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Evaluation of urine concentrations of amoxicillin and clavulanate in cats

Kate KuKanich[1] | Kallie Woodruff[1] | Zackery Bieberly[1] | Mark G. Papich[2] | Butch KuKanich[3]

[1]Department of Clinical Sciences, Kansas State University College of Veterinary Medicine, Manhattan, Kansas

[2]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina

[3]Department of Anatomy and Physiology, Kansas State University College of Veterinary Medicine, Manhattan, Kansas

Correspondence
Butch KuKanich, Kansas State University College of Veterinary Medicine, 228 Coles Hall, Manhattan, KS 66506.
Email: kukanich@ksu.edu

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Abstract

Background: To characterize urinary isolates, the Clinical and Laboratory Standards Institute (CLSI) uses an amoxicillin breakpoint for cats based on plasma (not urine) drug concentrations (≤0.25 μg/mL), but a urine-specific breakpoint for dogs exists (≤8 μg/mL).

Objectives: To measure urine concentrations of amoxicillin and clavulanate after PO administration of amoxicillin-clavulanate to cats, and to suggest updated urine-specific susceptibility breakpoints for PO amoxicillin and amoxicillin-clavulanate in cats.

Animals: Eleven healthy purpose-bred cats.

Methods: Cats were given 3 62.5 mg doses of amoxicillin-clavulanate PO q12h. After the third dose, urine was collected over 28 hours, recording urination time and volume. At least 3 urine samples were collected per cat. Liquid chromatography with mass spectrometry was used to determine the urine concentrations of amoxicillin and clavulanate.

Results: Amoxicillin concentrations were >8 μg/mL in all urine samples collected within 12 hours after administration (range, 31.6-1351 μg/mL), with means of 929 μg/mL (0-6 hours) and 532 μg/mL (6-12 hours). The mean half-life of amoxicillin in urine was 1.99 hours, and mean recovery was 30%. Clavulanate was detected in all urine samples, with mean half-life of 2.17 hours.

Conclusions and Clinical Importance: Orally administered amoxicillin-clavulanate resulted in urine amoxicillin concentrations above the cutoff (8 μg/mL) for wild-type *Escherichia coli* in all cats. Because urine-specific susceptibility testing breakpoints can be determined using urine concentrations, this information should allow new CLSI uropathogen susceptibility breakpoints for amoxicillin and amoxicillin-clavulanate in healthy cats, increasing the urine breakpoint from ≤0.25 to ≤8 μg/mL.

KEYWORDS
antimicrobial, pharmacokinetic, stewardship, urinary tract infection

Abbreviations: BUN, blood urea nitrogen; CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration; UTI, urinary tract infection.

1 | INTRODUCTION

Veterinarians can improve responsible use of antimicrobials by monitoring current regional antibiograms and following evidence-based recommendations for optimal prescribing.[1] Urinary tract infections (UTI) are a common indication for use of antimicrobials in small animals, and optimizing treatment has been a specific target for stewardship implementation in veterinary medicine.[2,3] In cats, the recommended empirical antimicrobial treatment for UTI is amoxicillin or amoxicillin-clavulanate.[3] A recent review of regional antibiograms in the midwestern United States determined that no urinary *Escherichia coli* isolates in cats were susceptible (S ≤ 0.25 μg/mL) to ampicillin (a surrogate for amoxicillin) or amoxicillin-clavulanate, despite higher susceptibility for isolates from dogs (amoxicillin, 53%; amoxicillin-clavulanate, 92%; S ≤ 8 μg/mL).[4] However, the minimum inhibitory concentration (MIC) distributions were similar between dogs and cats, and the lowest concentration of antimicrobial at which 90% of isolates from both dogs and cats were inhibited was 8 μg/mL.[4] The epidemiological cutoff for *E coli* is also 8 μg/mL.[5] This discrepancy in resistance between isolates from dogs and cats likely was caused by different Clinical and Laboratory Standards Institute (CLSI) breakpoints rather than true antimicrobial resistance. In a similar study reporting antibiograms from Europe, no urinary *E coli* isolates from cats were susceptible to amoxicillin-clavulanate using the breakpoint (S ≤ 0.25 μg/mL), but susceptibility to amoxicillin was 81% when using a higher breakpoint of (S ≤ 8 μg/mL), with isolates having MICs ranging from 2 to 64 μg/mL.[6]

The CLSI uses breakpoints to define interpretive categories for susceptibility testing. Breakpoints are established by analyzing MIC distribution of wild-type bacterial isolates, pharmacokinetics and pharmacodynamics of antimicrobial agents, and clinical studies assessing treatment effect of the antimicrobial for the intended infection.[7,8] Diagnostic laboratories use the CLSI standards to apply these interpretive categories and breakpoints to clinical isolates when performing antimicrobial susceptibility tests. This information provides guidance to clinicians for therapeutic decisions. The CLSI breakpoints in cats are the same for skin, soft tissue, and urinary tract *E coli* infections and are based on the approved label dosage of amoxicillin of 12.5 mg/kg PO q12h.[9,10] In dogs however, urine concentrations of amoxicillin have been documented to peak at >200 μg/mL, thus the approved UTI-specific breakpoint for dogs is S ≤ 8 μg/mL.[9-12] The basis for this decision is the long-held assumption that for uncomplicated infections of the lower urinary tract, urine concentrations rather than serum concentrations are predictive of clinical cure.[13,14] A recent pilot study evaluated urine concentrations of amoxicillin-clavulanate in azotemic and nonazotemic cats by collecting a single urine sample 1 to 2 hours after PO administration, 5 to 7 days after starting treatment.[15] A pharmacokinetic study in which urine is collected for 24 hours after dosing amoxicillin-clavulanate to cats will provide additional valuable data to guide development of a breakpoint specific for UTIs in cats.

Our objectives were to determine the urinary pharmacokinetics of PO amoxicillin and clavulanate in healthy cats and to establish UTI breakpoints for PO amoxicillin and amoxicillin-clavulanate specific for cats to be proposed to the Veterinary Antimicrobial Susceptibility Testing subcommittee of the CLSI. The hypothesis was that urine concentrations of amoxicillin and clavulanate in cats would be comparable to urine concentrations in dogs, which could lead to a revision of the CLSI breakpoints for *E coli* isolates from cats with UTI.

2 | MATERIALS AND METHODS

2.1 | Animals and study design

Ours was a prospective pharmacokinetic study approved by the Institutional Animal Care and Use Committee at Kansas State University. Twelve healthy cats from a research colony were targeted for enrollment. Typical plasma pharmacokinetic studies consist of between 4 and 8 animals.[16] Our study aimed to enroll 12 cats to account for greater variability in PO absorption and urine concentrations.[17,18] The increased sample size more accurately estimates mean and standard deviations and accounts for the expected variability within the population.[16] All cats had a complete physical examination as well as CBC, serum biochemistry profile, and urinalysis (voided) to determine their health before the study. Cats were excluded if they were deemed unhealthy on examination or had evidence of renal disease (based on concurrent urine-specific gravity <1.035 and increased serum creatinine concentration) or systemic illness evident from CBC, serum biochemistry, or urinalysis results.[19]

The cats were gradually transitioned over 3 days to nonabsorbent cat litter (NOSORB, Catco Inc, Cape Coral, Florida) and then given 7 to 10 days to adapt before sample collection. Cats became more accustomed to bladder palpation by the research team during this adaptation time. Throughout the study, the cats were housed individually with free access to water and kept on a standard dry maintenance diet for cats.

At 8 am the day before urine collection, each cat was given a 62.5 mg tablet (PO q12h) of amoxicillin-clavulanate (Putney, Portland, Maine), according to the label dosage instructions approved by the United States Food and Drug Administration. The mean (range) dose was 14.6 (11.4-18.1) mg/kg of amoxicillin-clavulanate at a fixed 4:1 ratio of amoxicillin: clavulanate. A second 62.5 mg dose was administered 12 hours later (8 pm), and the litterboxes and food were removed for the night. At 7 am on the morning of collection, a new litterbox was placed in each enclosure. The first urine sample of the morning was discarded and a fresh litterbox with 4 oz of nonabsorbent litter was placed in the enclosure. Immediately after the first urination of the morning, cats were given their third and final 62.5 mg tablet of amoxicillin-clavulanate, and the time was recorded. The cats were fed (4 oz of their standard diet) an hour after their morning dose was administered. The cats also were fed a 4 pm meal.

The cats were monitored continuously for 28 hours after the third dose of amoxicillin-clavulanate. The timing of urine sampling depended on voluntary urination by the cats. Thus, the samples were collected as available rather than at predetermined times. The cats' bladders were gently palpated every 2 to 4 hours when the cats were awake to stimulate urination, but bladders were not forcibly expressed. Urine was collected from the litterbox immediately after urination using a syringe and 2 to 4 pieces of preweighed 3 × 3 gauze.

The urine from both the syringe and gauze then was transferred into a collection tube, recording the time, weight and volume, and placed on ice. The total weight and volume of each sample consisted of both the urine from the syringe as well as the urine absorbed by the gauze. Once measurements were completed, the samples were separated into 2 2-mL sample tubes, labeled, and immediately stored at −80°C until analysis of amoxicillin and clavulanate concentrations. After each urine sample was collected, or after a defecation, the litterbox was cleaned with soap and water, rinsed well and dried, and a clean dry litterbox was placed in the cat's enclosure.

Three or more urine collections at least 2 hours apart were considered a complete dataset for each cat. Twenty-four hours after receiving the final amoxicillin-clavulanate dose, cats that had yet to produce 3 voided urine samples were sedated using alfaxalone 2 mg/kg IM (Alfaxan, Jurox Inc, North Kansas City, Missouri), midazolam 0.2 mg/kg IM (Hospira Inc, Lake Forest, Illinois), and butorphanol 0.2 mg/kg IM (Vetorphic, Vetone, Boise, Idaho). Cystocentesis then was performed, with palpation and ultrasound examination used to ensure minimal residual volume of the urinary bladder. Once recovered from sedation, the cats were returned to university animal housing, and efforts were made to adopt them.

2.2 | Urine drug analysis

Amoxicillin and clavulanate concentrations in the urine were measured by liquid chromatography with mass spectrometry (UltiMate 3000 Pump, Column Compartment and Autosampler and Q Exactive Focus, Thermo Scientific, San Jose, California) at a reference laboratory (Iowa State University Veterinary Diagnostic Laboratory).

The pH of urine samples was adjusted to 5.5 to 7.0 as needed using formic acid. Urine samples were processed by diluting urine (50 μL) with 75% acetonitrile containing amoxicillin d4 and ampicillin d5 (950 μL). If urine concentrations of amoxicillin exceeded the upper limit of the standard curve, the samples were diluted with untreated urine (10:1) with pH adjusted to 5.8 before sample preparation. The mobile phase consisted of A: 95% 25 mM ammonium formate in deionized water with 5% acetonitrile with pH of 5.7 and B: acetonitrile. The mobile phase flow rate was 0.3 mL/min and separation was achieved using a hydrophilic interaction liquid chromatography column (SeQuant ZIC-HILIC column, 150 mm × 2.1 mm, 5 μm particles, Merck KGaA, Darmstadt, Germany). The retention times of amoxicillin, amoxicillin d4, and clavulanate were 4.5, 4.5, and 2.8 minutes, respectively. Ampicillin d5 eluted as a doublet at 2 and 2.3 minutes. Amoxicillin, amoxicillin d4, and ampicillin d5 were monitored by parallel reaction monitoring using positive electrospray ion mode and clavulanate by negative electrospray ion mode. The following transitions *m/z* (mass/charge) were monitored on the mass spectrometry (qualifying ions→fragment ion[s]: amoxicillin 366.1118 → 114.001; amoxicillin-d4 370.1369 → 114.001; clavulanate 198.0408 → 136.039, 108.044, 82.082, and ampicillin-d5 355.1483 → 111.097). Standard curves in urine were linear between 0.1 and 200 μg/mL for amoxicillin and between 0.02 and 40 μg/mL for clavulanate. Standard curves were accepted if the correlation coefficient exceeded 0.99 and the runs accepted if the predicted concentrations in the quality control samples were within 15% of the actual concentration except at the lower limit of quantification, at which ±20% was acceptable. The accuracy of the assay for amoxicillin determined on 3 replicates at each concentration (0.5, 5, and 35 μg/mL) was 100.9% and the coefficient of variation was 4%. The quality control samples for clavulanate (n = 2 per concentration) at 0.1 μg/mL were 0.11 and 0.12 μg/mL, at 1 μg/mL were 1.11 and 1.14 μg/mL, and at 7 μg/mL were 7.33 and 7.85 μg/mL. The lower limits of quantification for amoxicillin and clavulanate were 0.1 and 0.02 μg/mL, respectively.

Before analysis, the stability of amoxicillin and clavulanate was evaluated and poor stability of clavulanate was identified. Amoxicillin was stable in urine at room temperature and when frozen at −20°C and −80°C. Clavulanate was stable in urine when frozen at −80°C, but at room temperature and pH of 9.3, clavulanate rapidly degraded within an hour. Similarly, potassium clavulanate in 50% acetonitrile degraded when stored at −20°C at approximately 10% per week. Clavulanate standards in 50% acetonitrile were stored at −80°C until analysis. Clavulanate was stable in refrigerated feline urine adjusted to pH of 6.6, 5.2, and 4.1. Clavulanate was stable for 3 hours in diluted feline urine (20:1) with 75% acetonitrile and maintained on the autosampler at 10°C. All analyses for clavulanate occurred within 3 hours of being placed on the autosampler.

2.3 | Pharmacokinetic analysis

Pharmacokinetic analysis of urine concentrations was carried out using noncompartmental methods (Phoenix 64, WinNonlin, Certara, Princeton, New Jersey). Because clavulanate does not alter the pharmacokinetics of amoxicillin, dosing with the combination of amoxicillin-clavulanate allowed determination of urine-specific breakpoints for amoxicillin and amoxicillin-clavulanate.[17,18] The elimination rate constant and half-life for reported pharmacokinetic parameters were calculated using the urine drug concentration (μg/mL), urine volume (mL) and time of collection (to determine mg/hour) with multiple (≥3) urine samples per cat. Because urine samples were collected as available, urine concentration at 12 hours was estimated by log-linear regression of the measured urine concentrations.

The measured urine concentrations were evaluated to assess the proportion of cats in our study that had urine concentrations above the MIC of 8 μg/mL during the 12-hour dosing interval, grouped as 0-6 and 6-12 hours. We used a 12-hour interval because the approved label dose interval is 12 hours. We used an MIC of 8 μg/mL because the epidemiological cutoff is 8 μg/mL for wild-type *E coli*.[5]

3 | RESULTS

Twelve research cats, 2 to 4 years old with body weights ranging from 4.14 to 5.86 kg (mean, 5.2 kg) initially were enrolled in the study. Eleven were male castrated and 1 was a spayed female. All had unremarkable physical examinations, CBC, and serum biochemistry

TABLE 1 Urine amoxicillin and clavulanate pharmacokinetic parameters from cats (n = 11) given amoxicillin (50 mg) with clavulanate (12.5 mg)

	Units	Geometric mean	Minimum	Maximum	CV%
Amoxicillin (n = 11)					
T 1/2 λz	hr	1.99	1.45	2.60	16.1
λz	1/hr	0.348	0.266	0.478	16.7
Percent recovery	%	30.2	19.9	38.1	22.1
Predicted 12-hour concentration	μg/mL	125	17	722	106
Clavulanate (n = 7)					
T 1/2 λz	hr	2.17	1.64	3.18	24.7
λz	1/hr	0.319	0.218	0.424	22.7
Percent recovery	%	2.8	1.4	5.1	41.2
Total urine volume	mL	58.5	24.2	122	48.3

Abbreviations: CV%, percent coefficient of variation; T 1/2 λz, half-life of the terminal slope; λz, terminal slope.

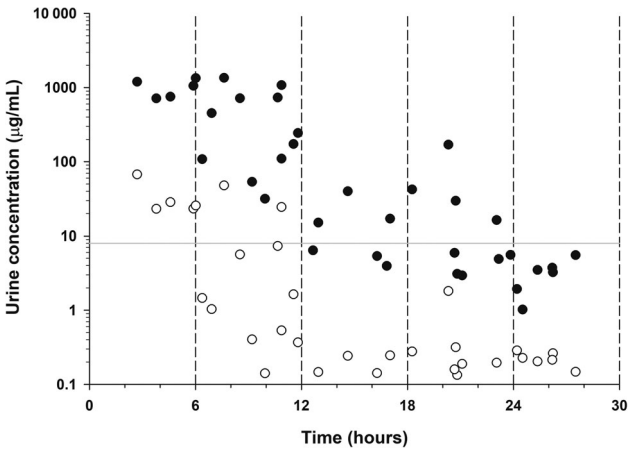

FIGURE 1 Urine concentrations of amoxicillin (black dots) and clavulanate (white dots) in cats after PO dosing of amoxicillin-clavulanate (time 0) to 11 cats, in relation to the previously established Clinical and Laboratory Standards Institute (CLSI) breakpoint (8 μg/mL) in dogs. All urine amoxicillin concentrations in cats were >8 μg/mL during the first 12 hours. Cats were given amoxicillin (50 mg) with clavulanate (12.5 mg)

profiles. No cat had evidence of renal disease. All cats had adequate urine concentrating ability (urine specific gravity range, 1.043 to >1.060). Blood urea nitrogen (BUN) concentrations were 21 to 33 mg/dL (reference interval, 15-31 mg/dL), and serum creatinine concentrations were 1.3 to 1.7 mg/dL (reference interval, 0.9-1.9 mg/dL).

The cats transitioned to nonabsorbent litter well, but urination times were unpredictable. One cat had not yet urinated by 5 pm of the day of urine sample collection (approximately 10 hours after drug administration), and thus was removed from the study. Of the remaining 11 cats, 1 cat produced 6 voided urine samples, 1 cat produced 4 voided urine samples, and 3 cats produced 3 voided urine samples in 24 hours. The remaining 6 cats produced 2 voided urine samples each and were sedated after 23 to 27.25 hours to collect a third sample by cystocentesis. All 6 cats recovered well from sedation, and all study cats were transferred back to their normal housing by 8 pm that same day.

Urine pharmacokinetic parameters for amoxicillin and clavulanate are presented in Table 1, and individual data are available in Supplementary Table 1. The mean (range) urine amoxicillin concentration from 0 to 6 hours was 929 (713-1196) μg/mL and from 6 to 12 hours was 532 (31.6-1351) μg/mL with a geometric mean (range) terminal half-life of 1.99 hours (1.45-2.60). Amoxicillin concentrations were >8 μg/mL in all urine samples collected before 12 hours in all cats (Figure 1). The mean predicted 12-hour urine concentration based on log-linear regression in all cats with 3 samples (n = 11) was 247 μg/mL, with a range of 17 to 722 μg/mL. Clavulanate was detected in all urine samples with a geometric mean (range) terminal half-life of 2.17 hours (1.64-3.18). Pharmacokinetic parameters for clavulanate are reported for 7/11 cats because of variability in elimination; 4/11 cats failed to have 3 decreasing urine clavulanate concentrations on the terminal slope.

4 | DISCUSSION

Our study provides important baseline data about urine concentrations of amoxicillin and clavulanate in cats, which is a common combination and safe and effective antimicrobial approved for used in cats. This information will help guide antimicrobial drug selection to treat UTIs in cats. We documented that amoxicillin-clavulanate administered at a dosage of 62.5 mg/cat at 4-to-1 ratio PO q12h produced urine amoxicillin concentrations above the cutoff (8 μg/mL) for wild-type *E coli* in cats without identifiable kidney disease. Because urine-specific susceptibility testing breakpoints can be determined using urine concentrations, our study provides evidence-based guidance and should allow for new CLSI breakpoints for testing uropathogen susceptibility to amoxicillin and amoxicillin-clavulanate in urine of cats without kidney disease, specifically increasing the urine breakpoint from ≤0.25 to ≤8 μg/mL. This recommendation follows the same standard used to interpret susceptibility of lower urinary tract pathogens in dogs and humans. For people and dogs, the CLSI standards for interpretation allow a higher breakpoint to be used for some antimicrobials that are excreted in the

urine in high concentrations. Until now, insufficient data were available in cats to allow the same standard to be applied.

Beta lactam antimicrobials, such as amoxicillin, are considered time-dependent antimicrobials.[8] Their efficacy is related to the duration of time their concentration exceeds the MIC of the bacteria being treated. Although many different recommendations are available, the urinary pharmacokinetics described in our study exceeded the MIC of wild-type *E coli* (8 μg/mL) for 100% of the dosing interval when amoxicillin/clavulanate was dosed at 62.5 mg/kg. These data support increasing the breakpoint of amoxicillin/clavulanate for treating *E coli* UTI in cats to an MIC ≤8 μg/mL.

This information is available for use by the CLSI veterinary subcommittee for Veterinary Antimicrobial Susceptibility Testing to apply a new CLSI breakpoint for interpretation of susceptibility to amoxicillin-clavulanate for lower urinary tract isolates of *E coli* from cats. This recommendation supports our goal of responsible stewardship to preserve the use of these commonly used antimicrobials in cats. Without this new breakpoint for lower urinary tract isolates from cats, most isolates would be reported as resistant using the former breakpoint of ≤0.25 μg/mL, which could lead to increased use of other valuable antimicrobial agents such as fluoroquinolones, 3rd generation cephalosporins, or carbapenems (meropenem and imipenem-cilastatin), among others.

Urine concentrations of amoxicillin in cats in our study (mean 6-12 hour concentration of 532 μg/mL) exceeded previously reported data from dogs that indicated mean 8-hour urine concentrations of 201 ± 93 μg/mL (range, 76-825 μg/mL) when amoxicillin was administered at a dosage of 33 mg/kg/day (divided q8h) PO to dogs.[11] Twelve hours after amoxicillin-clavulanate administration (a typical dosing interval for cats), all urine amoxicillin concentrations exceeded 8 μg/mL, suggesting that this urine-specific breakpoint would be appropriate for cats with normal renal function, as is currently recommended for dogs.

The percentage recovery of amoxicillin from cats in this study (30%) was lower than expected for a β-lactam antimicrobial agent. A similar percentage recovery (range, 9%-34%) was reported for dogs when collected during steady state, using an 8-hour dosing interval.[11] The cause for lower than expected recovery is undetermined, but might be caused by incomplete PO bioavailability, alternate excretion routes, and possible metabolism. One cat vomited 4.75 hours after antimicrobial administration, which might have resulted in decreased PO bioavailability, but urine recovery (19.9%) was still nearly within the range (20.4%-38.1%) of the remaining cats in the study.

Renal function is an important factor in determining the most appropriate breakpoints to guide management of UTI. Cats with decreased urine concentrating ability and azotemia are expected to have lower urine concentrations of antimicrobials, which was confirmed in a recent pilot study including 11 cats with and 50 cats without azotemic chronic kidney disease.[15] In that study, urine was collected by cystocentesis 1 to 2 hours after amoxicillin-clavulanate administration, and the median urine concentration of amoxicillin in cats with azotemic chronic kidney disease was 50.5 μg/mL (range, 8.1-158.5 μg/mL) compared to nonazotemic cats 328.3 μg/mL (range, 33.1-576.5 μg/mL).[15] Therefore, the same urine breakpoints that apply to healthy cats might not apply to cats with renal dysfunction. However, a limitation to that study was that a full urine elimination profile was not collected, only a single time point. Other factors could not be accounted for, such as absorption rates that could alter urine concentrations independent of elimination rate. Therefore, it was not possible to establish urine-specific breakpoints from those results. In our study, 1 cat had borderline azotemia (BUN concentration, 33 mg/dL; serum creatinine concentration, 1.6 mg/dL) that was deemed pre-renal based on adequate urine concentrating ability (urine-specific gravity, 1.060), and no cats had evidence of renal dysfunction. The total urine volume (mean, 58.5 mL per cat) was near the mean average daily urine volume for similar-sized healthy cats (61.5 mL) fed a dry diet.[20] Cats ingesting diets with different moisture content are known to have different drinking behavior and urine output, which also might influence urine drug concentrations.[20] An additional study is warranted to determine the full pharmacokinetic profile in azotemic cats compared to healthy cats so as to understand the effect of kidney disease on absorption and elimination of PO administered amoxicillin-clavulanate.

An approved urine-specific breakpoint for cats will enable laboratories to provide an accurate susceptibility test report for these antimicrobial agents. With a new CLSI amoxicillin breakpoint for uropathogenic *E coli* in cats, isolates with an MIC ≤ 8 μg/mL will be reported as susceptible. Our examination of the midwestern *E coli* antibiograms from cats showed that the percentage of isolates reported as susceptible would increase from 0% to 89% for ampicillin (amoxicillin) and from 0% to 99% for amoxicillin-clavulanate.[4] Similarly, applying a breakpoint of S ≤ 8 μg/mL to the European *E coli* antibiogram for cats would result in an increase from 0% to 92.5% of isolates being susceptible to amoxicillin-clavulanate.[6] This breakpoint information will allow updating of other existing and future antibiograms.

Determining the urine drug concentrations of amoxicillin and clavulanate in cats and creating a urine-specific breakpoint will have direct impact on antimicrobial selection for treating UTIs in cats. Our study results will allow amoxicillin and amoxicillin-clavulanate to remain acceptable as first-line PO options for treatment of UTIs by veterinary practitioners who utilize urine culture reports when prescribing antimicrobial treatment for cats.

A limitation of our study was the infrequent and inconsistent urination habits of the enrolled cats. Although 11 cats provided at least 3 urine samples, 1 cat was excluded because of infrequent urination. In anticipation of this possible complication, we intentionally enrolled more cats than typical for a pharmacokinetic study (6 cats), and as such we were still able to collect the necessary data to fulfill our study objectives.

5 | CONCLUSIONS

Our study in healthy cats showed that PO administration of amoxicillin-clavulanate at the approved label dose of 62.5 mg per cat q12h resulted in urine concentrations that exceeded the MIC of 8 μg/mL for wild-type strains of uropathogenic *E coli*. These results can be used by CLSI to add a new breakpoint to existing standards to include an interpretive category for urine isolates from cats that do not have evidence of kidney disease, specifically increasing the urine breakpoint from ≤0.25 to ≤8 μg/mL.[9,10] These evidence-based

recommendations support the stewardship goals of the veterinary profession and will improve clinical management of UTIs in cats.

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CONFLICT OF INTEREST DECLARATION

Mark G. Papich has provided consulting, accepted gifts, honoraria, and research support from Zoetis the sponsor of the Clavamox brand of amoxicillin-clavulanate, and Dechra, the sponsor of the generic form of amoxicillin-clavulanate. One of the authors (MGP) is a current member, and former chair holder of the CLSI-VAST subcommittee.

OFF-LABEL ANTIMICROBIAL DECLARATION

Amoxicillin-clavulanate was used in an approved species at an approved dosage, but for an unapproved condition.

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

Approved by the Kansas State University IACUC, Protocol #4200.

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Kate KuKanich https://orcid.org/0000-0002-0215-3284
Mark G. Papich https://orcid.org/0000-0002-7591-7898
Butch KuKanich https://orcid.org/0000-0002-4037-3472

REFERENCES

1. American Veterinary Medical Association Taskforce on Antimicrobial Stewardship in Companion Animal Practice. https://www.avma.org/KB/Resources/Reports/Documents/TFASCAP_Report.pdf. Accessed April 14, 2020.
2. Banfield Pet Hospital. Veterinary emerging topics report: A feline focus on antimicrobial usage. https://www.banfield.com/Banfield/media/contenthub/files/2018_VET_Report.pdf. Accessed April 14, 2020.
3. Weese JS, Blondeau J, Boothe D, et al. International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. *Vet J*. 2019;247:8-25.
4. KuKanich K, Lubbers B, Salgado B. Amoxicillin and amoxicillin-clavulanate resistance in urinary *Escherichia coli* antibiograms of cats and dogs from the Midwestern United States. *J Vet Intern Med*. 2020;34:227-231.
5. European Committee on Antimicrobial Susceptibility Testing (EUCAST). www.EUCAST.org. Accessed on April 14, 2020.
6. Moyaert H, Morrissey I, de Jong A, et al. Antimicrobial susceptibility monitoring of bacterial pathogens isolated from urinary tract infections in dogs and cats across Europe: ComPath results. *Microb Drug Resist*. 2017;23:391-403.
7. Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility patterns. *Clin Microbiol Rev*. 2007;20:391-408.
8. Papich MG. Antimicrobials, susceptibility testing, and minimum inhibitory concentrations (MIC) in veterinary infection treatment. *Vet Clin North Am Small Anim Pract*. 2013;43:1079-1089.
9. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*. 5th ed. Wayne, PA: CLSI standard VET01. Clinical and Laboratory Standards Institute; 2018.
10. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*. 4th ed. Wayne, PA: CLSI supplement VET08. Clinical and Laboratory Standards Institute; 2018.
11. Ling GV, Conzelman GM Jr, Franti CE, et al. Urine concentrations of five penicillins following oral administration to normal adult dogs. *Am J Vet Res*. 1980;41:1123-1125.
12. Osborne CA, Lees GE. Bacterial infections of the canine and feline urinary tract. In: Osborne CA, Finco DR, eds. *Canine and Feline Nephrology and Urology*. 1st ed. Baltimore, MD: Williams and Wilkins; 1995:783.
13. Stamey TA, Fair WR, Timothy MM, Millar MA, Mihara G, Lowery YC. Serum versus urinary antimicrobial concentrations in cure of urinary tract infections. *N Engl J Med*. 1974;291:1159-1163.
14. Frimodt-Møller N. Correlation between pharmacokinetic/pharmacodynamic parameters and efficacy for antibiotics in the treatment of urinary tract infection. *Int J Antimicrob Agents*. 2002;19:546-553.
15. Benson KK, Quimby JM, Dowers KL, et al. Pilot study of side effects and serum and urine concentrations of amoxicillin–clavulanic acid in azotemic and non-azotemic cats. *J Feline Med Surg*. 2019;22:729-735.
16. Riviere JE. Study Design and Data Analysis. In: Riviere JE, ed. *Comparative Pharmacokinetics: Principles, Techniques and Applications*. 1st ed. Ames, IA: Iowa State Press; 1999:239-258.
17. Vree TB, Dammers E, van Duuren E. Variable absorption of clavulanic acid after an oral dose of 25 mg/kg of Clavubactin and Synulox in healthy cats. *Scientific World Journal*. 2002;2:1369-1378.
18. Vree TB, Dammers E, Exler PS. Identical pattern of highly variable absorption of clavulanic acid from four different oral formulations of co-amoxiclav in healthy subjects. *J Antimicrob Chemother*. 2003;51:373-378.
19. International Renal Interest Society. IRIS staging of CKD. http://www.iris-kidney.com/guidelines/staging.html. Accessed April 14, 2020.
20. Thomas DG, Post M, Bosch G. The effect of changing the moisture levels of dry extruded and wet canned diets on physical activity in cats. *J Nutr Sci*. 2017;6:e9.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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