

STANDARD ARTICLE

Amoxicillin and amoxicillin-clavulanate resistance in urinary *Escherichia coli* antibiograms of cats and dogs from the Midwestern United States

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Abstract

Background: Antibiograms are stewardship tools that provide antimicrobial resistance data for regional bacterial isolates to guide treatment of infections.

Objectives: To develop regional antibiograms of urinary *Escherichia coli* isolates from cats and dogs.

Animals: *Escherichia coli* isolates cultured from feline (N = 143) and canine (640) urine from 2013 to 2017, from Kansas State University (N = 335) and private practice (N = 448) patients in the Midwestern United States.

Methods: Retrospective review of urine culture and susceptibility results. Antibiograms were created for 10 commonly used antimicrobial agents using Clinical and Laboratory Standards Institutes guidelines.

Results: No isolates from cats were susceptible to amoxicillin-clavulanate (susceptibility [S] ≤ 0.25/0.12) or amoxicillin (S ≤ 0.25); isolates from dogs had low susceptibility to amoxicillin 53% (S ≤ 8). Conversely, isolates from dogs had high susceptibility to amoxicillin-clavulanate 92% (S ≤ 8/4), despite equal 90th percentile minimum inhibitory concentrations (8 µg/mL) for feline and canine populations. Resistance to other antimicrobials was uncommon (≤7% for isolates from cats, ≤14% for isolates from dogs).

Conclusions and Clinical Importance: The disparity in susceptibility for amoxicillin and amoxicillin-clavulanate between isolates from cats and dogs likely reflects higher breakpoints for urinary tract infections (UTIs) in dogs. Urine concentration data for these antimicrobials in cats might support a UTI-specific breakpoint for cats and increase potential therapeutic options for managing UTIs in cats with first-line antimicrobials. Decreased susceptibility among isolates from dogs to amoxicillin (53%) compared to amoxicillin-clavulanate (92%) might support amoxicillin-clavulanate as a better empirical choice for UTIs in dogs in this geographical region.

KEYWORDS

amoxicillin, antibiograms, *Escherichia coli*, stewardship, urinary tract infection

Abbreviations: AMC, amoxicillin-clavulanate; AMP, ampicillin; CEF, cephalixin; CLSI, Clinical and Laboratory Standards Institute; MIC, minimal inhibitory concentration; MIC₉₀, 90th percentile of minimal inhibitory concentrations; UTI, urinary tract infection.

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1 | INTRODUCTION

Empirical antimicrobial treatment often is required to manage urinary tract infections (UTI) in veterinary patients, specifically for infections diagnosed by urinalysis or pending the results of culture and antimicrobial susceptibility testing. Making an educated antimicrobial choice to guide early treatment can decrease discomfort and minimize risk of ascending infection. Empirical treatment can be aided by urine sediment findings, prior history of UTI and response to treatment, knowledge of veterinary uropathogens, understanding of antimicrobial pharmacokinetics and pharmacodynamics, and review of consensus statements. Using evidence-based medicine to choose an antimicrobial likely to be effective the first time not only benefits the patient but also achieves overall stewardship goals of improving appropriate antimicrobial prescribing.¹

Antibiograms are a stewardship tool, both in human and veterinary medicine, to provide clinicians with updated information on local resistance patterns for bacterial isolates to help guide and improve successful treatment of various infections.^{2,3} In veterinary medicine, antimicrobial guideline statements, such as those available for UTIs, respiratory disease, and pyoderma, clarify that regional differences in susceptibility should be considered by veterinarians when deciding on the most appropriate antimicrobial protocol for a particular patient.⁴⁻⁷

Our objectives were to create antibiograms of urinary *Escherichia coli* isolates recovered from cats and dogs at the Kansas State Veterinary Diagnostic Laboratory from 2013 to 2017. These antibiograms then would be available as a regional stewardship tool to help guide evidence-based decisions on empirical treatment for UTIs for veterinary patients.

2 | MATERIALS AND METHODS

Ours was a retrospective review of aerobic urine culture results of cats and dogs from the Kansas State Veterinary Diagnostic Laboratory

between 2013 and 2017. Diagnostic results were used to create antibiograms for *E. coli* urinary isolates from cats and dogs according to Clinical and Laboratory Standards Institutes (CLSI) guidelines.² Feline and canine urine samples originating from both the Kansas State University Veterinary Health Center and regional midwestern private veterinary practices were eligible for inclusion in the study. Any feline and canine urine sample from which *E. coli* was isolated was included in the study, regardless of colony forming units per milliliter. Only the first positive culture per calendar year from an individual animal was included in the data set. Data collected included species (feline versus canine), year isolated, origination of sample (university versus private practice), and both minimal inhibitory concentration (MIC) and interpretive category (susceptible, intermediate, or resistant) for 10 antimicrobial agents. An antimicrobial panel change occurred in the diagnostic laboratory in mid-2016, adding cephalexin (CEF), orbifloxacin, and pradofloxacin. Because of the retrospective nature of the study, data regarding previous antimicrobial treatment and comorbidities were not consistently available.

Urine specimen handling before arrival at the laboratory was not standardized, but urine samples were plated within 4 hours of arrival. Culture, isolation, and identification procedures were conducted according to laboratory standard-operating procedure at the time of specimen submission but varied slightly over the course of this retrospective analysis. All urine specimens initially were plated on trypticase soy (with 5% sheep blood) agar and MacConkey agar using a 10 μ L calibrated loop. Cultures were incubated overnight (18-24 hours) at 37°C in 5% CO₂. Individual colonies with phenotypic appearance for any uropathogen were streaked for isolation onto nonselective agar, and isolates were identified using 1 of 2 automated systems. From January 2013 to June 2013, isolates were identified using the Omnilog system (Biolog, Hayward, California). From July 2013 through 2017, bacterial identification was performed using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (Bruker BioTyper, Billerica, Massachusetts).

TABLE 1 Breakpoints used to create antibiograms for urinary *Escherichia coli* isolates from cats and dogs, as recommended from Clinical and Laboratory Standards Institute

Antimicrobial	Minimal inhibitory concentration breakpoints (μ g/mL)								
	Cats				Breakpoints used [reference]	Dogs			Breakpoints used [reference]
	S	I	R	S		I	R		
Amoxicillin/clavulanate	$\leq 0.25/0.12$	0.5/0.25	$\geq 1.0/0.5$	Feline UTI [8]	$\leq 8/4$	N/A	≥ 16	Canine UTI [8]	
Ampicillin	≤ 0.25	0.5	≥ 1.0	Feline UTI [8]	≤ 8	N/A	≥ 16	Canine UTI [8]	
Cefovecin	≤ 2	4	≥ 8	Feline UTI [8]	≤ 2	4	≥ 8	Canine UTI [8]	
Cefpodoxime	≤ 2	4	≥ 8	Canine UTI [8]	≤ 2	4	≥ 8	Canine UTI [8]	
Cephalexin	≤ 16	N/A	≥ 32	Canine UTI [8]	≤ 16	N/A	≥ 32	Canine UTI [8]	
Enrofloxacin	≤ 0.5	1-2	≥ 4	Feline skin/soft tissue [8]	≤ 0.5	1-2	≥ 4	Canine UTI [8]	
Marbofloxacin	≤ 1	2	≥ 4	Feline skin/soft tissue [8]	≤ 1	2	≥ 4	Canine UTI [8]	
Orbifloxacin	≤ 1	2-4	≥ 8	Feline skin/soft tissue [8]	≤ 1	2-4	≥ 8	Canine UTI [8]	
Pradofloxacin	≤ 0.25	0.5-1	≥ 2	Feline skin, respiratory [8]	≤ 0.25	0.5-1	≥ 2	Canine UTI [8]	
Trimethoprim/sulfamethoxazole	$\leq 2/38$	N/A	$\geq 4/76$	Human [9]	$\leq 2/38$	N/A	$\geq 4/76$	Human [9]	

Abbreviations: I, intermediate; N/A, not applicable; R, resistant; S, susceptible.

TABLE 2 Urinary antibiograms for cats and dogs from 2013 to 2017 reporting percent susceptibility among urinary *Escherichia coli* isolates from cats and dogs originating from either the university hospital or private practices to 10 antimicrobial agents

Antimicrobial	N	AMC (%)	AMP (%) ^a	CFV (%)	CPD (%)	CEF (%) ^b	ENR (%)	MBF (%)	ORF (%) ^b	PRF (%) ^b	SXT (%)
Feline total	143	0	0	93	94	99	96	96	97	97	96
University	62	0	0	94	94	100	95	95	96	96	97
Private	81	0	0	93	94	98	96	96	98	98	96
Canine total	640	92	53	87	86	86	90	91	89	90	92
University	273	92	63	85	84	84	90	91	90	90	92
Private	367	92	46	88	88	88	89	91	88	90	92

Abbreviations: AMC, amoxicillin-clavulanate; AMP, ampicillin; CEF, cephalexin; CFV, cefovecin; CPD, cefpodoxime; ENR, enrofloxacin; MBF, marbofloxacin; ORF, orbifloxacin; PRF, pradofloxacin; SXT, trimethoprim-sulfamethoxazole.

^aIndicates a significant difference in proportion of canine isolates that were reported susceptible (versus not susceptible) to AMP from the university versus private practice populations ($P < .001$).

^bIndicates that sample size is lower for cephalexin, orbifloxacin, and pradofloxacin (85 total feline, 302 total canine) because the laboratory panel was changed in 2016 to add cephalexin and these fluoroquinolones.

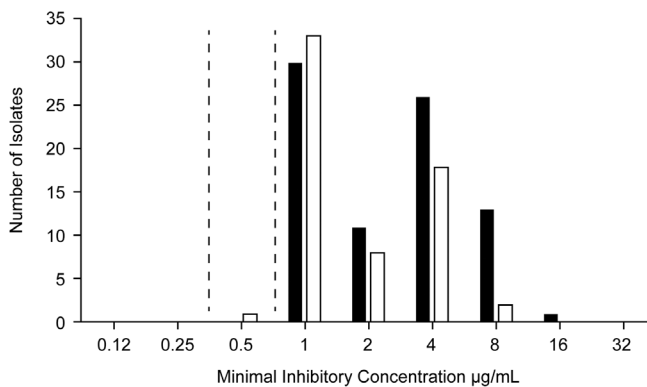


FIGURE 1 Distribution of minimal inhibitory concentration ($\mu\text{g}/\text{mL}$) for amoxicillin-clavulanate among urinary *Escherichia coli* isolates from cats ($N = 143$). The dashed vertical lines represent the CLSI breakpoints, with susceptible isolates having $\text{MIC} \leq 0.25 \mu\text{g}/\text{mL}$ and resistant isolates $\geq 1 \mu\text{g}/\text{mL}$. Black bars represent isolates submitted from private practices and white bars represent isolates submitted from the university hospital

Antimicrobial susceptibility was performed using microwell dilution testing according to CLSI recommendations, using the breakpoints listed in Table 1.⁸⁻¹⁰ When breakpoints were not available for *E. coli* UTIs for isolates from cats and dogs specifically, breakpoints for UTIs in dogs were used for cefpodoxime and CEF in cats, soft tissue infection breakpoints were used for fluoroquinolones in cats, and non-site specific *Enterobacteriaceae* breakpoints for humans were used for trimethoprim-sulfamethoxazole for both isolates from cats and dogs.^{8,9} Antibiograms were created by calculating the percentage of isolates susceptible to each antimicrobial agent; isolates reported as intermediate were not considered susceptible.⁸ The 90th percentile of minimal inhibitory concentrations (MIC_{90}) was calculated for isolates recovered from submissions from cats and dogs.¹¹ A chi-square test with Yates correction was performed to test the proportion of isolates reported susceptible (versus not susceptible) between university and private practice populations for antimicrobials that appeared to have discordant results; significance was set at $P < .05$.

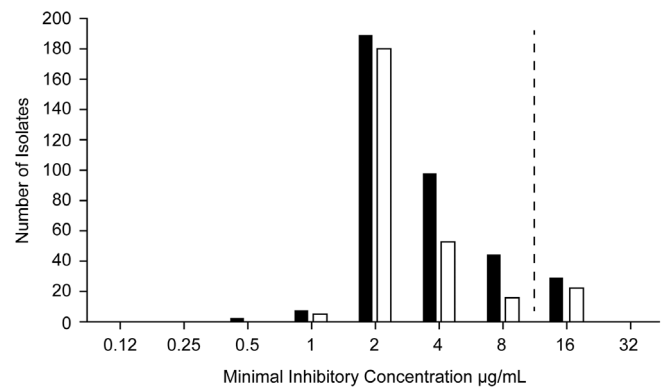


FIGURE 2 Distribution of minimal inhibitory concentration ($\mu\text{g}/\text{mL}$) for amoxicillin-clavulanate among urinary *Escherichia coli* isolates from dogs ($N = 640$). The dashed vertical line represents the CLSI breakpoint for urinary tract infections, with susceptible isolates having $\text{MIC} \leq 8 \mu\text{g}/\text{mL}$. Black bars represent isolates submitted from private practices and white bars represent isolates submitted from the university hospital

3 | RESULTS

Six-hundred forty urinary *E. coli* isolates were recovered from dogs, with 273 (43%) from the university and 367 (57%) from private practice. One-hundred forty-three urinary *E. coli* isolates were recovered from cats, with 62 (43%) from the university and 81 (57%) from private practice. Despite efforts to exclude patients with >1 submission in a calendar year, 2 isolates from cats (1.4%) and 11 isolates from dogs (1.7%) were considered possible repeats but were included because of unconfirmed identification and lack of unique patient identifiers.

Antibiogram results for urinary *E. coli* isolates from cats and dogs are listed in Table 2. No isolates from cats were reported susceptible to amoxicillin-clavulanate (AMC) or ampicillin (AMP), a surrogate for amoxicillin. Among the isolates from dogs, susceptibility to AMC was high (92%) but susceptibility to amoxicillin, a surrogate for amoxicillin, was lower (53%). Minimal inhibitory concentration distributions for

AMC for isolates from cats and dogs are presented in Figures 1 and 2. The MIC₉₀ of urinary *E. coli* isolates from both cats and dogs for AMC was 8 µg/mL. Resistance in isolates from cats and dogs to other antimicrobials was uncommon (Table 2), with <6% of isolates from cats and <14% of isolates from dogs showing resistance to any other antimicrobial agent.

When comparing between university and private practice isolates from both cats and dogs, percentages of isolates susceptible to each antimicrobial were numerically similar (Table 2). The exception was AMP in dogs, where more isolates from dogs from the university hospital (172/273, 63%) population were susceptible to AMP than from the private practice (169/367, 46%) population; this difference was significant ($P < .001$).

4 | DISCUSSION

A high level of resistance was reported among urinary *E. coli* isolates from cats to ampicillin and AMC, compared with isolates from dogs. With a similar MIC distribution and MIC₉₀ between isolates of cats and dogs, this discrepancy likely is reflective of the application of different (higher) breakpoints for UTIs in dogs ($S \leq 8$ µg/mL), but not in cats ($S \leq 0.25$ µg/mL). Rather than viewing these data as a reason to avoid prescribing amoxicillin and AMC, both first-line antimicrobial agents recommended in the guidelines for empirical treatment of UTIs in cats and dogs,⁵ our study emphasizes the need to reevaluate the breakpoint used to determine susceptibility in cats.

Beta lactam antimicrobials are recognized to have good penetration into the urine, yet published data on concentrations for amoxicillin and AMC in feline urine have not been reported. Therefore, ampicillin and AMC breakpoints in cats were determined from plasma drug concentrations, as compared to UTI-specific breakpoints in dogs based on urine concentration data.⁸ Consequently, ampicillin and AMC breakpoint for cats are conservative (low), resulting in more isolates from cats being reported resistant than isolates from dogs, despite the populations having very similar MIC distributions. Determination of urine concentration data for these antimicrobials in cats would support the development of a UTI-specific breakpoint for cats.

A less conservative, UTI-specific breakpoint for *E. coli* infections in cats would increase the percentage of isolates reported as susceptible, and veterinarians would have additional therapeutic options for successfully managing these UTIs with PO, affordable, first-line antimicrobial agents. For example, if the breakpoint of $S \leq 8$ µg/mL in dogs was applied to the population of isolates from cats in our study, the percentage of isolates from cats reported susceptible would increase from 0 to 89% for AMP, and from 0 to 99% for AMC. The data set for our study did not delineate isolates from cases of subclinical bacteriuria versus clinical UTI, nor from azotemic versus non-azotemic cats; thus, these results might not apply to each of these populations of cats similarly. Ideally, unique antibiograms would be created for each of these populations to allow clinicians to make the most appropriate conclusions and therapeutic decisions.

A study comparing susceptibility of *E. coli* UTI isolates from cats and dogs throughout Europe had very similar findings to ours, with 0% susceptibility to AMC among isolates from cats, with an MIC₉₀ of 8 µg/mL, when using the same breakpoint of $S \leq 0.25$ µg/mL.¹² In that study, however, the breakpoint for ampicillin used was the breakpoint from dogs ($S \leq 8$ µg/mL), thus they found 81.3% susceptibility despite MIC₉₀ >32 µg/mL.¹² This highlights the importance of noting the exact breakpoint used when reviewing susceptibility data, the challenges of comparing antibiograms and susceptibility results among studies, and the need for universal feline-specific UTI breakpoints.

It was also noteworthy that among the urinary *E. coli* isolates from dogs in our study, susceptibility to ampicillin (53%) was decreased compared with AMC (92%). This finding could suggest to regional veterinarians that AMC might be a better empirical choice than amoxicillin for treatment of *E. coli* UTIs in dogs. Although a regional finding, it emphasizes the need for evidence-based research to determine whether a benefit might exist for treating UTIs in dogs with AMC rather than amoxicillin alone.^{4,5} For comparison, in the study from Europe, 79% of *E. coli* UTI isolates from dogs were susceptible to AMP, whereas 98% were susceptible to AMC.¹² The potential confounding effect of prior antimicrobial treatment should be taken into consideration when interpreting these results and making treatment decisions for future canine patients. Decisions for empirical treatment ideally would be made based on regional antibiograms from patients having received no prior antimicrobial treatment. Without these historical data, it is unclear to what extent ampicillin resistance seen in isolates from dogs in our study was influenced by previous beta-lactam exposure. This population could have been skewed with a higher proportion of isolates from cats and dogs with a history of beta-lactam antimicrobial use or of those having received multiple antimicrobial courses leading to greater resistance, because aerobic urine cultures might have been submitted more often in animals with recurrent UTI or previous therapeutic failure.

Our study also was limited by its retrospective nature which did not allow for consistent handling of urine samples before submission or for consistent time between urine collection and plating, because specimen shipment was required for most of the private practice submissions. It is also possible that, despite efforts, >1 urine sample per animal was included in the data set, but this would represent <2% of samples if it occurred and thus would have limited impact on the clinical interpretation of the results. Medical records were not reviewed to identify clinical signs and information on urine collection method, and all positive samples were included without consideration for bacterial load (colony forming units). Therefore, distinguishing among subclinical bacteriuria, contamination, and infection was not possible. In the future, exclusion of subclinical bacteriuria when determining antibiograms could be beneficial for stewardship efforts.

Our results highlight the importance of understanding the relevance of antibiogram development on individual patient management. Factors, such as specimen origin (practice type in our study), geographical location, and which interpretive criteria (breakpoints) are applied can impact cumulative antimicrobial susceptibility testing data. Additional factors such as whether isolates come from clinical or subclinical and healthy or azotemic patients also may influence

antibiogram results and applicability to individual patients. Although the current findings may not reflect the prevalence of *E. coli* antimicrobial resistance in other parts of the United States, these results can be used as part of an overall veterinary antimicrobial stewardship plan by guiding the selection of empirical antimicrobial treatment for UTIs in dogs and cats in the Midwestern United States.

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CONFLICT OF INTEREST DECLARATION

BV Lubbers has received funding for consultation, research and speaking honoraria from the following: Bayer Animal Health, Boehringer Ingelheim Vetmedica, Merck Animal Health, Zoetis.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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