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Trends of feline *Escherichia coli* minimum inhibitory concentrations over 14 years illustrate the need for judicious antimicrobial use in cats

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OBJECTIVE
This study aims to assess the antimicrobial resistance (AMR) trends among *Escherichia coli* isolated from cats between 2008 and 2022, utilizing MIC data, within a one-health framework.

SAMPLE
The study analyzed MIC results from 1,477 feline *E coli* isolates that were obtained from samples submitted to the Cornell University Animal Health Diagnostic Center, primarily from the northeastern US.

METHODS
MIC values were categorized as susceptible or not susceptible using the Clinical and Laboratory Standards Institute breakpoints. Multidrug resistance (MDR) was analyzed using a Poisson regression model. Additionally, accelerated failure time models were employed to analyze MIC values.

RESULTS
Out of the 1,477 *E coli* isolates examined, 739 (50%) showed susceptibility to all tested antimicrobials. Among the tested antimicrobials, cefazolin (69%) and ampicillin (74% for urinary tract isolates) exhibited the lowest susceptibility. Overall, 15% of isolates were not susceptible to cefovecin. *E coli* isolates were highly susceptible (> 95%) to antibiotics typically reserved for human use. Almost one-third of the isolates were classified as MDR, with nonurinary isolates more likely to exhibit an MDR pattern. A decrease in MICs for fluoroquinolones and gentamicin in recent years was identified. However, MICs for cephalexin increased from 2016 to 2022 and cefovecin from 2012 to 2019.

CLINICAL RELEVANCE
This study highlights the challenge of AMR in feline medicine, emphasizing the importance of responsible antimicrobial use and surveillance to address *E coli* AMR. The related Currents in One Health by Cazer et al, *JAVMA*, December 2023, addresses additional feline antimicrobial stewardship topics.

Keywords: *Escherichia coli*, antimicrobial resistance, feline, multidrug resistance, minimum inhibitory concentration

Antimicrobials are important for human and veterinary medicine. However, the emergence of antimicrobial resistance (AMR) significantly threatens our ability to treat infectious diseases. AMR occurs when microorganisms adapt and acquire the ability to survive exposure to antimicrobials that were clinically effective previously.[1] Infections caused by resistant pathogens lead to an elevated risk of disease transmission, severe illness, and mortality, thereby increasing the societal and economic burdens of disease.[2] The CDC estimates that AMR contributes to over $4.6 billion in annual healthcare costs in the US alone.[1]

To address this growing concern, health authorities have called for the implementation of antimicrobial stewardship programs in both human and veterinary medicine. These programs aim to ensure responsible antimicrobial use and reduce the

selection and dissemination of resistant bacteria.[3] While most initiatives have traditionally focused on food-producing animals to prevent the entry of antimicrobials and AMR genes into the food chain,[4] mounting evidence suggests that nonfoodborne transmission of AMR is on the rise. Companion animals, due to their close contact with humans and frequent exposure to antimicrobials, are now considered potential reservoirs of AMR.[5]

Although *Escherichia coli* is a commensal bacteria present in the intestinal environment of humans and warm-blooded animals, it can cause severe and life-threatening infections.[6,7] Pathogenic *E coli* can cause enteric and extraenteric diseases in humans and animals. In cats, *E coli* is a major pathogen in genital, systemic, and urinary tract infections.[8,9]

The prevalence of AMR varies across different host species and antimicrobial compounds. For instance, 1 study[10] conducted in Atlantic Canada found susceptibility rates in *E coli* isolated from cats were greater than 85% for all antimicrobials except ampicillin (74% susceptible) and cephalexin (61% susceptible). In Spain, fewer than 60% of *E coli* isolated from cats and dogs were susceptible to ampicillin and first-generation cephalosporins.[11] A recent study[12] in the US reported that 33% of urinary *E coli* isolated from dogs and cats are not susceptible to amoxicillin and 18% are not susceptible to cefovecin.

Moreover, *E coli* exhibits the capacity to acquire and accumulate resistance genes from other bacteria, resulting in multidrug resistance (MDR).[13] In companion animals, AMR and MDR *E coli* infections pose an increased risk of treatment failure and heightened disease-related costs and raise public health concerns regarding zoonotic transmission of AMR.[14]

The numerous pathways for direct and indirect transmission of *E coli* between humans and animals are a major concern in human and veterinary medicine, leading to increased risk of transmission of AMR determinants.[6] There are reported genotypic similarities between uropathogenic *E coli* isolates from domestic cats and those from humans, suggesting bidirectional transmission between the 2 populations.[15] Furthermore, a recent study[16] found a higher likelihood of vaginal *E coli* colonization in pregnant women who owned cats, as compared to those without feline companions, underscoring the potential for bacterial transmission from pets to their owners. These findings underscore the pressing need for AMR surveillance in cats.

Furthermore, local or regional AMR data can empower prescribers by providing relevant resistance patterns to guide empiric therapeutic decisions.[17] Additionally, these data assist in the development of antimicrobial stewardship guidelines and inform public policy regarding antimicrobial use.[10] To address these pressing concerns, this study aims to examine the trends in AMR among *E coli* isolates from cats between 2008 and 2022, utilizing MIC data collected at the Cornell University Animal Health Diagnostic Center (AHDC).

Methods

Study design and microbiological procedures

This retrospective study analyzed data from antimicrobial susceptibility testing of 1,654 *E coli* isolates obtained from clinical samples of cats. The isolates were cultured by the Cornell University AHDC from 2008 to 2022, using standard bacteriologic culture methods previously described.[18] Briefly, sample material was inoculated onto Columbia agar with 5% sheep blood, eosin methylene blue agar, and trypticase soy agar with 5% sheep blood. Individual colonies were then chosen as presumptive for *E coli* based on morphology from eosin methylene blue agar. The identity of isolates was confirmed as *E coli* using either the Sensititre Automated Microbiology System (TREK Diagnostic Systems) or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI Biotyper; Bruker). All procedures were performed in accordance with standardized operating procedures in a laboratory accredited by the American Association of Veterinary Laboratory Diagnosticians.

Antimicrobial susceptibility testing of *E coli* isolates was carried out using the broth microdilution method as previously described.[14] Nonurinary *E coli* isolates collected between 2007 and November 2016 were tested using 2 Thermo Scientific Sensititre MIC susceptibility plates (COMPAN1F and COMPAN2F panels), after which a different plate (COMPGN1F panel) was adopted. Urinary isolates were subjected to testing with the CMV1BURF panel, or occasionally the COMPGN1F panel if they were MDR on the urine panel.

Statistical analysis

All the analyses were performed using the software Stata SE v18. Duplicate laboratory accessions were identified and eliminated, and the first isolate per accession was selected, leaving a total of 1,477 isolates for subsequent data analysis. The analysis started generating frequency tables to explore the distribution of each variable.

The MICs necessary to inhibit the growth of 50% and 90% of the tested isolates, denoted as MIC_{50} and MIC_{90}, respectively, were calculated. Subsequently, isolates were classified into susceptible and not susceptible (including intermediate and resistant) according to the Clinical and Laboratory Standards Institute breakpoints **(Supplementary Table S1)**,[19,20] and the proportion of susceptible isolates to each antimicrobial was calculated. Susceptibility to doxycycline was not calculated because most of the MICs were not interpretable due to recent changes in the Clinical and Laboratory Standards Institute breakpoints **(Supplementary Table S2)**.

Multidrug resistance (MDR) was defined as not susceptible to at least 1 agent in 3 or more antimicrobial categories (Supplementary Table S1), following the international standard definitions for acquired resistance.[21] Doxycycline interpretations

were excluded from the MDR analysis. We used a Poisson regression with robust variance to model MDR, including site of isolation, year, exhibiting beta-hemolysis, and the number of antimicrobials tested as predictors and the presence of MDR as the outcome. The possible interactions were explored and retained only if the interaction term was significant ($P < .05$). The Deviance and Pearson tests were used to test goodness of fit, which indicated no lack of fit in the model ($P = 1.00$). Finally, coefficients, SEs, and CIs of the Poisson regression were exponentiated to express them as prevalence ratios.

An interval for each MIC was created, using the antimicrobial concentration in which there was no bacterial growth (the reported MIC) as the upper limit and the next lowest concentration as the lower limit of the interval. For example, an MIC of = 2 would generate an interval of (1, 2). In the case of the maximum concentration tested (eg, > 4), the maximum concentration constituted the lower limit, and the interval was treated as right censored (eg, [4, infinity]). When the MIC was the minimum concentration tested (eg, ≤ 1) the lower limit of the interval was a random number between 0 and the minimum concentration tested (eg, [random value, 1]). This approach mitigated high levels of left censoring, which could result in predicted MIC values of 0. These intervals were used to fit an interval-censored parametric survival model in which, instead of time to event, the concentration to event (ie, inhibition of bacterial growth) was analyzed, as reported elsewhere.[18,22]

Four types of parametric survival models were explored: exponential, Weibull, lognormal, and log logistic. Based on the distribution of MICs in the baseline hazard (ie, isolates from 2008 and urinary tract isolates [UTIs]) and the Akaike's information criterion statistic. The log-logistic was selected as the model that best fits our data. One model per antimicrobial tested on our database was set. All the models included year as a categorical variable (reference level, 2008 to 2011) and site of isolation (classified into UTI as the reference and non-UTI) and whether the isolate exhibited beta-hemolysis. The interactions between year and being a UTI were examined and retained the significant terms ($P < .05$). Coefficients were exponentiated to obtain concentration ratios, representing the expected proportional change in the median antimicrobial concentration required for bacterial growth inhibition for a 1-unit change in the predictor.[23]

Results

In total, 1,477 *E coli* isolates were used in the data analysis. Most isolates were recovered from the urinary tract (n = 1,030; 70%) (**Table 1**; Supplementary Table S2). About one-third of the isolates (n = 411; 28%) were beta-hemolytic. Isolates were not consistently tested against the same antimicrobials **(Table 2)**. Trimethoprim-sulfamethoxazole (n = 1,476 isolates), enrofloxacin (n = 1,472), amoxicillin-clavulanic acid (n = 1,472), ampicillin (n = 1,396), tetracycline (n = 1,263), cephalexin (n = 1,217), and ceftiofur (n = 1,146) were the most consistently tested. Antimicrobial susceptibility panels changed during the study period, resulting in different MIC dilutions and, in some cases, MIC values that cannot be interpreted with current breakpoints **(Supplementary Table S3)**.

Table 1—Distribution of isolates included in the analysis over the year periods and site of isolation.

Year	Urinary tract	Nonurinary tract
2008–2011	163	91
2012–2015	303	90
2016–2019	358	131
2020–2022	206	135
Total	**1,030**	**447**

Antimicrobial susceptibility

Of 1,477 isolates, 739 (50%) were susceptible to all the tested antimicrobials. No susceptibility to 1 antimicrobial was observed in 175 isolates (12%), and 562 (38%) isolates were not susceptible to 2 or more antimicrobials. Cephalexin MIC_{50} and MIC_{90} for non-UTIs were not calculated for the years 2008 to 2011 and 2012 to 2015 because there were only 2 observations for each of those periods. The antimicrobials with the highest susceptibility rates were antibiotics typically reserved for human use imipenem (99%), amikacin (98%), and piperacillin-tazobactam (95%). High susceptibility to trimethoprim-sulfamethoxazole (94%) and ceftiofur (94%) was also observed (Table 2). Excluding penicillins for non-UTIs, which are expected to be not susceptible, ampicillin (74% for UTI) and cefazolin (69%) had the lowest rates of susceptibility **(Figure 1)**. There were no apparent linear trends over time in MIC_{50} or MIC_{90} or the percent susceptibility for most antimicrobials. There was a decrease in susceptibility to enrofloxacin, gentamicin, tetracycline, and trimethoprim-sulfamethoxazole from 2017 to 2019 compared to the years before and after. Susceptibility to third-generation cephalosporins (cefovecin, cefpodoxime, and ceftiofur) similarly increased in recent years (eg, 2020 to 2022), and a decrease in their MIC_{90} was observed in this period.

Multidrug resistance

In total, 413 (28%) isolates were classified as MDR. Year, sample site, and beta-hemolysis were significantly associated with the probability of MDR, and there was a significant interaction between the year and sample site **(Table 3)**. MDR trends diverged among UTI and non-UTI *E coli*. Non-UTIs exhibited an increase in MDR over time, whereas UTIs had a decreasing probability of MDR over time **(Figure 2)**.

Accelerated failure time survival models

Predicted MICs for all fluoroquinolones and gentamicin significantly decreased in the period from

Table 2—Distribution of minimum inhibitory concentrations necessary to inhibit the growth of 50% and 90% (MIC_{50} and MIC_{90}, respectively) of *E coli* isolates recovered from cats from 2008 to 2022 and the overall percentage of susceptible (S) isolates.

		Years								
		2008–2011		2012–2015		2016–2019		2020–2022		Overall
Antimicrobial	n	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	S (%)
Amikacin	590	4	4	4	4	4	4	4	4	98
Amoxicillin-clavulanic acid UTI	1,030	4	16	4	8	4	8	4	8	90
Amoxicillin/clavulanic acid non-UTI	442	4	16	1	1	4	8	4	8	< 1
Ampicillin UTI	1,011	2	256	2	256	4	256	4	256	74
Ampicillin non-UTI	385	4	16	1	1	2	8	4	8	< 1
Cefazolin	590	4	16	2	8	2	32	2	8	69
Cefovecin	541	0.5	4	0.5	4	0.5	8	0.5	2	84
Cefpodoxime	586	2	16	2	16	1	8	1	4	82
Ceftazidime	328					4	16	4	4	90
Ceftiofur	1,146	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	94
Cephalexin UTI	965	4	8	4	8	4	16	4	16	92
Cephalexin non-UTI	252					4	16	4	16	2
Chloramphenicol	590	4	16	4	16	4	8	8	8	90
Doxycycline	545	2	8	2	8	2	8	1	4	*
Enrofloxacin	1,472	0.06	0.5	0.03	0.25	0.03	0.25	0.12	0.12	93
Gentamicin	590	1	2	1	2	1	2	0.5	1	94
Imipenem	586	1	1	1	1	1	1	1	1	99
Marbofloxacin	586	0.25	2	0.25	2	0.12	4	0.12	0.12	87
Orbifloxacin	374	1	4			1	8	1	1	87
Piperacillin-tazobactam	329					8	8	8	8	95
Pradofloxacin	330					0.25	2	0.25	0.25	89
Tetracycline	1,263	2	8	2	2	2	16	4	4	90
Trimethoprim-sulfamethoxazole	1,476	2	2	2	2	2	4	0.5	2	94

UTI = Urinary tract isolates.

*The true susceptibility prevalence is unknown because the smallest MIC intervals (eg, ≤ 0.25 and ≤ 2) included possible MIC values in both the susceptible and not susceptible categories; therefore, no isolates could be classified as susceptible to doxycycline (Supplementary Table S3).

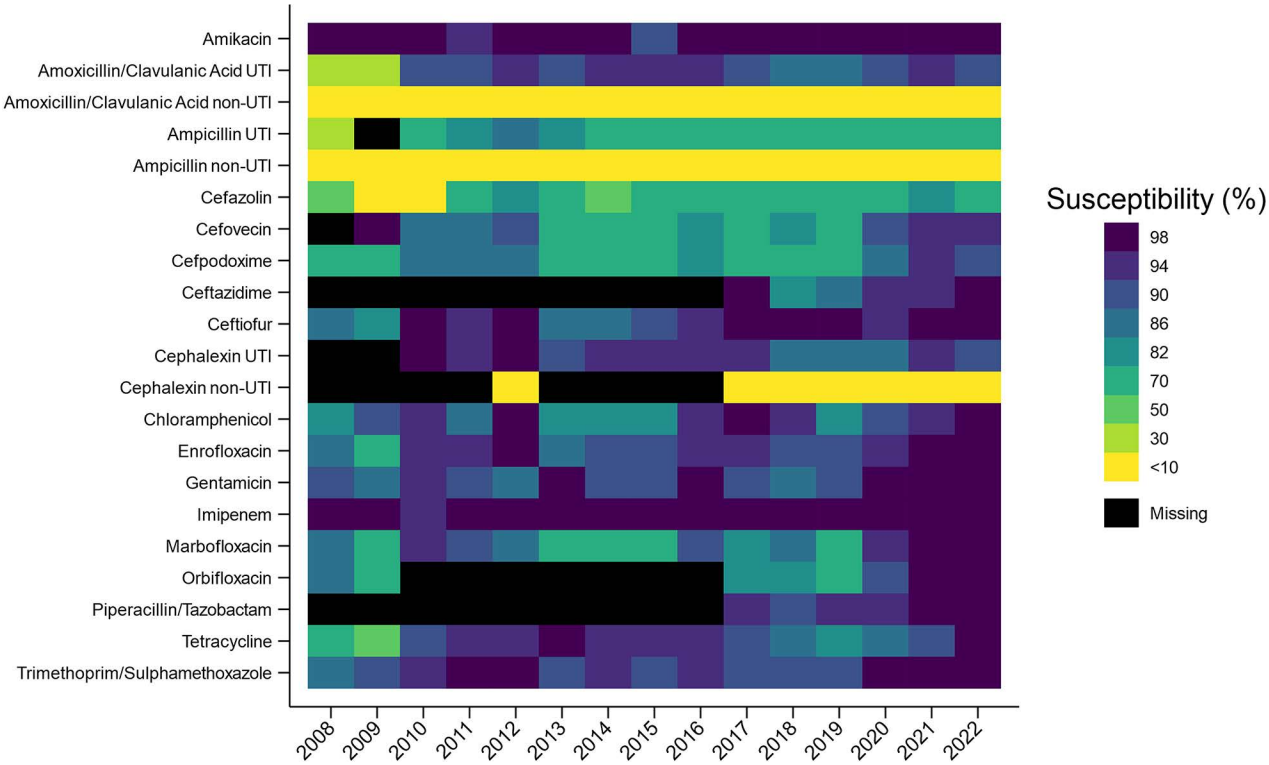

Figure 1—Proportion of susceptible isolates per year for *E coli* isolated from cats between 2008 and 2022. UTI = Urinary tract isolates.

Table 3—Poisson regression model for the prevalence ratio for multidrug resistance from 1,477 feline *E coli* isolates at the Cornell University Animal Health Diagnostic Center between 2008 and 2022.

Variable	Prevalence ratio	*P* value	95% CI
Sample site (UTI) and year	1.39	.260	0.78–2.48
2012–2015	1.60	.075	0.95–2.68
2016–2019	1.64	.053	1.00–2.71
2020–2022	1.76	.027	1.07–2.91
Interaction between sample site (UTI) and year			
2012–2015	0.60	.164	0.30–1.23
2016–2019	0.46	.012	0.25–0.84
2020–2022	0.27	< .001	0.14–0.54
Beta-hemolytic isolate	0.90	.103	0.79–1.02
Number of antimicrobials tested	1.21	< .001	1.16–1.26
Constant	0.02	< .001	0.01–0.03

2020 to 2022 when compared to the reference period from 2008 to 2011 **(Table 4)**. For marbofloxacin, pradofloxacin, and orbifloxacin, this was driven by a decrease in UTI MICs, whereas enrofloxacin UTI MICs increased over time and non-UTI MICs decreased **(Supplementary Figure S1)**. MIC trends were variable for the cephalosporins. There was a decrease in cefazoline and ceftazidime UTI MICs from the 2016–2019 period to the 2020–2022 period. Cephalexin MICs were higher estimates in the periods from 2016 to 2019 and 2020 to 2022 compared to the reference years. Cefovecin MICs were higher from 2012 to 2015 and 2016 to 2019 compared to 2008 to 2011, but the trend did not continue in 2020 to 2022.

The predicted MIC_{50} for UTIs is significantly higher for all the antimicrobials analyzed except for ampicillin, enrofloxacin, and tetracycline (Table 4). Beta-hemolytic isolates were associated with reduced MICs for cephalexin, ceftiofur, enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin, and tetracycline. The interaction between the sample site (UTI vs. non-UTI) and year category was significant for several antimicrobials. For most, the predicted MIC_{50} for non-UTIs was relatively stable over time, while the MIC_{50} for UTIs declined (Supplementary Figure S1; cefazolin, ceftazidime, marbofloxacin, orbifloxacin, and pradofloxacin). Notably, the non-UTI predicted chloramphenicol MIC_{50} increased over time while the UTI MIC_{50} was stable. Over 95% of the MICs for amikacin, cefpodoxime, and imipenem were concentrated within a single category (Supplementary Table S3). Consequently, these MICs were not suitable for inclusion in our MIC modeling analysis.

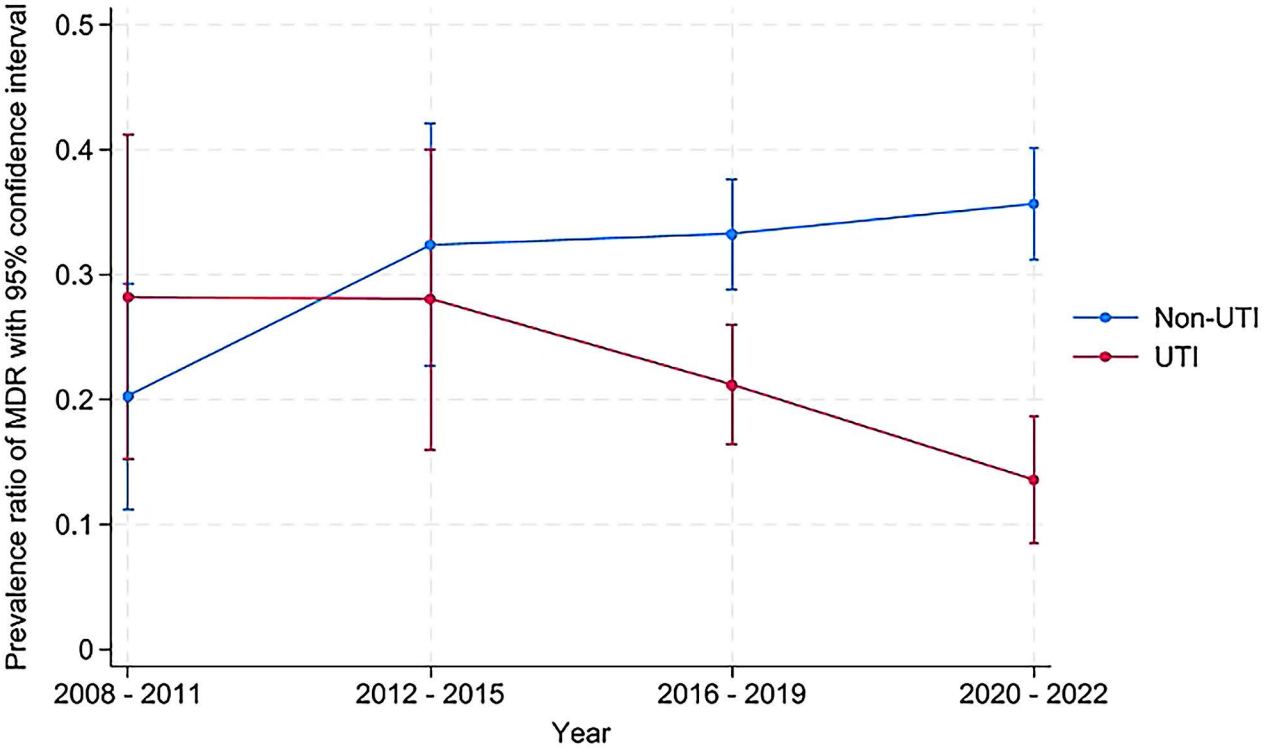

Figure 2—Probability of multidrug resistance (MDR) for urinary tract *E coli* isolates (UTI) and nonurinary *E coli* isolates (Non-UTI) based on the Poisson regression model.

Table 4—Concentration ratios (95% CI) obtained from log-logistic accelerated failure time models of MIC of *E coli* isolated from cats at the Cornell University Animal Health Diagnostic Center between 2008 and 2022.

	Year of isolation (reference, 2008–2011)					
Antibiotic	**2012–2015**	**2016–2019**	**2020–2022**	**Urinary tract isolate**	**Beta-hemolytic isolate**	**Baseline MIC_{50}**
Amoxicillin-clavulanic acid	0.99 (0.87–1.12)	1.09 (0.97–1.24)	1.15* (1.00–1.30)	0.93 (0.85–1.02)	0.92 (0.83–1.00)	3.21 (2.86– 3.62)
Ampicillin	1.44 (0.93–2.24)	1.77* (1.14–2.75)	1.64* (1.03–2.60)	0.57** (0.42–0.77)	0.77 (0.57– 1.05)	3.49 (2.28–5.36)
Cefazolin[a]	0.79 (0.54–1.14)	0.76 (0.54–1.07)	0.87 (0.61–1.23)	3.02** (1.51–6.03)	0.77 (0.59–1.00)	1.91 (1.44–2.52)
Cefazolin: interaction UTI and year	1.04 (0.41–2.62)	1.75 (0.74–4.11)	0.35** (0.57–0.77)			
Cephalexin	1.20 (0.95–1.51)	1.74** (1.39–2.18)	2.01** (1.59–2.54)	0.90 (0.78–1.03)	0.73** (0.64–0.83)	2.44 (1.92–3.09)
Cefovecin	1.47* (1.06–2.19)	1.49** (1.10–2.02)	1.18 (0.87–1.59)	2.41* (1.89–3.06)	0.79 (0.63–1.00)	0.39 (0.30–0.50)
Ceftiofur	1.09 (0.98–1.22)	1.06 (0.93–1.20)	1.10 (0.94–1.30)	1.14* (1.03–1.26)	0.87* (0.78–0.98)	0.26 (0.24–0.29)
Ceftazidime[a,b]			1.01 (0.92–1.11)	1.48** (1.25–1.77)	0.91 (0.83–1.00)	3.34 (3.10–3.59)
Ceftazidime: interaction between UTI and year			0.69** (0.56–1.00)			
Chloramphenicol	0.91 (0.75–1.12)	1.16 (0.96–1.38)	1.30** (1.09–1.55)	1.76** (1.28–2.40)	0.89 (0.78–1.02)	3.37 (2.93–3.88)
Chloramphenicol: interaction between UTI and year	1.07 (0.69–1.67)	0.70 (0.48–1.01)	0.66* (0.45–0.96)			
Doxycycline	1.36 (0.90–2.04)	1.30 (0.90–1.88)	1.06 (0.74–1.52)	1.97** (1.58–2.45)	0.82 (0.66–1.03)	0.78 (0.55–1.09)
Enrofloxacin	1.16 (0.74–1.81)	0.61* (0.41–0.90)	0.57** (0.39–0.84)	0.30** (0.21–0.43)	0.73** (0.64–0.84)	0.09 (0.70 -0.13)
Enrofloxacin: interaction between UTI and year	0.79 (0.49–1.28)	1.89** (1.22–2.90)	2.12* (1.38–3.29)			
Gentamicin	1.11 (0.87–1.42)	0.91 (0.74–1.13)	0.75** (0.60–0.92)	1.29** (1.12–1.48)	0.98 (0.84–1.14)	0.52 (0.43–0.63)
Marbofloxacin[a]	1.18 (085–1.63)	0.73 (0.55–0.98)	0.70* (0.52–0.93)	2.71** (1.46–5.04)	0.65** (0.51–0.83)	0.15 (0.12–0.18)
Marbofloxacin: interaction between UTI and year	1.81 (0.73–4.48)	1.59 (0.74–3.42)	0.37** (0.18–0.76)			
Orbifloxacin[a,b]			0.97 (0.83–1.14)	3.79** (2.52–5.70)	0.72** (0.61–0.85)	0.85 (0.75–0.96)
Orbifloxacin: interaction between UTI and year			0.27** (0.17–0.43)			
Pradofloxacin[a,b]			0.89 (0.76–1.04)	3.49** (2.31–5.83)	0.77** (0.66–0.91)	0.22 (0.19–0.24)
Pradofloxacin: interaction between UTI and year			0.35** (0.22–0.55)			
Tetracycline	0.93 (0.77–1.11)	1.15 (0.96–1.38)	1.16 (0.96–1.42)	0.74** (0.64–0.87)	0.82** (0.72–0.94)	1.69 (1.40–2.04)
Trimethoprim-sulfamethoxazole	1.14 (1.00–1.29)	1.09 (0.96–1.24)	0.95 (0.83–1.08)	1.82** (1.67–1.98)	0.94 (0.85–1.04)	0.39 (0.35–0.43)

A concentration ratio > 1 indicates larger MICs compared to the reference level; a concentration ratio < 1 indicates smaller MICs compared to the reference level. Baseline represents median MIC in the baseline population.

*P < .05. **P < .01. [a]Model for these antimicrobials included a significant interaction that is presented in the row below. [b]Reference year period for these antimicrobials was 2016–2019 due to lack of data in the previous years.

Discussion

E coli is recognized as a sentinel microorganism for AMR surveillance against gram-negative active antimicrobials.[6] Its ability to thrive in various hosts and environments underscores its significance in the one-health context.[6–9] Therefore, it is essential to analyze longitudinal AMR trends beyond susceptible-resistant classifications. In this study, survival accelerated failure time models were used as an innovative approach to assess MIC data and detect changes in bacterial tolerance to antimicrobials over time, a phenomenon known as "MIC creep."[24]

An increase in cephalexin MICs from 2016 to 2022 and cefovecin MICs from 2012 to 2019 was observed, mirroring similar cephalosporin susceptibility trends observed in *E coli* isolated from canines in the same geographic area.[18] Interestingly, cefovecin MICs increased from 2016 to 2019 followed by a decrease from 2020 to 2022 (Table 1; Table 4). Cefovecin is a long-acting injectable third-generation cephalosporin. This class of antimicrobials is critically important in human medicine.[25] While cefovecin is not employed in human medicine, it is important to note that resistance to cephalosporins typically stems from a limited number of mechanisms that can also provide resistance to other cephalosporins,[26] some of which are used in human medicine. This raises concerns about the fact that cefovecin is one of the most frequently prescribed antimicrobials in cats for common conditions such as abscesses, wounds, dermatitis, and urinary tract infections,[27–29] despite the guidelines indicating that its use should be restricted only when oral treatment is not possible and after

culture and susceptibility testing.[30,31] This practice likely exerts increased selective pressure on feline pathogens and commensal bacteria, which could result in higher cefovecin resistance rates and elevated cefovecin MICs. The reasons behind the lower MICs after 2020 are unknown and were not evaluated in canine isolates[18]; data on cefovecin use associated with these bacterial infections are not available. We found a higher overall *E coli* susceptibility to cefovecin than a study[9] of Spanish feline isolates from 2016 to 2018 (75% susceptible) and a United Kingdom study[32] of feline isolates from 2011 to 2012 (41% susceptible). A recent study[12] in the US reported high *E coli* overall susceptibility to third-generation cephalosporins (83%), with significant differences between states. A similar overall susceptibility to cefovecin and third-generation cephalosporins (Table 2) was found in this study, although susceptibility dipped below 80% from 2013 to 2019 (Figure 1). These geographical differences in susceptibility highlight the importance of frequently analyzing regional data to inform empiric antimicrobial treatment choices.

Furthermore, the extensive use of cephalosporins, including cefovecin, has been a major contributor to the development of extended-spectrum β-lactamase bacteria and MDR.[6] extended-spectrum β-lactamase bacteria often exhibit coresistance to many other classes of antibiotics, severely limiting therapeutic options in veterinary and human medicine.[33] Alarmingly, almost one-third of the isolates in this study were classified as multidrug resistant, a similar proportion to the 26% reported in canine isolates from the same laboratory during a similar time period,[18] but higher than reported in feline isolates from Saudi Arabia (18%).[34] MDR *E coli* poses a global health threat, rapidly spreading through horizontal gene transfer of mobile genetic elements.[35] The rise of MDR *E coli* causing urinary tract infections is currently a global one-health issue.[36] Interestingly, a decreasing trend of MDR from feline UTIs over the years was identified (Figure 2). This finding aligns with recent research on human isolates from the US.[37] Large-scale studies of *E coli* using the one-health approach are necessary to gain a more comprehensive understanding of AMR dynamics associated with interspecies transmission. The clinical implications of MDR are substantial, including delays in initiating appropriate antimicrobial therapy, as empirically prescribed antibiotics may prove ineffective.[6]

Most of the *E coli* isolates in this study were obtained from UTIs, consistently displaying higher MIC values for most of the antimicrobials tested compared to isolates from other body sites (Table 4). *E coli* is the leading cause of urinary tract infections not only in dogs and cats,[38] but also in humans,[36] and is often associated with resistant and complicated urinary tract infections.[32,36] The first-line treatments for urinary tract infections in companion animals typically include amoxicillin or amoxicillin-clavulanic acid, as well as trimethoprim-sulfamethoxazole.[30] The results from this analysis suggest that amoxicillin may not be an appropriate empiric option because only 74% of UTIs were susceptible to ampicillin (Table 2). Amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole remain appropriate initial or empirical options for bacterial urinary tract infections in cats (overall susceptibility of 90% and 94%, respectively). The amoxicillin-clavulanic acid susceptibility found in this analysis is higher than the approximately 80% susceptibility reported in other studies,[9,12,32] which could raise concerns about amoxicillin-clavulanic acid as empiric therapy for urinary tract infections.

It is important to note that cumulative AMR data from diagnostic laboratories may underestimate the real susceptibility because diagnostic laboratory culture submissions for companion animals may be biased toward resistant isolates. Considering that the cost of the culture is borne by the animal owners, sporadic infections may be less likely to be cultured than complicated, recurrent, or refractory infections, which may have a higher prevalence of resistance. Culture and susceptibility testing was offered free to all clients at the Cornell University Hospital for Animals from June 2021 to July 2022, which comprises a subset (18%) of all the AHDC susceptibility data that we analyzed. Therefore, we expect the AHDC susceptibility data from this time period to have reduced bias towards complicated infections; approximately 90% to 94% of UTIs were susceptible to amoxicillin-clavulanic acid during 2021 and 2022 (Figure 1).

The lack of trimethoprim-sulfamethoxazole cat-specific breakpoints (Supplementary Table S1), as well as the potential adverse events, should be considered when using this drug as a first-line empiric choice for feline urinary tract infections. This analysis uses a human-specific breakpoint for trimethoprim-sulfamethoxazole, and drug pharmacokinetics may be different in cats, leading to incongruence between in vitro susceptibility test results and clinical outcomes. The susceptibility prevalence to trimethoprim-sulfamethoxazole in our study is similar to a previous study in the United Kingdom (92%)[32] but greater than the 77% reported in Switzerland[39] and 86% susceptible reported in Hong Kong,[40] indicating a possible emergence of resistance to this antimicrobial in other countries and posing a threat to feline *E coli* urinary tract infection treatment.

Finally, beta-hemolytic *E coli* isolates exhibited significantly lower MIC values for cephalexin, ceftiofur, fluoroquinolones, and tetracycline (Table 4). This is an underreported feature; most studies about resistance in *E coli* do not mention any association with beta-hemolysis. One study[41] from Spain reported a lower proportion of resistance to quinolones and tetracyclines in human clinical beta-hemolytic isolates. Hemolysin is a virulence factor in strains causing different extraintestinal infections and it has been studied in the context of disease pathogenesis.[42] Additional research about the association of hemolysis and AMR is needed to understand the implications of this result.

Despite these insights, this study has limitations, including the inability to ensure only 1 sample per patient due to the lack of specific patient identifiers

and the lack of population demographics and exposure to risk factors, such as antimicrobial use, in the dataset. In addition, extrapolating breakpoints from humans and other animal species to classify feline-associated bacteria as susceptible or resistant could lead to misclassification.[43] Changes in MIC breakpoints, as well as changes in dilutions and antimicrobials from commercial plates over time, make it difficult to establish comparisons and trends over the years. For example, it was not possible to classify any isolates as susceptible to doxycycline due to a recent change in the breakpoints that is not reflected in the historical commercial testing panels.

In conclusion, the use of survival accelerated failure time models allowed a nuanced assessment of MIC data, beyond the susceptible-resistant classification, revealing MIC changes over time and associations with sample site and beta-hemolysis. There were variable trends in cephalosporin MICs that need to be monitored given the frequency of third-generation cephalosporin use in cats. Feline *E coli* therapeutic options may be compromised due to the high proportion of MDR, although amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole remain appropriate first-line treatments for urinary tract infections.

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Supplementary Materials

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