335	0.861	0.446	1.659	0.199	0.656	-	-0.388	0.454	0.679	0.279	1.653	0.730	0.393	-
Fed dry Mixer		0.055	0.409	1.057	0.474	2.357	0.018	0.894	-	-0.187	0.589	0.829	0.261	2.632	0.102	0.749	-
Fed complete dry food		0.086	0.439	1.090	0.461	2.574	0.037	0.847	-	0.403	0.641	1.497	0.426	5.258	0.407	0.523	-

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Fed raw food		0.555	0.619	1.742	0.518	5.865	0.795	0.373	-	1.800	0.737	6.051	1.428	25.637	5.715	0.017	-
Fed cooked meat		-0.362	0.628	0.696	0.203	2.385	0.325	0.569	-	0.171	0.793	1.187	0.251	5.614	0.046	0.830	-
Housed indoors		-0.220	0.444	0.802	0.336	1.915	0.238	0.626	-	-0.419	0.699	0.658	0.167	2.588	0.348	0.555	-
Housed outdoors		0.377	0.586	1.457	0.462	4.600	0.408	0.523	-	1.389	0.788	4.011	0.856	18.805	3.087	0.079	-
Housed in kennels		0.139	0.473	1.149	0.455	2.903	0.085	0.770	-	0.438	0.730	1.550	0.371	6.478	0.365	0.546	-
Multi-dog household		0.113	0.348	1.120	0.567	2.213	0.105	0.746	-	0.260	0.471	1.297	0.516	3.262	0.303	0.582	-
Number of dogs in household	1	(Ref)	-	-	-	-	2.459	0.483	-	(Ref)	-	-	-	-	3.089	0.378	-
	2-3	-0.184	0.419	0.832	0.366	1.889	-	-	-	-0.132	0.555	0.876	0.295	2.602	-	-	-
	4-5	0.088	0.759	1.092	0.247	4.832	-	-	-	1.664	1.016	5.281	0.721	38.682	-	-	-
	6+	0.942	0.656	2.566	0.709	9.288	-	-	-	0.689	0.979	1.991	0.293	13.553	-	-	-
In-contact dogs on antimicrobials		-0.269	0.582	0.764	0.244	2.392	0.212	0.645	-	-0.348	0.935	0.706	0.113	4.412	0.143	0.705	-
In-contact dogs hospitalised		-0.668	0.894	0.513	0.089	2.955	0.572	0.449	-	0.173	1.182	1.189	0.117	12.065	0.021	0.884	-
Contact with animals other than dogs		0.285	0.367	1.329	0.648	2.729	0.598	0.439	-	-2.139	0.497	1.341	0.506	3.555	0.343	0.558	-
Cats		0.472	0.440	1.603	0.676	3.800	1.157	0.282	-	0.041	0.597	1.042	0.323	3.358	0.005	0.946	-
Horses		0.646	0.534	1.908	0.670	5.432	1.487	0.223	-	0.553	0.783	1.738	0.374	8.071	0.487	0.485	-
Small Mammals		1.294	0.785	3.649	0.784	16.995	2.996	0.083	-	0.954	0.878	2.597	0.464	14.525	1.136	0.286	-
Farm Animals		1.748	0.697	5.743	1.465	22.515	7.090	0.008	-	1.059	0.832	2.884	0.565	14.732	1.471	0.225	-
Wildlife		0.827	0.559	2.287	0.765	6.837	2.241	0.134	-	0.303	0.752	1.353	0.310	5.909	0.157	0.692	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; Ab Tx= antimicrobial treatment; Hosp= hospitalisation

Table III-b Results of univariable analysis for risk factors associated with third generation cephalosporin and ESBL-mediated resistance in faecal samples obtained from 292 kennelled dogs in the North of England

Variable	Category	Third Generation Cephalosporin Resistance								ESBL Phenotype							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Premises type	Boarding kennel	(Ref)	-	-	-	-	0.351	0.554	-	(Ref)	-	-	-	-	0.049	0.824	0.016
	Rescue centre	0.433	0.723	1.541	0.373	6.362	-	-	-	0.485	2.231	1.624	0.020	128.821	-	-	-
Age	<1	(Ref)	-	-	-	-	1.464	0.481	0.106	-	-	-	-	-	-	-	1.000
	1-6years	1.146	1.117	3.146	0.352	28.108	-	-	-	-	-	-	-	-	-	-	-
	7+ years	1.194	1.121	3.300	0.367	29.688	-	-	-	-	-	-	-	-	-	-	-
Sex	Female	(Ref)	-	-	-	-	4.558	0.033	-	(Ref)	-	-	-	-	1.495	0.221	-
	Male	-0.783	0.373	0.457	0.220	0.949	-	-	-	-1.327	1.129	0.265	0.029	2.427	-	-	-
Neutered		0.612	0.489	1.844	0.707	4.808	1.658	0.198	-	3.775	3.524	43.581	0.044	4.36E+04	2.430	0.119	-
Sex	Male entire	(Ref)	-	-	-	-	7.135	0.068	-	(Ref)	-	-	-	-	10.341	0.016	0.024
	Male neutered	0.946	0.703	2.576	0.649	10.225	-	-	-	-0.001	6.101	0.999	0.000	1.56E+05	-	-	-
	Female entire	1.405	0.747	4.077	0.942	17.637	-	-	-	-3.737	4.716	0.024	0.000	246.162	-	-	-
	Female neutered	1.548	0.686	4.702	1.226	18.028	-	-	-	10.222	6.007	2.75E+04	0.212	3.57E+09	-	-	-
Pure breed		-0.134	0.417	0.875	0.386	1.980	0.103	0.749	-	0.671	2.823	1.956	0.008	494.41	0.065	0.798	-
Size	Small/Toy	(Ref)	-	-	-	-	5.353	0.069	-	-11.010	2.844	0.000	0.000	0.004	0.888	0.641	0.205
	Medium	1.220	0.573	3.388	1.102	10.422	-	-	-	0.186	1.743	1.205	0.040	36.716	-	-	-
	Giant	0.644	0.574	1.904	0.617	5.869	-	-	-	1.146	1.589	3.144	0.140	70.843	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	0.755	0.860	-	(Ref)	-	-	-	-	3.029	0.387	0.000
	Overnight	0.108	0.685	1.114	0.291	4.269	-	-	-	-2.533	0.002	0.079	0.079	0.080	-	-	-
	Day	0.448	1.069	1.565	0.193	12.715	-	-	-	-0.287	0.002	0.751	0.747	0.754	-	-	-
	Rescue	0.618	0.832	1.855	0.363	9.468	-	-	-	0.203	0.002	1.225	1.219	1.231	-	-	-
Duration of kennelling	(days)	0.000	0.001	1.000	0.998	1.001	0.090	0.764	-	-	-	-	-	-	-	-	-
Duration of kennelling (piecewise)	<100 days	-	-	-	-	-	-	-	-	0.029	0.083	0.507	0.467	0.548	7.005	0.220	-
	100-500 days	-	-	-	-	-	-	-	-	-0.018	0.050	0.496	0.471	0.520	-	-	-
	≥500 days	-	-	-	-	-	-	-	-	-0.005	0.031	0.499	0.484	0.514	-	-	-
Duration of kennelling (categorical)	Up to 2 weeks	(Ref)	-	-	-	-	2.732	0.604	-	(Ref)	-	-	-	-	1.469	0.832	0.117
	2 w-1m	-1.298	0.914	0.273	0.046	1.636	-	-	-	1.288	2.150	3.625	0.054	245.330	-	-	-
	1-3m	-0.630	0.744	0.533	0.124	2.292	-	-	-	2.642	2.928	14.035	0.045	4.36E+03	-	-	-
	4-6m	-0.478	0.974	0.620	0.092	4.182	-	-	-	3.128	3.223	22.825	0.041	1.26E+04	-	-	-
	>6 m	-0.427	0.561	0.652	0.217	1.960	-	-	-	1.086	1.713	2.964	0.103	85.109	-	-	-
Sharing kennel		0.017	0.542	1.017	0.352	2.941	0.001	0.975	-	-1.906	3.215	0.149	0.000	81.080	0.593	0.441	-

Variable	Category	Third Generation Cephalosporin Resistance								ESBL Phenotype							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Working Dog		-1.663	1.318	0.190	0.014	2.509	1.995	0.158	-	-1.778	1.942	0.169	0.004	7.599	0.886	0.347	-
Life Owner		-0.250	0.506	0.779	0.289	2.099	0.243	0.622	-	0.084	1.133	1.088	0.118	10.026	0.006	0.940	-
Origin	Rescue	-2.215	0.706	0.109	0.027	0.435	2.686	0.612	-	(Ref)	-	-	-	-	2.611	0.625	0.137
	Breeder	-0.577	0.602	0.562	0.172	1.829	-	-	-	-0.032	1.568	0.968	0.045	20.919	-	-	-
	Home bred	0.377	1.137	1.458	0.157	13.540	-	-	-	3.869	3.173	47.871	0.095	2.40E+04	-	-	-
	Previous owner	-0.761	0.654	0.467	0.130	1.684	-	-	-	1.168	2.827	3.214	0.013	820.076	-	-	-
	Other	-0.905	0.830	0.405	0.079	2.060	-	-	-	2.181	3.734	8.858	0.006	1.34E+04	-	-	-
Veterinary treatment within 6 months		1.370	0.503	3.937	1.470	10.542	8.052	0.005	-	0.035	1.128	1.036	0.114	9.444	0.001	0.975	-
Last vet treatment	>6m	-3.247	0.638	0.039	0.011	0.136	8.869	0.031	-	-	-	-	-	-	-	-	0.344
	<1m	1.492	0.561	4.446	1.479	13.361	-	-	-	-	-	-	-	-	-	-	-
	2-3m	1.421	0.655	4.141	1.148	14.945	-	-	-	-	-	-	-	-	-	-	-
	4-6m	0.462	0.804	1.587	0.328	7.675	-	-	-	-	-	-	-	-	-	-	-
Antimicrobials within 6 months		0.802	0.464	2.231	0.899	5.537	2.920	0.087	-	-0.961	1.452	0.382	0.022	6.582	0.478	0.489	0.218
Last antimicrobial	>6m	-1.442	0.734	0.237	0.056	0.996	4.531	0.210	-	-	-	-	-	-	-	-	0.847
	<1m	-1.018	0.967	0.361	0.054	2.403	-	-	-	-	-	-	-	-	-	-	-
	2-3m	-1.044	1.097	0.352	0.041	3.027	-	-	-	-	-	-	-	-	-	-	-
	4-6m	-1.310	0.612	0.270	0.081	0.895	-	-	-	-	-	-	-	-	-	-	-
Hospitalisation within 6 months		1.792	0.883	6.000	1.063	33.875	3.985	0.046	-	-	-	-	-	-	-	-	1.000
Polytomous variables									-	-	-	-	-	-	-	-	-
Vet Treatment, antimicrobials and hospitalisation	No Treatment	(Ref)	-	-	-	-	9.341	0.053	-	-	-	-	-	-	-	-	-
	No Ab Tx or Hosp	1.089	0.553	2.970	1.004	8.787	-	-	-	-	-	-	-	-	-	-	-
	Ab Tx	1.317	0.636	3.732	1.073	12.982	-	-	-	-	-	-	-	-	-	-	-
	Hosp	2.035	1.475	7.654	0.425	137.90	-	-	-	-	-	-	-	-	-	-	-
	Ab Tx and Hosp	2.447	1.076	11.553	1.403	95.132	-	-	-	-	-	-	-	-	-	-	-
Vet treatment and antimicrobials	No treatment	(Ref)	-	-	-	-	8.429	0.015	-	-	-	-	-	-	-	-	-
	No Ab Tx	1.259	0.536	3.523	1.231	10.081	-	-	-	-	-	-	-	-	-	-	-
	Ab Tx	1.558	0.587	4.749	1.504	14.994	-	-	-	-	-	-	-	-	-	-	-
Vet treatment and hospitalisation	No treatment	(Ref)	-	-	-	-	9.098	0.011	-	-	-	-	-	-	-	-	-
	No Hosp	1.163	0.524	3.199	1.146	8.925	-	-	-	-	-	-	-	-	-	-	-
	Hospitalisation	2.323	0.912	10.209	1.707	61.052	-	-	-	-	-	-	-	-	-	-	-
Veterinary treatment alone most significant so included																	

Variable	Category	Third Generation Cephalosporin Resistance								ESBL Phenotype							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Fed wet food		0.095	0.485	1.100	0.425	2.848	0.038	0.845	-	10.042	5.331	2.30E+04	0.666	7.93E+08	6.665	0.010	-
Fed dry Mixer		-1.352	0.818	0.259	0.052	1.285	3.379	0.066	-	0.896	1.576	2.450	0.112	53.811	0.315	0.575	-
Fed complete dry		1.263	0.832	3.537	0.692	18.073	2.775	0.096	-	-0.917	1.579	0.400	0.018	8.824	0.328	0.567	-
Fed raw food		1.722	0.743	5.598	1.304	24.026	5.035	0.025	-	1.726	1.837	5.616	0.154	205.467	0.859	0.354	-
Fed cooked meat		-0.961	1.178	0.383	0.038	3.852	0.782	0.376	-	-	-	-	-	-	-	-	1.000
Housed indoors		-0.122	0.718	0.885	0.217	3.611	0.028	0.866	-	1.825	3.191	6.206	0.012	3227.93	0.460	0.498	-
Housed outdoors		-0.473	1.056	0.623	0.079	4.930	0.220	0.639	-	1.439	3.552	4.215	0.004	4450.56	0.127	0.721	-
Housed in kennels		0.278	0.747	1.320	0.306	5.706	0.138	0.710	-	2.349	2.774	10.480	0.046	2406.87	0.943	0.331	-
Multi-dog household		0.823	0.551	2.277	0.774	6.701	2.318	0.128	-	1.158	1.349	3.184	0.226	44.800	0.763	0.382	-
Number of dogs in household	1	(Ref)	-	-	-	-	2.626	0.453	-	(Ref)	-	-	-	-	4.672	0.197	-
	2-3	0.799	0.612	2.224	0.670	7.382	-	-	-	0.628	1.697	1.875	0.067	52.128	-	-	-
	4-5	0.228	1.424	1.256	0.077	20.459	-	-	-	8.255	6.673	3846.371	0.008	1.84E+09	-	-	-
	6+	1.118	1.012	3.059	0.421	22.219	-	-	-	-0.795	5.031	0.451	0.000	8658.720	-	-	-
In-contact dogs on		-0.793	1.267	0.453	0.038	5.427	0.438	0.508	0.031	-	-	-	-	-	-	-	1.000
In-contact dogs		0.588	1.456	1.800	0.104	31.270	0.155	0.694	-	-	-	-	-	-	-	-	1.000
Contact with animals		0.689	0.554	1.992	0.672	5.903	1.536	0.215	-	0.894	1.349	2.444	0.174	34.364	0.448	0.504	-
Cats		0.695	0.655	2.004	0.555	7.228	1.114	0.291	-	1.558	1.401	4.747	0.305	73.956	1.270	0.260	-
Horses		-0.533	0.938	0.587	0.093	3.690	0.342	0.559	-	0.060	1.564	1.062	0.050	22.764	0.001	0.969	-
Small Mammals		-0.903	1.274	0.405	0.033	4.925	0.568	0.451	-	-0.340	1.648	0.712	0.028	17.989	0.043	0.835	-
Farm Animals		0.863	0.904	2.369	0.403	13.940	0.841	0.359	-	-0.932	3.375	0.394	0.001	293.890	0.092	0.761	-
Wildlife		0.660	0.757	1.934	0.439	8.525	0.707	0.401	-	1.403	1.604	4.068	0.175	94.442	0.730	0.393	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; Ab Tx= antimicrobial treatment; Hosp= hospitalisation

Table III-c Results of univariable analysis for risk factors associated with ampicillin and amoxycyclav resistance in faecal samples obtained from 292 kennelled dogs in the North of England

Variable	Category	Ampicillin Resistance								Amoxycyclav Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Premises type	Boarding kennel	(Ref)	-	-	-	-	0.401	0.526	-	(Ref)	-	-	-	-	1.473	0.225	-
	Rescue centre	0.478	0.743	1.613	0.376	6.924	-	-	-	0.747	0.600	2.110	0.651	6.842	-	-	-
Age	<1	(Ref)	-	-	-	-	0.457	0.796	-	(Ref)	-	-	-	-	0.694	0.707	0.152
	1-6years	0.186	0.621	1.204	0.357	4.067	-	-	-	0.513	0.838	1.670	0.323	8.631	-	-	-
	7+ years	-0.030	0.631	0.970	0.281	3.344	-	-	-	0.266	0.852	1.304	0.246	6.928	-	-	-
Sex	Female	(Ref)	-	-	-	-	1.363	0.243	-	(Ref)	-	-	-	-	0.665	0.415	-
	Male	-0.344	0.290	0.709	0.401	1.253	1.363	0.243	-	-0.283	0.347	0.754	0.381	1.489	-	-	-
Neutered		0.247	0.334	1.280	0.664	2.465	0.530	0.467	-	0.316	0.438	1.371	0.581	3.237	0.529	0.467	-
Sex	Male entire	(Ref)	-	-	-	-	3.604	0.308	-	(Ref)	-	-	-	-	1.567	0.667	-
	Male neutered	0.615	0.441	1.850	0.779	4.394	-	-	-	0.505	0.589	1.656	0.523	5.250	-	-	-
	Female entire	0.902	0.511	2.466	0.906	6.708	-	-	-	0.618	0.653	1.856	0.516	6.673	-	-	-
	Female neutered	0.603	0.432	1.828	0.783	4.266	-	-	-	0.665	0.578	1.945	0.627	6.039	-	-	-
Pure breed		0.168	0.338	1.183	0.610	2.297	0.240	0.624	-	0.116	0.400	1.123	0.513	2.458	0.085	0.771	-
Size	Small/Toy	(Ref)	-	-	-	-	5.356	0.069	-	(Ref)	-	-	-	-	5.887	0.053	-
	Medium	0.443	0.406	1.557	0.703	3.448	-	-	-	0.931	0.506	2.537	0.940	6.844	-	-	-
	Giant	0.934	0.412	2.546	1.136	5.708	-	-	-	0.080	0.527	1.083	0.386	3.044	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	5.786	0.123	-	(Ref)	-	-	-	-	2.226	0.527	-
	Overnight	-0.795	0.514	0.452	0.165	1.236	-	-	-	0.413	0.689	1.511	0.392	5.832	-	-	-
	Day	-2.050	0.906	0.129	0.022	0.761	-	-	-	0.541	1.059	1.718	0.215	13.690	-	-	-
	Rescue	-0.275	0.699	0.759	0.193	2.991	-	-	-	1.086	0.773	2.963	0.652	13.471	-	-	-
Duration of kennelling	(days)	0.001	0.001	1.001	1.000	1.003	3.609	0.057	-	-0.001	0.001	0.999	0.998	1.001	0.592	0.442	-
Duration of kennelling	Up to 2 weeks	(Ref)	-	-	-	-	4.539	0.338	-	(Ref)	-	-	-	-	2.857	0.582	-
	2 w-1m	0.161	0.637	1.175	0.337	4.097	-	-	-	-1.232	0.895	0.292	0.050	1.686	-	-	-
	1-3m	0.687	0.575	1.988	0.644	6.138	-	-	-	-0.043	0.649	0.958	0.269	3.416	-	-	-
	4-6m	0.910	0.792	2.485	0.526	11.743	-	-	-	-0.461	0.928	0.630	0.102	3.887	-	-	-
	>6 m	0.849	0.431	2.338	1.004	5.444	-	-	-	-0.517	0.539	0.596	0.207	1.716	-	-	-
Sharing kennel		0.213	0.415	1.238	0.549	2.791	0.256	0.613	-	-0.140	0.520	0.870	0.314	2.412	0.073	0.787	-
Working Dog		0.654	0.755	1.923	0.438	8.446	0.722	0.395	-	-1.867	1.237	0.155	0.014	1.745	3.081	0.079	0.030
Life Owner		-0.403	0.384	0.668	0.315	1.419	1.057	0.304	-	-0.398	0.472	0.672	0.266	1.695	0.705	0.401	-

Variable	Category	Ampicillin Resistance								Amoxycylav Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Origin	Rescue	(Ref)	-	-	-	-	12.858	0.012	-	(Ref)	-	-	-	-	1.098	0.895	-
	Breeder	-0.577	0.002	0.561	0.560	0.563	-	-	-	-0.478	0.550	0.620	0.211	1.822	-	-	-
	Home bred	-0.557	0.002	0.573	0.571	0.575	-	-	-	-0.734	1.230	0.480	0.043	5.353	-	-	-
	Previous owner	-2.134	0.002	0.118	0.118	0.119	-	-	-	-0.408	0.597	0.665	0.207	2.143	-	-	-
	Other	-2.143	0.002	0.117	0.117	0.118	-	-	-	-0.444	0.780	0.642	0.139	2.962	-	-	-
Veterinary treatment within 6 months		0.705	0.348	2.025	1.023	4.007	4.754	0.029	-	1.573	0.484	4.819	1.868	12.435	11.750	0.0006	
Last vet treatment	>6m	(Ref)	-	-	-	-	5.672	0.129	-	(Ref)	-	-	-	-	10.191	0.017	-
	<1m	0.485	0.392	1.624	0.753	3.502	-	-	-	1.492	0.500	4.445	1.669	11.837	-	-	-
	2-3m	1.191	0.509	3.292	1.213	8.934	-	-	-	1.284	0.598	3.611	1.118	11.665	-	-	-
	4-6m	0.429	0.599	1.536	0.475	4.969	-	-	-	0.491	0.772	1.634	0.360	7.423	-	-	-
Antimicrobials within 6 months		0.566	0.402	1.761	0.801	3.871	1.943	0.163	-	0.673	0.443	1.960	0.823	4.666	2.237	0.135	
Last antimicrobial treatment	>6m	(Ref)	-	-	-	-	2.210	0.530	-	(Ref)	-	-	-	-	6.228	0.101	-
	<1m	0.756	0.548	2.130	0.728	6.235	-	-	-	1.364	0.576	3.913	1.265	12.106	-	-	-
	2-3m	0.375	0.708	1.455	0.363	5.831	-	-	-	-0.484	0.888	0.616	0.108	3.514	-	-	-
4-6m	0.364	0.811	1.439	0.293	7.053	-	-	-	0.345	0.939	1.411	0.224	8.882	-	-	-	
Hospitalisation within 6 months		2.439	1.023	11.464	1.542	85.211	7.471	0.006	-	2.269	0.842	9.634	1.850	50.176	7.255	0.007	
Polytomous variables									-								
Vet Treatment, antimicrobials and hospitalisation	No Treatment	(Ref)	-	-	-	-	10.876	0.028	-	(Ref)	-	-	-	-	14.369	0.006	-
	No Ab Tx or Hosp	0.510	0.392	1.666	0.772	3.592	-	-	-	1.284	0.537	3.612	1.260	10.354	-	-	-
	Ab Tx	0.651	0.487	1.917	0.739	4.975	-	-	-	3.370	1.283	29.093	2.355	359.362	-	-	-
	Hosp	17.595	323.817	4.38E+07	0.000	1.91E+283	-	-	-	1.351	0.619	3.860	1.147	12.988	-	-	-
	Ab Tx and Hosp	2.148	1.110	8.564	0.973	75.394	-	-	-	2.540	1.032	12.680	1.679	95.779	-	-	-
Vet treatment and antimicrobials	No treatment	(Ref)	-	-	-	-	5.081	0.079	-	(Ref)	-	-	-	-	11.781	0.003	-
	No antimicrobials	0.669	0.384	1.952	0.920	4.143	-	-	-	1.543	0.512	4.680	1.716	12.763	-	-	-
	Ab Tx	0.931	0.449	2.537	1.052	6.115	-	-	-	1.624	0.563	5.074	1.682	15.307	-	-	-
Vet treatment and hospitalisation	No treatment	-0.308	0.414	0.735	0.326	1.656	9.775	0.008	-	(Ref)	-	-	-	-	14.052	0.0008	-
	No	0.541	0.360	1.718	0.848	3.481	-	-	-	1.302	0.511	3.677	1.352	10.006	-	-	-
	Hospitalisation	2.642	1.025	14.042	1.884	104.647	-	-	-	2.855	0.865	17.376	3.189	94.666	-	-	-
Hospitalisation alone most significant so included in multivariable model									Veterinary treatment alone most significant so included								
Fed wet food		-0.528	0.372	0.590	0.285	1.223	1.960	0.162	-	-0.126	0.456	0.882	0.360	2.157	0.076	0.782	-
Fed dry Mixer		-0.103	0.442	0.903	0.380	2.146	0.051	0.822	-	-0.799	0.694	0.450	0.115	1.753	1.461	0.227	-
Fed complete dry food		0.335	0.484	1.398	0.541	3.613	0.457	0.499	-	0.690	0.704	1.993	0.502	7.920	1.038	0.308	-

Variable	Category	Ampicillin Resistance									Amoxycylav Resistance						
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Fed raw food		0.957	0.677	2.603	0.690	9.822	1.976	0.160	-	0.830	0.718	2.294	0.561	9.376	1.245	0.264	-
Fed cooked meat		0.178	0.713	1.195	0.295	4.834	0.059	0.808	-	-0.025	0.884	0.975	0.173	5.513	0.001	0.978	-
Housed indoors		-0.229	0.528	0.795	0.282	2.240	0.179	0.673	-	-0.473	0.583	0.623	0.199	1.956	0.613	0.433	-
Housed outdoors		0.288	0.663	1.334	0.364	4.888	0.181	0.670	-	-0.348	0.902	0.706	0.120	4.137	0.159	0.690	-
Housed in kennels		0.402	0.582	1.494	0.477	4.680	0.461	0.497	-	0.229	0.627	1.258	0.368	4.302	0.130	0.718	-
Multi-dog household		0.353	0.386	1.423	0.668	3.030	0.810	0.368	-	0.215	0.499	1.240	0.467	3.298	0.187	0.666	-
Number of dogs in household	1	(Ref)	-	-	-	-	4.131	0.248	-	(Ref)	-	-	-	-	2.252	0.522	0.174
	2-3	-0.084	0.459	0.920	0.374	2.262	-	-	-	0.396	0.547	1.486	0.509	4.339	-	-	-
	4-5	1.049	0.908	2.856	0.481	16.945	-	-	-	-0.752	1.326	0.472	0.035	6.338	-	-	-
	6+	1.351	0.767	3.860	0.859	17.349	-	-	-	0.954	0.814	2.596	0.527	12.798	-	-	-
In-contact dogs on antimicrobials		-0.417	0.666	0.659	0.179	2.430	0.386	0.535	-	-1.037	1.187	0.355	0.035	3.635	0.909	0.341	0.030
In-contact dogs hospitalised		0.181	1.024	1.199	0.161	8.921	0.029	0.865	-	0.225	1.320	1.253	0.094	16.642	0.028	0.866	-
Contact with animals other than dogs		0.299	0.402	1.349	0.613	2.966	0.538	0.463	-	0.638	0.523	1.892	0.679	5.274	1.484	0.223	-
Cats		0.226	0.467	1.253	0.502	3.130	0.225	0.635	-	0.639	0.618	1.894	0.564	6.366	1.050	0.030	-
Horses		0.715	0.602	2.043	0.628	6.650	1.352	0.245	-	-0.408	0.878	0.665	0.119	3.720	0.226	0.230	-
Small Mammals		1.152	0.806	3.166	0.652	15.364	2.040	0.153	-	-0.724	1.215	0.485	0.045	5.244	0.400	0.527	0.227
Farm Animals		1.525	0.743	4.593	1.072	19.685	4.253	0.039	-	0.807	0.833	2.242	0.438	11.476	0.876	0.349	-
Wildlife		0.913	0.608	2.493	0.757	8.206	2.214	0.137	-	1.008	0.659	2.739	0.752	9.973	2.152	0.142	-
Poytomous variable																	
Non-canine contact and contact with wildlife	No contact	-	-	-	-	-	-	-	-	(Ref)	-	-	-	-	2.813	0.421	-
	No wildlife	-	-	-	-	-	-	-	-	0.256	0.643	1.292	0.367	4.553	-	-	-
	Wildlife	-	-	-	-	-	-	-	-	1.138	0.686	3.121	0.813	11.982	-	-	-

Contact with wildlife alone most significant so included in multivariable model

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; Ab Tx= antimicrobial treatment; Hosp= hospitalisation

Table III-d Results of univariable analysis for risk factors associated with chloramphenicol and ciprofloxacin resistance in faecal samples obtained from 292 kennelled dogs in the North of England

Variable	Category	Chloramphenicol Resistance								Ciprofloxacin Resistance							
		β	se	OR	L95%CI	U95%CI	LRT	P	FE	β	se	OR	L95%CI	U95%CI	LRT	P	FE
Premises type	Boarding kennel	(Ref)	-	-	-	-	0.760	0.383	-	(Ref)	-	-	-	-	0.160	0.690	-
	Rescue centre	0.534	0.601	1.705	0.525	5.541	-	-	-	0.898	2.218	2.454	0.032	189.728	-	-	-
Age	<1	(Ref)	-	-	-	-	3.652	0.161	0.040	(Ref)	-	-	-	-	4.733	0.094	0.058
	1-6years	1.438	1.092	4.212	0.495	35.817	-	-	-	0.199	0.001	1.220	1.216	1.223	-	-	-
	7+ years	0.932	1.107	2.539	0.290	22.245	-	-	-	-1.277	0.001	0.279	0.278	0.280	-	-	-
Sex	Female	(Ref)	-	-	-	-	1.156	0.282	-	(Ref)	-	-	-	-	1.497	0.221	-
	Male	0.374	0.350	1.454	0.732	2.889	-	-	-	0.776	0.653	2.172	0.604	7.809	-	-	-
Neutered		0.319	0.436	1.375	0.585	3.231	0.548	0.459	-	-0.006	0.826	0.994	0.197	5.024	0.000	0.996	-
Sex	Male entire	(Ref)	-	-	-	-	2.743	0.433	-	(Ref)	-	-	-	-	1.188	0.756	-
	Male neutered	0.395	0.531	1.485	0.525	4.202	-	-	-	0.233	0.956	1.262	0.194	8.216	-	-	-
	Female entire	-0.492	0.687	0.612	0.159	2.349	-	-	-	-0.344	1.218	0.709	0.065	7.711	-	-	-
	Female neutered	-0.167	0.555	0.846	0.285	2.512	-	-	-	-0.564	1.030	0.569	0.076	4.282	-	-	-
Pure breed		0.498	0.406	1.646	0.743	3.647	1.564	0.211	-	0.940	0.714	2.560	0.632	10.370	1.875	0.171	-
Size	Small/Toy	(Ref)	-	-	-	-	4.086	0.130	-	(Ref)	-	-	-	-	1.578	0.454	-
	Medium	1.019	0.534	2.771	0.973	7.892	-	-	-	0.448	0.847	1.565	0.297	8.237	-	-	-
	Giant	0.840	0.541	2.317	0.802	6.692	-	-	-	-0.474	0.918	0.622	0.103	3.763	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	5.046	0.168	-	(Ref)	-	-	-	-	0.654	0.884	0.048
	Overnight	-0.052	0.662	0.949	0.260	3.472	-	-	-	-0.082	1.938	0.921	0.021	41.126	-	-	-
	Day	1.459	0.936	4.303	0.687	26.960	-	-	-	0.414	2.278	1.513	0.017	131.369	-	-	-
	Rescue	0.801	0.728	2.227	0.535	9.274	-	-	-	1.127	1.880	3.085	0.077	122.996	-	-	-
Duration of kennelling	(days)	-0.001	0.001	0.999	0.997	1.001	1.900	0.168	-	-	-	-	-	-	-	-	-
Duration of kennelling (piecewise)	<100 days	-	-	-	-	-	-	-	-	-0.023	0.028	0.977	0.925	1.032	7.447	0.190	-
	100-450 days	-	-	-	-	-	-	-	-	0.019	0.018	1.019	0.984	1.056	-	-	-
	≥450 days	-	-	-	-	-	-	-	-	-0.005	0.014	0.995	0.967	1.024	-	-	-
Duration of kennelling	Up to 2 weeks	(Ref)	-	-	-	-	0.746	0.946	-	(Ref)	-	-	-	-	2.493	0.646	0.147
	2 w-1m	0.230	0.664	1.259	0.342	4.628	-	-	-	-1.434	1.238	0.238	0.021	2.699	-	-	-
	1-3m	-0.340	0.662	0.712	0.195	2.603	-	-	-	-1.409	1.254	0.244	0.021	2.857	-	-	-
	4-6m	0.011	0.860	1.011	0.188	5.453	-	-	-	-0.791	1.485	0.453	0.025	8.328	-	-	-
	>6 m	-0.254	0.519	0.775	0.280	2.145	-	-	-	-0.510	1.018	0.601	0.082	4.419	-	-	-
Sharing kennel		-0.297	0.524	0.743	0.266	2.078	0.332	0.565	-	0.829	1.093	2.292	0.269	19.508	0.547	0.459	-
Working Dog		-1.930	1.246	0.145	0.013	1.668	3.387	0.066	-	-0.183	3.014	0.833	0.002	306.329	0.004	0.951	-
Life Owner		-0.768	0.432	0.464	0.199	1.081	3.238	0.072	-	-0.318	1.006	0.728	0.101	5.228	0.100	0.752	-

Variable	Category	Chloramphenicol Resistance								Ciprofloxacin Resistance								
		β	se	OR	L95%CI	U95%CI	LRT	P	FE	β	se	OR	L95%CI	U95%CI	LRT	P	FE	
Origin	Rescue	(Ref)	-	-	-	-	1.348	0.853	-	-	-	-	-	-	-	-	0.333	
	Breeder	-0.450	0.502	0.638	0.239	1.704	-	-	-	-	-	-	-	-	-	-	-	
	Home bred	-0.304	0.945	0.738	0.116	4.706	-	-	-	-	-	-	-	-	-	-	-	
	Previous owner	-0.478	0.549	0.620	0.211	1.820	-	-	-	-	-	-	-	-	-	-	-	
	Other	-0.628	0.740	0.533	0.125	2.273	-	-	-	-	-	-	-	-	-	-	-	
Veterinary treatment										0.791	0.921	2.206	0.363	13.417	0.764	0.382	-	
Last vet treatment	>6m	(Ref)	-	-	-	-	4.073	0.254	-	(Ref)	-	-	-	-	1.727	0.631	-	
	<1m	0.871	0.452	2.388	0.986	5.786	-	-	-	1.176	0.973	3.240	0.481	21.837	-	-	-	
	2-3m	0.212	0.589	1.237	0.389	3.926	-	-	-	0.545	1.140	1.724	0.185	16.095	-	-	-	
	4-6m	0.488	0.624	1.629	0.480	5.531	-	-	-	0.327	1.325	1.387	0.103	18.632	-	-	-	
Antimicrobials within 6 months										-0.842	0.880	0.431	0.077	2.417	1.022	0.312	-	
Last antimicrobial	>6m	(Ref)	-	-	-	-	4.873	0.181	-	-	-	-	-	-	-	-	0.811	
	<1m	1.064	0.573	2.897	0.943	8.898	-	-	-	-	-	-	-	-	-	-	-	
	2-3m	-0.550	0.848	0.577	0.110	3.038	-	-	-	-	-	-	-	-	-	-	-	
	4-6m	-0.820	1.119	0.440	0.049	3.949	-	-	-	-	-	-	-	-	-	-	-	
Hospitalisation within 6 months										0.831	1.318	2.296	0.173	30.373	0.364	0.546	-	
Fed wet food										0.296	0.906	1.344	0.228	7.937	0.108	0.742	-	
Fed dry Mixer										0.958	1.100	2.607	0.302	22.499	0.740	0.390	-	
Fed complete dry food										-0.551	1.321	0.577	0.043	7.684	0.165	0.685	-	
Fed raw food										0.099	2.212	1.104	0.014	84.324	0.002	0.967	-	
Fed cooked meat										1.517	1.446	4.559	0.268	77.603	0.992	0.319	-	
Housed indoors										-1.540	2.167	0.214	0.003	14.997	0.492	0.483	-	
Housed outdoors										1.933	1.370	6.909	0.472	101.218	1.786	0.181	-	
Housed in kennels										0.312	2.229	1.366	0.017	107.761	0.019	0.890	-	
Multi-dog household										-0.453	1.130	0.636	0.069	5.823	0.168	0.682	-	
Number of dogs in household	1	(Ref)	-	-	-	-	0.022	0.999	-	(Ref)	-	-	-	-	0.962	0.810	0.000	
	2-3	0.018	0.543	1.018	0.351	2.953	-	-	-	-0.707	1.231	0.493	0.044	5.502	-	-	-	
	4-5	-0.025	0.974	0.975	0.145	6.577	-	-	-	0.969	2.657	2.635	0.014	481.418	-	-	-	
	6+	0.119	0.829	1.127	0.222	5.724	-	-	-	1.734	2.209	5.664	0.075	429.888	-	-	-	
In-contact dogs on antimicrobials										-1.257	1.119	0.285	0.032	2.554	1.604	0.205	0.018	-
In-contact dogs hospitalised										-0.314	1.188	0.731	0.071	7.498	0.073	0.787	-	1.000

Variable	Category	Chloramphenicol Resistance							Ciprofloxacin Resistance								
		β	se	OR	L95%CI	U95%CI	LRT	P	FE	β	se	OR	L95%CI	U95%CI	LRT	P	FE
Contact with animals other than dogs		0.359	0.466	1.431	0.574	3.565	0.583	0.445	-	-2.613	2.210	0.073	0.001	5.575	2.758	0.097	-
Cats		0.240	0.581	1.272	0.407	3.972	0.169	0.681	-	-0.677	1.293	0.508	0.040	6.404	0.298	0.585	0.051
Horses		0.116	0.676	1.123	0.298	4.227	0.029	0.865	-	1.010	1.682	2.746	0.102	74.138	0.362	0.548	0.240
Small Mammals		-1.117	1.200	0.327	0.031	3.438	1.062	0.303	-	-0.769	3.315	0.464	0.001	307.725	0.060	0.807	-
Farm Animals		0.905	0.671	2.473	0.664	9.211	1.629	0.202	-	0.165	2.229	1.180	0.015	93.245	0.005	0.946	-
Wildlife		1.428	0.586	4.169	1.322	13.150	5.447	0.020	-	-0.461	2.181	0.631	0.009	45.386	0.050	0.824	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; Ab Tx= antimicrobial treatment; Hosp= hospitalisation

Table III-e Results of univariable analysis for risk factors associated with nalidixic acid and tetracycline resistance in faecal samples obtained from 292 kennelled dogs in the North of England

Variable	Category	Nalidixic Acid Resistance								Tetracycline Resistance							
		β	se	OR	L95%CI	U95%CI	LRT	P	FE	β	se	OR	L95%CI	U95%CI	LRT	P	FE
Premises type	Boarding kennel	(Ref)	-	-	-	-	0.132	0.716	-	(Ref)	-	-	-	-	0.046	0.830	-
	Rescue centre	0.564	1.537	1.758	0.086	35.763	-	-	-	0.133	0.621	1.143	0.338	3.862	-	-	-
Age	<1	(Ref)	-	-	-	-	1.801	0.406	0.219	(Ref)	-	-	-	-	0.554	0.758	-
	1-6years	0.887	1.202	2.428	0.230	25.589	-	-	-	0.501	0.698	1.651	0.421	6.480	-	-	-
	7+ years	0.283	1.213	1.328	0.123	14.316	-	-	-	0.500	0.700	1.649	0.418	6.504	-	-	-
Sex	Female	(Ref)	-	-	-	-	0.378	0.538	-	(Ref)	-	-	-	-	0.672	0.412	-
	Male	-0.279	0.453	0.757	0.311	1.839	0.378	0.538	-	-	0.296	0.782	0.437	1.398	-	-	-
Neutered		0.374	0.645	1.454	0.411	5.151	0.348	0.555	-	0.391	0.360	1.478	0.730	2.994	1.172	0.279	-
Sex	Male entire	(Ref)	-	-	-	-	1.094	0.779	-	(Ref)	-	-	-	-	2.348	0.503	-
	Male neutered	0.427	0.809	1.533	0.314	7.484	-	-	-	0.696	0.474	2.005	0.792	5.075	-	-	-
	Female entire	0.595	0.945	1.814	0.285	11.550	-	-	-	0.576	0.548	1.779	0.608	5.205	-	-	-
	Female	0.746	0.792	2.109	0.447	9.954	-	-	-	0.564	0.470	1.757	0.699	4.416	-	-	-
Pure breed		0.305	0.526	1.356	0.484	3.805	0.340	0.560	-	-	0.335	0.770	0.400	1.484	0.590	0.442	-
Size	Small/Toy	(Ref)	-	-	-	-	1.813	0.404	-	(Ref)	-	-	-	-	-	-	-
	Medium	0.669	0.737	1.952	0.461	8.272	-	-	-	0.162	0.425	1.176	0.511	2.705	1.754	0.416	-
	Giant	0.942	0.727	2.566	0.617	10.672	-	-	-	0.506	0.417	1.658	0.732	3.756	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	0.940	0.816	0.024	(Ref)	-	-	-	-	0.400	0.940	-
	Overnight	-0.699	0.901	0.497	0.085	2.907	-	-	-	-	0.002	0.769	0.767	0.772	-	-	-
	Day	-0.949	1.481	0.387	0.021	7.064	-	-	-	-	0.002	0.613	0.611	0.615	-	-	-
	Rescue	0.207	1.199	1.230	0.117	12.895	-	-	-	-	0.002	0.925	0.922	0.928	-	-	-
Duration of kennelling (days)		-	-	-	-	-	-	-	-	0.000	0.001	1.000	0.999	1.002	0.300	0.584	-
Duration of kennelling (piecewise)	<100 days	-0.028	0.022	0.973	0.932	1.016	6.388	0.270	-	-	-	-	-	-	-	-	-
	100-450 days	0.013	0.012	1.013	0.990	1.038	-	-	-	-	-	-	-	-	-	-	-
	≥450 days	0.006	0.009	1.006	0.988	1.024	-	-	-	-	-	-	-	-	-	-	-
Duration of kennelling	Up to 2 weeks	(Ref)	-	-	-	-	6.861	0.143	-	(Ref)	-	-	-	-	1.501	0.826	-
	2 w-1m	-2.096	1.238	0.123	0.011	1.392	-	-	-	-	0.682	0.467	0.123	1.777	1.501	0.826	-
	1-3m	-1.450	1.026	0.235	0.031	1.754	-	-	-	-	0.582	0.784	0.250	2.454	-	-	-
	4-6m	-2.149	1.419	0.117	0.007	1.880	-	-	-	-	0.820	0.673	0.135	3.356	-	-	-
	>6 m	-0.289	0.762	0.749	0.168	3.337	-	-	-	-	0.426	0.962	0.417	2.215	-	-	-
Sharing kennel		-0.008	0.803	0.992	0.205	4.786	0.000	0.993	-	-	0.422	0.846	0.369	1.935	0.155	0.694	-
Working Dog		-0.583	2.119	0.558	0.009	35.513	0.079	0.779	-	-	0.002	0.572	0.570	0.573	0.494	0.482	-
Life Owner		-0.620	0.756	0.538	0.122	2.366	0.679	0.410	-	-	0.002	0.648	0.646	0.650	1.210	0.271	-

Variable	Category	Nalidixic Acid Resistance								Tetracycline Resistance							
		β	se	OR	L95%CI	U95%CI	LRT	P	FE	β	se	OR	L95%CI	U95%CI	LRT	P	FE
Origin	Rescue	(Ref)	-	-	-	-	10.203	0.037	-	(Ref)	-	-	-	-	11.884	0.018	-
	Breeder	0.486	0.880	1.626	0.290	9.114	-	-	-	-	0.457	0.372	0.152	0.912	-	-	-
	Home bred	-0.031	1.533	0.969	0.048	19.563	-	-	-	-	0.976	0.181	0.027	1.226	-	-	-
	Previous owner	-1.465	0.889	0.231	0.040	1.321	-	-	-	-	0.559	0.192	0.064	0.574	-	-	-
	Other	-2.929	1.076	0.053	0.006	0.440	-	-	-	-	0.755	0.232	0.053	1.019	-	-	-
Veterinary treatment within 6 months		0.013	0.660	1.013	0.278	3.691	0.000	0.984	-	0.488	0.359	1.629	0.805	3.295	1.847	0.174	-
Last vet treatment	>6m	(Ref)	-	-	-	-	1.640	0.650	-	(Ref)	-	-	-	-	6.235	0.101	-
	<1m	0.271	0.001	1.311	1.308	1.314	-	-	-	-	0.438	0.744	0.315	1.756	-	-	-
	2-3m	-0.630	0.001	0.532	0.531	0.534	-	-	-	0.604	0.522	1.829	0.657	5.090	-	-	-
	4-6m	-0.364	0.001	0.695	0.693	0.697	-	-	-	1.007	0.559	2.738	0.915	8.195	-	-	-
Antimicrobials within 6 months		-0.068	0.566	0.935	0.308	2.833	0.014	0.905	-	0.501	0.002	1.650	1.644	1.655	1.591	0.207	-
Last antimicrobial	>6m	(Ref)	-	-	-	-	2.238	0.524	0.160	(Ref)	-	-	-	-	10.544	0.014	-
	<1m	0.698	0.672	2.009	0.538	7.500	-	-	-	-	0.629	0.477	0.139	1.637	-	-	-
	2-3m	-0.968	1.188	0.380	0.037	3.898	-	-	-	1.164	0.676	3.201	0.850	12.052	-	-	-
	4-6m	-0.403	1.225	0.668	0.061	7.371	-	-	-	1.768	0.763	5.859	1.313	26.151	-	-	-
Hospitalisation within 6 months		1.306	0.001	3.690	3.681	3.699	1.281	0.258	-	1.016	0.002	2.763	2.754	2.771	1.876	0.171	-
Fed wet food		0.490	0.001	1.633	1.629	1.637	0.560	0.454	-	0.045	0.002	1.046	1.042	1.049	0.013	0.910	-
Fed dry Mixer		0.874	0.824	2.396	0.476	12.053	1.110	0.292	-	1.115	0.461	3.049	1.236	7.523	6.121	0.013	-
Fed complete dry food		-1.170	0.893	0.310	0.054	1.784	1.702	0.192	-	-	0.002	0.797	0.794	0.800	0.230	0.632	-
Fed raw food		2.920	1.020	18.549	2.514	136.87	8.026	0.005	-	1.458	0.002	4.297	4.281	4.313	5.224	0.022	-
Fed cooked meat		1.106	1.410	3.023	0.191	47.951	0.571	0.450	-	0.832	0.002	2.297	2.290	2.305	1.648	0.199	-
Housed indoors		-0.300	1.479	0.741	0.041	13.453	0.039	0.843	-	0.270	0.002	1.310	1.306	1.315	0.248	0.618	-
Housed outdoors		1.584	0.001	4.874	4.862	4.885	1.524	0.217	-	1.358	0.002	3.889	3.876	3.902	4.157	0.041	-
Housed in kennels		1.288	1.499	3.626	0.192	68.421	0.784	0.376	-	-	0.002	0.816	0.813	0.819	0.144	0.704	-
Multi-dog household		-0.383	0.759	0.682	0.154	3.019	0.262	0.609	-	0.171	0.384	1.187	0.559	2.518	0.189	0.664	-
Number of dogs in household	1	(Ref)	-	-	-	-	2.753	0.431	-	(Ref)	-	-	-	-	3.086	0.379	-
	2-3	-0.857	0.906	0.424	0.072	2.504	-	-	-	-	0.002	0.781	0.778	0.783	-	-	-
	4-5	2.858	2.487	17.432	0.133	2284	-	-	-	0.914	0.002	2.495	2.487	2.504	-	-	-
	6+	0.100	2.321	1.105	0.012	104.45	-	-	-	0.978	0.002	2.658	2.649	2.667	-	-	-
In-contact dogs on antimicrobials		-	-	-	-	-	-	-	0.309	-	0.002	0.879	0.877	0.882	0.033	0.856	-
In-contact dogs hospitalised		-	-	-	-	-	-	-	0.610	0.573	0.964	1.773	0.268	11.726	0.329	0.566	-

Variable	Category	Nalidixic Acid Resistance								Tetracycline Resistance							
		β	se	OR	L95%CI	U95%CI	LRT	P	FE	β	se	OR	L95%CI	U95%CI	LRT	P	FE
Contact with animals other than dogs		0.335	0.001	1.397	1.394	1.401	0.209	0.648	-	0.231	0.411	1.259	0.563	2.817	0.299	0.584	-
Cats		-0.258	0.857	0.772	0.144	4.145	0.093	0.761	-	0.119	0.002	1.126	1.123	1.130	0.058	0.810	-
Horses		-0.296	1.383	0.744	0.049	11.184	0.047	0.828	-	0.410	0.002	1.507	1.502	1.511	0.418	0.518	-
Small Mammals		2.630	1.325	13.877	1.034	186.156	4.059	0.044	-	1.882	0.770	6.568	1.453	29.683	6.259	0.012	-
Farm Animals		-0.206	1.829	0.814	0.023	29.313	0.013	0.908	-	1.494	0.650	4.457	1.247	15.930	4.980	0.026	-
Wildlife		0.391	0.001	1.479	1.476	1.483	0.119	0.730	-	-	0.002	0.968	0.965	0.971	0.003	0.957	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; Ab Tx= antimicrobial treatment; Hosp= hospitalisation

Table III-f Results of univariable analysis for risk factors associated with nalidixic acid and tetracycline resistance in faecal samples obtained from 292 kennelled dogs in the North of England

Variable	Category	β	se	OR	Trimethoprim Resistance		LRT	P	FE
					L95%CI	U95%CI			
Premises type	Boarding kennel	(Ref)	-	-	-	-	3.616	0.057	-
	Rescue centre	1.224	0.637	3.401	0.975	11.860	-	-	-
Age	<1	(Ref)	-	-	-	-	3.092	0.213	-
	1-6years	-0.098	0.600	0.906	0.280	2.936	-	-	-
	7+ years	-0.643	0.615	0.526	0.157	1.756	-	-	-
Sex	Female	-0.421	0.355	0.656	0.327	1.316	2.189	0.139	-
	Male	-0.436	0.291	0.647	0.366	1.144	-	-	-
Neutered		-0.197	0.344	0.821	0.418	1.612	-	-	-
Sex	Male entire	(Ref)	-	-	-	-	2.296	0.513	-
	Male neutered	-0.081	0.452	0.922	0.380	2.237	-	-	-
	Female entire	0.638	0.511	1.892	0.695	5.148	-	-	-
	Female	0.172	0.437	1.187	0.504	2.794	-	-	-
Pure breed		0.225	0.330	1.253	0.656	2.394	0.455	0.500	-
Size	Small/Toy	(Ref)	-	-	-	-	4.844	0.089	-
	Medium	0.694	0.414	2.002	0.889	4.506	-	-	-
	Giant	0.907	0.420	2.478	1.087	5.648	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	8.099	0.044	-
	Overnight	-1.159	0.002	0.314	0.313	0.315	-	-	-
	Day	-1.246	0.002	0.288	0.287	0.289	-	-	-
	Rescue	0.351	0.002	1.420	1.415	1.425	-	-	-
Duration of kennelling	(days)	0.001	0.001	1.001	1.000	1.002	1.833	0.176	-
Duration of kennelling	Up to 2 weeks	(Ref)	-	-	-	-	4.332	0.363	-
	2 w-1m	0.287	0.613	1.332	0.401	4.425	-	-	-
	1-3m	0.539	0.553	1.714	0.580	5.063	-	-	-
	4-6m	1.421	0.747	4.140	0.958	17.896	-	-	-
	>6 m	0.568	0.413	1.764	0.786	3.961	-	-	-
Sharing kennel		-0.086	0.407	0.917	0.413	2.038	0.044	0.834	-
Working Dog		0.104	0.722	1.110	0.269	4.573	0.019	0.890	-
Life Owner		-0.559	0.376	0.572	0.274	1.194	2.113	0.146	-
Origin	Rescue	(Ref)	-	-	-	-	6.341	0.175	-
	Breeder	-0.531	0.457	0.588	0.240	1.440	-	-	-
	Home bred	-0.733	0.843	0.481	0.092	2.509	-	-	-
	Previous owner	-1.115	0.525	0.328	0.117	0.917	-	-	-

Variable	Category	Trimethoprim Resistance							
		β	se	OR	L95%CI	U95%CI	LRT	P	FE
	Other	-1.563	0.760	0.209	0.047	0.928	-	-	-
Veterinary treatment within 6 months		0.285	0.346	1.330	0.675	2.622	0.651	0.420	-
Last vet treatment	(Ref)	-	-	-	-	-	1.089	0.780	-
		0.042	0.388	1.043	0.487	2.234	-	-	-
		0.422	0.474	1.525	0.602	3.862	-	-	-
		0.345	0.569	1.412	0.463	4.305	-	-	-
Antimicrobials within 6 months		0.493	0.382	1.638	0.774	3.465	1.623	0.203	-
Last antimicrobial	(Ref)	-	-	-	-	-	4.731	0.193	-
		0.786	0.528	2.194	0.780	6.172	-	-	-
		-0.442	0.674	0.643	0.171	2.410	-	-	-
		1.143	0.807	3.137	0.646	15.247	-	-	-
Hospitalisation within 6 months		1.636	0.798	5.135	1.075	24.533	4.745	0.029	-
Fed wet food		-0.244	0.357	0.783	0.389	1.577	0.452	0.501	-
Fed dry Mixer		-0.286	0.447	0.751	0.313	1.806	0.396	0.529	-
Fed complete dry food		0.559	0.499	1.748	0.658	4.648	1.255	0.263	-
Fed raw food		0.672	0.644	1.958	0.554	6.918	1.038	0.308	-
Fed cooked meat		-0.225	0.637	0.798	0.229	2.780	0.121	0.728	-
Housed indoors		-1.019	0.002	0.361	0.360	0.362	4.060	0.044	-
Housed outdoors		0.379	0.676	1.461	0.388	5.493	0.293	0.588	-
Housed in kennels		1.002	0.554	2.725	0.920	8.068	3.329	0.068	-
Multi-dog household		0.319	0.380	1.375	0.653	2.895	0.683	0.409	-
Number of dogs in household	(Ref)	-	-	-	-	-	1.986	0.575	-
		0.090	0.451	1.095	0.452	2.648	-	-	-
		0.220	0.859	1.246	0.231	6.704	-	-	-
		0.991	0.697	2.695	0.688	10.558	-	-	-
In-contact dogs on antimicrobials		0.241	0.646	1.273	0.358	4.519	0.134	0.715	-
In-contact dogs hospitalised		-1.699	0.002	0.183	0.182	0.184	2.152	0.142	-
Contact with animals other than dogs		-0.173	0.399	0.841	0.385	1.840	0.182	0.669	-
Cats		-0.063	0.467	0.939	0.376	2.345	0.018	0.894	-
Horses		0.480	0.570	1.616	0.529	4.940	0.680	0.410	-
Small Mammals		1.239	0.742	3.453	0.807	14.782	2.826	0.093	-
Farm Animals		0.548	0.652	1.730	0.481	6.212	0.672	0.412	-
Wildlife		0.408	0.586	1.503	0.477	4.737	0.459	0.498	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval

Residual plots

Residual plots were utilised to assess if any premises were exerting an increased influence on the mixed effect models constructed for each outcome. Where a significant difference was detected, data from these premises were checked for any errors.

Figure III-b Premises level residuals plotted against the overall mean for each mixed effect model (n=32 premises).

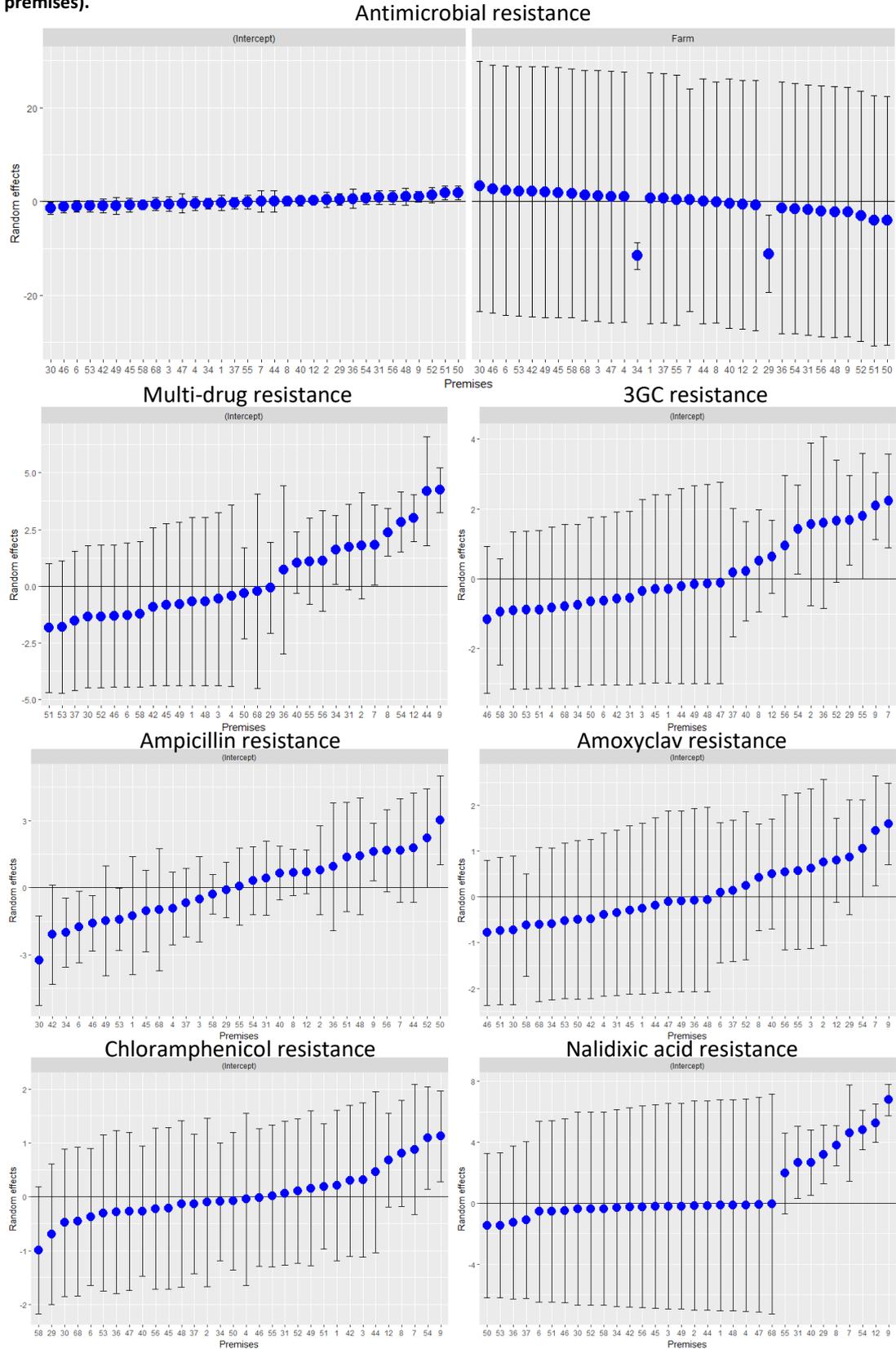
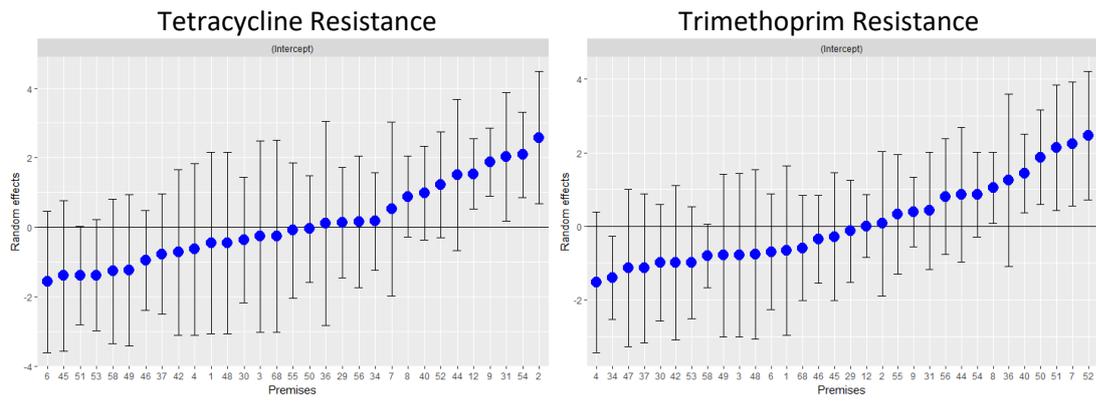


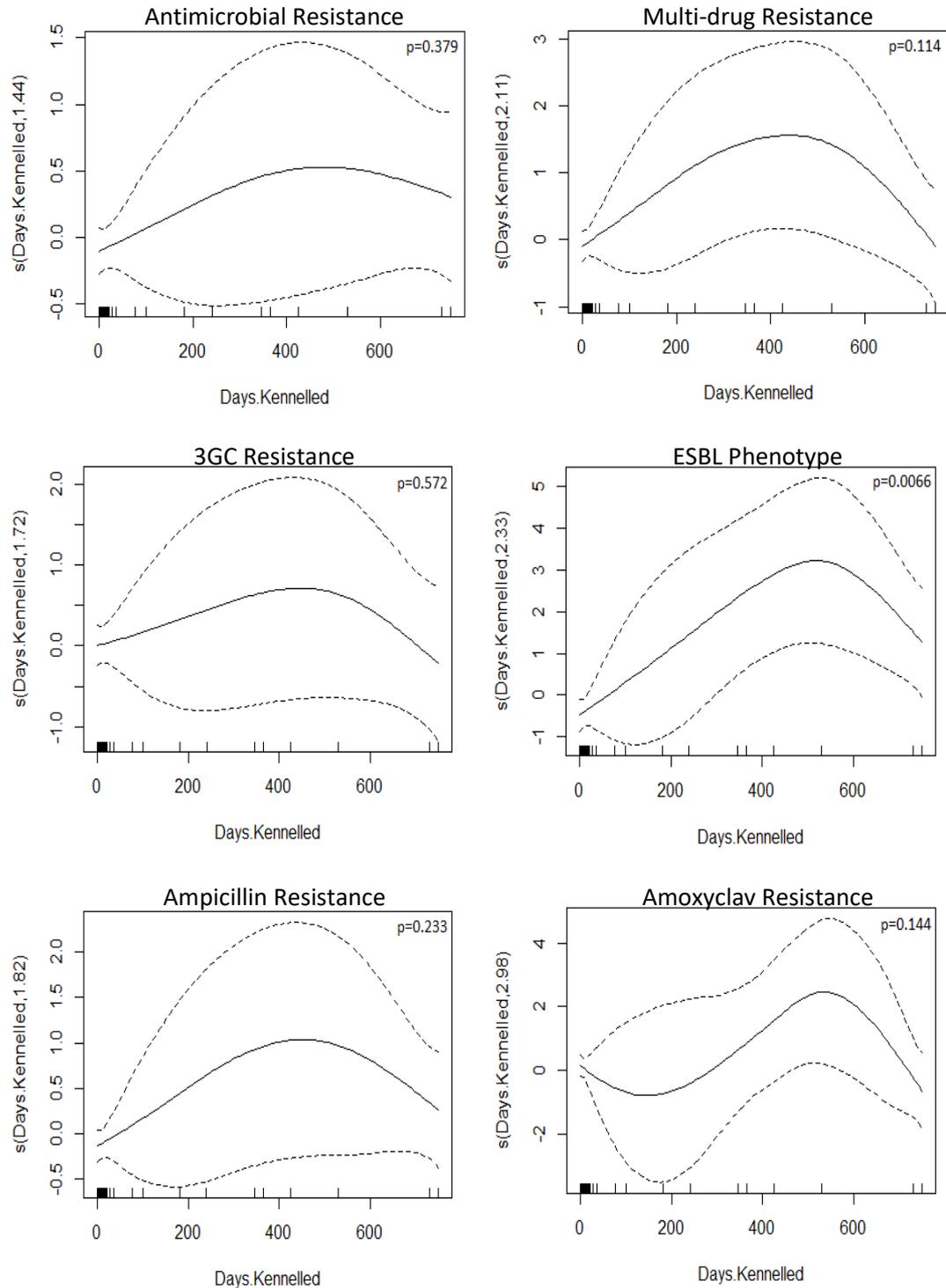
Figure III-b cont.



Owned Dog Population

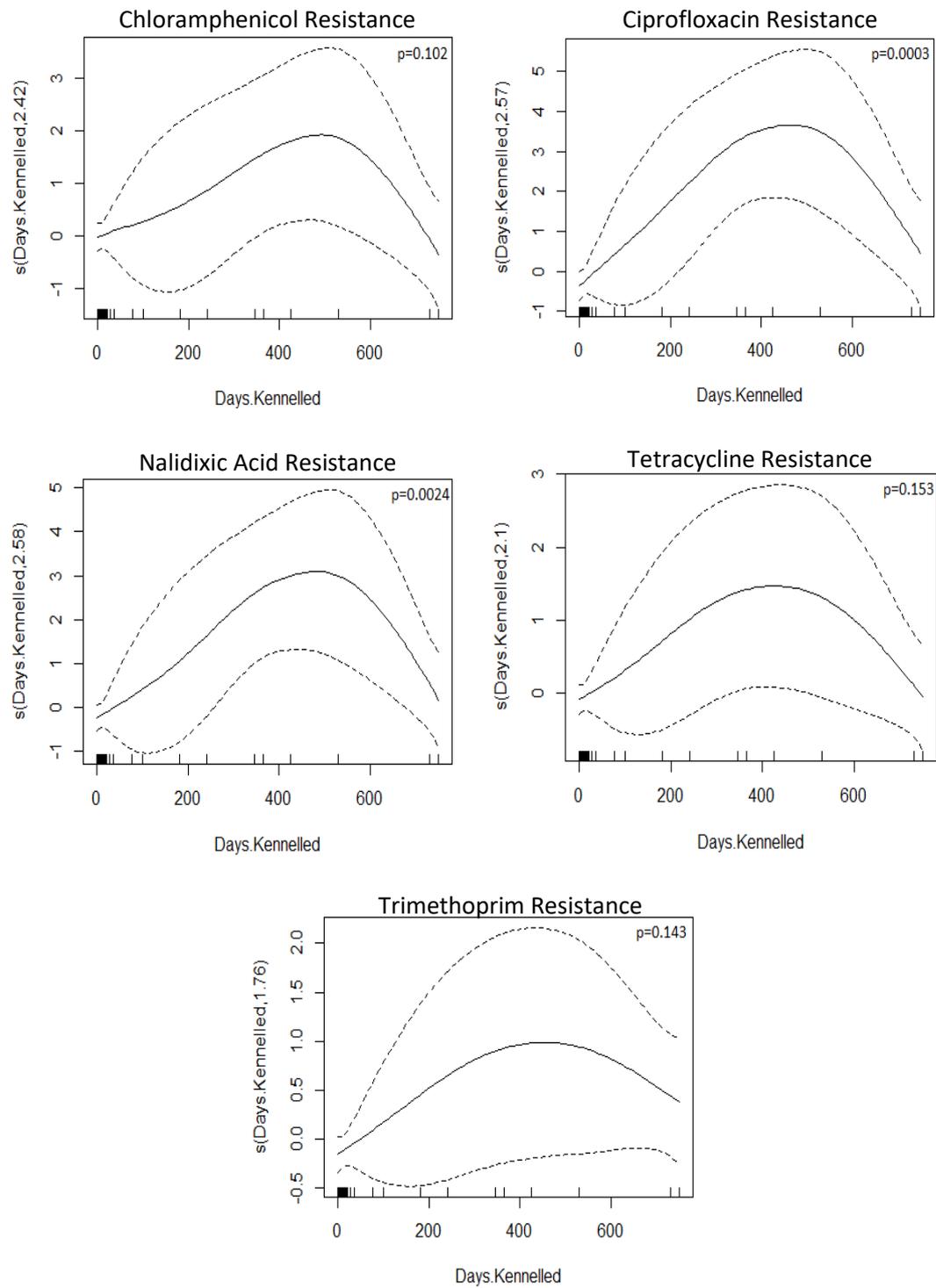
Generalised Additive Model (GAM) plots for duration of kennelling

Figure III-c Plots indicating the functional form of duration of kennelling modelled in generalised additive models for each outcome of interest utilising spline smoothers. The plots provide an estimate of the shape of the relationship between the explanatory variable on the x axis and the modelled outcome variable plotted on a centred log scale on the y axis



Key: Dashed lines indicate 95% confidence intervals; Rug plots on x-axis indicate data points; P values indicate the significance of smooth terms calculated using Chi squared test

Figure III-c cont.



Dashed lines indicate 95% confidence intervals; Rug plots on x-axis indicate data points; P values indicate the significance of smooth terms calculated using Chi squared test

Results of univariable analysis

Complete results of univariable analysis for all outcomes of interest are presented on pages 298 to 315

Table III-g Results of univariable analysis for risk factors associated with antimicrobial and multi-drug resistance in faecal samples from 185 owned kennelled dogs in the North of England

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age	<1	(Ref)	-	-	-	-	0.248	0.883	-	(Ref)	-	-	-	-	0.108	0.947	-
	1-6years	-0.187	0.761	0.829	0.187	3.683	-	-	-	-0.264	0.918	0.768	0.127	4.642	-	-	-
	7+ years	-0.017	0.755	0.983	0.224	4.321	-	-	-	-0.142	0.894	0.868	0.150	5.009	-	-	-
Sex	Female	(Ref)	-	-	-	-	2.092	0.148	-	(Ref)	-	-	-	-	1.575	0.210	-
	Male	-0.495	0.341	0.610	0.312	1.190	-	-	-	-0.602	0.486	0.548	0.211	1.419	-	-	-
Neutered		0.166	0.369	1.181	0.573	2.435	0.202	0.653	-	-0.183	0.546	0.833	0.286	2.427	0.112	0.738	-
Sex	Male entire	(Ref)	-	-	-	-	1.824	0.610	-	(Ref)	-	-	-	-	1.194	0.754	-
	Male neutered	0.209	0.500	1.232	0.462	3.286	-	-	-	-0.239	0.729	0.787	0.189	3.282	-	-	-
	Female entire	0.533	0.577	1.705	0.550	5.286	-	-	-	0.531	0.820	1.700	0.341	8.476	-	-	-
	Female neutered	0.583	0.495	1.792	0.679	4.727	1.824	0.610	-	0.263	0.707	1.301	0.325	5.204	1.194	0.754	-
Pure breed		-0.062	0.456	0.940	0.385	2.295	0.019	0.892	-	-0.892	0.570	0.410	0.134	1.252	2.386	0.122	-
Size	Small/Toy	(Ref)	-	-	-	-	10.212	0.006	-	(Ref)	-	-	-	-	3.919	0.141	-
	Medium	0.207	0.486	1.230	0.474	3.189	-	-	-	1.113	0.715	3.043	0.750	12.349	-	-	-
	Giant	1.312	0.480	3.712	1.448	9.513	-	-	-	1.283	0.707	3.609	0.903	14.420	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	2.996	0.224	-	(Ref)	-	-	-	-	0.511	0.775	-
	Overnight	-0.636	0.474	0.530	0.209	1.342	-	-	-	-0.393	0.690	0.675	0.175	2.611	-	-	-
	Day	-1.251	0.786	0.286	0.061	1.336	-	-	-	-0.735	1.090	0.479	0.057	4.059	-	-	-
Duration of kennelling (days)		0.001	0.001	1.001	1.000	1.002	2.027	0.155	-	0.000	0.001	1.000	0.998	1.002	0.116	0.734	-
Duration of kennelling	Up to 2 weeks	-	-	-	-	-	-	-	0.392	-	-	-	-	-	-	-	0.790
	2 w-1m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1-3m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4-6m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	>6 m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sharing kennel		0.352	0.408	1.421	0.639	3.164	0.728	0.393	-	-0.109	0.565	0.897	0.296	2.711	0.038	0.846	-
Working Dog		0.202	0.754	1.224	0.279	5.364	0.071	0.790	-	-1.423	1.284	0.241	0.019	2.986	1.409	0.235	-
Life Owner		-0.358	0.391	0.699	0.325	1.504	0.831	0.362	-	-1.102	0.527	0.332	0.118	0.933	4.517	0.034	-
Origin	Rescue	(Ref)	-	-	-	-	0.352	0.950	-	(Ref)	-	-	-	-	2.342	0.505	-
	Breeder	-0.021	0.459	0.979	0.398	2.406	-	-	-	-0.831	0.611	0.435	0.131	1.443	-	-	-
	Home bred	-0.233	0.806	0.792	0.163	3.845	-	-	-	-0.642	1.138	0.526	0.057	4.893	-	-	-
	Previous owner	-0.349	0.694	0.705	0.181	2.749	-	-	-	-1.079	0.918	0.340	0.056	2.054	-	-	-

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Veterinary treatment within 6 months		0.919	0.384	2.506	1.180	5.319	5.852	0.016	-	0.417	0.521	1.518	0.547	4.213	0.642	0.423	-
Last vet treatment		(Ref)	-	-	-	-	5.602	0.133	-	(Ref)	-	-	-	-	6.507	0.089	-
		1.112	0.504	3.042	1.133	8.168	-	-	-	-0.996	0.786	0.369	0.079	1.723	-	-	-
		0.579	0.621	1.785	0.529	6.027	-	-	-	-0.300	0.919	0.741	0.122	4.488	-	-	-
		0.568	0.687	1.764	0.459	6.779	-	-	-	1.461	0.781	4.310	0.932	19.928	-	-	-
Antimicrobials within 6 months		0.600	0.534	1.822	0.640	5.187	1.280	0.258	-	-0.278	0.781	0.757	0.164	3.499	0.131	0.718	-
Last antimicrobial		0.188	0.318	1.207	0.647	2.251	4.383	0.223	-	-	-	-	-	-	-	-	0.489
		1.705	0.908	5.502	0.928	32.609	-	-	-	-	-	-	-	-	-	-	-
		-0.279	1.009	0.757	0.105	5.463	-	-	-	-	-	-	-	-	-	-	-
		-0.102	0.944	0.903	0.142	5.748	-	-	-	-	-	-	-	-	-	-	-
Hospitalisation within 6 months		1.845	1.189	6.329	0.615	65.107	3.115	0.078	0.016	1.222	0.975	3.396	0.502	22.948	1.547	0.214	-
Polytomous variable																	
Vet Treatment and Hospitalisation	No treatment	(Ref)	-	-	-	-	6.1909	0.04525	-	-	-	-	-	-	-	-	-
	No hospitalisation	0.68	0.393	1.974	0.914	4.263	-	-	-	-	-	-	-	-	-	-	-
	Hospitalisation	2.076	1.195	7.969	0.766	82.965	-	-	-	-	-	-	-	-	-	-	-
Veterinary treatment alone most significant so included in multivariable model																	
Fed wet food		-0.228	0.388	0.796	0.372	1.701	0.342	0.559	-	-0.791	0.552	0.453	0.154	1.339	2.077	0.150	-
Fed dry Mixer		-0.115	0.423	0.891	0.389	2.044	0.073	0.787	-	-0.044	0.603	0.957	0.294	3.120	0.005	0.942	-
Fed complete dry		0.288	0.457	1.334	0.545	3.265	0.395	0.530	-	0.253	0.660	1.288	0.353	4.692	0.150	0.699	-
Fed raw food		0.634	0.617	1.884	0.562	6.318	1.034	0.309	-	1.948	0.732	7.018	1.670	29.493	6.457	0.011	-
Fed cooked meat		-0.278	0.726	0.757	0.182	3.144	0.143	0.706	-	-0.134	0.939	0.874	0.139	5.507	0.021	0.886	-
Housed indoors		-0.267	0.589	0.766	0.241	2.431	0.200	0.654	-	-0.519	0.938	0.595	0.095	3.739	0.294	0.588	-
Housed outdoors		0.584	0.609	1.793	0.543	5.918	0.914	0.339	-	1.723	0.873	5.602	1.012	31.010	4.104	0.043	-
Housed in kennels		0.127	0.809	1.135	0.233	5.541	0.024	0.876	-	1.072	1.282	2.920	0.237	36.036	0.702	0.402	-
Multi-dog household		0.257	0.382	1.293	0.611	2.736	0.445	0.505	-	0.091	0.538	1.095	0.381	3.146	0.028	0.866	-
Number of dogs in household	One	(Ref)	-	-	-	-	3.742	0.291	-	(Ref)	-	-	-	-	3.487	0.322	-
	2-3	-0.142	0.454	0.868	0.356	2.112	3.487	0.322	-	-0.318	0.605	0.728	0.222	2.380	-	-	-
	4-5	0.443	0.935	1.557	0.249	9.722	3.487	0.322	-	2.123	1.391	8.354	0.547	127.541	-	-	-
	6+	1.245	0.701	3.473	0.879	13.717	3.487	0.322	-	0.951	1.055	2.588	0.328	20.452	-	-	-
In-contact dogs on antibiotics		-0.187	0.647	0.829	0.234	2.945	0.083	0.773	-	-1.052	1.245	0.349	0.030	4.009	0.809	0.368	-
In-contact dogs hospitalised		-0.749	1.196	0.473	0.045	4.929	0.402	0.526	-	-1.189	2.005	0.305	0.006	15.487	0.388	0.533	-

Variable	Category	Antimicrobial Resistance								Multi-drug Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Contact with animals other than dogs																	
Cats		0.452	0.446	1.571	0.656	3.763	1.039	0.308	-	0.033	0.617	1.033	0.309	3.460	0.003	0.958	-
Horses		0.930	0.607	2.536	0.771	8.339	2.468	0.116	-	0.425	0.934	1.529	0.245	9.534	0.204	0.652	-
Small Mammals		2.070	1.151	7.926	0.831	75.594	4.495	0.034	-	0.657	1.060	1.930	0.242	15.418	0.373	0.541	-
Farm Animals		3.175	1.140	23.929	2.563	223.430	13.121	0.000	-	1.124	1.050	3.078	0.393	24.115	0.952	0.329	-
Wildlife		1.033	0.617	2.810	0.839	9.415	2.931	0.087	-	-0.065	0.912	0.937	0.157	5.598	0.005	0.943	-
Owner works in Healthcare																	
Hospital work		0.367	0.503	1.444	0.539	3.872	0.535	0.464	-	-0.695	0.777	0.499	0.109	2.288	0.861	0.353	-
GP work		1.688	0.875	5.408	0.973	30.070	4.361	0.037	-	-0.837	1.188	0.433	0.042	4.446	0.557	0.456	-
Care home work		-1.302	1.269	0.272	0.023	3.270	1.167	0.280	-	-	-	-	-	-	-	-	1.000
Owner works with animals																	
Farming		0.947	0.531	2.579	0.911	7.302	3.261	0.071	-	0.973	0.678	2.646	0.701	9.992	1.946	0.163	-
Veterinary		2.351	1.252	10.500	0.903	122.135	4.119	0.042	-	3.873	1.235	48.108	4.275	541.428	9.185	0.002	-
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polytomous variable																	
Working with animals	No	(Ref)	-	-	-	-	5.283	0.0713	-	(Ref)	-	-	-	-	9.252	0.010	-
	Non-Farming	0.600	0.567	1.821	0.600	5.531	-	-	-	0.186	0.720	1.205	0.294	4.938	-	-	-
	Farming	2.447	1.269	11.557	0.961	139.028	-	-	-	3.912	1.250	49.994	4.311	579.723	-	-	-
Farming alone most significant so included in multivariable model									Farming alone most significant so included in multivariable model								
Owner has visited hospital in last month		0.087	0.396	1.091	0.502	2.369	0.048	0.826	-	0.201	0.542	1.222	0.423	3.533	0.138	0.711	-
Admitted		-0.612	0.852	0.542	0.102	2.879	0.524	0.469	-	1.500	1.153	4.479	0.467	42.941	1.625	0.202	-
Visiting patient		-0.431	0.626	0.650	0.190	2.219	0.471	0.493	-	-2.299	1.313	0.100	0.008	1.315	4.614	0.032	-
Outpatient appointment		0.759	0.518	2.136	0.774	5.900	2.226	0.136	-	-0.043	0.714	0.958	0.237	3.880	0.004	0.952	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; GP= General Practice

Table III-h Results of univariable analysis for risk factors associated with third generation cephalosporin and ESBL-mediated resistance in faecal samples from 185 owned kennelled dogs in the North of England

Variable	Category	Third Generation Cephalosporin Resistance							ESBL Phenotype								
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age	<1	-	-	-	-	-	-	-	0.45	-	-	-	-	-	-	-	0.696
	1-6years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	7+ years	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sex	Female	(Ref)	-	-	-	-	0.767	0.381	-	(Ref)	-	-	-	-	1.493	0.222	-
	Male	-0.412	0.472	0.663	0.263	1.671	-	-	-	-1.320	1.124	0.267	0.030	2.421	-	-	-
Neutered		0.210	0.574	1.234	0.401	3.798	0.138	0.711	-	3.628	3.400	37.630	0.048	2.95E+04	2.335	0.127	-
Sex	Male entire	(Ref)	-	-	-	-	2.023	0.568	-	(Ref)	-	-	-	-	10.058	0.018	-
	Male neutered	0.707	0.811	2.027	0.414	9.936	-	-	-	-0.001	5.889	0.999	0.000	1.03E+05	-	-	0.052
	Female entire	1.148	0.854	3.153	0.591	16.821	-	-	-	-3.663	4.568	0.026	0.000	198.315	-	-	-
	Female neutered	0.775	0.798	2.170	0.454	10.366	-	-	-	10.005	5.919	2.21E+04	0.203	2.42E+09	-	-	-
Pure breed Size		-0.401	0.610	0.670	0.203	2.215	0.418	0.518	-	0.229	2.677	1.258	0.007	238.985	0.008	0.930	-
	Small/Toy	(Ref)	-	-	-	-	5.237	0.073	-	(Ref)	-	-	-	-	0.837	0.658	0.090
	Medium	1.542	0.733	4.676	1.111	19.678	-	-	-	0.252	1.734	1.287	0.043	38.513	-	-	-
Boarder	Giant	0.833	0.727	2.300	0.553	9.556	-	-	-	1.141	1.572	3.130	0.144	68.124	-	-	-
	Resident	(Ref)	-	-	-	-	0.105	0.949	-	(Ref)	-	-	-	-	3.440	0.179	0.001
Duration of kennelling (days)	Overnight	-0.073	0.656	0.930	0.257	3.366	-	-	-	-2.588	1.497	0.075	0.004	1.413	-	-	-
	Day	0.214	1.043	1.239	0.160	9.569	-	-	-	-0.460	1.699	0.631	0.023	17.632	-	-	-
Duration of kennelling (piecewise)	(days)	0	0.001	1.000	0.998	1.002	0.058	0.810	-	-	-	-	-	-	-	-	-
Duration of kennelling (piecewise)	<525days	-	-	-	-	-	-	-	-	-0.006	0.100	0.994	0.834	1.232	6.695	0.082	-
	≥525 days	-	-	-	-	-	-	-	-	-0.019	0.098	0.981	0.810	1.189	-	-	-
Duration of kennelling	Up to 2 weeks	-	-	-	-	-	-	-	0.95	(Ref)	-	-	-	-	5.627	0.229	0.042
	2 w-1m	-	-	-	-	-	-	-	-	3.136	1.980	23.003	0.475	1115	-	-	-
	1-3m	-	-	-	-	-	-	-	-	4.931	3.417	138.56	0.171	1.12E+05	-	-	-
	4-6m	-	-	-	-	-	-	-	-	5.405	3.977	222.60	0.092	5.41E+05	-	-	-
	>6 m	-	-	-	-	-	-	-	-	2.765	1.894	15.884	0.388	650.19	-	-	-
Sharing kennel		0.225	0.551	1.252	0.425	3.686	0.162	0.687	-	-1.854	2.969	0.157	0.000	52.713	0.645	0.422	-
Working Dog		-1.434	1.358	0.238	0.017	3.416	1.326	0.249	-	-1.768	1.922	0.171	0.004	7.386	0.902	0.342	-
Life Owner		-0.120	0.551	0.887	0.302	2.610	0.047	0.828	-	-0.064	1.122	0.938	0.104	8.459	0.003	0.955	-

Variable	Category	Third Generation Cephalosporin Resistance								ESBL Phenotype							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Origin	Rescue	(Ref)	-	-	-	-	0.964	0.810	-	(Ref)	-	-	-	-	-	-	0.859
	Breeder	-0.008	0.692	0.992	0.256	3.850	-	-	-	-	-	-	-	-	-	-	-
	Home bred	1.128	1.255	3.091	0.264	36.200	-	-	-	-	-	-	-	-	-	-	-
	Previous owner	0.264	1.009	1.303	0.180	9.414	-	-	-	-	-	-	-	-	-	-	-
Veterinary treatment within 6 months		1.032	0.546	2.807	0.962	8.187	3.685	0.055	-	0.100	1.115	1.106	0.124	9.840	0.008	0.928	-
Last vet treatment	(Ref)	-	-	-	-	-	2.735	0.434	-	-	-	-	-	-	-	-	0.853
		0.734	0.709	2.083	0.519	8.361	-	-	-	-	-	-	-	-	-	-	-
		1.256	0.820	3.511	0.704	17.509	-	-	-	-	-	-	-	-	-	-	-
		0.448	0.936	1.565	0.250	9.809	-	-	-	-	-	-	-	-	-	-	-
Antimicrobials within 6 months		0.066	0.753	1.069	0.244	4.677	0.008	0.930	-	-	-	-	-	-	-	-	0.361
Last antimicrobial	(Ref)	-	-	-	-	-	0.236	0.972	-	-	-	-	-	-	-	-	1.000
		-0.286	1.213	0.751	0.070	8.101	-	-	-	-	-	-	-	-	-	-	-
		0.565	1.336	1.759	0.128	24.148	-	-	-	-	-	-	-	-	-	-	-
		0.062	1.259	1.063	0.090	12.539	-	-	-	-	-	-	-	-	-	-	-
Hospitalisation within 6 months		2.135	1.017	8.455	1.152	62.043	4.258	0.039	-	-	-	-	-	-	-	-	1.000
Polytomous variable																	
Vet Treatment and Hospitalisation	No treatment	(Ref)	-	-	-	-	5.565	0.062	-	-	-	-	-	-	-	-	-
	No hospitalisation	0.686	0.590	1.986	0.625	6.314	-	-	-	-	-	-	-	-	-	-	-
	Hospitalisation	2.365	1.042	10.644	1.382	81.984	-	-	-	-	-	-	-	-	-	-	-
Hospitalisation alone most significant so included in multivariable model																	
Fed wet food		-0.300	0.590	0.741	0.233	2.357	0.257	0.612	-	9.771	0.003	1.75E+04	1.74E+	1.76E+04	6.447	0.011	-
Fed dry Mixer		-1.195	0.823	0.303	0.060	1.519	2.528	0.112	-	0.741	1.553	2.098	0.100	44.075	0.224	0.636	-
Fed complete dry food		1.089	0.839	2.970	0.574	15.379	1.966	0.161	-	-0.771	1.556	0.462	0.022	9.756	0.241	0.623	-
Fed raw food		1.902	0.702	6.701	1.692	26.542	6.377	0.012	-	1.742	1.789	5.709	0.171	190.241	0.920	0.338	-
Fed cooked meat		-0.815	1.198	0.442	0.042	4.629	0.527	0.468	-	-	-	-	-	-	-	-	0.600
Housed indoors		0.310	1.118	1.364	0.153	12.199	0.083	0.774	-	1.035	0.003	2.817	2.802	2.831	0.118	0.731	-
Housed outdoors		-0.338	1.135	0.713	0.077	6.590	0.096	0.757	-	0.943	3.293	2.567	0.004	1631.887	0.069	0.793	-
Housed in kennels		-0.229	1.386	0.796	0.053	12.035	0.028	0.868	-	3.014	2.392	20.375	0.188	2213.884	1.802	0.179	-
Multi-dog household		1.210	0.607	3.353	1.021	11.013	4.221	0.040	-	1.023	1.346	2.781	0.199	38.922	0.605	0.437	-

Variable	Category	Third Generation Cephalosporin Resistance								ESBL Phenotype							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Number of dogs in household	One	(Ref)	-	-	-	-	4.553	0.208	-	(Ref)	-	-	-	-	4.361	0.225	-
	2-3	1.121	0.654	3.068	0.851	11.058	-	-	-	0.633	1.694	1.882	0.068	52.092	-	-	-
	4-5	0.834	1.491	2.302	0.124	42.747	-	-	-	7.532	7.507	1867.467	0.001	4.59E+09	-	-	-
	6+	1.657	1.020	5.245	0.711	38.715	-	-	-	-0.760	4.671	0.468	0.000	4422.732	-	-	-
In-contact dogs on antibiotics		-0.580	1.277	0.560	0.046	6.834	0.222	0.637	0.13	-	-	-	-	-	-	-	0.602
In-contact dogs hospitalised		1.249	1.606	3.488	0.150	81.266	0.566	0.452	-	-	-	-	-	-	-	-	1.000
Contact with animals other than dogs																	
Cats		0.810	0.684	2.247	0.588	8.585	1.407	0.235	-	1.401	1.385	4.061	0.269	61.328	1.065	0.302	-
Horses		-0.361	0.961	0.697	0.106	4.584	0.146	0.702	-	0.019	1.539	1.020	0.050	20.829	0.000	0.990	-
Small Mammals		-0.761	1.308	0.467	0.036	6.060	0.373	0.542	-	-0.336	1.642	0.715	0.029	17.840	0.043	0.836	-
Farm Animals		1.313	0.901	3.717	0.635	21.745	1.795	0.180	-	-0.805	3.203	0.447	0.001	238.344	0.076	0.783	-
Wildlife		0.985	0.777	2.679	0.584	12.288	1.406	0.236	-	1.347	1.556	3.844	0.182	81.084	0.713	0.399	-
Owner works in Healthcare		-0.033	0.793	0.968	0.205	4.574	0.002	0.967	-	-2.368	2.811	0.094	0.000	23.153	1.267	0.260	-
Hospital work		1.539	0.972	4.660	0.694	31.294	2.411	0.120	-	-1.357	2.876	0.258	0.001	72.305	0.295	0.587	-
GP work		-	-	-	-	-	-	-	0.37	-	-	-	-	-	-	-	1.000
Care home work		-	-	-	-	-	-	-	1.00	-	-	-	-	-	-	-	1.000
Owner works with animals		-0.377	0.889	0.686	0.120	3.917	0.192	0.661	-	-2.496	3.234	0.082	0.000	46.674	1.046	0.307	-
Farming		1.953	1.167	7.050	0.716	69.456	2.143	0.143	-	2.626	3.822	13.817	0.008	2.47E+04	0.354	0.552	-
Veterinary		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Owner has visited hospital in last month		0.470	0.597	1.601	0.497	5.158	0.626	0.429	-	-1.109	1.502	0.330	0.017	6.260	0.579	0.447	-
Admitted		0.641	1.026	1.898	0.254	14.176	0.373	0.541	-	1.100	1.554	3.004	0.143	63.144	0.476	0.490	-
Visiting patient		-0.218	0.945	0.804	0.126	5.125	0.055	0.815	-	-0.556	2.833	0.574	0.002	147.968	0.043	0.836	-
Outpatient appointment		-0.610	0.863	0.543	0.100	2.947	0.541	0.462	-	-0.491	1.572	0.612	0.028	13.340	0.101	0.751	0.211

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; GP= General Practice

Table III-i Results of univariable analysis for risk factors associated with ampicillin and amoxycyclav resistance in faecal samples obtained from 185 owned kennelled dogs in the North of England

Variable	Category	Ampicillin Resistance							Amoxycyclav Resistance								
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95% CI	U95% CI	LRT statistic	P	FE
Age	<1	(Ref)	-	-	-	-	0.075	0.963	-	-	-	-	-	-	-	-	0.571
	1-6years	-0.205	0.794	0.814	0.172	3.862	-	-	-	-	-	-	-	-	-	-	-
	7+ years	-0.214	0.784	0.807	0.174	3.752	-	-	-	-	-	-	-	-	-	-	-
Sex	Female	(Ref)	-	-	-	-	3.924	0.048	-	(Ref)	-	-	-	-	0.001	0.972	-
	Male	-0.729	0.366	0.483	0.235	0.989	-	-	-	-0.016	0.455	0.984	0.403	2.402	-	-	-
Neutered		0.112	0.401	1.119	0.510	2.453	0.077	0.782	-	-0.194	0.507	0.824	0.305	2.226	0.144	0.705	-
Sex	Male entire	(Ref)	-	-	-	-	-	-	-	(Ref)	-	-	-	-	0.917	0.821	-
	Male neutered	0.152	0.546	1.165	0.400	3.394	-	-	-	0.212	0.698	1.236	0.314	4.859	0.917	0.821	-
	Female entire	0.839	0.629	2.315	0.675	7.941	-	-	-	0.545	0.745	1.724	0.401	7.422	-	-	-
	Female neutered	0.761	0.535	2.139	0.749	6.110	-	-	-	-0.088	0.711	0.915	0.227	3.691	-	-	-
Pure breed		-0.254	0.477	0.776	0.305	1.975	0.278	0.598	-	0.017	0.615	1.017	0.305	3.397	0.001	0.978	-
Size	Small/Toy	(Ref)	-	-	-	-	8.554	0.014	-	(Ref)	-	-	-	-	9.009	0.011	-
	Medium	0.508	0.525	1.662	0.594	4.648	-	-	-	1.713	0.700	5.545	1.405	21.881	-	-	-
	Giant	1.393	0.512	4.027	1.476	10.991	-	-	-	0.532	0.726	1.702	0.410	7.060	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	6.136	0.047	-	(Ref)	-	-	-	-	0.075	0.963	-
	Overnight	-0.853	0.526	0.426	0.152	1.194	-	-	-	0.133	0.614	1.142	0.343	3.806	-	-	-
	Day	-2.123	0.905	0.120	0.020	0.705	-	-	-	0.246	0.984	1.279	0.186	8.791	-	-	-
Duration of kennelling	(days)	0.001	0.001	1.001	1.000	1.003	3.693	0.055	-	0.000	0.001	1.000	0.998	1.001	0.255	0.616	-
Duration of kennelling	Up to 2 weeks	-	-	-	-	-	-	-	0.422	-	-	-	-	-	-	-	0.893
	2 w-1m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1-3m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4-6m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	>6 m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sharing kennel		0.184	0.439	1.202	0.509	2.840	0.172	0.679	-	0.312	0.504	1.366	0.509	3.669	0.364	0.546	-
Working Dog		0.604	0.857	1.830	0.341	9.813	0.489	0.485	-	-1.166	1.176	0.312	0.031	3.123	1.226	0.268	0.133
Life Owner		-0.488	0.415	0.614	0.272	1.385	1.338	0.247	-	-0.142	0.523	0.867	0.311	2.416	0.074	0.786	-
Origin	Rescue	(Ref)	-	-	-	-	3.988	0.263	-	(Ref)	-	-	-	-	0.195	0.978	-
	Breeder	-0.268	0.495	0.765	0.290	2.018	-	-	-	0.213	0.657	1.237	0.341	4.485	-	-	-
	Home bred	-0.009	0.861	0.991	0.183	5.362	-	-	-	-0.082	1.272	0.921	0.076	11.137	-	-	-
	Previous owner	-1.529	0.804	0.217	0.045	1.048	-	-	-	0.315	0.975	1.370	0.203	9.259	-	-	-

Variable	Category	Ampicillin Resistance								Amoxycylav Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95% CI	U95% CI	LRT statistic	P	FE
Veterinary treatment within 6 months		0.605	0.407	1.831	0.824	4.067	2.143	0.092	-	1.105	0.521	3.019	1.087	8.387	4.650	0.031	-
Last vet treatment	>6m	(Ref)	-	-	-	-	2.111	0.550	-	(Ref)	-	-	-	-	1.318	0.725	-
	<1m	0.581	0.500	1.788	0.671	4.766	-	-	-	0.595	0.640	1.813	0.517	6.357	-	-	-
	2-3m	0.736	0.665	2.087	0.566	7.689	-	-	-	0.656	0.786	1.928	0.413	8.995	-	-	-
	4-6m	0.312	0.708	1.367	0.341	5.472	-	-	-	0.496	0.891	1.642	0.286	9.410	-	-	-
Antimicrobials within 6 months		0.503	0.546	1.654	0.567	4.823	0.832	0.362	-	-0.367	0.817	0.693	0.140	3.439	0.215	0.643	
Last antimicrobial	>6m	(Ref)	-	-	-	-	1.421	0.701	-	-	-	-	-	-	-	-	-
	<1m	0.922	0.783	2.514	0.542	11.673	-	-	-	-	-	-	-	-	-	-	-
	2-3m	0.210	1.104	1.233	0.142	10.736	-	-	-	-	-	-	-	-	-	-	-
	4-6m	0.053	0.975	1.054	0.156	7.125	-	-	-	-	-	-	-	-	-	-	-
Hospitalisation within 6 months		2.246	1.249	9.446	0.817	109.234	4.344	0.037	0.005	2.893	0.990	18.043	2.594	125.493	8.962	0.003	
Polytomous variable																	
Vet Treatment and Hospitalisation	No treatment	(Ref)	-	-	-	-	5.131	0.077		-2.864	0.552	0.057	0.019	0.168	9.920	0.007	
	No hospitalisation	0.355	0.009	1.427	1.403	1.452				0.600	0.587	1.823	0.577	5.757			
	Hospitalisation	2.397	0.009	10.991	10.804	11.182				3.080	1.004	21.749	3.038	155.676			
Hospitalisation alone most significant so included in multivariable model									Hospitalisation alone most significant so included in multivariable model								
Fed wet food		-0.569	0.420	0.566	0.249	1.289	1.790	0.181	-	-0.620	0.561	0.538	0.179	1.613	1.243	0.265	-
Fed dry Mixer		-0.252	0.454	0.777	0.319	1.893	0.298	0.585	-	-0.616	0.695	0.540	0.138	2.111	0.844	0.358	-
Fed complete dry		0.523	0.503	1.688	0.629	4.527	1.060	0.303	-	0.481	0.703	1.618	0.408	6.417	0.494	0.482	-
Fed raw food		1.026	0.676	2.791	0.742	10.494	2.271	0.132	-	1.178	0.626	3.247	0.952	11.068	2.917	0.088	-
Fed cooked meat		-0.089	0.789	0.915	0.195	4.298	0.011	0.916	-	0.199	0.885	1.220	0.215	6.910	0.049	0.825	-
Housed indoors		-0.250	0.661	0.779	0.213	2.849	0.136	0.713	-	-0.208	0.792	0.812	0.172	3.837	0.066	0.798	-
Housed outdoors		0.419	0.681	1.520	0.400	5.769	0.546	0.460	-	0.284	0.839	1.329	0.257	6.879	0.107	0.744	-
Housed in kennels		0.738	0.908	2.091	0.353	12.390	0.653	0.419	-	-	-	-	-	-	-	-	0.373
Multi-dog household		0.448	0.410	1.565	0.700	3.497	1.155	0.283	-	0.725	0.543	2.064	0.711	5.988	1.787	0.181	-
Number of dogs in household	One	(Ref)	-	-	-	-	4.268	0.234	-	(Ref)	-	-	-	-	3.874	0.275	0.060
	2-3	0.042	0.477	1.043	0.409	2.658	-	-	-	0.699	0.592	2.012	0.630	6.424	-	-	-
	4-5	0.926	1.069	2.524	0.310	20.529	-	-	-	0.075	1.200	1.078	0.103	11.330	-	-	-
	6+	1.555	0.807	4.735	0.974	23.015	-	-	-	1.393	0.716	4.029	0.991	16.383	-	-	-
In-contact dogs on antibiotics		-0.711	0.724	0.491	0.119	2.030	0.971	0.325	-	-0.653	1.160	0.521	0.054	5.058	0.353	0.552	0.221
In-contact dogs hospitalised		-0.602	1.375	0.548	0.037	8.109	0.186	0.666	-	0.838	1.348	2.312	0.165	32.483	0.353	0.552	-

Variable	Category	Ampicillin Resistance								Amoxyclav Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95% CI	U95% CI	LRT statistic	P	FE
Contact with animals other than dogs																	
Cats		0.186	0.476	1.204	0.474	3.058	0.147	0.701	-	0.765	0.636	2.148	0.618	7.470	1.440	0.230	-
Horses		0.618	0.644	1.855	0.525	6.555	0.893	0.345	-	-0.172	0.878	0.842	0.151	4.703	0.039	0.843	-
Small Mammals		0.931	0.898	2.537	0.436	14.752	1.092	0.296	-	-	-	-	-	-	-	-	0.601
Farm Animals		1.654	0.835	5.229	1.018	26.873	3.999	0.046	-	1.414	0.712	4.113	1.019	16.596	3.077	0.079	-
Wildlife		0.788	0.642	2.199	0.625	7.738	1.489	0.222	-	1.761	0.618	5.819	1.734	19.526	6.042	0.014	-
Owner works in Healthcare																	
Hospital work		-0.184	0.781	0.832	0.180	3.844	0.054	0.816	-	0.595	0.900	1.812	0.310	10.585	0.406	0.524	-
GP work		1.818	0.961	6.158	0.937	40.470	4.255	0.039	-	-0.169	1.169	0.845	0.085	8.355	0.021	0.884	-
Care home work		-0.783	1.319	0.457	0.034	6.058	0.361	0.548	0.122	-	-	-	-	-	-	-	1.000
Owner works with animals																	
Farming		2.598	1.401	13.437	0.862	209.463	3.584	0.058	-	1.816	0.726	6.145	1.480	25.519	4.047	0.044	-
Veterinary		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polytomous variable																	
Working with animals	No	(Ref)	-	-	-	-	4.287	0.117	-	-	-	-	-	-	-	-	-
	Non-Farming	0.514	0.618	1.672	0.498	5.612	-	-	-	-	-	-	-	-	-	-	-
	Farming	2.677	1.415	14.540	0.908	232.922	-	-	-	-	-	-	-	-	-	-	-
Working in farming alone most significant so included in multivariable model																	
Owner has visited hospital in last month																	
Admitted		-0.294	0.880	0.745	0.133	4.184	0.111	0.739	-	0.934	0.947	2.544	0.398	16.267	0.884	0.347	-
Visiting patient		-0.939	0.722	0.391	0.095	1.611	1.727	0.189	-	-0.961	1.111	0.382	0.043	3.371	0.915	0.339	-
Outpatient appointment		0.441	0.541	1.555	0.538	4.493	0.647	0.421	-	-0.687	0.819	0.503	0.101	2.506	0.789	0.374	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; GP= General Practice

Table III-j Results of univariable analysis for risk factors associated with chloramphenicol and ciprofloxacin resistance in faecal samples obtained from 185 owned kennelled dogs in the North of England

Variable	Category	Chloramphenicol Resistance								Ciprofloxacin Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age	<1	-	-	-	-	-	-	-	0.250	(Ref)	-	-	-	-	4.545	0.103	0.054
	1-6years	-	-	-	-	-	-	-	-	-0.776	1.415	0.460	0.029	7.362	-	-	-
	7+ years	-	-	-	-	-	-	-	-	-2.764	1.696	0.063	0.002	1.750	-	-	-
Sex	Female	(Ref)	-	-	-	-	1.010	0.315	-	(Ref)	-	-	-	-	5.531	0.019	-
	Male	0.473	0.475	1.604	0.632	4.073	-	-	-	3.697	2.661	40.343	0.219	7427	-	-	-
Neutered		0.016	0.535	1.017	0.356	2.901	0.001	0.976	-	-1.145	1.196	0.318	0.031	3.315	0.899	0.343	-
Sex	Male entire	(Ref)	-	-	-	-	1.690	0.639	-	(Ref)	-	-	-	-	3.183	0.364	0.002
	Male	-0.091	0.648	0.913	0.256	3.249	-	-	-	-0.185	1.286	0.831	0.067	10.347	-	-	-
	Female	-0.914	0.882	0.401	0.071	2.259	-	-	-	-3.239	2.854	0.039	0.000	10.542	-	-	-
Pure breed	Female	-0.510	0.686	0.600	0.157	2.301	-	-	-	-1.322	1.510	0.267	0.014	5.140	-	-	-
		-0.171	0.593	0.843	0.264	2.693	0.082	0.775	-	-0.754	1.045	0.471	0.061	3.652	0.501	0.479	-
	Size	Small/Toy	(Ref)	-	-	-	-	7.940	0.019	-	(Ref)	-	-	-	-	1.171	0.557
Boarder	Medium	2.034	0.832	7.642	1.495	39.055	-	-	-	1.132	1.330	3.101	0.229	42.058	-	-	-
	Giant	1.559	0.838	4.753	0.920	24.548	-	-	-	0.049	1.493	1.051	0.056	19.616	-	-	-
	Resident	(Ref)	-	-	-	-	3.401	0.183	-	(Ref)	-	-	-	-	0.098	0.952	0.009
Duration of kennelling	Overnight	-0.311	0.649	0.733	0.205	2.616	-	-	-	-0.428	2.441	0.652	0.005	77.910	-	-	-
	Day	1.150	0.922	3.157	0.518	19.231	-	-	-	-0.026	2.756	0.974	0.004	215.968	-	-	-
	(days)	0.000	0.001	1.000	0.998	1.002	0.154	0.695	-	0.000	0.004	1.001	0.992	1.009	0.016	0.899	-
Duration of kennelling (piecewise)	<450 days	-	-	-	-	-	-	-	-	0.006	0.347	1.062	0.538	2.098	0.441	0.932	-
	≥450 days	-	-	-	-	-	-	-	-	-0.025	0.347	0.975	0.494	1.925	-	-	-
Duration of kennelling	Up to 2	-	-	-	-	-	-	-	0.566	(Ref)	-	-	-	-	3.711	0.447	0.055
	2 w-1m	-	-	-	-	-	-	-	-	1.637	1.718	5.139	0.177	149.008	-	-	-
	1-3m	-	-	-	-	-	-	-	-	4.099	2.966	60.296	0.180	2.02E+04	-	-	-
	4-6m	-	-	-	-	-	-	-	-	1.597	6.095	4.936	0.000	7.61E+05	-	-	-
	>6 m	-	-	-	-	-	-	-	-	0.695	2.616	2.004	0.012	337.656	-	-	-
Sharing kennel		0.001	0.556	1.001	0.336	2.975	0.000	0.999	-	0.915	1.224	2.497	0.227	27.517	0.540	0.463	-
Working Dog		-1.701	1.267	0.183	0.015	2.188	2.410	0.121	-	0.108	4.472	1.114	0.000	7130	0.001	0.981	-
Life Owner		-0.679	0.477	0.507	0.199	1.293	2.011	0.156	-	-0.211	1.089	0.810	0.096	6.843	0.037	0.847	-
Origin	Rescue	(Ref)	-	-	-	-	0.363	0.948	-	-	-	-	-	-	-	-	0.490
	Breeder	0.018	0.590	1.018	0.320	3.235	-	-	-	-	-	-	-	-	-	-	-
	Home bred	0.355	1.031	1.426	0.189	10.751	-	-	-	-	-	-	-	-	-	-	-
	Previous	-0.357	0.932	0.700	0.113	4.349	-	-	-	-	-	-	-	-	-	-	-

Variable	Category	Chloramphenicol Resistance							Ciprofloxacin Resistance								
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Veterinary treatment within 6 months		1.068	0.483	2.909	1.128	7.504	5.033	0.025	-	1.213	4.609	0.428	49.693	1.794	0.180	-	
Last vet treatment	>6m	(Ref)	-	-	-	-	2.446	0.485	-	-	-	-	-	-	-	0.729	
	<1m	0.423	0.640	1.527	0.435	5.354	-	-	-	-	-	-	-	-	-	-	
	2-3m	0.414	0.776	1.513	0.331	6.924	-	-	-	-	-	-	-	-	-	-	
	4-6m	1.153	0.745	3.167	0.735	13.647	-	-	-	-	-	-	-	-	-	-	
Antimicrobials within 6 months		(Ref)	-	-	-	-	0.427	0.513	-	-	-	-	-	-	-	0.372	
Last antimicrobial	>6m	-	-	-	-	-	-	-	0.894	-	-	-	-	-	-	1.000	
	<1m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2-3m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	4-6m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Hospitalisation within 6 months		0.970	0.974	2.638	0.391	17.810	0.915	0.339	-	1.636	1.560	5.136	0.241	109.274	1.028	0.311	-
Fed wet food		-1.095	0.539	0.335	0.116	0.963	4.413	0.036	-	-0.333	1.105	0.716	0.082	6.247	0.091	0.763	-
Fed dry Mixer		-1.092	0.677	0.336	0.089	1.266	3.059	0.080	-	1.223	1.166	3.397	0.346	33.375	1.083	0.298	-
Fed complete dry food		0.939	0.689	2.557	0.663	9.860	2.123	0.145	-	-0.891	1.384	0.410	0.027	6.180	0.388	0.534	-
Fed raw food		1.466	0.591	4.334	1.360	13.813	5.067	0.024	-	-0.577	3.077	0.561	0.001	233.620	0.040	0.841	-
Fed cooked meat		-0.222	0.873	0.801	0.145	4.435	0.067	0.796	-	1.812	1.549	6.122	0.294	127.366	1.261	0.262	-
Housed indoors		-0.936	0.644	0.392	0.111	1.386	1.839	0.175	-	-1.752	3.711	0.173	0.000	250.058	0.194	0.660	-
Housed outdoors		1.128	0.648	3.091	0.868	11.009	2.543	0.111	-	2.056	1.566	7.811	0.363	168.004	1.633	0.201	-
Housed in kennels		0.756	1.050	2.129	0.272	16.666	0.505	0.477	-	-	-	-	-	-	-	-	0.601
Multi-dog household		0.239	0.510	1.269	0.468	3.447	0.217	0.641	-	-0.471	1.221	0.624	0.057	6.830	0.155	0.693	-
Number of dogs in household	One	(Ref)	-	-	-	-	0.841	0.840	-	(Ref)	-	-	-	-	0.760	0.859	0.000
	2-3	0.019	0.592	1.019	0.320	3.252	-	-	-	-0.584	1.270	0.558	0.046	6.715	-	-	-
	4-5	0.744	1.072	2.104	0.257	17.213	-	-	-	1.821	3.876	6.178	0.003	1.23E+04	-	-	-
	6+	0.575	0.858	1.778	0.331	9.549	-	-	-	2.092	3.108	8.099	0.018	3579	-	-	-
In-contact dogs on antibiotics		-1.070	1.154	0.343	0.036	3.294	1.038	0.308	0.077	-	-	-	-	-	-	-	0.366
In-contact dogs hospitalised		0.689	1.326	1.991	0.148	26.758	0.252	0.616	-	-	-	-	-	-	-	-	1.000
Contact with animals other than dogs																	
Cats		0.330	0.599	1.391	0.430	4.496	0.301	0.583	-	-0.129	1.326	0.879	0.065	11.813	0.010	0.922	0.038
Horses		0.538	0.725	1.713	0.414	7.090	0.537	0.464	-	7.892	6.462	2677	0.008	8.47E+08	2.721	0.099	0.218
Small Mammals		-0.717	1.248	0.488	0.042	5.633	0.371	0.543	-	-1.031	4.337	0.357	0.000	1754	0.066	0.797	-
Farm Animals		1.784	0.646	5.955	1.678	21.133	5.670	0.017	-	-0.519	3.117	0.595	0.001	267.662	0.031	0.859	-
Wildlife		2.032	0.590	7.631	2.401	24.250	10.159	0.001	-	-1.220	3.042	0.295	0.001	114.676	0.224	0.636	-

Variable	Category	Chloramphenicol Resistance								Ciprofloxacin Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Owner works in Healthcare		-0.766	0.824	0.465	0.092	2.339	0.974	0.324	-	0.013	1.421	1.013	0.063	16.407	0.000	0.993	0.221
Hospital work		-0.410	1.146	0.664	0.070	6.275	0.138	0.711	-	-	-	-	-	-	-	-	1.000
GP work		-0.565	1.174	0.568	0.057	5.671	0.254	0.614	-	-	-	-	-	-	-	-	0.602
Care home work		-	-	-	-	-	-	-	1.000	-	-	-	-	-	-	-	1.000
Owner works with animals		0.902	0.577	2.464	0.795	7.636	2.212	0.137	-	-1.992	3.060	0.136	0.000	54.891	0.740	0.390	-
Farming		2.673	0.742	14.490	3.383	62.065	9.413	0.002	-	4.060	4.187	57.983	0.016	2.13E+05	0.723	0.395	-
Veterinary		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polytomous variable																	
Working with animals	No	(Ref)	-	-	-	-	9.413	0.009	-	-	-	-	-	-	-	-	-
	Non-Farming	0.006	0.694	1.006	0.258	3.917			-	-	-	-	-	-	-	-	-
	Farming	2.674	0.751	14.504	3.326	63.253			-	-	-	-	-	-	-	-	-
Working in farming alone most significant so included in multivariable model																	
Owner has visited hospital in last month		-0.409	0.536	0.665	0.232	1.902	0.589	0.443		0.218	1.103	1.244	0.143	10.816	0.039	0.843	-
Admitted		-	-	-	-	-	-	-	0.356	0.461	1.417	1.586	0.099	25.482	-	-	1.000
Visiting patient		-0.551	0.869	0.576	0.105	3.165	0.435	0.509		0.314	1.345	1.369	0.098	19.118	0.053	0.818	-
Outpatient appointment		0.081	0.666	1.085	0.294	4.002	0.015	0.903		0.083	0.655	1.092	0.302	4.010	0.102	0.750	0.126

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; GP= General Practice

Table III-k Results of univariable analysis for risk factors associated with nalidixic acid and tetracycline resistance in faecal samples obtained from 185 owned kennelled dogs in the North of England

Variable	Category	Nalidixic Acid Resistance								Tetracycline Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Age	<1	-	-	-	-	-	-	-	0.351	(Ref)	-	-	-	-	0.855	0.652	-
	1-6years	-	-	-	-	-	-	-	-	0.327	0.002	1.387	1.382	1.393	-	-	-
	7+ years	-	-	-	-	-	-	-	-	0.624	0.002	1.867	1.860	1.874	-	-	-
Sex	Female	(Ref)	-	-	-	-	0.317	0.573	-	(Ref)	-	-	-	-	2.746	0.098	-
	Male	-0.378	0.671	0.685	0.184	2.551	-	-	-	-0.663	0.002	0.515	0.514	0.517	-	-	-
Neutered		0.480	0.910	1.616	0.272	9.612	0.298	0.585	-	0.017	0.002	1.017	1.013	1.021	0.001	0.973	-
Sex	Male entire	(Ref)	-	-	-	-	1.677	0.642	-	(Ref)	-	-	-	-	2.523	0.471	-
	Male neutered	-0.184	1.090	0.832	0.098	7.053	-	-	-	-0.303	0.002	0.739	0.736	0.742	-	-	-
	Female entire	-1.111	1.924	0.329	0.008	14.292	1.677	0.642	-	0.287	0.002	1.333	1.328	1.338	-	-	-
	Female	0.605	1.015	1.831	0.251	13.381	-	-	-	0.475	0.002	1.609	1.602	1.615	-	-	-
Pure breed		-0.325	0.859	0.722	0.134	3.892	0.139	0.709	-	-1.138	0.484	0.320	0.124	0.828	5.474	0.019	-
Size	Small/Toy	(Ref)	-	-	-	-	3.451	0.178	-	(Ref)	-	-	-	-	2.524	0.283	-
	Medium	1.924	1.204	6.848	0.647	72.454	-	-	-	-0.037	0.002	0.964	0.960	0.967	-	-	-
	Giant	1.622	1.211	5.066	0.472	54.415	-	-	-	0.645	0.002	1.907	1.900	1.914	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	1.003	0.606	0.016	(Ref)	-	-	-	-	0.473	0.790	-
	Overnight	-0.889	0.904	0.411	0.070	2.415	-	-	-	-0.307	0.002	0.736	0.733	0.739	-	-	-
	Day	-1.150	1.476	0.317	0.018	5.716	-	-	-	-0.566	0.002	0.568	0.566	0.570	-	-	-
Duration of kennelling (days)		-	-	-	-	-	-	-	-	0.000	0.001	1.000	0.999	1.001	0.012	0.912	-
		(Ref)	-	-	-	-	0.653	0.884	-	-	-	-	-	-	-	-	-
Duration of kennelling(piecewise)	<425 days	0.004	0.012	1.01	0.986	1.034	-	-	-	-	-	-	-	-	-	-	-
	>425 days	-0.006	0.010	0.994	0.975	1.013	-	-	-	-	-	-	-	-	-	-	-
Duration of kennelling	Up to 2 weeks	(Ref)	-	-	-	-	2.900	0.575	0.197	-	-	-	-	-	-	-	0.762
	2 w-1m	0.793	1.408	2.210	0.140	34.912	-	-	-	-	-	-	-	-	-	-	-
	1-3m	3.258	2.231	25.991	0.328	2061	-	-	-	-	-	-	-	-	-	-	-
	4-6m	1.128	3.193	3.088	0.006	1614	-	-	-	-	-	-	-	-	-	-	-
	>6 m	0.301	1.054	1.351	0.171	10.675	-	-	-	-	-	-	-	-	-	-	-
Sharing kennel		0.018	0.881	1.018	0.181	5.726	0.000	0.989	-	-0.467	0.002	0.627	0.625	0.629	0.911	0.340	-
Working Dog		-0.409	2.572	0.664	0.004	102.783	0.026	0.872	-	-1.099	1.026	0.333	0.045	2.492	1.254	0.263	-
Life Owner		-0.619	0.802	0.539	0.112	2.596	0.597	0.440	-	-0.564	0.443	0.569	0.239	1.355	1.608	0.205	-

Variable	Category	Nalidixic Acid Resistance								Tetracycline Resistance							
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
Origin	Rescue	-	-	-	-	-	-	-	0.486	(Ref)	-	-	-	-	3.793	0.285	-
	Breeder	-	-	-	-	-	-	-	-	-0.835	0.507	0.434	0.161	1.172	-	-	-
	Home bred	-	-	-	-	-	-	-	-	-1.282	1.042	0.278	0.036	2.141	-	-	-
	Previous	-	-	-	-	-	-	-	-	-1.069	0.786	0.343	0.074	1.603	-	-	-
Veterinary treatment within 6 months		0.194	0.793	1.214	0.257	5.749	0.060	0.807	-	0.555	0.436	1.743	0.741	4.100	1.620	0.203	-
Last vet treatment	>6m	-	-	-	-	-	-	-	0.543	(Ref)	-	-	-	-	2.003	0.572	-
	<1m	-	-	-	-	-	-	-	-	0.014	0.566	1.014	0.334	3.072	-	-	-
	2-3m	-	-	-	-	-	-	-	-	0.443	0.705	1.558	0.391	6.208	-	-	-
	4-6m	-	-	-	-	-	-	-	-	0.943	0.708	2.568	0.641	10.283	-	-	-
Antimicrobials within 6 months		0.169	1.011	1.184	0.163	8.593	0.028	0.868	-	0.380	0.587	1.463	0.463	4.619	0.412	0.521	-
Last antimicrobial	>6m	-	-	-	-	-	-	-	0.548	(Ref)	-	-	-	-	2.788	0.425	-
	<1m	-	-	-	-	-	-	-	-	-0.653	1.035	0.520	0.068	3.957	-	-	-
	2-3m	-	-	-	-	-	-	-	-	0.472	1.107	1.603	0.183	14.041	-	-	-
	4-6m	-	-	-	-	-	-	-	-	1.428	0.981	4.168	0.610	28.496	-	-	-
Hospitalisation within 6 months		0.950	1.408	2.587	0.164	40.829	0.422	0.516	-	2.168	0.982	8.741	1.274	59.961	5.485	0.019	-
Polytomous variable																	
Vet Treatment and Hospitalisation	No treatment	-	-	-	-	-	-	-	-	-	-	-	-	-	5.789	0.055	-
	No	-	-	-	-	-	-	-	-	0.294	0.472	1.341	0.532	3.384	-	-	-
	Hospitalisation	-	-	-	-	-	-	-	-	2.246	0.995	9.454	1.344	66.504	-	-	-
Hospitalisation alone most significant so included in multivariable model																	
Fed wet food		-0.331	0.791	0.719	0.152	3.388	0.175	0.676	-	0.179	0.464	1.196	0.481	2.971	0.149	0.699	-
Fed dry Mixer		1.029	0.844	2.797	0.535	14.633	1.469	0.226	-	1.144	0.500	3.138	1.177	8.367	5.437	0.020	-
Fed complete dry		-1.370	0.919	0.254	0.042	1.540	2.212	0.137	-	-0.058	0.523	0.944	0.339	2.629	0.012	0.912	-
Fed raw food		3.207	1.003	24.703	3.460	176.382	8.460	0.004	-	1.496	0.650	4.466	1.250	15.956	4.797	0.029	-
Fed cooked meat		1.397	1.456	4.042	0.233	70.103	0.846	0.358	-	0.854	0.731	2.349	0.561	9.845	1.360	0.243	-
Housed indoors		0.174	2.194	1.191	0.016	87.726	0.006	0.937	-	0.640	0.849	1.896	0.359	10.016	0.617	0.432	-
Housed outdoors		1.683	1.334	5.382	0.394	73.510	1.471	0.225	-	1.696	0.782	5.453	1.177	25.267	5.122	0.024	-
Housed in kennels		2.283	2.073	9.804	0.169	570.163	1.262	0.261	-	-0.276	1.030	0.759	0.101	5.710	0.073	0.787	-
Multi-dog household		-0.394	0.796	0.674	0.142	3.211	0.251	0.616	-	0.002	0.460	1.002	0.406	2.471	0.000	0.996	-
Number of dogs in household	One	(Ref)	-	-	-	-	3.761	0.288	-	(Ref)	-	-	-	-	3.702	0.295	-
	2-3	-0.864	0.926	0.422	0.069	2.591	-	-	-	-0.468	0.540	0.626	0.217	1.805	-	-	-
	4-5	4.330	3.498	75.970	0.080	7.22E+04	-	-	-	1.031	1.167	2.805	0.285	27.604	-	-	-
	6+	0.096	2.795	1.101	0.005	263.498	-	-	-	1.135	0.839	3.111	0.601	16.104	-	-	-

Variable	Category	Nalidixic Acid Resistance							Tetracycline Resistance								
		β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE	β	se	OR	L95%CI	U95%CI	LRT statistic	P	FE
In-contact dogs on antibiotics		-0.798	1.528	0.450	0.023	8.989	0.301	0.583	0.221	-0.342	0.842	0.710	0.136	3.698	0.168	0.682	-
In-contact dogs hospitalised		-	-	-	-	-	-	-	1.000	0.273	1.477	1.315	0.073	23.773	0.034	0.854	-
Contact with animals other than dogs																	
Cats		-0.264	0.878	0.768	0.137	4.293	0.092	0.761		0.069	0.516	1.071	0.389	2.947	0.018	0.894	-
Horses		-0.141	1.451	0.868	0.051	14.912	0.010	0.922		0.379	0.721	1.461	0.355	6.008	0.276	0.600	-
Small Mammals		2.936	1.398	18.832	1.216	291.769	4.573	0.032		2.110	0.932	8.250	1.329	51.220	5.630	0.018	-
Farm Animals		-0.508	2.126	0.602	0.009	38.832	0.063	0.801		1.712	0.771	5.540	1.223	25.100	4.417	0.036	-
Wildlife		0.295	1.170	1.343	0.136	13.304	0.061	0.805		-0.445	0.810	0.641	0.131	3.137	0.322	0.571	-
Owner works in Healthcare		-1.433	0.002	0.239	0.238	0.239	1.832	0.176	0.045	-0.574	0.632	0.563	0.163	1.944	0.862	0.353	-
Hospital work		-	-	-	-	-	-	-	0.614	0.123	0.882	1.131	0.201	6.369	0.019	0.889	-
GP work		-	-	-	-	-	-	-	0.364	-0.517	0.934	0.596	0.096	3.719	0.320	0.571	-
Care home work		-	-	-	-	-	-	-	1.000	-	-	-	-	-	-	-	0.323
Owner works with animals		0.014	1.151	1.014	0.106	9.681	0.000	0.993		0.102	0.628	1.107	0.323	3.788	0.026	0.872	-
Farming		4.100	2.497	60.338	0.452	8054.696	1.656	0.198		3.720	1.269	41.265	3.433	496.029	9.229	-	-
Veterinary		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Owner has visited hospital in last month		-1.043	0.822	0.352	0.070	1.763	1.735	0.188		-0.109	0.461	0.897	0.363	2.213	0.056	0.813	-
Admitted		-	-	-	-	-	-	-	0.599	1.521	0.989	4.578	0.659	31.801	2.338	0.126	-
Visiting patient		-1.038	1.337	0.354	0.026	4.865	0.712	0.399	0.226	-1.438	0.925	0.237	0.039	1.454	2.910	0.088	-
Outpatient appointment		0.452	0.992	1.571	0.225	10.977	0.201	0.654		-0.156	0.585	0.856	0.272	2.693	0.071	0.790	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; GP= General Practice

Table III-I Results of univariable analysis for risk factors associated with trimethoprim resistance in faecal samples obtained from 185 owned kennelled dogs in the North of England

Variable	Category	Trimethoprim Resistance					LRT statistic	P	FE
		β	se	OR	L95%CI	U95%CI			
Age	<1	(Ref)	-	-	-	-	0.270	0.874	-
	1-6years	-0.287	0.002	0.751	0.748	0.753	-	-	-
	7+ years	-0.391	0.002	0.676	0.674	0.679	-	-	-
Sex	Female	(Ref)	-	-	-	-	4.171	0.041	-
	Male	-0.823	0.002	0.439	0.438	0.441	-	-	-
Neutered		-0.227	0.002	0.797	0.794	0.800	0.254	0.614	-
Sex	Male entire	(Ref)	-	-	-	-	3.555	0.314	-
	Male neutered	-0.242	0.624	0.785	0.231	2.668	-	-	-
	Female entire	0.855	0.706	2.351	0.590	9.372	-	-	-
	Female	0.449	0.595	1.567	0.488	5.029	-	-	-
Pure breed		-0.518	0.002	0.596	0.594	0.598	1.057	0.304	-
Size	Small/Toy	(Ref)	-	-	-	-	7.528	0.023	-
	Medium	1.101	0.591	3.007	0.944	9.571	-	-	-
	Giant	1.495	0.583	4.457	1.423	13.962	-	-	-
Boarder	Resident	(Ref)	-	-	-	-	5.893	0.053	-
	Overnight	-1.301	0.567	0.272	0.090	0.827	-	-	-
	Day	-1.454	0.889	0.234	0.041	1.336	-	-	-
Duration of	(days)	0.002	0.001	1.002	1.000	1.003	4.675	0.031	-
Duration of	Up to 2 weeks	-	-	-	-	-	-	-	0.285
	2 w-1m	-	-	-	-	-	-	-	-
	1-3m	-	-	-	-	-	-	-	-
	4-6m	-	-	-	-	-	-	-	-
	>6 m	-	-	-	-	-	-	-	-
Sharing kennel		0.027	0.447	1.028	0.428	2.467	0.003	0.956	-
Working Dog		0.246	0.002	1.279	1.274	1.284	0.076	0.782	-
Life Owner		-0.383	0.002	0.682	0.679	0.684	0.783	0.376	-
Origin	Rescue	(Ref)	-	-	-	-	0.560	0.905	-
	Breeder	0.030	0.516	1.031	0.375	2.832	-	-	-
	Home bred	0.211	0.955	1.235	0.190	8.029	-	-	-
	Previous owner	-0.449	0.760	0.638	0.144	2.828	-	-	-
Veterinary treatment within 6 months		0.238	0.429	1.268	0.547	2.943	0.305	0.581	-

Variable	Category	Trimethoprim Resistance						LRT statistic	P	FE
		β	se	OR	L95%CI	U95%CI				
Last vet treatment	>6m	(Ref)	-	-	-	-	1.233	0.745	-	
	<1m	-0.073	0.002	0.930	0.926	0.933	-	-	-	
	2-3m	0.705	0.002	2.025	2.017	2.032	-	-	-	
	4-6m	0.083	0.002	1.086	1.082	1.090	-	-	-	
Antimicrobials within 6 months		0.597	0.576	1.817	0.587	5.619	1.067	0.302	-	
Last antimicrobial	>6m	(Ref)	-	-	-	-	1.492	0.684	-	
	<1m	0.272	0.854	1.312	0.246	7.001	-	-	-	
	2-3m	0.559	1.090	1.749	0.207	14.807	-	-	-	
	4-6m	1.122	1.039	3.073	0.401	23.561	-	-	-	
Hospitalisation within 6 months		2.461	1.241	11.712	1.028	133.461	5.464	0.019	-	
Fed wet food		-0.441	0.002	0.643	0.641	0.646	1.008	0.315	-	
Fed dry Mixer		-0.449	0.002	0.639	0.636	0.641	0.860	0.354	-	
Fed complete dry		0.807	0.002	2.240	2.232	2.248	2.231	0.135	-	
Fed raw food		0.887	0.674	2.428	0.648	9.099	1.656	0.198	-	
Fed cooked meat		-0.271	0.002	0.763	0.760	0.765	0.132	0.716	-	
Housed indoors		-0.869	0.667	0.419	0.113	1.550	1.650	0.199	-	
Housed outdoors		0.959	0.002	2.609	2.599	2.619	1.762	0.184	-	
Housed in kennels		1.096	0.002	2.992	2.982	3.003	1.236	0.266	-	
Multi-dog household		0.532	0.431	1.703	0.732	3.963	1.533	0.216	-	
Number of dogs in household	One	(Ref)	-	-	-	-	3.724	0.293	-	
	2-3	0.217	0.493	1.243	0.473	3.267	-	-	-	
	4-5	0.545	1.119	1.724	0.192	15.448	-	-	-	
	6+	1.470	0.775	4.350	0.953	19.855	-	-	-	
In-contact dogs on antibiotics		0.400	0.002	1.492	1.487	1.498	0.275	0.600	-	
In-contact dogs hospitalised		2.333	2.395	10.312	0.094	1126	0.712	0.399	-	
Contact with animals other than dogs										
Cats		0.069	0.002	1.071	1.067	1.075	0.019	0.890	-	
Horses		0.761	0.667	2.141	0.579	7.913	1.324	0.250	-	
Small Mammals		1.556	0.904	4.741	0.806	27.908	3.172	0.075	-	
Farm Animals		0.890	0.782	2.435	0.526	11.265	1.222	0.269	-	
Wildlife		0.512	0.002	1.668	1.662	1.674	0.562	0.453	-	

Variable	Category	Trimethoprim Resistance						P	FE
		β	se	OR	L95%CI	U95%CI	LRT statistic		
Owner works in Healthcare		0.712	0.002	2.039	2.032	2.046	1.579	0.209	-
Hospital work		-0.073	0.002	0.929	0.926	0.933	0.007	0.932	-
GP work		1.582	0.897	4.865	0.838	28.228	3.472	0.062	-
Care home work		0.088	0.002	1.092	1.088	1.096	0.004	0.948	-
Polytomous variable									
Owners works in human healthcare	No	(Ref)	-	-	-	-	3.483	0.175	-
	Non-GP	0.080	0.753	1.083	0.248	4.737			-
	GP	1.589	0.900	4.901	0.839	28.622			-
Working in GP practice alone most significant so included in multivariable model									
Owner works with animals		0.831	0.569	2.296	0.753	7.004	2.092	0.148	-
Farming		2.594	1.189	13.383	1.302	137.592	4.558	0.033	-
Veterinary		0.357	0.002	1.429	1.424	1.434	0.319	0.572	-
Polytomous variable									
Working with animals	No	(Ref)	-	-	-	-	5.031	0.081	-
	Non-Farming	0.419	0.607	1.520	0.462	4.998	-	-	-
	Farming	2.667	1.201	14.396	1.366	151.665	-	-	-
Working in farming alone most significant so included in multivariable model									
Owner has visited hospital in last month		0.293	0.002	1.340	1.335	1.345	0.408	0.523	-
Admitted		1.212	0.952	3.362	0.521	21.704	1.586	0.208	-
Visiting patient		-0.555	0.753	0.574	0.131	2.512	0.560	0.454	-
Outpatient appointment		0.483	0.002	1.620	1.614	1.626	0.725	0.394	-

P values quoted in bold are from the likelihood ratio test, all other P values are from the Wald Chi squared test; OR=Odds Ratio; 95% CI= 95% Confidence Interval; GP= General Practice

Residual plots

Residual plots were utilised to assess if any premises were exerting an increased influence on the mixed effect models constructed for each outcome. Where a significant difference was detected, data from these premises were checked for any errors

Figure III-d Premises level residuals plotted against the overall mean for each mixed effect model (n=24 premises).

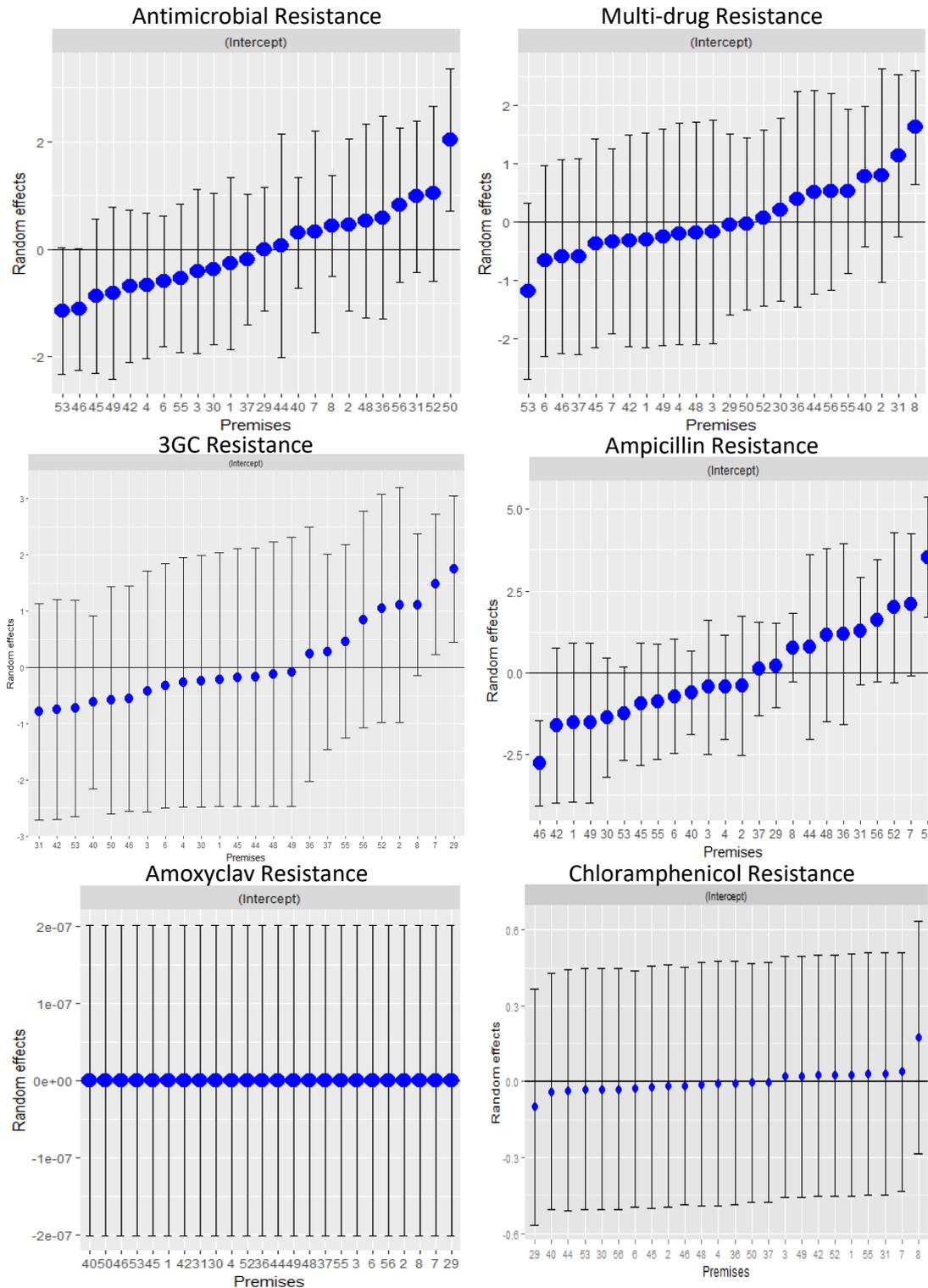
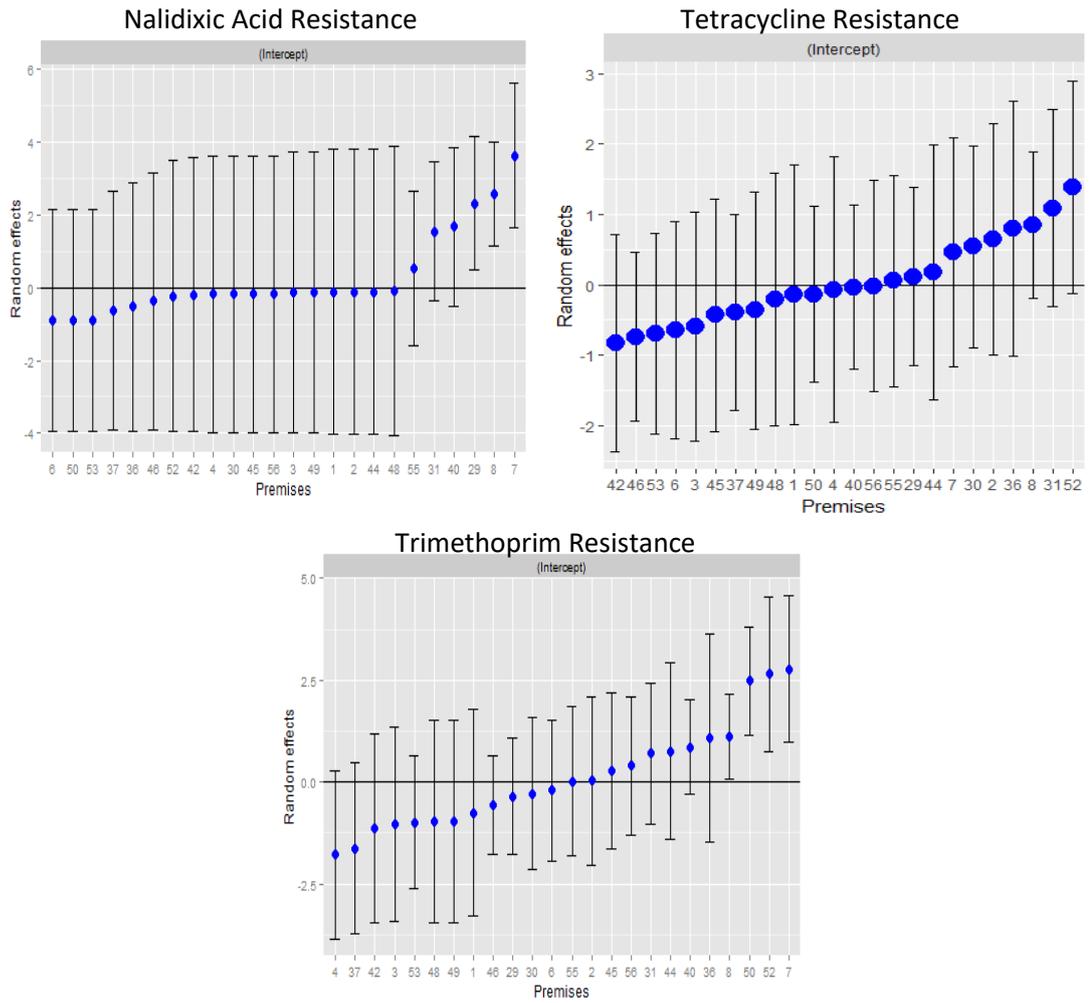


Figure III-d cont.



Appendix IV

Material pertaining to Chapter Six

Table IV-a Association of phylogenetic groups and the presence or absence of one of eleven antimicrobial resistance outcomes for 491 canine faecal *E. coli* isolates assigned to Phylogroups A-F

Resistance outcome	Phylogenetic Group																				
	Phylogroup A (n=96)			Phylogroup B1 (n=180)			Phylogroup B2 (n=152)			Phylogroup C (n=19)			Phylogroup D (n=27)			Phylogroup E (n=8)		Phylogroup F (n=19)			
	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P [†]	n	Prevalence (95% CI)	P [†]
Any resistance	75	78.1 (65.2-82.6)	0.0051	108	60.0 (49.7-70.3)	0.63	85	55.9 (48.0-63.8)	0.096	19	100.0 (100.0)	0.00043	15	55.6 (36.8-74.3)	0.52	2	25.0 (0.0-55.0)	0.060	4	44.4 (12.0-76.9)	0.31
MDR	26	27.1 (18.2-36.0)	0.0076	37	20.6 (14.1-27.0)	0.21	4	2.6 (0.1-5.2)	<0.0001	13	68.4 (47.5-89.3)	<0.0001[†]	5	18.5 (3.9-33.2)	0.80 [†]	1	12.5 (0.0-35.4)	1	2	22.2 (0.0-49.4)	0.66
3GC	11	11.5 (5.1-17.8)	0.76	25	13.9 (8.8-18.9)	0.45	10	6.6 (2.6-10.5)	0.0093	8	42.1 (19.9-64.3)	0.00091 [†]	6	22.2 (6.5-37.9)	0.1286 [†]	1	12.5 (0.0-35.4)	1	2	22.2 (0.0-49.4)	0.31
Ampicillin	45	46.9 (36.9-56.9)	0.50	95	52.8 (45.5-60.1)	0.35	73	48.0 (40.1-56.0)	0.56	16	84.2 (67.8-100.0)	0.0023	14	51.9 (33.0-70.7)	0.84	2	25.0 (0.0-55.0)	0.29	4	44.4 (12.0-76.9)	1
Amoxycylav	7	7.3 (2.1-12.5)	0.092	23	12.8 (7.9-17.7)	0.85	14	9.2 (4.6-13.8)	0.015	10	52.6 (30.2-75.1)	0.00016[†]	6	22.2 (6.5-37.9)	0.13 [†]	0	0.0 (0.0)	0.60	1	11.1 (0.0-31.6)	1
Chloramphenicol	14	14.6 (7.5-21.6)	0.52	24	13.3 (8.4-18.3)	0.71	12	7.9 (3.6-12.2)	0.037	7	36.8 (15.2-58.5)	0.0053 [†]	4	14.8 (1.4-28.2)	0.76 [†]	0	0.0 (0.0)	0.60	1	11.1 (0.0-31.6)	1
Ciprofloxacin	7	7.3 (2.1-12.5)	0.19 [†]	11	6.1 (2.6-9.6)	0.28	1	0.7 (0.0-1.9)	0.0048	2	10.5 (0.0-24.3)	0.22 [†]	1	3.7 (0.0-10.8)	1 [†]	0	0.0 (0.0)	1	1	11.1 (0.0-31.6)	0.36
Nalidixic Acid	11	11.5 (5.1-17.8)	0.36	16	8.9 (4.7-13.0)	0.92	3	2.0 (0.0-4.2)	0.00028	9	47.4 (24.9-69.8)	<0.0001[†]	2	7.4 (0.0-17.3)	1 [†]	0	0.0 (0.0)	1	4	44.4 (12.0-76.9)	0.0053
Tetracycline	59	56.3 (46.3-66.2)	<0.0001	39	21.7 (15.6-27.7)	0.12	19	12.5 (7.2-17.8)	<0.0001	8	42.1 (19.9-64.3)	0.0013	7	25.9 (9.4-42.5)	0.99	2	25.0 (0.0-55.0)	1	0	0.0 (0.0)	0.12
Trimethoprim	46	47.9 (37.9-57.9)	0.00049	51	28.3 (21.8-34.9)	0.11	43	28.3 (21.1-35.4)	0.15	16	84.2 (67.8-100.0)	<0.0001	6	22.2 (6.5-37.9)	0.23	1	12.5 (0.0-35.4)	0.28	2	22.2 (0.0-49.4)	0.73
ESBL phenotype	4	4.2 (0.2-8.2)	0.25 [†]	4	2.2 (0.1-4.1)	1 [†]	0	0.0 (0.0)	0.022 [†]	1	5.3 (0.0-15.3)	0.37 [†]	0	0.0 (0.0)	1 [†]	1	12.5 (0.0-35.4)	0.18	2	22.2 (0.0-49.4)	0.017

P=P value calculated using Chi-squared test; † P value calculated using Fisher's Exact method; 95% CI=95% confidence interval; MDR= multi-drug resistant phenotype; 3GC= third generation cephalosporin resistance; Significance set at P<0.00066 (Sidak-Bonferroni correction).

Table IV-b Association of phylogenetic groups and the presence or absence of one of eleven antimicrobial resistance outcomes for of 358 human faecal *E. coli* isolates assigned to Phylogroups A-F

Resistance outcome	Phylogenetic Group																				
	Group A (n=62)			Group B1 (n=82)			Group B2 (n=142)			Group C (n=3)			Group D (n=46)			Group E (n=6)			Group F (n=17)		
	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P [†]	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	P [†]	n	Prevalence (95% CI)	P
Any resistance	42	67.7 (56.1-79.4)	0.18	40	48.8 (38.8-59.3)	0.017	89	62.7 (54.7-76.0)	0.43	3	100.00 (100.0)	0.28	29	63.0 (49.1-77.0)	0.67	4	66.7 (28.9-100.0)	1	10	58.8 (35.4-82.2)	0.91
MDR	16	25.8 (14.9-36.7)	0.052	11	13.4 (7.8-19.0)	0.29	15	10.6 (5.5-15.6)	0.0065	0	0.0 (0.0)	1	17	37.0 (23.0-50.9)	0.00016	1	16.7 (0.0-46.5)	1	2	11.8 (0.0-27.1)	0.75 [†]
3GC	5	8.1 (1.3-14.8)	0.36 [†]	3	3.7 (0.0-7.7)	0.58 [†]	7	4.9 (1.4-8.5)	0.71	1	33.3 (0.0-86.7)	0.16	1	2.2 (0.0-6.4)	0.49 [†]	0	0.00 (0.0)	1	3	17.6 (0.0-35.8)	0.059 [†]
Ampicillin	30	48.4 (35.9-60.8)	0.56	30	36.6 (26.2-47.0)	0.080	68	47.9 (39.7-56.1)	0.39	2	66.7 (13.3-100.0)	0.59	25	54.3 (40.0-68.7)	0.18	0	0.00 (0.0)	0.035	8	47.1 (23.3-70.8)	0.86
Amoxyclav	0	0.0 (0.0-0.0)	0.85 [†]	3	3.7 (0.0-7.7)	1 [†]	8	5.6 (1.8-9.4)	0.36 [†]	1	33.3 (0.0-86.7)	0.13	0	0.00 (0.0)	0.24 [†]	0	0.00 (0.0)	1	3	17.6 (0.0-35.8)	0.033 [†]
Chloramphenicol	8	12.9 (4.6-21.2)	0.14	4	4.9 (0.2-9.5)	0.21	11	7.7 (3.3-12.1)	0.78	0	0.0 (0.0)	1	5	10.9 (1.9-19.9)	0.56 [†]	1	16.7 (0.0-46.5)	0.41	1	5.9 (0.0-17.1)	1 [†]
Ciprofloxacin	5	8.1 (1.3-14.8)	0.21 [†]	3	3.7 (0.0-7.7)	0.77 [†]	6	4.2 (0.9-7.5)	0.61	0	0.0 (0.0)	1	4	8.7 (0.6-16.8)	0.26 [†]	0	0.00 (0.0)	1	0	0.00 (0.0)	1 [†]
Nalidixic Acid	11	17.7 (8.2-27.3)	0.21	6	7.3 (1.7-13.0)	0.086	13	9.2 (4.4-13.9)	0.087	0	0.0 (0.0)	1	14	30.4 (17.1-43.7)	0.00015	3	50.0 (10.0-90.0)	0.031	0	0.00 (0.0)	0.14 [†]
Tetracycline	21	33.9 (22.1-45.7)	0.043	20	24.4 (15.1-33.7)	0.91	22	15.5 (9.5-21.4)	0.0026	0	0.0 (0.0)	1	18	39.1 (25.0-53.2)	0.0096	1	16.7 (0.0-46.5)	01	3	17.6 (0.0-35.8)	0.77
Trimethoprim	20	32.3 (20.6-43.9)	0.18	15	18.3 (9.9-26.7)	0.087	32	22.5 (15.7-29.4)	0.29	2	66.7 (13.3-100.0)	0.16	14	30.4 (17.1-43.7)	0.42	3	50.0 (10.0-90.0)	0.18	5	29.4 (7.8-51.1)	0.78 [†]
ESBL phenotype	5	8.1 (1.3-14.8)	0.0089 [†]	2	2.4 (0.0-5.8)	1 [†]	1	0.7 (0.0-2.1)	0.10 [†]	0	0.00 (0.0)	1	1	2.2 (0.0-6.4)	1 [†]	0	0.00 (0.0)	1	0	0.00 (0.0)	1 [†]

P value calculated using Chi-squared test; † P value calculated using Fisher's Exact method; 95% CI=95% confidence interval; MDR= multi-drug resistant phenotype; 3GC= third generation cephalosporin resistance; Significance set at P<0.00066 (Sidak-Bonferroni correction).

Multiple Correspondence Analysis

Table IV-c Correlation of variables with dimensions included in multiple correspondence analysis

Categorical Variable	Correlation (eta ²)					
	Dimensions					
	1	2	3	4	5	6
Species	0.010	0.079	0.267	0.228	0.000	0.001
Ampicillin resistance	0.353	0.052	0.011	0.092	0.021	0.024
Amoxyclav resistance	0.123	0.676	0.009	0.002	0.001	0.003
Chloramphenicol resistance	0.313	0.005	0.000	0.021	0.018	0.024
Ciprofloxacin resistance	0.266	0.039	0.233	0.162	0.005	0.017
Nalidixic Acid resistance	0.423	0.033	0.245	0.063	0.006	0.001
Tetracycline resistance	0.377	0.128	0.084	0.026	0.026	0.021
Trimethoprim resistance	0.376	0.027	0.127	0.046	0.041	0.002
MDR	0.717	0.057	0.010	0.004	0.000	0.000
Phylogroup	0.165	0.165	0.306	0.492	0.903	0.900
3GC Resistance	0.145	0.602	0.011	0.005	0.012	0.034

Key: MDR= multi-drug resistance; 3GC= third generation cephalosporin resistance

Table IV-d Contribution of each variable to the dimensions included in multiple correspondence analysis

Variable	Category	Contribution to dimension					
		Dimension					
		1	2	3	4	5	6
Species	Dog	0.134	1.783	8.649	8.427	0.002	0.053
	Human	0.184	2.445	11.862	11.558	0.003	0.073
Phylogroup	A	0.704	2.598	5.787	0.279	1.716	15.15
	B1	0.017	0.537	1.574	21.823	12.232	0.203
	B2	1.27	0.079	0.592	6.505	9.77	23.852
	C	2.769	4.27	0.336	0.001	20.878	0.988
	D	0.236	0.768	9.094	6.981	7.686	0.214
	E	0.043	0.229	0.294	3.689	35.146	24.808
Ampicillin	F	0.004	0.384	5.802	3.828	0.008	22.482
	Resistant	5.564	1.45	0.445	4.141	1.037	1.206
Amoxyclav	Sensitive	5.246	1.367	0.42	3.904	0.978	1.137
	Resistant	3.439	33.017	0.66	0.184	0.102	0.262
Ciprofloxacin	Sensitive	0.338	3.246	0.065	0.018	0.01	0.026
	Resistant	7.74	2.003	16.991	13.506	0.42	1.544
Nalidixic Acid	Sensitive	0.393	0.102	0.862	0.685	0.021	0.078
	Resistant	11.548	1.601	16.745	4.951	0.523	0.05
Chloramphenicol	Sensitive	1.403	0.195	2.035	0.602	0.064	0.006
	Resistant	8.53	0.223	0.001	1.664	1.557	2.108
Tetracycline	Sensitive	1.037	0.027	0.000	0.202	0.189	0.256
	Resistant	8.624	5.12	4.832	1.674	1.902	1.502
Trimethoprim	Sensitive	2.907	1.725	1.629	0.564	0.641	0.506
	Resistant	8.034	0.998	6.793	2.821	2.77	0.148
MDR	Sensitive	3.468	0.431	2.932	1.218	1.196	0.064
	Resistant	18.061	2.533	0.616	0.307	0.006	0.004
3GC	Sensitive	3.876	0.544	0.132	0.066	0.001	0.001
	Resistant	3.998	29.165	0.767	0.363	1.031	2.958
	Sensitive	0.433	3.16	0.083	0.039	0.112	0.321

Key: MDR= multi-drug resistance; 3GC= third generation cephalosporin

Table IV-e The prevalence of isolates of human and canine origin in clusters formed by hierarchical clustering of the results of multiple correspondence analysis

Cluster	Human isolates (n=358)			Dog isolates (n=491)			P
	n	Prevalence	95% CI	n	Prevalence	95% CI	
1 (n=620)	272	43.8	39.9-47.7	348	56.1	52.3-60.1	0.098
2 (n=85)	16	18.8	10.5-27.1	69	81.2	72.9--89.5	<0.0001
3 (n=144)	70	48.6	40.4-56.8	74	51.0	45.7-66.7	0.086

Key: p= P value calculated using Chi-squared test

Table IV-f The prevalence of isolates of *E. coli* belonging to Phylogroups A-F in clusters formed by hierarchical clustering of the results of multiple correspondence analysis

Phylogroup	Cluster 1 (n=620)			Cluster 2 (n=85)			Cluster 3 (n=144)		
	n	Prevalence (95% CI)	p	n	Prevalence (95% CI)	P	n	Prevalence (95% CI)	p
A	103	16.6 (13.7-19.5)	0.014	10	11.8 (5.0-18.5)	0.087	45	31.3 (23.7-38.8)	<0.0001
B1	189	30.5 (26.9-34.1)	0.74	26	30.6 (23.1-38.1)	0.95	47	32.6 (25.0-40.3)	0.61
B2	251	40.5 36.6-44.3	<0.0001	22	25.9 (16.6-35.2)	0.074	21	14.6 (8.8-20.3)	<0.0001
C	5	0.8 (0.1-1.5)	<0.0001[†]	14	16.5 (8.6-24.4)	<0.0001[†]	3	2.1 (0.0-4.4)	1 [†]
D	43	6.9 (4.9-8.9)	0.0044	7	8.2 (2.4-14.1)	0.90	23	16.0 (10.0-22.0)	0.00053
E	11	1.8 (0.7-2.8)	0.77 [†]	1	1.2 (0.0-3.5)	1 [†]	2	1.4 (0.0-3.3)	1 [†]
F	18	2.9 (1.6-4.2)	0.65	5	5.9 (0.9-10.9)	0.17 [†]	3	2.1 (0.0-4.4)	0.60

Key: P value calculated using Chi-squared test; [†] P value calculated using Fisher's Exact method; 95% CI=95% confidence interval; Significance set at p<0.0024 (Sidak-Bonferroni correction).

Whole Genome Sequencing

Table IV-g Details of sequences obtained during whole genome sequencing of 132 isolates of *E. coli*

Isolate	Species	Source Details	Number of contigs	Genome Size	N50
H77	<i>Escherichia coli</i>	Human	346	5,014,693	28,408
H93	<i>Escherichia coli</i>	Human	181	4,980,773	65,546
H133	<i>Escherichia coli</i>	Human	321	4,793,339	31,789
H190	<i>Escherichia coli</i>	Human	557	5,273,843	19,736
H198	<i>Escherichia coli</i>	Human	151	4,909,568	88,681
H200	<i>Escherichia coli</i>	Human	195	4,671,871	46,386
H206	<i>Escherichia coli</i>	Human	632	5,161,947	16,840
H211	<i>Escherichia coli</i>	Human	209	4,982,125	59,672
H223	<i>Escherichia coli</i>	Human	218	5,158,589	80,011
H269	<i>Escherichia coli</i>	Human	205	5,636,190	79,715
H288	<i>Escherichia coli</i>	Human	221	4,696,731	63,786
H338	<i>Escherichia coli</i>	Human	1,348	6,143,444	26,111
H381	<i>Escherichia coli</i>	Human	183	4,859,266	98,117
H399	<i>Escherichia coli</i>	Human	527	5,421,105	40,683
H418	<i>Escherichia coli</i>	Human	2,108	4,276,652	2,762
H421	<i>Escherichia coli</i>	Human	336	5,096,493	41,372
H422	<i>Escherichia coli</i>	Human	234	5,125,801	63,461
H430	<i>Escherichia coli</i>	Human	357	5,253,170	34,885
H432	<i>Escherichia coli</i>	Human	188	4,735,867	62,006
H437	<i>Escherichia coli</i>	Human	337	5,263,386	35,019
H439	<i>Escherichia coli</i>	Human	245	5,278,274	52,526
H444	<i>Escherichia coli</i>	Human	194	5,247,687	83,580
H449	<i>Escherichia coli</i>	Human	707	5,498,316	35,970
H481	<i>Escherichia coli</i>	Human	232	5,262,402	82,819
H484	<i>Escherichia coli</i>	Human	436	5,285,322	29,226
H669	<i>Escherichia coli</i>	Human	289	5,268,114	46,118
H677	<i>Escherichia coli</i>	Human	293	5,057,169	50,788
H690	<i>Escherichia coli</i>	Human	173	4,889,179	81,270
H697	<i>Escherichia coli</i>	Human	248	5,112,879	61,080
H698	<i>Escherichia coli</i>	Human	277	5,249,952	51,441
H712	<i>Escherichia coli</i>	Human	266	5,236,482	57,443
H766	<i>Escherichia coli</i>	Human	171	5,153,745	79,379
H774	<i>Escherichia coli</i>	Human	221	5,314,921	69,962
H798	<i>Escherichia coli</i>	Human	292	5,229,187	41,236
H933	<i>Escherichia coli</i>	Human	191	5,248,924	62,531
H953	<i>Escherichia coli</i>	Human	136	4,891,013	124,667
H971	<i>Escherichia coli</i>	Human	317	4,898,749	35,002
H976	<i>Escherichia coli</i>	Human	162	4,930,448	67,804
H1023	<i>Escherichia coli</i>	Human	236	5,159,774	55,952
H1046	<i>Escherichia coli</i>	Human	199	5,210,569	69,039
H1071	<i>Escherichia coli</i>	Human	195	5,119,847	66,991
H1118	<i>Escherichia coli</i>	Human	590	5,368,145	26,031
H1162	<i>Escherichia coli</i>	Human	275	5,143,154	43,731
H1170	<i>Escherichia coli</i>	Human	177	4,874,868	70,968
D29	<i>Escherichia coli</i>	Dog	175	4,648,396	65,503
D41	<i>Escherichia coli</i>	Dog	119	5,017,048	123,279
D93	<i>Escherichia coli</i>	Dog	333	4,941,964	33,898
D138	<i>Escherichia coli</i>	Dog	131	4,692,399	99,536
D142	<i>Escherichia coli</i>	Dog	167	4,918,864	63,253
D151	<i>Escherichia coli</i>	Dog	363	4,888,488	28,883

Isolate	Species	Source Details	Number of contigs	Genome Size	N50
D157	<i>Escherichia coli</i>	Dog	169	4,900,118	65,956
D159	<i>Escherichia coli</i>	Dog	242	4,700,520	46,856
D164	<i>Escherichia coli</i>	Dog	213	4,892,639	63,184
D171	<i>Escherichia coli</i>	Dog	182	4,790,044	81,017
D173	<i>Escherichia coli</i>	Dog	201	4,706,542	63,054
D178	<i>Escherichia coli</i>	Dog	267	5,239,913	63,006
D182	<i>Escherichia coli</i>	Dog	150	4,914,395	78,264
D189	<i>Escherichia coli</i>	Dog	146	4,916,015	86,212
D191	<i>Escherichia coli</i>	Dog	424	5,030,660	29,501
D193	<i>Escherichia coli</i>	Dog	223	4,693,547	53,017
D194	<i>Escherichia coli</i>	Dog	294	4,568,128	31,142
D216	<i>Escherichia coli</i>	Dog	421	5,021,576	30,509
D272	<i>Escherichia coli</i>	Dog	136	4,997,535	94,989
D289	<i>Escherichia coli</i>	Dog	297	5,477,609	46,212
D306	<i>Escherichia coli</i>	Dog	205	5,036,373	85,113
D319	<i>Escherichia coli</i>	Dog	282	4,887,010	43,048
D320	<i>Escherichia coli</i>	Dog	249	4,886,430	52,795
D330	<i>Escherichia coli</i>	Dog	218	5,032,289	73,917
D333	<i>Escherichia coli</i>	Dog	214	5,000,007	48,717
D341	<i>Escherichia coli</i>	Dog	217	5,202,134	65,627
D346	<i>Escherichia coli</i>	Dog	232	5,377,888	130,818
D350	<i>Escherichia coli</i>	Dog	251	4,594,263	44,015
D355	<i>Escherichia coli</i>	Dog	305	5,186,721	43,096
D361	<i>Escherichia coli</i>	Dog	231	5,326,355	52,828
D362	<i>Escherichia coli</i>	Dog	274	5,229,407	49,205
D369	<i>Escherichia coli</i>	Dog	235	4,841,057	50,254
D375	<i>Escherichia coli</i>	Dog	198	4,831,362	52,543
D396	<i>Escherichia coli</i>	Dog	263	5,190,265	63,261
D405	<i>Escherichia coli</i>	Dog	247	4,774,392	39,833
D414	<i>Escherichia coli</i>	Dog	1,644	9,171,102	10,326
D419	<i>Escherichia coli</i>	Dog	207	5,129,021	59,099
D420	<i>Escherichia coli</i>	Dog	222	5,000,224	66,794
D424	<i>Escherichia coli</i>	Dog	2,111	8,089,639	23,945
D426	<i>Escherichia coli</i>	Dog	276	5,020,164	40,768
D432	<i>Escherichia coli</i>	Dog	235	5,195,614	72,939
D435	<i>Escherichia coli</i>	Dog	306	4,854,252	82,778
D436	<i>Escherichia coli</i>	Dog	245	4,950,162	54,627
D445	<i>Escherichia coli</i>	Dog	359	4,856,620	37,404
D446	<i>Escherichia coli</i>	Dog	203	4,998,506	68,288
D457	<i>Escherichia coli</i>	Dog	923	5,242,123	26,410
D460	<i>Escherichia coli</i>	Dog	1,426	5,956,052	23,117
D471	<i>Escherichia coli</i>	Dog	268	5,137,586	42,624
D483	<i>Escherichia coli</i>	Dog	288	5,186,521	37,602
D491	<i>Escherichia coli</i>	Dog	644	5,392,406	65,509
D503	<i>Escherichia coli</i>	Dog	512	5,197,153	23,851
D512	<i>Escherichia coli</i>	Dog	474	5,539,929	36,111
D514	<i>Escherichia coli</i>	Dog	2,660	3,897,655	1,772
D524	<i>Escherichia coli</i>	Dog	205	5,235,758	69,154
D528	<i>Escherichia coli</i>	Dog	208	5,066,248	67,913
D540	<i>Escherichia coli</i>	Dog	228	5,216,361	75,581
D552	<i>Escherichia coli</i>	Dog	129	5,057,540	146,291
D558	<i>Escherichia coli</i>	Dog	227	5,240,031	58,814

Isolate	Species	Source Details	Number of contigs	Genome Size	N50
D570	<i>Escherichia coli</i>	Dog	263	5,017,660	47,316
D579	<i>Escherichia coli</i>	Dog	328	4,723,170	35,253
D582	<i>Escherichia coli</i>	Dog	187	5,026,500	54,194
D587	<i>Escherichia coli</i>	Dog	512	5,294,208	96,795
D591	<i>Escherichia coli</i>	Dog	507	4,995,717	22,623
D592	<i>Escherichia coli</i>	Dog	1,014	4,455,515	7,088
D598	<i>Escherichia coli</i>	Dog	414	5,207,701	44,798
D699	<i>Escherichia coli</i>	Dog	129	5,288,507	139,097
D714	<i>Escherichia coli</i>	Dog	1,451	5,883,724	25,752
D754	<i>Escherichia coli</i>	Dog	1,273	5,925,525	19,902
D783	<i>Escherichia coli</i>	Dog	242	5,281,367	56,314
D977	<i>Escherichia coli</i>	Dog	161	4,862,047	74,703
D1040	<i>Escherichia coli</i>	Dog	143	4,861,957	64,739
D1045	<i>Escherichia coli</i>	Dog	220	4,597,914	52,088
D1109	<i>Escherichia coli</i>	Dog	241	5,220,401	48,796
D1110	<i>Escherichia coli</i>	Dog	590	5,267,006	21,387
D1127	<i>Escherichia coli</i>	Dog	378	5,149,112	34,098
D1201	<i>Escherichia coli</i>	Dog	263	4,924,496	49,117
D1202	<i>Escherichia coli</i>	Dog	170	5,027,324	92,904
D1206	<i>Escherichia coli</i>	Dog	102	4,700,364	129,269
D1224	<i>Escherichia coli</i>	Dog	253	4,767,434	40,691
D1227	<i>Escherichia coli</i>	Dog	2,429	4,147,023	2,188
D1240	<i>Escherichia coli</i>	Dog	225	4,988,984	56,715
D1245	<i>Escherichia coli</i>	Dog	209	4,693,126	57,687
D1248	<i>Escherichia coli</i>	Dog	165	4,701,842	66,636
D1324	<i>Escherichia coli</i>	Dog	244	5,080,689	47,659
D1328	<i>Escherichia coli</i>	Dog	320	5,145,978	49,142
D1330	<i>Escherichia coli</i>	Dog	194	5,089,009	81,462
D1363	<i>Escherichia coli</i>	Dog	133	4,872,134	83,624
D1404	<i>Escherichia coli</i>	Dog	637	5,080,452	40,213
		Average	387	5,103,363	56,112

Table IV-h The prevalence of virulence genes among *E. coli* isolates of canine and human origin carrying *bla*_{ESBL} and *bla*_{AmpC} genes

Gene	Function	Isolate		Phylogroup					
		Canine (n=34)	Human (n=14)	A (n=14)	B1 (n=12)	B2 (n=11)	D (n=6)	E (n=1)	F (F=4)
<i>aap</i>	Dispersin	0	2	0.0	8.3	0.0	16.7	0.0	0.0
<i>air</i>	Enteroaggregative immunoglobulin repeat protein	4	4	0.0	0.0	9.1	83.3	0.0	50.0
<i>astA</i>	EAST-1 heat-stable toxin	2	1	0.0	0.0	18.2	16.7	0.0	0.0
<i>capU</i>	Hexosyltransferase homolog	1	1	0.0	16.7	0.0	0.0	0.0	0.0
<i>cba</i>	Colicin B	2	1	0.0	0.0	9.1	33.3	0.0	0.0
<i>cma</i>	Colicin M	3	1	0.0	8.3	9.1	16.7	100.0	0.0
<i>cnf1</i>	Cytotoxic necrotizing factor	4	1	0.0	0.0	45.5	0.0	0.0	0.0
<i>eae</i>	Intimin adherence protein	1	1	7.1	8.3	0.0	0.0	0.0	0.0
<i>eilA</i>	Salmonella <i>HilA</i> homolog (adherence)	3	2	0.0	0.0	0.0	66.7	0.0	25.0
<i>espA</i>	Type III secretions system (epithelial cell signal induction)	1	1	7.1	8.3	0.0	0.0	0.0	0.0
<i>espF</i>	Type III secretion system (increased tight junction permeability and apoptosis)	1	1	7.1	8.3	0.0	0.0	0.0	0.0
<i>gad</i>	Glutamate decarboxylase acid survival	32	14	92.9	100.0	90.9	100.0	100.0	100.0
<i>iha</i>	Adherence protein	0	2	0.0	8.3	9.1	0.0	0.0	0.0
<i>iroN</i>	Siderophore receptor protein (iron acquisition)	10	4	7.1	25.0	81.8	0.0	100.0	0.0
<i>iss</i>	Increased serum survival (complement resistance)	15	6	28.6	50.0	90.9	0.0	100.0	0.0
<i>lpfA</i>	Long polar fimbriae colonisation	15	5	7.1	91.7	27.3	33.3	0.0	75.0
<i>mchB</i>	Microcin H47 part of colicin H	5	4	7.1	8.3	63.6	0.0	0.0	0.0
<i>mchC</i>	MchC protein	6	4	7.1	8.3	72.7	0.0	0.0	0.0
<i>mchF</i>	ABC transporter protein MchF	7	5	7.1	8.3	90.9	0.0	0.0	0.0
<i>mcmA</i>	Microcin M part of colicin H	7	2	0.0	8.3	72.7	0.0	0.0	0.0
<i>nleB</i>	Non-LEE encoded effector B (inhibition of NF-κB activation)	1	1	7.1	8.3	0.0	0.0	0.0	0.0
<i>sat</i>	Secreted autotransporter toxin (vacuolating cytotoxin)	0	2	0.0	8.3	9.1	0.0	0.0	0.0
<i>sfaS</i>	S-fimbriae minor subunit adhesin	2	0	0.0	0.0	18.2	0.0	0.0	0.0
<i>tir</i>	Translocated intimin receptor protein Adherence	1	1	7.1	8.3	0.0	0.0	0.0	0.0
<i>vat</i>	Vacuolating autotransporter toxin	5	0	0.0	0.0	45.5	0.0	0.0	0.0

