Prevalence of bacteria and changes in trends in antimicrobial resistance of *Escherichia coli* isolated from positive canine urinary samples from an Australian referral hospital over a 5-year period (2013–2017)

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ABSTRACT

Lower urinary tract disease is common in dogs with approximately 14% developing a bacterial lower urinary tract infection (UTI) during their lifetime. Empirical antimicrobials are often prescribed while waiting urine culture and susceptibility results. Regional knowledge of bacterial prevalence and antimicrobial resistance patterns aids veterinarians in antimicrobial choice. This study aimed to identify the prevalence of uropathogens in canine urine tract isolates and to assess for changes in antimicrobial resistance of *Escherichia coli* (*E. coli*) over a 5-year study period at a large multidisciplinary private referral hospital in Australia (January 2013–December 2017). The proportion of resistant isolates was compared across 5 years (Fisher's exact test and Cochran Armitage test for trend) for select antimicrobials towards *E. coli*. A total of 246 positive urine cultures were included. *E. coli* was the most prevalent uropathogen at 64%, followed by *Proteus* sp., *Staphylococcus* sp. and *Enterococcus* sp., respectively (9%, 8% and 7%). *E. coli* was most commonly resistant to amoxicillin at 41%. There was no statistically significant difference, nor trend, in resistance of *E. coli* isolates towards the selected antimicrobials over the 5 years. Resistance towards trimethoprim–sulfonamide was lower at 15%. This information will aid local veterinarians in selecting empirical antimicrobials pending culture results for the treatment of UTIs in dogs.

INTRODUCTION

Lower urinary tract disease is common in dogs with approximately 14% of dogs developing a bacterial urinary tract infection (UTI) during their lifetime.[1–6] The recommended method for the diagnosis of a UTI is the combination of consistent clinical signs and urinalysis, culture and susceptibility results from a sample collected by cystocentesis.[1] *Escherichia coli* is the most frequently isolated uropathogen in dogs, with prevalence between 35% and 70%.[1–3 5] Other common urinary bacterial isolates include *Staphylococcus* sp., other *Enterobacteriaceae* (*Proteus* sp., *Klebsiella* sp.), *Enterococcus* sp. and *Streptococcus* sp. The majority of UTIs are classified as sporadic bacterial infections that occur as a single episode in the absence of any underlying disease and resolve with appropriate antimicrobial therapy.[1]

To minimise treatment failure and the development of antimicrobial resistance, selection of antimicrobials should be based on results of in vitro susceptibility testing.[1 2 4 7] Pending culture and susceptibility results, and empirical antimicrobial therapy are often instituted. Prudent empirical antimicrobial choice requires knowledge of the prevalence of bacterial pathogens within the facility or region and the likely susceptibility patterns.[1 3 6]

The International Society for Companion Animal Infectious Disease Committee (ISCAID) and other national veterinary bodies have formulated guidelines with recommend first-line antimicrobials for UTIs to promote rational antimicrobial prescribing practices.[1 8 9] Current ISCAID recommendations for empirical treatment for bacterial UTIs in dogs include amoxicillin and trimethoprim–sulfonamide (TMS).[1] These differ from the Australasian Infectious Diseases Advisory Panel (AIDAP), with recommendations for amoxicillin and amoxicillin–clavulanic acid (AMC) as first-line treatment.[9]

Empirical antimicrobial administration may select for multidrug-resistant organisms and disturb normal flora.[3 6 10–13] To detect emerging antimicrobial resistance, it is recommended that the prevalence of bacterial pathogens and changes in resistance patterns are monitored.[1 3 14 15] This form of antimicrobial stewardship is frequently used in medical

facilities to inform empirical antimicrobial use. A change in empirical treatment guidelines is recommended when there is a 10% increase in resistance within the population from baseline.[1 3]

Bacterial prevalence and antimicrobial resistance can vary between medical facilities and also by geographical regions. There are several longitudinal studies in the veterinary literature describing the prevalence of canine uropathogens and antimicrobial resistance patterns in North America, New Zealand and Europe.[3 5 6] Data from Australian institutions are lacking.

The aims of this study were to identify (i) the prevalence of canine uropathogens and (ii) changes in the antimicrobial resistance pattern of the most prevalent uropathogen over a 5-year period at a large, multidisciplinary private referral hospital (2013–2017).

MATERIALS AND METHODS

Positive canine urine culture results submitted from our facility were obtained from three external veterinary clinical pathology laboratories between January 2013 and December 2017 inclusive. These submissions were cross referenced with a retrospective search of electronic medical records to ensure all positive canine urine culture results were included.

Data retrieved included date of collection, collection method (submission form or clinical records), microorganism(s) isolated and antimicrobial susceptibility results. Only samples collected via cystocentesis were included. To limit the study to resistance patterns of non-recurrent UTIs, only the initial positive urine culture from each patient was included. Where multiple organisms were cultured, the isolate with the heaviest growth was included in the analysis.

Microbiological methods

Samples were determined to be culture positive when one or more microorganism was detected following aerobic culture. Isolates were evaluated at 24 hours from plating, and again between 72 and 120 hours if there was no initial growth. All laboratories used the Kirby-Bauer disk-diffusion method to determine antimicrobial susceptibility patterns during the study period. Isolates were classified as susceptible, intermediate or resistant based on published serum breakpoints as per the relevant Clinical Laboratory Standard Institute Guidelines.[16] Intermediate and resistant isolates were reclassified to a single resistant category based on published clinical standard guidelines.

Each laboratory tested a panel of antimicrobials that included AMC, ampicillin/amoxicillin, TMS, doxycycline, a cephalosporin (first, second or third generation) and enrofloxacin. Additional antimicrobials were included based on the discretion of the veterinary microbiologist. An extended antimicrobial panel was performed either by request or when multidrug resistance patterns were identified (resistance to greater than three antibacterial classes).

Statistical methods

Data were analysed using R V.3.5.1 (R Development Core Team 2018; R Foundation for Statistical Computing, Vienna, Austria). The proportion of susceptible isolates was compared across the 5 years using Fisher's exact test for absolute differences and a Cochran Armitage test to assess for a trend in susceptibility patterns across the 5-year study period for select antimicrobials for *E. coli*. SEs and a CI were calculated to provide a measure of the precision of the prevalence estimate. For all comparisons, a p value <0.05 was considered statistically significant.

Table 1 Prevalence of bacterial species isolated from 246 dogs with bacteriuria between January 2013 and December 2017 at a private referral hospital in New South Wales, Australia

	Positive isolates	Prevalence (%)	SE (%)	95% CI
Gram-negative bacteria				
Escherichia coli	158	64	3.1	58.1 to 70
Other *Enterobacteriaceae*	38	15		
Proteus species	22	9	1.8	6.0 to 13.2
Klebsiella species	11	4	1.3	2.5 to 7.8
Enterobacter species	3	1	0.7	0.4 to 3.5
Serratia species	2	1	0.6	0.2 to 2.9
Pseudomonas aeruginosa	7	3	11.0	1.4 to 5.8
Pasteurella species	2	1	0.6	0.2 to 2.9
Gram-positive bacteria				
Staphylococcus species	19	8	1.7	5.0 to 11.7
Enterococcus species	18	7	1.7	4.7 to 11.3
Mycoplasma species	3	1	0.7	0.4 to 3.5
Corynebacterium species	1	<1	0.4	0.1 to 2.3

Table 2 The antimicrobial resistance of 158 canine *Escherichia coli* urinary isolates to the four most routinely evaluated antimicrobials between January 2013 and December 2017

| Antibiotic | 2013 | | 2014 | | 2015 | | 2016 | | 2017 | | Total period |
	Tested	% resistant (n)	Tested	% resistant (n)	Tested	% resistant (n)	Tested	% resistant (n)	Tested	% resistant (n)	% resistant (n)
AMC	33	21 (7)	27	18 (5)	48	25 (12)	30	20 (6)	20	25 (5)	24 (35)
Amoxicillin	33	48 (16)	26	23 (6)	46	43 (20)	30	46 (14)	20	30 (6)	41 (62)
Enrofloxacin	33	1 (3)	27	7 (2)	45	7 (2)	27	7 (2)	18	11 (2)	8 (10)
TMS	33	15 (5)	27	18 (5)	44	8 (4)	30	16 (5)	20	20 (4)	15 (23)

P values >0.05 for absolute differences in proportion susceptible (Fisher's exact test) and trend over time (Cochrane Armitage test) for all antimicrobials evaluated.
AMC, amoxicillin–clavulanate; TMS, trimethoprim–sulfonamide.

RESULTS

In all, 507 positive urine cultures were identified (January 2013–December 2017). When including samples collected only by cystocentesis and excluding those of repeat submissions (n=20) and cultures other than bacterial pathogens (n=2), a study pool of 246 cases was determined.

From the 246 positive cultures, 243 were single bacterial isolates (99%) and three were dual isolates (1%). *E. coli* accounted for 158 (64%) of all isolates. Other frequently isolated bacteria included *Proteus* sp. (22; 9%), *Staphylococcus* sp. (19; 8%), *Enterococcus* sp. (18; 7%), *Klebsiella* sp. (11; 4%) and *Pseudomonas* sp. (7; 3%), respectively (table 1).

The most commonly tested antimicrobials (>100 *E. coli* isolates tested) included AMC (158 isolates), TMS (158), amoxicillin (155) and enrofloxacin (149).

E. coli was most commonly resistant to amoxicillin (resistance 41%). *E. coli* resistance towards the selected antimicrobials AMC, enrofloxacin and TMS was 22%, 15% and 8%, respectively (table 2). There was no statistically significant difference or trend to difference in the resistance of *E. coli* isolates towards the selected antimicrobials between 2013 and 2017. The percentage of *E. coli* resistant to AMC and enrofloxacin increased during the study period; however, these changes were not statistically significant.

DISCUSSION

This study confirms that *E. coli* is the most common uropathogen in dogs and describes contemporary antimicrobial resistance patterns. There was no statistically significant change in antimicrobial resistance over 5 years in this referral veterinary hospital. *E. coli* isolates displayed the highest level of resistance towards amoxicillin, a first-line ISCAID recommended antimicrobial for uncomplicated canine UTIs, highlighting the need for appropriate antimicrobial stewardship in the face of the global trend for increasing antimicrobial resistance.[1 9 17]

E. coli was the most frequently isolated uropathogen in this study with a prevalence similar to that reported elsewhere (35%–70%).[3 6 13 15 18 19] The prevalence of *Staphylococcus* sp., *Proteus* sp. and *Enterococcus* sp. in this population is also in accordance with previous reports.[6]

The variation in the prevalence of canine *E. coli* urinary isolates reported in the literature may be due to differences in hospital demographics or geographical location. For example, the prevalence of *E. coli* in this study is similar to that reported in two North American referral institutions (51% and 53%, respectively) but is higher than a veterinary referral laboratory from New Zealand at 35%, which included samples from both first opinion and referral practices.[3 5 18] It is possible that more patients at referral hospitals have been treated with antimicrobials prior to referral which influenced the uropathogens identified and their resistance patterns. Geographical variability has been documented in a recent pan

European antimicrobial surveillance programme of canine uropathogens, where the prevalence of *E. coli* ranged from 35% to 70%.[15] Whether these differences reflect true geographical variability in uropathogens or are merely the consequence of small sample size and non-standardised study design is unknown. Further epidemiological research comparing the prevalence in our institution to the region, similarly across different countries, would aid in further describing the prevalence of canine urinary *E. coli* isolates.

In this study, *E. coli* isolates were most likely to be resistant to amoxicillin when compared with other antimicrobials. This result is similar to some previous studies in dogs and in people in our region, but differs from other studies where *E. coli* resistance to amoxicillin was lower.[5 20] Possible explanations for the difference in antimicrobial resistance include case selection from referral hospitals and geographical variability in antimicrobial prescribing guidelines. Geographical variability in resistance of urinary *E. coli* towards antimicrobials has been demonstrated in feline studies.[10 13 21 22] This variability emphasises the need for geographical specific prevalence and antimicrobial resistance data to guide treatment choices. This is of particular importance in situations when empirical therapy is employed pending antimicrobial susceptibility results.

ISCAID currently recommends amoxicillin and TMS as first-line empirical antimicrobials for uncomplicated UTIs.[1] This contrasts to regional-specific guidelines (AIDAP) for amoxicillin or AMC.[9] In a recent survey of antimicrobial prescribing habits of veterinarians within our region, amoxicillin and AMC were the most routinely prescribed empirical antimicrobial for canine UTIs at 10% and 71%, respectively.[23] These two antimicrobials had the highest level of resistance towards *E. coli* in this study.

Historically, AMC was considered a first-line antimicrobial for UTIs in dogs. AMC Is listed by WHO critically important antimicrobial (CIA) in people, and in veterinary guidelines listed as a drug to use with caution and not recommended for first-line empirical therapy.[17 24] Urinary isolates of *E. coli* are as likely to be resistant to AMC to amoxicillin in some studies; thus, the addition of clavulanic acid may provide unnecessary additional selection pressures towards resistant uropathogens.[1 3 21] TMS, not listed as a CIA by WHO, displayed the second lowest level of resistance in this study.[17] Given this and ISCAID recommendations clinicians in this region could consider TMS, a reasonable empirical treatment for canine UTIs while pending culture results.

Monitoring for emergence of resistance is a key component of surveillance systems to keep resistance levels low.[3 14 17 21] A change in empirical treatment guidelines is recommend when there is an increase in antimicrobial resistance in a non-biased population of 10% from baseline.[1 3] This study did not detect any statistically significant change in antimicrobial resistance in the antimicrobials evaluated over 5 years. However, prudent surveillance and monitoring are warranted, as recent work elsewhere documented increases in resistance of urinary *E. coli* in dogs towards AMC and cephalothin over a 7-year study period.[3 10]

One study limitation is that the laboratories did not routinely perform quantitative colony counts. Quantitative colony counts in addition to consideration of lower urinary tract signs can aid in determining whether a true UTI is present compared with subclinical bacteriuria or contaminant.[3 25] To minimise over-representation of lower urinary tract contaminants, only cystocentesis samples were included in analysis. Similarly, bacteriuria does not always equate to clinical disease. A retrospective study has limited ability to distinguish UTIs from subclinical bacteriuria. The performance standards for antimicrobial disk and dilution susceptibility tests changed in the first year of this study (2013).[16] This may have contributed to an initial change in resistance from 2013 to 2014 of analysis; however, no statistical significant difference of resistance of *E. coli* towards any of the selected antimicrobials was noted. In comparison to the disk diffusion, minimum inhibitory concentration (MIC) reports are the preferred method suited for detecting subtle changes in antimicrobial resistance. Future studies should be based on bacteriological analysis to determine the MIC of antimicrobials for urinary bacteria species.

A referral population is likely to be biased towards patients with recurrent, resistant or complicated UTIs. We eliminated subsequent submissions from patients with multiple submissions to limit the study to resistance patterns of non-recurrent uropathogens. Evaluation of recurrent samples in this dataset, and future datasets could be of value to evaluate their resistance patterns as these could differ.[5]

CONCLUSIONS

This is the first study to describe prevalence of canine uropathogens at a referral institution in Australia. Consistent with other studies, *E. coli* was the most commonly identified urinary isolate. There were no statistically significant trends in resistance of *E. coli* towards the most routinely evaluated antimicrobials over the study period. TMS, recommended by ISCAID as a first-line empirical antimicrobial, had lower level of resistance compared with amoxicillin. This information will aid veterinarians in this region in selecting appropriate empirical antimicrobials pending culture results for the treatment of UTI in dogs. Ongoing monitoring and surveillance of bacterial prevalence and resistance patterns of urinary isolates in our facility and greater region are recommended to detect any future trends.

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