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Antimicrobial resistance in clinical *Escherichia coli* isolated from companion animals in Australia

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Highlights

- Resistance to critically important antimicrobials registered in human medicine such as carbapenems and amikacin are rare among clinical *E. coli* isolated (n=883) from dogs, cats and horses in Australia
- Frequency of resistance to critically important antimicrobials registered for veterinary use in dog isolates was classified as low for fluoroquinolones (9.1%-9.3%) and moderate for 3rd generation cephalosporins (10.1%-10.9%)
- Frequency of resistance to critically important antimicrobials registered for veterinary use was low among clinical *E. coli* isolates from cats (fluoroquinolones 3.2%-5%; 3rd generation cephalosporins (5.6%-6.5%).
- Chronic and/or recurrent disease and prior antimicrobial treatments were the main risk factors for the isolation of multi-drug resistant (MDR) *E. coli* from urinary tract infections in both dogs and cats.

Abstract

Multidrug-resistant (MDR) *Escherichia coli* have become a major public health concern to both humans and animal health. While the frequency of antimicrobial resistance (AMR) in clinical *E. coli* is monitored regularly in human medicine, current frequency of AMR in companion animals remains unknown in Australia. In this study we conducted antimicrobial susceptibility testing (AST) and where possible, determined potential risk factors for MDR infection among 883 clinical *Escherichia coli* isolated from dogs (n=514), cats (n=341) and horses (n=28). AST was undertaken for 15 antimicrobial agents according to the Clinical Laboratory Standards Institute (CLSI) guidelines and interpreted using epidemiological cut-off values (ECOFFs) as well as CLSI veterinary and human clinical breakpoints. The AST revealed complete absence of resistance to carbapenems while resistance to amikacin was observed at a low level in isolates from dogs (1.6%) and cats (1.5%) compared to horses (10.7%). Among dog isolates, resistance to fluoroquinolones ranged from 9.1%-9.3% whereas among cat isolates, it ranged from 3.2%-5%. Among dog isolates, the proportion showing a 3rd generation cephalosporin (3GC) non-wild type phenotype was significantly higher ($P<0.05$) in skin and soft tissue infection (SSTI, n=122) isolates (17.2%-20.5%) compared to urinary tract infection (UTI, n=392) isolates (9.9%-10.2%). The frequency of multidrug resistance was 18.1%, 11.7% and 42.9% in dog, cat and horse isolates, respectively. Risk factor analysis revealed that MDR *E. coli* isolated from UTI were positively associated with chronicity of infection and previous antimicrobial treatment. Dogs and cats with chronic UTI that had been previously treated with antimicrobials were eight times and six times more likely to be infected with MDR *E. coli* compared to dogs and cats with non-chronic UTI, and no history of antimicrobial treatment, respectively. This study revealed that pre-existing disease condition

and prior antimicrobial use were the major risks associated with UTI with MDR *E. coli* in companion animals.

Keywords: *E. coli*, antimicrobial resistance, risk factors, urinary tract infections, companion animals, dogs, cats, horses

Introduction

Escherichia coli, a Gram-negative bacteria normally residing in the intestinal tract, is among the most common pathogenic agents in humans and animals. It is classified into various pathotypes, causing intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infections (UTI), skin and soft tissue infections (SSTI), and septicaemia (Hammerum and Heuer, 2009). Infections are usually less responsive to treatment when multidrug-resistant (MDR) *E. coli* are encountered, especially when they are resistant to critically important antimicrobials (CIA), including extended-spectrum β-lactams (e.g. 3rd and 4th generation cephalosporins and carbapenems) and fluoroquinolones (FQN) (Abraham et al. (2014b). Therefore, effectiveness of treatment of bacterial disease is becoming a challenge because of emerging MDR strains contributing to more costly and protracted infections.

Antimicrobial resistance (AMR) in bacteria that are well adapted for colonisation of both humans and animals is also a significant concern as shared environments provide the opportunity for rapid dissemination of resistant strains from one host to the other (Guardabassi et al., 2004). Environmental exposure and direct exposure to companion animals play important roles in transmission of resistant bacteria (Abraham et al., 2014b; Groves et al., 2016; Guardabassi et al., 2004). Frequent or intimate contact between companion animals and humans increases potential for transmission of resistant bacteria to humans, as readily transferable strains have been documented in several studies (Johnson et al., 2009).

MDR *E. coli* is increasing in frequency in both human and companion animal settings, including strains possessing extended-spectrum β-lactamases (ESBLs). Public health implications of ESBLs in animal isolates have mainly been considered in food-producing animals (Abraham et al., 2014a; Jahanbakhsh et al., 2016), but should also be extended to companion animals. Similarly, human-to-animal transmission is just as significant an issue for

strains that predominately infect humans, such as 025b:ST131 and O75:ST1193 (Platell et al., 2011; Platell et al., 2012).

Monitoring of ongoing emergence and dissemination of resistance is a critical component of management systems that aim to keep resistance at low levels (WHO, 2014). Many countries have established surveillance programs to understand the emergence and severity of resistance in major pathogenic bacteria in both and humans and animals (EFSA and ECDC, 2015). These activities enable the early detection of the acquisition and spread of antimicrobial resistance. In Australia, surveillance of human pathogens conducted by the Australian Group of Antimicrobial Resistance (AGAR) shows an increasing trend in the frequency of resistance amongst *E. coli,* especially involving the major classes of antibiotics used for treatment in humans (Turnidge et al., 2014). Recently, AMR in clinical *E. coli* isolated from food-producing animals in Australia has been reported (Abraham et al., 2015). However, current frequency of AMR in companion animals (defined here as dogs, cats and horses) remains unknown although the presence of multidrug resistance in pathogenic *E. coli* from dogs has been reported in some localised studies (Guo et al., 2013; Guo et al., 2015). Therefore, as part of the first Australia-wide survey of antimicrobial resistance in pathogens from animals, we generated antimicrobial susceptibility data for putative pathogenic *E. coli* isolated from companion animals with clinical illness. We used epidemiological cut-off values (ECOFFs) recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to define wild type populations and the Clinical Laboratory Standards Institute (CLSI) veterinary and human breakpoints to define the frequency of AMR from both a veterinary and public health standpoint. We then used these data to determine multiple resistance patterns and identify potential risk factors for UTI by strains defined as MDR *E. coli* in dogs and cats.

Material and Methods

Isolates collections and identifications

Clinical *E. coli* isolates were obtained from 22 government, private and university veterinary diagnostic laboratories throughout Australia from January 2013-January 2014. Isolates were collected as part of the first nation-wide survey of antimicrobial resistance in animals in Australia (Abraham et al., 2015; Saputra et al., 2017). These isolates were accompanied by clinical history and a laboratory submission report with the details of the client having been de-identified. Attributes of each case (age, gender, infection site, prior antimicrobial treatment) were extracted from clinical histories to use in the study of risk factors for MDR *E. coli* infection. Although all isolates had been identified as *E. coli* by submitting laboratories, this was repeated by detection of the *E. coli*-specific universal stress protein A (*uspA*) gene (Chen and Driffiths, 1998), performing the indole spot test (BactiDrop™ Spot Indole, Thermofisher Scientific) as well as observing colony morphology pattern on Sheep Blood Agar (SBA) prior to cryopreservation of isolates in 20% glycerol broth.

Antimicrobial susceptibility testing (AST)

Minimum inhibitory concentrations (MICs) were determined using broth microdilution performed in 96 well plates by the method of CLSI (CLSI, 2013). Susceptibility to a total of 15 antimicrobial agents from 9 antimicrobial categories was assessed, including: amikacin (AMK) and gentamicin (GNT), from the aminoglycosides (AMG); amoxicillin-clavulanic acid (AMC) from β-lactam/β-lactamase inhibitor combinations (BLI); ampicillin (AMP) from the β-lactam (BLA) group; imipenem (IMP) from carbapenems (CRB), cephalothin (CEF), from the 1st generation cephalosporins (1GC); cefoxitin (FOX) a 2nd generation cephamycin (2GC); cefovecin (CVN), ceftiofur (CTR) and ceftriaxone (CRO), representing 3rd generation cephalosporins (3GC); ciprofloxacin (CIP), enrofloxacin (ENR), marbofloxacin (MRB) and orbifloxacin (ORB), representing fluoroquinolones (FQN); and tetracycline (TET). The

antimicrobials were all obtained from Sigma Aldrich except for CVN and CTR, which were obtained from Zoetis (Australia). Quality controls were monitored on every MIC testing by using *E. coli* ATCC 25922 as a control strain.

Interpretation of antimicrobial susceptibility profiles

Each isolate was designated as non-wild type (non-WT) to each antimicrobial based on ECOFFs published by the EUCAST or assessment using actual MIC distribution with ECOFFinder (Turnidge et al., 2006). This interpretation aimed to assess "microbiological resistance" for detection emerging resistance in the community. Further, to assess "clinical resistance" (to advise on therapy in the patients) and public health significance, MIC results were also interpreted based on both veterinary and human clinical breakpoints according to CLSI VET01S (CLSI, 2015a) and CLSI M100-S25 (CLSI, 2015b) as listed in Table 1. Additionally, AGAR have used the term "non-susceptibility" to include both intermediate and resistance isolates interpreted based on CLSI clinical breakpoints (Turnidge et al., 2014). Note that non-WT isolates as defined by ECOFFs may or may not respond clinically to antimicrobial treatment while resistant isolates defined by CLSI clinical breakpoints are not likely to respond clinically to the usually achievable concentrations of the agent with normal dosage regiments (CLSI, 2015a; Silley, 2012).

The frequency of non-WT, non-susceptibility or resistance for each antimicrobial was described as rare: <0.1 %; very low: 0.1 % to 1.0 %; low: >1 % to 10.0 %; moderate: >10.0 % to 20.0 %; high: >20.0 % to 50.0 %; very high: >50.0 % to 70.0 %; and extremely high: >70.0 %; according to the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) (EFSA and ECDC, 2015). Resistance profiles were generated using CLSI clinical breakpoints which include intermediately resistant and resistant isolates (i.e. non-susceptible isolates), and this interpretation was retained in order to classify isolates as MDR for the risk factors study. Given the differences in ampicillin, amoxicillin-

clavulanate and 1st generation cephalosporin clinical breakpoints established on the basis of pharmacokinetics for skin and soft tissue vs urinary tract infection sites in companion animals, strains were defined as MDR if they showed resistance to at least one drug in three or more antimicrobial classes using human clinical breakpoints, as previously described (Magiorakos et al., 2012).

Detection of β-lactamase genes

Following phenotypic detection of non-WT isolates to either carbapenems or 3rd cephalosporins/cephamycins (MICs for imipenem ≥1 µg/mL; ceftriaxone ≥0.25 µg/mL; ceftiofur ≥2 µg/mL; cefovecin ≥4 µg/mL and/or cefoxitin ≥16 µg/mL), isolates were screened by PCR for the major groups of genes encoding β-lactamases including *bla*$_{IMP}$, *bla*$_{CTX-M}$, *bla*$_{CMY-2}$ and *bla*$_{TEM}$ (Abraham et al., 2015; Dallenne et al., 2010).

Statistical analysis and risk factors study for multidrug-resistant *E. coli*

All statistical analyses were performed using Stata 14.0 (Stata Corp., College Station, TX, USA) (Barlow et al., 2015). MIC distributions were produced per animal category with corresponding 95% confidence intervals (CIs) for the proportion of isolates showing non-susceptibility to each antimicrobial. For the assessment of risk factors, only *E. coli* isolates originating from UTI in dogs (n=366) and cats (n=306) were analysed for factors contributing to isolation of a MDR *E. coli* strain since there were too few isolates from SSTI and from horses to be included. AMC was excluded during risk factor analysis for the cat isolates because the veterinary clinical breakpoint is actually lower than the wild-type ECOFFs. The proportion of *E. coli* isolates expressing multidrug resistance was derived for combinations of possible risk factors, including animal (dogs and cats), age group (<2, 2-10 years and >10 years), sex (male and female), chronic and recurrent diseases (yes/no), concurrent diseases, such as diabetes, kidney and immune disease (yes/no), and the source case having received prior antimicrobial treatment (yes/no). Univariate and multivariate analyses based on logistic regression were used

to assess the effect of various factors on the proportion of resistant vs susceptible isolates. MDR *E. coli* isolates (interpreted by human clinical breakpoints) were used as the outcome. In multivariate logistic regression, variables with a significant result from univariate analysis ($P<0.2$) were included in the model to obtain a main effects model. Two way interactions arising from the main effects were retained where significant ($p<0.05$).

Results

Isolate MIC distributions according to host species

Overall, 883 isolates were confirmed as *E. coli* using the *uspA* PCR and spot indole test. Among these clinical *E. coli*, 514 isolates (58.2%) originated from dogs, 341 isolates (38.6%) from cats, and only 28 isolates (3.2%) from horses. Isolates were obtained from all mainland states of Australia, with over 45% of the isolates originating from New South Wales (NSW) (Table 2). The distribution of MIC values and overall frequency of non-WT and resistance based on ECOFFs, veterinary and human clinical breakpoints are presented for all antimicrobials in Table 3.

Frequency of resistance among clinical *E. coli* according to CLSI breakpoints

Most notably, resistance to carbapenems (imipenem) was not detected in any isolates (0/883, 0%). Among the dog isolates (n=514), the highest frequency of resistance based on veterinary breakpoints was to CEF (n=486, 94.6%) followed by AMC (n=234, 45.5%) and AMP (n=227, 44.2%). However, if human clinical breakpoints were used, the percentage of isolates exhibiting resistance to CEF, AMC and AMP was reduced to 21.6% (n=111), 27.8% (n=143) and 11.3% (n=58), respectively. The frequency of resistance to 3GC used in veterinary (CTR and CVN) and human medicine (CRO) was moderate with frequencies of 10.1% (n=52), 10.9% (n=56) and 10.3% (n=53), respectively. Resistance frequency to FQN was slightly lower

at 9.1% (n=47) for CIP and ENR and 9.3% (n=51) for MRB and ORB. Low levels of resistance to AMK (n=8, 1.6%) and GEN (n=25, 4.9%) were also recorded.

Among cat isolates (n=341), all isolates (100%) would be classified as resistant to AMC based on recently approved veterinary specific breakpoints, but if human clinical breakpoints were used, the frequency of resistance was markedly lower (n=26, 7.6%). The frequency of resistance to 3GC (n=29, 5.6% for CTR and CRO; n=33, 6.5% for CVN), FQN (n=11, 3.2%) and GEN (n=6, 1.8%) was significantly lower among cat isolates compared to dog isolates ($P<0.05$).

With such a low number of isolates obtained from horses in the nation-wide study (n= 28), confidence intervals were large, however, the frequency of resistance to CIA was generally higher than observed for cat and dog isolates including aminoglycosides (AMK n=3, 10.7%; GEN n=11, 39.3%), 3GC (n=9, 32.1%), and FQN (n=7, 25%) based on veterinary specific breakpoints.

For the remainder of the antimicrobials, interpretation of MIC results using either ECOFFs, or human and veterinary specific breakpoints did not differ significantly.

Frequency of resistance and non-WT among clinical *E. coli* according to site of infection

Generally, the proportion of isolates showing resistance to each antimicrobial was lower among *E. coli* obtained from canine UTI (n=392, 76.3%) compared to SSTI (n=122, 23.7%) but did not reach significance. However, based on EUCAST ECOFFs, among dog isolates, a significantly higher proportion of non-WT isolates from SSTI was observed for CEF, 3GC and TET ($P<0.05$) compared to UTI. Among cat isolates, the proportion of non-WT isolates from SSTI (n=21, 6.2%) was significantly higher compared to UTI (n=320, 93.8%) in FQN and TET ($P<0.05$) (Table 4).

Phenotypic resistance patterns derived from veterinary and human clinical breakpoints

Overall, 541 isolates (61.3%) were susceptible to all antimicrobial agents, interpreted according to CLSI clinical breakpoints. A moderate proportion of dog isolates (n=93, 18.1%) and cat isolates (n=40, 11.7%) and a high proportion of horse isolates (n=12, 42.9%) were classified as MDR (Table 5). The most common resistance profile was BLA-BLI detected in 71 isolates while the most common MDR profile was BLA-BLI-2GC-3GC observed in 20 dog isolates and 8 cat isolates. A total 13 isolates, including 10 isolates from dogs, two isolates from cats and one isolate from a horse shared a MDR profile to seven antimicrobial classes (AMG-BLA-BLI-2GC-3GC-FQN-TET).

Presence of β-lactamase genes

Corresponding with the interpretation of imipenem susceptibilities, *bla*$_{IMP}$ was not detected in any isolate with an MIC >0.5 µg/mL. Among 112 isolates that satisfied the selection criteria and were screened for β-lactamase genes, *bla*$_{CMY-2}$ was dominant, having been detected in 58 isolates (51.8%), followed by *bla*$_{TEM}$ (n=48; 42.9%) and *bla*$_{CTX-M}$ (n=23; 20.5%). A total of 28 isolates (25%) contained both *bla*$_{CMY-2}$ and *bla*$_{TEM}$; 13 isolates (11.6%) contained *bla*$_{CTX-M}$ and *bla*$_{TEM}$ and four isolates (3.6%) contained *bla*$_{CMY-2}$ and *bla*$_{CTX-M}$. A total of 27 isolates that satisfied the β-lactamase screening criteria were negative for all resistance genes tested.

Risk factors associated with MDR *E. coli* in urinary tract infections

The epidemiological data and univariate analysis for the risk factors study are shown in Table 6. *E. coli* urinary tract infections were more common in female dogs (n=264, 72.1%) and cats (n=222, 72.5%). There was a significant difference in the proportion of *E. coli* isolates expressing multidrug resistance in two potential risk factor groups, those reported to have chronic infection and prior antimicrobial treatment. A significant association of MDR *E. coli* with age groups including 2-10 years and >10 years, concurrent disease and prior antimicrobial

treatment was observed in dog isolates only. In multivariate analysis, chronicity of UTI was the factor that remained significantly associated with MDR *E. coli* isolates in dog (OR 4.3; 95%CI 2.1-9; P<0.0001) and cat isolates (OR 3; 95%CI 1.2-7.4; P<0.02). However, after inclusion of interaction terms, dogs and cats with chronic UTI that had been previously treated with antimicrobials were eight times and six times more likely to be infected with MDR *E. coli* compared to dogs and cats with acute UTI and/or no history of prior antimicrobial treatment, respectively (Table 7).

Discussion

In this study, we report the findings of the first nation-wide survey of antimicrobial resistance in clinical *E. coli* isolated from dogs, cats and horses in Australia. Major findings from this study are: 1) the overall frequency of resistance to CIA registered for veterinary use in dog isolates was classified as low for FQN (9.1%-9.3%) and moderate for 3GC (10.1%-10.9%), whilst it was low among cat isolates (FQN 3.2%-5%; 3GC 5.6%-6.5%); (2) among dog and cat isolates, resistance to CIA registered in human medicine was either not reported (carbapenems) or low (amikacin; 1.5%-1.6%) ; and 3) chronic and/or recurrent disease and prior antimicrobial treatments were the main risk factors for the isolation of MDR *E. coli* from UTI in both dogs and cats.

To date, information related to the frequency of resistance among companion animal clinical *E. coli* to CIA classes in Australia is very limited. Since large scale surveys of companion animals for AMR have not been performed previously, it is uncertain over what timescale resistance has evolved. Ideally, resistance to CIA would be detected early and this may only happen if such surveys are performed at regular time intervals. In contrast to pathogenic *E. coli* in companion animals, more information is available for commensal *E. coli* (Barlow et al., 2015) and for some pathogenic *E. coli* (Abraham et al., 2015) from Australian livestock, but once again, these studies are very recent and require ongoing surveillance to

determine trends. For example, the frequency of resistance to CIA in both commensal and pathogenic *E. coli* from livestock is negligible in Australia, possibly because of strict regulation of antimicrobials in food animals and/or animal management systems that do not favour bacterial disease (Barlow et al., 2015; Cheng et al., 2012). By comparison, treatment of infections in companion animals closely mirrors human medicine, with veterinarians able to prescribe 3GC and FQN registered for use in dogs and cats, largely without any restrictions other than the availability of prudent use guidelines (AIDAP, 2016). Furthermore, companion animal veterinarians in Australia do have access to off-label use of human formulations (e.g. amikacin and carbapenems), and although the numbers of animals actually treated with these drug classes is thought to be very limited (Gibson et al., 2008), antimicrobial stewardship programmes governing use are still in their infancy (Abraham et al., 2014b).

International comparison of the results obtained in this study are difficult to interpret due to differences in study design, drugs tested, breakpoint determination, and temporal or geographic variation. In comparison with an analogous study in the USA conducted over a five year period (2008-2013; dog isolates n=2390; cat isolates n=780) using CLSI breakpoints (Thungrat et al., 2015), a moderately high level of resistance was observed among dog isolates to FQN (CIP 10.7%; ENR 11.7%) and a low level among cat isolates (CIP 5.3%; ENR 5.9%). In our Australian study, when we applied the same clinical breakpoints to our data, we observed a similar though slightly lower level of resistance to CIP and ENR among dog (both 9.1%) and cat isolates (both 3.2%). Resistance to GEN in the Australian study was also lower (dog isolates 4.9%, cat isolates 1.8%) compared to the US study (dog isolates 8.5%; cat isolates 5.9%).

Additionally, using cefotaxime and cefpodoxime as representatives of the 3GC class, the proportion of resistant isolates in the US study ranged from 13.4%-13.9% among dog isolates and 7.6%-9.5% among cat isolates, which is analogous to our Australian study (dog isolates 11.5%-12.6%, cat isolates 6-7%-8.2%). However, in contrast to these two studies, a

surveillance study conducted in Sweden among clinical *E. coli* isolated from UTI in dogs (n=943) and cats (n=461) in 2014, showed a very low to low frequency of non-WT isolates for the critically important antimicrobials such as 3GC (cefotaxime: dogs 0.7%, cats 1%) and FQN (dogs 7%; cats 7%) (Swedres-Svarm, 2015) when ECOFFs were used. In our study, low and moderately high proportions of isolates from UTI cases were classified as non-WT for both 3GC (CRO: dogs 10.2%, cats 8.1%) and FQN (dogs 12%, cats 4.7%) using the same breakpoints.

Comparison of AMR among human isolates from Australia obtained over a similar time scale is difficult, as in 2013, AGAR switched to surveying AMR in blood sepsis isolates only, whereas in previous surveys they alternated each year between hospital and community sourced isolates. Nevertheless, comparison with data from the AGAR 2012 report, confirmed that the proportion of non-susceptibility for some CIA was generally higher among dog isolates and lower among cat isolates. The non-susceptibility rate of CRO and CIP in *E. coli* isolates causing UTI in humans (n= 2025) was 4.2% and 6.9%, respectively (Turnidge et al., 2014) while in our study, the proportion of non-susceptibility among dog isolates was 8.9% and 8.2%, respectively. Resistance to CRO in cat isolates was also slightly higher (5.3%) but much lower for CIP (2.8%) when compared to human isolates. By using the same definition, the proportion of multidrug resistance (non-susceptibility to three or more antimicrobial classes) among human isolates (n=1871) was 13.8%, while among dog isolates it was much higher (18.1%) and slightly lower among cat isolates (11.7%).

Although the frequency of multidrug resistance among horse *E. coli* isolates was much higher compared to dog and cat isolates, the sample size was extremely small compared to the total population of horses in Australia. Nevertheless, the fact that a number of equine isolates were resistant to amikacin, a critically important drug only registered for use in humans in Australia, raises some concerns that should be followed up with a further survey on larger

numbers of horses. However, a study in the UK recommended that WHO-designated and prioritised critically important antimicrobials should be discouraged from use as first-line therapies in horses, especially with the significant increase in resistance among clinical *E.coli* to these drug classes and reports of extended-spectrum β-lactamase (ESBL) genes being identified in equine isolates (Johns and Adams, 2015).

As reported previously, *bla*$_{\text{CMY-2}}$ and *bla*$_{\text{CTX-M}}$ genes are the most frequent AmpC β-lactamase and ESBL encoding genes identified in both human and veterinary medicine (Abraham et al., 2015; Sidjabat et al., 2014), and are often associated with *bla*$_{\text{TEM}}$ on MDR plasmids (Hordijk et al., 2013). In this study, *bla*$_{\text{CMY-2}}$ and *bla*$_{\text{CTX-M}}$ were collectively identified in 68.7% (77/112) of *E. coli* isolates with a 3GC and 38.4% (43/94) of isolates with FQN non-WT phenotype. A comparative genomics study is currently underway to confirm phylogenetic groups, multilocus sequence types, plasmids, and resistance genes in selected MDR *E. coli* isolates from this study.

Owing to the much larger number of dog and cat isolates, it was possible to identify potential risk factors for isolation of MDR *E. coli* from UTI, with the most significant factors being the presence of chronic and/or recurrent disease and prior use of antimicrobial agents. Although UTIs are positively associated with older aged and/or female dogs and cats (Thompson et al., 2011), we found that there was no significant correlation between these two variables and frequency of antimicrobial resistance. Another study identified that the proportion of MDR *E. coli* from dogs with complicated UTI was significantly higher (36%) compared to isolates from dogs with uncomplicated infections (21%) (Wong et al., 2015). Previous antimicrobial treatments also significantly influenced the likelihood of the *E. coli* isolate to exhibit a MDR phenotype, in particular resistance to 3GC and FQN (Leite-Martins et al., 2014). A study in cats also showed that prior antimicrobial treatment significantly influenced the risk of AMR while the type of infection did not reach significance (Hernandez

et al., 2014). Other factors in addition to predisposing disease condition and prior treatment with antimicrobials that could not be examined in the present study include number of previous hospitalizations and length of hospitalization which have both been associated with carriage of MDR extraintestinal pathogenic *E. coli* in dogs (Gibson et al., 2008).

This study has some limitations. One major factor is the current differences in interpretative criteria applied to AST results. Much higher rates of resistance were observed for some antimicrobials (AMP, AMC and CEF), according to the latest veterinary specific breakpoints (CLSI), particularly according to body site-specific breakpoints (SSTI compared to UTI breakpoints), which is in contrast with an interpretation based on EUCAST ECOFFs or human specific CLSI clinical breakpoints (Table 3). If only veterinary specific clinical breakpoints were applied, many SSTI isolates would be classified as resistant even though their ECOFFs indicate that they are wild-type *E. coli*. This is important from a PK/PD and antibiotic stewardship perspective, but it gives neither an indication of the isolate's propensity to carry β-lactamase genes nor its public health significance. Further, the estimates of frequency of resistance amongst horse isolates had very wide confidence limits owing to the small sample size. Future studies should therefore focus on achieving a sufficiently large collection of isolates from horses to increase the accuracy of frequency estimates beyond that obtained here. Caution must therefore be exercised in making any public health recommendations based on the resistance frequencies generated in this study using veterinary specific breakpoints. Despite these shortfalls, we are unaware of any collection of *E. coli* isolates that is as representative of the Australian population of dogs and cats, both in terms of size and geographic source.

Conclusion

In conclusion, using a combination of ECOFFs and CLSI clinical breakpoints, the first Australia-wide survey of antimicrobial resistance in pathogenic *E. coli* originating from companion animals identified no resistance to carbepenems and low to moderate levels of

resistance to other CIA (amikacin, 3GC and FQN) in cat and dog isolates. Pathogenic *E. coli* isolates from horses represented only a small fraction of the total and it is recommended this group of animals is more appropriately targeted in future surveys. Dogs and cats with chronic and/or recurrent UTI or that had previous antimicrobial treatment/s were at increased risk of yielding a MDR *E. coli* isolate on a urine culture and susceptibility test. To maintain or even lower these levels of resistance, it is crucial for all veterinarians and pet owners to reduce inappropriate antimicrobial use by following prudent use guidelines and reduce the potential for transmission by applying biosecurity, infection control and antimicrobial stewardship strategies in companion animal practice.

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Table 1. Determination of non-wild type (non-WT) organisms based on epidemiological cut-off values (ECOFFs) and resistant (R) organisms based on clinical breakpoints according to CLSI documents.

Antimicrobials	Code	Non-WT	R[1]	R[2]
Amikacin	AMK	≥16	≥64	≥16
Ampicillin	AMP	≥16	≥32	≥1 and ≥16[a]; ≥32[b,c]
Amoxicillin-clavulanate	AMC	≥32	≥32	≥1/0.5 and ≥16/8[a,b]; ≥32/16[c]
Cefoxitin	FOX	≥16	≥32	-
Ceftiofur	CTR	≥2	-	≥8
Ceftriaxone	CRO	≥0.25	≥4	-
Cefovecin	CVN	≥4	-	≥4
Cephalothin	CEF	≥32	≥32	≥8[a]: ≥32[b,c]
Ciprofloxacin	CIP	≥0.12	≥4	-
Enrofloxacin	ENR	≥0.25	-	≥4
Gentamicin	GEN	≥4	≥16	≥8[a,c]; ≥16[b]
Imipenem	IPM	≥2	≥4	≥4
Marbofloxacin	MRB	≥0.25	-	≥4
Orbifloxacin	ORB	≥1	-	≥8
Tetracycline	TET	≥4	≥16	≥16

[1]Clinical breakpoints were adapted from human interpretative criteria according to CLSI M100 S25. [2]Veterinary clinical breakpoints were adapted according to CLSI VET01S. [a]Breakpoints used for dog isolates; [b]breakpoints used for cat isolates; [c]breakpoints used for horse isolates. For dog isolates, an AMP breakpoint ≥1 µg/mL was used for SSTI and ≥16 µg/mL for UTI, an AMC breakpoint ≥1/0.5 µg/mL was used for SSTI and ≥16/8 µg/mL for UTI. An AMC breakpoint ≥1/0.5 µg/mL was used for both UTI and SSTI isolates from cats. A CEF resistance breakpoint ≥8 µg/mL was used for isolates from dogs and was adapted from veterinary breakpoints established for bovine mastitis. Proposed breakpoints for cefovecin were established in this study.

Table 2. Number of total *E. coli* isolated from clinical infections in dogs, cats and horses from five regions in Australia

Region	No. of isolates	%	Animal origin		
			Dog	Cat	Horse
New South Wales (NSW)	403	45.6	233	157	13
Queensland (QLD)	236	26.7	130	100	6
Victoria (VIC)	212	24.0	124	82	6
South Australia (SA)	25	2.8	21	2	2
Western Australia (WA)	7	0.8	6	0	1
Total	883	100	514	341	28

Table 3. MIC distribution and frequency of non-wild type (non-WT, based on ECOFFs) and resistant isolates (R, based on CLSI breakpoints) among *E. coli* obtained from dogs (n=514), cats (n=341) and horses (n=28) in Australia.

Antimicrobials	Animal	% non-WT	% R[1]	% R[2]	Percentage of isolates with indicate d MIC (µg/mL)													
					≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	≥128
Amikacin	Dog	1.6	0.2	1.6							0.2	7	42.4	40.1	8.8	1.4		0.2	
	Cat	1.5	0	1.5							0.3	5	45.2	41.1	7	1.5			
	Horse	10.7	0	10.7								3.6	25	46.4	14.3	10.7			
Ampicillin	Dog	28.8	27.8	44.2								1.4	15.6	46.7	7.6	1	0.2	0.4	27.2
	Cat	26.7	26.7	26.7								1.8	29	37	5.6			0.9	25.8
	Horse	50	50	50									7.1	42.9				3.6	46.4
Amoxicillin-clavulanate	Dog	11.3	11.3	45.5								0.4	1.6	14.6	53.1	19.1	2.7	8.6	
	Cat	7.6	7.6	100									2.6	25.5	47.8	16.4	2.3	5.3	
	Horse	17.9	17.9	7.8									3.6	10.7	50	17.9		17.9	
Cefoxitin	Dog	13	10.5	-								1	16.9	54.1	15	2.5	1.2	1.8	7.6
	Cat	9.1	5.9	-								2.1	32.8	44	12	3.2	1.2	1.8	2.9
	Horse	10.7	7.1	-								3.6	7.1	46.4	32.1	3.6			7.1
Ceftiofur	Dog	12.3	-	10.1				0.4	3.5	24.7	48.8	10.3	1.9	0.2		1.6	2.1	6.4	
	Cat	6.7	-	5.6				0.6	7.6	40.8	36.7	7.6	1.2			0.9	0.6	4.1	
	Horse	32.1	-	32.1				3.6		35.7	25	3.6						32.1	
Ceftriaxone	Dog	12.6	10.3	-			26.7	48.1	12.6	1.4	0.4	0.2	0.4		0.2	0.6	9.5		
	Cat	8.2	5.6	-			43.4	41.1	7.3	2.1		0.3	0.3			0.3	5.3		
	Horse	35.7	32.1	-			28.6	32.1	3.6	3.6							32.1		
Cefovecin	Dog	11.5	-	10.9					0.8	10.1	41.8	31.3	4.5	0.6	0.6		0.4	0.2	9.7
	Cat	7.3	-	6.5					3.8	19.4	46.6	19.1	3.8	0.9	0.6	0.3	0.3	0.6	4.7
	Horse	28.6	-	28.6					3.6	10.7	32.1	25						3.6	25
Cephalothin	Dog	21.6	21.6	94.6									1.2	4.3	34.2	38.7	8.9	0.2	12.5
	Cat	15	15	15									0.9	11.7	43.4	29	6.5	0.3	8.2
	Horse	39.3	39.3	39.3									7.1	3.6	32.1	17.9			39.3
Ciprofloxacin	Dog	10.5	9.1	-	30.7	49.6	6.2	0.6	2.3	0.8	0.2	0.4		1	8.2				
	Cat	3.8	3.2	-	44.9	44.6	4.7	0.9	1.2		0.6				3.2				
	Horse	25	25	-	35.7	35.7		3.6							25				
Enrofloxacin	Dog	12.8	-	9.1	0.2	10.5	55.1	20	1.4	1.2	2.1	0.2	0.2		9.1				
	Cat	5	-	3.2	0.9	24	56.9	11.7	1.5	0.3	1.2	0.3			3.2				
	Horse	28.6	-	25		7.1	46.4	17.9		3.6					25				
Gentamicin	Dog	4.9	4.5	4.9						5.3	50.6	35.2	4.1		0.4	0.6	3.5	0.4	
	Cat	2.6	1.8	1.8					0.6	2.6	49.3	39.3	5.6	0.9			1.5	0.3	
	Horse	39.3	39.3	39.3						3.6	17.9	39.3					25	14.3	

Imipenem	Dog	0	0	0				3.1	31.3	44.6	16.1	4.9							
	Cat	0	0	0				4.4	29.6	44.3	18.8	2.9							
	Horse	0	0	0				10.7	42.9	28.6	10.7	7.1							
Marbofloxacin	Dog	12.8	-	9.3	0.2	9.9	62.3	14.2	0.6	1.8	1.4		0.4	0.8	8.6				
	Cat	5	-	3.2		20.8	61.6	11.4	1.2	0.9	0.9				3.2				
	Horse	28.6	-	25		14.3	46.4	10.7		3.6					25				
Orbifloxacin	Dog	12.5	-	9.3		0.2	1	25.3	49.8	9.9	1.4	1.4	1.4	0.4	0.2	9.1			
	Cat	4.4	-	3.2		0.3	3.8	37.8	42.5	10	1.2	0.3	0.6	0.3		3.2			
	Horse	25	-	25				21.4	46.4	3.6	3.6					25			
Tetracycline	Dog	17.1	16.7	16.7					0.2	0.6	8.9	57.6	15.6	0.2	0.2	0.2	1	2.9	12.6
	Cat	11.7	10	10						0.6	21.1	60.7	5.9	1.5	0.3			1.5	8.5
	Horse	50	50	50						0	7.1	35.7	7.1				3.6	10.7	35.7

*Unshaded areas show the dilution range for each drug. Epidemiological cut-off values (ECOFFs) are indicated as vertical dotted lines. [1]Frequency of resistance according to human clinical breakpoints (CLSI M100 S25), indicated by double vertical solid lines. [2]Frequency of resistance based on veterinary clinical breakpoints (CLSI VET01S), indicated by vertical solid lines.

Table 4. Percentage of non-WT (based on ECOFFs) and resistance (based on CLSI clinical breakpoints) in *E. coli* obtained from dogs (n=514), cats (n=341) and horses (n=28) by site of infection.

Antimicrobial agents	Dogs				Cats				Horses			
	ECOFF		CLSI		ECOFF		CLSI		ECOFF		CLSI	
	UTI n=392	SSTI n=122	UTI n=392	SSTI n=122	UTI n=320	SSTI n=21	UTI n=320	SSTI n=21	UTI n=4	SSTI n=24	UTI n=4	SSTI n=24
Amikacin	1.8	0.8	1.8	0.8	1.2	4.8	1.2	4.8	0	12.5	0	12.5
Ampicillin	26.7	35.2	26.8	100	26.3	31.8	26.3	33.3	0	58.3	0	58.3
Amoxicillin-clavulanate	10.2	14.6	28.6	100	7.8	4.8	100	100	0	20.8	0	20.8
Cefoxitin	12.2	15.6	9.2	14.8	9	9.5	5.6	9.5	0	41.7	0	41.7
Ceftiofur	10.2[a]	18.9	8.7	14.8	6.6	9.5	5.3	9.5	0	37.5	0	37.5
Ceftriaxone	10.2[a]	20.5	8.9	14.8	8.1	9.5	5.3	9.5	0	41.6	0	41.6
Cefovecin	9.9[a]	17.2	9.2	16.4	7.2	9.5	6.3	9.5	0	33.3	0	33.3
Cephalothin	11[a]	18	95.4	92.6	8.6	9.5	15	14.3	0	45.8	0	45.8

Ciprofloxacin	9.4	13.9	8.2	12.3	3.1[b]	14.3	2.8	9.5	0	29.2	0	29.2
Enrofloxacin	12	15.6	8.2	12.3	4.7[b]	14.3	2.8	9.5	0	33.3	0	33.3
Gentamicin	4.3	6.6	4.3	6.6	2.5	4.8	1.6	4.8	0	45.8	0	45.8
Imipenem	0	0	0	0	0	0	0	0	0	0	0	0
Marbofloxacin	11.7	16.4	8.4	12.3	4.4[b]	14.3	2.8	9.5	0	33.3	0	33.3
Orbifloxacin	11.7	14.6	8.4	12.3	3.8[b]	14.3	2.8	9.5	0	29.2	0	29.2
Tetracycline	14.3[a]	26.2	16.3	25.4	10.9[b]	23.8	9.1	23.8	25	54.2	25	54.2

*Clinical breakpoints for FOX, CRO and CIP were adapted from CLSI M100 S25. [a] A significantly lower proportion (P<0.05) of non-WT was observed in dog isolates from UTI compared to SSTI (CTR, CRO, CVN, CEF and TET). [b] A significantly lower proportion (P<0.05) of non-WT was observed in cat isolates from UTI compared to SSTI (CIP, ENR, MRB, ORB and TET) .

Table 5. The most prevalent resistance profile per antimicrobial category found in clinical *E. coli* isolated from dogs (n=514), cats (n=341) and horses (n=28) in Australia based on CLSI human clinical breakpoint data.

No. antimicrobial category	No. of isolates (%)	Resistance pattern (no. of isolates)		
		Dogs	Cats	Horses
All susceptible	541 (61.3)	302	227	12
1	83 (9.4)	BLI (29)	BLA (15)	TET (2)
2	95 (10.8)	BLA-BLI (38)	BLA-BLI (32)	BLA-BLI (1)
3	42 (4.8)	BLA-BLI-TET (14)	BLA-BLI-TET (9)	BLA-BLI-2GC (1)
4	43 (4.9)	BLA-BLI-2GC-3GC (20)	BLA-BLI-2GC-3GC (8)	AMG-BLA-BLI-TET (1)
5	26 (2.9)	AMG-BLA-BLI-FQN-TET (5)	AMG-BLA-BLI-FQN-TET (5)	AMG-BLA-BLI-FQN-TET (2)
6	21 (2.4)	AMG-BLA-BLI-3GC-FQN-TET (4)	AMG-BLA-BLI-2GC-3GC-FQN (2)	AMG-BLA-BLI-3GC-FQN-TET (4)
7	13 (1.5)	AMG-BLA-BLI-2GC-3GC-FQN-TET (10)	AMG-BLA-BLI-2GC-3GC-FQN-TET (2)	AMG-BLA-BLI-2GC-3GC-FQN-TET (1)
Non-MDR	738 (83.6)	420	301	16
MDR	145 (16.4)	93	40	12

*Antimicrobial categories included: aminoglycosides, AMG (AMK and GEN); penicillin, BLA (AMP), beta-lactam/inhibitors, BLI (AMC), 2nd generation cephalosporins, 2GC (FOX); 3rd generation cephalosporins, 3GC (CVN, CTR, CRO), fluoroquinolones, FQN (CIP, ENR, MRB and ORB); and tetracycline, TET.

Table 6. Univariate analysis of risk-factor variables from MDR *E. coli* isolated from UTI in dogs (n=366) and cats (n=306). Odds ratios define the risk of isolates being classified as MDR.

Risk factor	Dogs					Cats				
	n	%MDR	OR	P value	95% CI	n	%MDR	OR	P value	95% CI
Age in years										
<2	45	4.4	Ref			9	11.1	Ref		
2-10	154	18.9	5	0.033	1.1-21.8	78	9	0.8	0.834	0.1-7.3
<10	167	17.4	4.5	0.045	1-19.7	219	12.3	1.1	0.913	1.1-9.3
Sex										
Male	102	16.7	Ref			84	13.1	Ref		
Female	264	16.3	1	0.93	0.5-1.8	222	10.8	0.8	0.576	0.4-1.7
Chronic and recurrent diseases										
No	317	12.6	Ref			276	10.5	Ref		
Yes	49	40.8	4.8	<0.0001	2.5-9.2	30	20	3.2	0.011	0.8-5.6
Concurrent diseases										
No	323	14.6	Ref			268	11.6	Ref		
Yes	43	30.2	2.5	0.011	1-3-5.2	38	10.5	0.9	0.85	0.3-2.7
Prior antimicrobial treatment										
No	301	13.6	Ref			275	10.5	Ref		
Yes	65	29.2	2.6	0.003	1.4-5	31	19.4	2	0.151	0.8-5.4

Table 7. Odds ratios showing the likelihood of *E. coli* isolates obtained from UTI in dogs and cats being MDR using chronicity of infection and exposure of the host to prior antimicrobial treatment as the variables.

Prior antimicrobial treatment	Dogs				Cats			
	Chronic		Non chronic		Chronic		Non chronic	
	n	OR; 95%CI	n	OR; 95%CI	n	OR; 95%CI	n	OR; 95%CI
Yes	22	8.3; 3.2-21.3	43	1.8; 0.7-4.3	6	4.8; 0.8-28	25	2; 0.6-6.5
No	27	4.2; 1.7-10.5	274	Ref.	25	23.1; 1.1-8.7	250	Ref.