



# Detection of *Escherichia coli* and *Enterococcus* spp. in dogs with polymicrobial urinary tract infections: A 5-year retrospective study

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## Abstract

**Background:** Urinary tract infections (UTI) caused by *Escherichia coli* and *Enterococcus* spp., which are frequently coisolated in polymicrobial UTI, cause morbidity among dogs and warrant antimicrobial therapy.

**Objectives:** To evaluate clinical features of dogs with polymicrobial *E. coli* and Enterococcal UTI.

**Animals:** Forty-four client-owned dogs with polymicrobial bacteriuria and groups of 100 client-owned dogs with *E. coli* and Enterococcal monomicrobial bacteriuria.

**Methods:** Retrospective cohort study of medical records of dogs at a university teaching hospital from 2014 to 2019. Prevalence of recurrent UTI and isolate antimicrobial resistance were determined. Clinical outcomes of dogs with recurrent UTI from groups including cost and hospital visits were compared.

**Results:** Recurrent UTI was more prevalent ( $P = .05$ ) in dogs with polymicrobial bacteriuria (57%, 95% confidence interval [95% CI]: 42%-70%) compared to the Enterococcal monomicrobial group (40%, 95% CI: 31%-50%). *Escherichia coli* from polymicrobial bacteriuria were more frequently resistant to doxycycline ( $P < .01$ , 43%, 95% CI: 29%-58%) and gentamicin ( $P = .03$ , 17%, 95% CI: 9%-31%) compared to *E. coli* from monomicrobial bacteriuria (17% and 5%, 95% CI: 11%-26% and 2%-11% for doxycycline and gentamicin, respectively). Dogs with recurrent UTI from the polymicrobial UTI group had significantly ( $P = .05$ ) more hospital visits (mean = 6 visits, 95% CI: 1.7-9.8) compared to recurrent monomicrobial UTI dogs (mean = 4 and 3 visits, 95% CI: 1.0 to 4.4 and -0.7 to 7.7 for *E. coli* and Enterococcal monomicrobial UTI, respectively).

**Abbreviations:** CFU, colony forming units; MDR, multidrug resistance; MIC, minimum inhibitory concentration; NCSU-VH, North Carolina State University Veterinary Hospital; PB, polymicrobial bacteriuria; SEC, single agent *E. coli*; SENT, single agent *Enterococcus* spp.; UPEC, uropathogenic *E. coli*; UTI, urinary tract infection.

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**Conclusions and Clinical Importance:** *Escherichia coli* and *Enterococcus* spp. polymicrobial UTI had more frequent adverse clinical outcomes for dogs.

**KEYWORDS**

antibiotics, bacteriuria, cystitis, urine culture

## 1 | INTRODUCTION

Bacterial cystitis in dogs due to urinary tract infections (UTIs) is an important cause of morbidity and therefore warranting antimicrobial therapy.<sup>1,2</sup> Affected dogs present with varying underlying conditions, frequency of recurrence, and clinical signs.<sup>1</sup> Urinary tract infection can be categorized as sporadic, recurrent, or upper/ascending UTI (pyelonephritis); each have different therapeutic guidelines and sequelae.<sup>1</sup> Recurrent UTI in otherwise healthy dogs can be frustrating to treat and costly for pet owners and clinicians due to multiple rounds of therapy.<sup>2</sup> Of the many causative agents of UTI, *E. coli* and *Enterococcus* are among the most common in dogs as they are isolated in 55% and 23% of all urine cultures from dogs with UTI, respectively.<sup>3,4</sup> Antimicrobial resistance is common among *E. coli* and *Enterococcus* isolated from the urine of dogs with UTI, which further complicates therapy and can even result in zoonotic, community acquired UTI when pet owners are exposed.<sup>5-7</sup> *Escherichia coli* and *Enterococcus* are frequently coisolated in urine cultures from dogs with polymicrobial and recurrent UTI,<sup>2,3</sup> pyelonephritis,<sup>8</sup> and catheter-associated urinary tract infections.<sup>9</sup>

Although coisolation of *E. coli* with other uropathogens occurs in dogs with UTI (21%-34%),<sup>4,10</sup> it is unclear if the clinical features and outcomes of dogs with polymicrobial UTI differ from those where *E. coli* is the only isolated agent. One treatment recommendation for polymicrobial UTI where *Enterococcus* spp. are coisolated is to target the other agent(s) with antimicrobial therapy and allow the *Enterococcus* spp. infection to self-resolve.<sup>11</sup> However, Enterococci are intrinsically resistant to many drugs used to treat UTI in dogs<sup>1</sup> and experimental evidence demonstrates that of *Enterococcus faecalis* promotes growth and immune evasion of uropathogenic *E. coli* (UPEC) strains in vitro and in vivo.<sup>12,13</sup> Despite the frequency of coinfection with these 2 common causes of UTI in dogs, it remains unclear if or how these interactions impact the clinical presentation and outcomes of dogs with polymicrobial UTI. No study to date has retrospectively examined polymicrobial *E. coli* and *Enterococcus* spp. UTI in dogs.

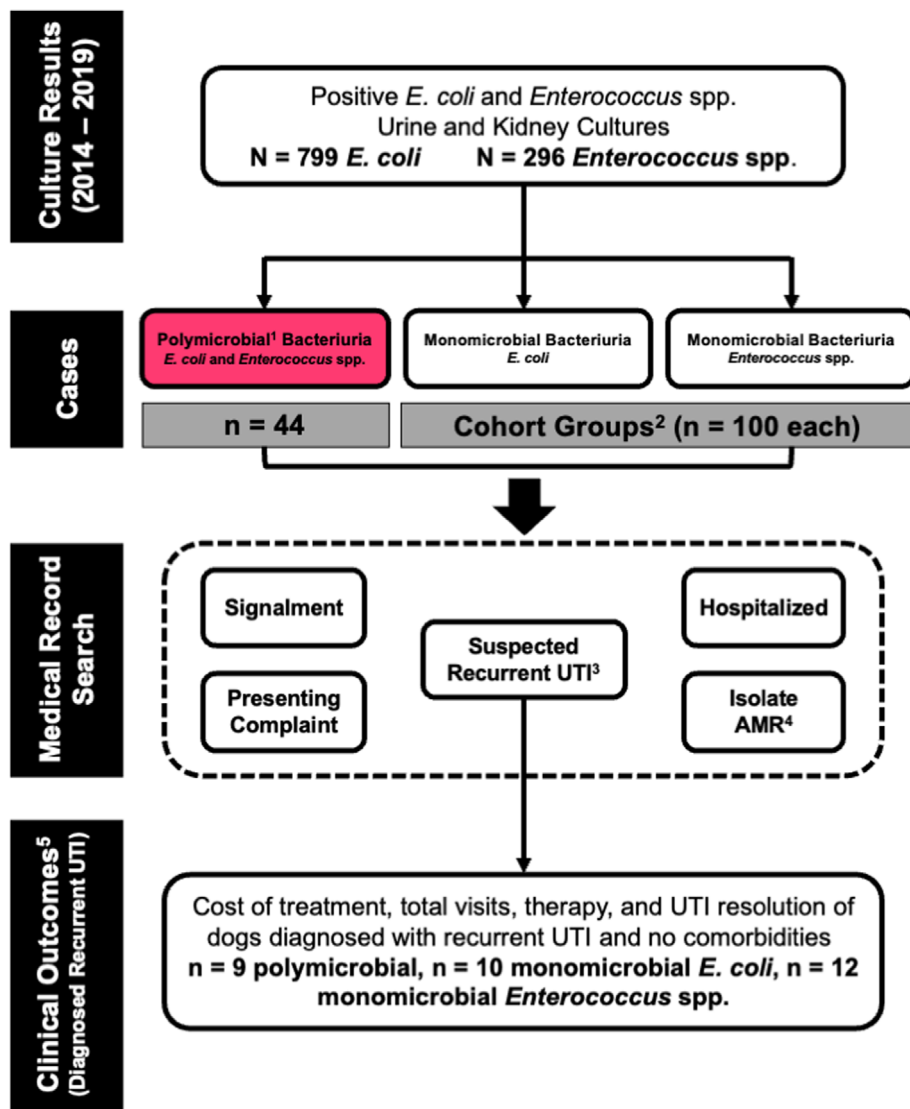
We hypothesize that coinfection of *E. coli* and *Enterococcus* is more likely to result in treatment difficulties such as greater rates of recurrent UTI and increased cost when compared to single agent UTI. The goal of the present 5-year retrospective study is to determine the clinical presentations and outcomes of therapy in dogs initially diagnosed with polymicrobial bacteriuria where *E. coli* and *Enterococcus* were isolated, as well as to further characterize antimicrobial resistance phenotypes among isolates.

## 2 | MATERIALS AND METHODS

The overall study design and procedure for data extraction are indicated in Figure 1. To identify dogs with urine or postmortem kidney cultures where *E. coli* and *Enterococcus* spp. were recovered, aerobic culture results for 5 calendar years (October 2014 to October 2019) from the Microbiology and Molecular Diagnostic Laboratory at the North Carolina State University (Raleigh, North Carolina) Veterinary Hospital (NCSU-VH) were searched. Associated medical records were reviewed and dogs were categorized as having either polymicrobial bacteriuria (containing only *E. coli* and *Enterococcus* spp., [PB]) or single agent *E. coli* ( $S_{EC}$ ) or *Enterococcus* spp. ( $S_{ENT}$ ) in pure culture (monomicrobial) bacteriuria. Dogs were categorized as having polymicrobial bacteriuria if (1) mixed cultures of *E. coli* and *Enterococcus* spp. were recovered simultaneously in a single culture or (2) single agents were recovered independently in serial cultures within a 1-week period to account for dogs that either had undetected polymicrobial bacteriuria at first culture or developed this condition. Control cohorts of 100 dogs each with single agent *E. coli* and *Enterococcus* spp. bacteriuria were selected during the same 5-year period. Control cohorts were selected using computerized random selection of dogs diagnosed with a monomicrobial urine culture result from a sample obtained by catheterization (>1000 CFU/mL) or cystocentesis (any growth). Dogs were initially assigned to these study groups based on urine culture results independent of clinical signs of UTI. Thereafter, dogs with suspected recurrent UTI were defined as (1) having at least 2 UTIs caused by either *E. coli* or *Enterococcus* spp. (confirmed by aerobic bacterial culture) with associated clinical signs of UTI such as hematuria and stranguria within a 6-month period<sup>3</sup> or (2) if recurrent UTI was noted in the history by the attending clinician. For comparisons of overall treatment costs and clinical outcome after therapy, nonhospitalized dogs were designated as having confirmed UTI if they met the following criteria: had suspected recurrent UTI (as defined above), presented primarily for overt clinical signs associated with UTI, and had no comorbidities. Antimicrobial drugs administered to treat recurrent UTI were recorded for these dogs, and follow up cultures were used to measure treatment efficacy (determined by presence or absence of bacteriuria) at these follow up appointments.

### 2.1 | Signalment and presenting complaint

Breeds were initially assigned to 1 of 7 breed groups defined by the American Kennel Club breed groups and consolidated as follows: sport (sporting and hound), toy (toy and terrier), work (working and



**FIGURE 1** Retrospective cohort study design and data collection.

<sup>1</sup>Polymicrobial UTI were identified if (1) mixed cultures of *Escherichia coli* and *Enterococcus* spp. were recovered simultaneously in a single culture or (2) single agents were recovered independently in serial cultures within a 1-week period. <sup>2</sup>Cohort groups were assigned by randomly selecting dogs diagnosed with a monomicrobial urine culture result from a sample obtained by catheterization (>1000 CFU/mL) or cystocentesis (any growth). <sup>3</sup>Dogs with suspected recurrent UTI were defined as (1) having at least 2 UTIs caused by either *E. coli* or *Enterococcus* spp. (confirmed by aerobic bacterial culture) within a 6-month period<sup>3</sup> or (2) if recurrent UTI was noted in the dogs' history by the attending clinician. <sup>4</sup>AMR = antimicrobial resistance as determined by minimum inhibitory concentrations (MIC) for amoxicillin/clavulanic acid, ampicillin, doxycycline, enrofloxacin, and gentamicin. <sup>5</sup>Clinical outcomes of nonhospitalized dogs diagnosed with recurrent UTI and no known comorbidities including costs of UTI-related treatment (normalized for inflation), number of hospital visits, antimicrobial therapy, and UTI resolution diagnosed by a negative follow-up culture

herding), and other (including mixed breed groups). Dogs were assigned to the following groups based on presenting complaint and the receiving hospital service when the first UTI was diagnosed: urinary tract infections, neurology, oncology, or other (cardiology, internal medicine, and multiple services). Dogs were designated as hospitalized if the duration from receiving to discharge exceeded 24 hours.

## 2.2 | Urine collection, culture, and isolate species

Urine samples were collected by cystocentesis or sterile catheterization by the attending clinician as part of the normal case work-up. Voided urine and postmortem kidney samples were included in special cases as follows. Due to the potential for contamination, voided samples were only included if they were collected midstream by the attending clinician and the urine culture resulted in growth that exceeded 100 000 CFU/mL for either *E. coli*, *Enterococcus* spp., or

both as consistent with previously published guidelines for UTI diagnosis based on bacteriuria in voided samples.<sup>14</sup> Voided samples were not included in cohort control groups, and postmortem kidney samples were kept consistent in all 3 groups examined (1 per group). All samples were transported to the onsite laboratory in a universal transport medium (Port-A-Cul, BD, Franklin Lakes, New Jersey) at ambient temperature per the manufacturer quality control instructions until plating. Ten microliters of the sample was aseptically plated on 5% sheep blood agar and MacConkey agar and incubated for 24 hours at 37°C after which purity was assessed by visual inspection. When mixed cultures were identified based on visual observation of inconsistent phenotypic characteristics, each suspected species was subcultured until pure cultures were obtained before independent speciation of each. Isolate speciation was achieved by matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry or automated biochemistry panels according to the manufacturer's protocol (Vitek MS, Biomerieux; Marcy-l'Étoile, France).

## 2.3 | Urinalysis

Urinalyses were conducted during the same visit as urine culture sample collection for each dog in the Clinical Pathology and Immunology Laboratory at the NCSU-VH. Urine specific gravity was measured using a digital refractometer (Pal Abbe Digital Refractometer #PA202, MISCO Cleveland, Ohio) and chemical analysis performed using commercially available dipsticks (Chemstrip 10, Roche Diagnostics, Mannheim Germany) with a calibrated digital analyzer. Sediment examination was conducted by centrifuging 5 mL of the urine sample for 5 minutes at 1600 rpm, removing the supernatant, resuspending the sediment, and examining an unstained aliquot (10-15 microscopic fields at 50 $\times$  and 400 $\times$  magnification). The following information was included in the final analysis: pH, urine specific gravity (USG), proteinuria (mg/dL), hematuria (RBC/ $\mu$ L), pyuria (WBC/HPF), and bacteriuria. For statistical analysis, data were consolidated and converted to categorical variables as follows: proteinuria (negative/trace or 30-500 mg/dL), hematuria ( $\leq 5$  or  $> 5$  RBC/ $\mu$ L), pyuria ( $\leq 5$  or  $> 5$  WBC/HPF) and gross bacteriuria (negative/trace/1+ or 2+ to 4+).

## 2.4 | Bacterial isolates and susceptibility testing

Antimicrobial susceptibility was determined for each isolate using the broth microdilution method on a commercial system (Sensititre; ThermoFisher Scientific, Waltham, Massachusetts). Companion-animal-based panels (typically CMV3AGNF and CMV3AGPF; ThermoFisher Scientific, Waltham, Massachusetts) were used for *E. coli* and *Enterococcus* spp. isolates, respectively. The minimum inhibitory concentrations (MIC) were recorded for amoxicillin/clavulanic acid, ampicillin, doxycycline, enrofloxacin, gentamicin, and trimethoprim/sulfadimethoxole as these were the most consistently reported and represent multiple classes of antimicrobial drugs used to treat UTI in dogs.<sup>15</sup> Isolates were designated as susceptible or resistant based on breakpoints available from the Clinical & Laboratory Standards Institute (CLSI) at the time of culture over the 5-year period.<sup>16</sup> Isolates that exhibited intermediate resistance were designated as susceptible. MIC<sub>50</sub> values were calculated according to criteria for quantitative interpretation of the results.<sup>17</sup> Clinical breakpoints for treating UTI in dogs were used when available.<sup>16</sup> Otherwise, interpretations were based on human breakpoints. An isolate was denoted as multidrug resistant (MDR) if it exhibited resistance to 3 or more classes of antimicrobial drugs.

## 2.5 | Statistical analysis

Comparisons between groups were conducted using 2-sided hypothesis tests and a *P* value of  $\leq .05$  as the criterion for determining associations. Analyses were conducted by comparing the polymicrobial group (*E. coli* and *Enterococcus* spp.) to the monomicrobial control groups independently. A 1-way ANOVA was used to analyze urine specific gravity and pH. Categorical data were analyzed via the Fisher exact

test for 2 variables and 95% confidence intervals (95% CI) calculated for proportions where appropriate. Accrued UTI-related medical costs (eg, urine culture, prescriptions) for selected dogs with recurrent UTI were adjusted for inflation by converting US dollar values to October 2019 values using data from the US Bureau of Labor Statistics.<sup>18</sup> Distribution normality of outcome variables were assessed by the goodness-of-fit test (Shapiro-Wilk) and means from nonnormal distributions (adjusted cost and number of visits) compared using the non-parametric Kruskal-Wallis test by ranks and 95% CI. All analyses were performed using commercially available software (JMP, Version 15. SAS Institute Inc, Cary, North Carolina).

## 3 | RESULTS

### 3.1 | Study cohort

Forty-four dogs with bacteriuria due to *E. coli* and *Enterococcus* spp. met the inclusion criteria for PB. Each control cohort consisted of 100 randomly selected dogs with monomicrobial bacteriuria for a total of 244 dogs included in this study. The demographics, recurrent UTI prevalence, and hospitalization status of all dogs are shown in Table 1. Spayed female dogs were most prevalent in each group, with 73% ( $n = 32/44$ ) in the PB group; 65% ( $n = 65/100$ ) in the *S*<sub>EC</sub> group, and 73% ( $n = 73/100$ ) in the *S*<sub>ENT</sub> group. Intact male and female dogs were least common among the 3 groups ( $< 10\%$  in all groups). Sporting breeds (sporting and hound) were most prevalent in the PB (30%,  $n = 13/44$ ) and *S*<sub>EC</sub> (31%,  $n = 31/100$ ) groups while the toy breeds (toy and terrier) were slightly more prevalent (30%,  $n = 30/100$ ) in the *S*<sub>ENT</sub> group than the sporting breeds (29%,  $n = 29/100$ ). There were no effects of age ( $P = .94$  and  $.52$ ), sex and intact status ( $P = .45$  and  $.58$ ), or breed group ( $P = .77$  and  $.82$ ) when comparing PB group to each of the control groups. However, there was a significantly ( $P = .05$ ) greater prevalence of suspected recurrent UTI (57%,  $n = 25/44$ , 95% CI: 42%-70%) and greater ( $P < .01$ ) hospitalization (43%,  $n = 19/44$ , 95% CI: 30%-58%) among dogs with PB only when compared to the *S*<sub>ENT</sub> group (40% ( $n = 40/100$ , 95% CI: 31%-50%) suspected recurrent UTI, 10% ( $n = 10/100$ , 95% CI: 5%-17%) hospitalized). There were no differences among distributions of the receiving hospital service (data not shown).

### 3.2 | Isolate characterization and antimicrobial resistance

Forty-four each of *E. coli* and *Enterococcus* spp. were coisolated (PB) from urine samples of 44 different dogs. These were compared to *E. coli* or *Enterococcus* spp. pure culture (*S*<sub>EC</sub> and *S*<sub>ENT</sub>, respectively) isolates from 2 cohorts of 100 dogs. The PB group consisted of 21 *Enterococcus faecium* (48%), 18 *E. faecalis* (41%), and other species including *Enterococcus durans*, *Enterococcus gallinarum*, and *Enterococcus hirae* (1 of each, 2%). The species distribution in the *S*<sub>ENT</sub> group consisted of 51 *E. faecalis* (56%), 27 *E. faecium* (30%), 2 *E. durans* (2%),

|                            |                 | PB<br>No. (%)        | S <sub>EC</sub><br>No. (%) | P<br>PB vs S <sub>ENT</sub> | S <sub>ENT</sub><br>No. (%) | P<br>PB vs S <sub>ENT</sub> |
|----------------------------|-----------------|----------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Age (years)                | ≤5              | 16 (36)              | 37 (37)                    | .94                         | 42 (42)                     | .52                         |
|                            | >5              | 28 (64)              | 63 (63)                    |                             | 58 (58)                     |                             |
| Sex                        | Female, intact  | 1 (2)                | 7 (7)                      | .45                         | 4 (4)                       | .58                         |
|                            | Female, spayed  | 32 (73)              | 65 (65)                    |                             | 73 (73)                     |                             |
|                            | Male, intact    | 2 (5)                | 9 (9)                      |                             | 1 (1)                       |                             |
|                            | Male, castrated | 9 (20)               | 19 (19)                    |                             | 22 (22)                     |                             |
| Breed grouping             | Sport           | 13 (30)              | 31 (31)                    | .77                         | 29 (29)                     | .82                         |
|                            | Toy             | 10 (23)              | 21 (21)                    |                             | 30 (30)                     |                             |
|                            | Work            | 9 (20)               | 27 (27)                    |                             | 18 (18)                     |                             |
|                            | Other           | 12 (27)              | 21 (21)                    |                             | 23 (23)                     |                             |
| Recurrent UTI <sup>a</sup> |                 | 25 (57) <sup>A</sup> | 50 (50)                    | .45                         | 40 (40) <sup>B</sup>        | .04                         |
| Hospitalized               |                 | 19 (43) <sup>A</sup> | 44 (44)                    | .93                         | 10 (10) <sup>B</sup>        | <.01                        |

Note: <sup>A,B</sup> Means within a row with no common superscripts differ significantly ( $P \leq .05$ ).

<sup>a</sup>Dogs were designated as having suspected recurrent UTI if at least 2 UTIs of either *E. coli* or *Enterococcus* spp. were confirmed by aerobic bacterial culture within a 6-month period or if recurrent UTI was noted in the dog's history by the attending clinician.

<sup>b</sup>Dogs were designated as hospitalized if the time from receiving to discharge exceeded 24 hours.

**TABLE 1** Signalment (age, sex, and breed grouping), prevalence of suspected recurrent UTI<sup>a</sup>, and hospitalization status<sup>b</sup> of dogs with polymicrobial (PB) or monomicrobial bacteriuria due to *Escherichia coli* (S<sub>EC</sub>) and *Enterococcus* spp. (S<sub>ENT</sub>) over a 5-year period

**TABLE 2** Prevalence of drug-resistant *Escherichia coli* and *Enterococcus* spp. isolates from polymicrobial (n = 44) or monomicrobial (n = 100) bacteriuria of dogs over a 5-year period

| Drug                        | <i>E. coli</i>          |   |                         |                            | P    | <i>Enterococcus</i> spp. |                            |                       |                            | P   |
|-----------------------------|-------------------------|---|-------------------------|----------------------------|------|--------------------------|----------------------------|-----------------------|----------------------------|-----|
|                             | Polymicrobial           |   | Monomicrobial           |                            |      | Polymicrobial            |                            | Monomicrobial         |                            |     |
|                             | Resistance<br>No. (%)   | MIC <sub>50</sub> <sup>a</sup><br>μg/mL | Resistance<br>No. (%)   | MIC <sub>50</sub><br>μg/mL |      | Resistance<br>No. (%)    | MIC <sub>50</sub><br>μg/mL | Resistance<br>No. (%) | MIC <sub>50</sub><br>μg/mL |     |
| Amoxicillin/CA <sup>b</sup> | N/A <sup>c</sup>        | >1                                      | N/A                     | >1                         | .12  | 4/28 (14)                | 1                          | 7/77 (9)              | 1                          | .46 |
| Ampicillin                  | 21/22 (95)              | 8                                       | 48/48 (100)             | 4                          | .13  | 4/25 (16)                | 1                          | 8/76 (11)             | 1                          | .48 |
| Doxycycline                 | 17/40 (43) <sup>A</sup> | 4                                       | 17/98 (17) <sup>B</sup> | 2                          | <.01 | 4/28 (14)                | ≤2                         | 12/72 (17)            | ≤2                         | .77 |
| Enrofloxacin                | 14/40 (35)              | ≤0.25                                   | 22/99 (22)              | ≤0.25                      | .13  | 12/21 (57)               | 2                          | 23/52 (44)            | 1                          | .32 |
| Gentamicin                  | 7/41 (17) <sup>A</sup>  | ≤1                                      | 5/99 (5) <sup>B</sup>   | ≤1                         | .03  | 21/32 (66)               | >8                         | 53/79 (67)            | >8                         | .88 |
| TMS <sup>d</sup>            | 11/41 (27)              | ≤0.5                                    | 23/98 (23)              | ≤0.5                       | .68  | 20/20 (100)              | >2                         | 43/48 (90)            | >2                         | .06 |

Note: <sup>A,B</sup> Means within a column with no row superscripts differ significantly ( $P \leq .05$ ).

<sup>a</sup>MIC<sub>50</sub> = minimum inhibitory concentration value at which 50% of isolates in a group were inhibited by the selected drug.

<sup>b</sup>CA = clavulanic acid.

<sup>c</sup>N/A = not applicable; these data were generated with breakpoints that are no longer used to interpret urine MICs of Amoxicillin/CA for *E. coli*.

<sup>d</sup>TMS = trimethoprim/sulfamethoxazole.

and 1 each of *Enterococcus cecorum* and *Enterococcus avium* (1%). Eleven *Enterococcus* isolates were unable to be identified at the species level.

Designation as MDR did not differ between isolate groups (data not shown). Antimicrobial resistance phenotypes of isolates are shown in Table 2. Using a MIC of 8 μg/mL, *E. coli* isolated from PB were more resistant ( $P < .05$ ) to doxycycline (43%, n = 17/40, 95% CI: 29%-58%) and gentamicin (17%, n = 7/41, 95% CI: 9%-31%) when compared to single agent isolates (doxycycline: 17%, n = 17/98, 95% CI: 11%-26%; gentamicin: 5%, n = 5/99, 95% CI: 2%-11%). Resistance to selected drugs was not different between the PB and S<sub>ENT</sub> groups. Enterococcal resistance to trimethoprim/sulfamethoxazole was 100%

and 90% for polymicrobial and monomicrobial groups, respectively. Of note, in vitro antimicrobial resistance phenotypes for this drug do not correspond to in vivo resistance because Enterococci can incorporate exogenous folates from host urine and reverse its effects.<sup>19</sup>

### 3.3 | Urinalysis

Urinalyses of dogs from each group are shown in Table 3. Dogs with PB had significantly reduced prevalence of pyuria (45%, n = 17/38, 95% CI: 30%-60%,  $P = .04$ ) and gross bacteriuria (53%, n = 20/38, 95% CI: 37%-67%,  $P = .02$ ) when compared to S<sub>EC</sub> (pyuria: 63%,

**TABLE 3** Urinalysis variables of dogs with polymicrobial *Escherichia coli* and *Enterococcus* spp. (PB, n = 44) or monomicrobial *E. coli* ( $S_{EC}$ ) and *Enterococcus* spp. ( $S_{ENT}$ ; n = 100 each) urine culture results over a 5-year period

| Variable                | Degree         | PB                      | $S_{EC}$                | P<br>PB vs $S_{EC}$ | $S_{ENT}$  | P<br>PB vs $S_{ENT}$ |
|-------------------------|----------------|-------------------------|-------------------------|---------------------|------------|----------------------|
| Specific gravity        | -              | 1.025                   | 1.021                   | .07                 | 1.028      | .27                  |
| pH                      | -              | 6.68                    | 6.87                    | .36                 | 6.76       | .68                  |
|                         |                | No. (%)                 |                         |                     | No. (%)    |                      |
| Protein<br>(mg/dL)      | Negative/trace | 18/38 (47)              | 51/94 (54)              | .47                 | 32/84 (38) | .34                  |
|                         | 1+ to 3+       | 20/38 (53)              | 43/94 (46)              |                     | 52/84 (62) |                      |
| Blood<br>(RBC/ $\mu$ L) | 0-5            | 10/38 (26)              | 18/94 (19)              | .37                 | 34/84 (40) | .13                  |
|                         | 5 to >50       | 28/38 (74)              | 76/94 (81)              |                     | 50/84 (60) |                      |
| WBC<br>(WBC/hpf)        | 0-5            | 21/38 (55)              | 35/94 (37)              | .04                 | 51/84 (61) | .57                  |
|                         | 5 to >50       | 17/38 (45) <sup>B</sup> | 59/94 (63) <sup>A</sup> |                     | 33/84 (39) |                      |
| Bacteria                | Negative/1+    | 18/38 (47)              | 24/94 (26)              | .02                 | 44/84 (52) | .60                  |
|                         | 2+ to 4+       | 20/38 (53) <sup>B</sup> | 70/94 (74) <sup>A</sup> |                     | 40/84 (48) |                      |

Note: <sup>A,B</sup> Means within a row with no common superscripts differ significantly ( $P \leq .05$ ).

**TABLE 4** Total cost of therapy and clinical outcomes of dogs that presented with recurrent UTI that was either polymicrobial (*Escherichia coli* and *Enterococcus* spp., PB), monomicrobial *E. coli* ( $S_{EC}$ ), or monomicrobial *Enterococcus* spp. ( $S_{ENT}$ ) over a 5-year period

| Group     | Dogs | Adjusted cost <sup>a</sup> /dog    | P   | Hospital visits <sup>b</sup> /dog | P   | UTI resolved <sup>c</sup> |
|-----------|------|------------------------------------|-----|-----------------------------------|-----|---------------------------|
|           | n    | Mean $\pm$ SD (95% CI)             |     | Mean $\pm$ SD (95% CI)            |     |                           |
| PB        | 9    | \$1457 $\pm$ \$1295 (\$462-\$2452) | .78 | 6 <sup>A</sup> $\pm$ 5 (2-10)     | .05 | 2/6                       |
| $S_{EC}$  | 10   | \$1082 $\pm$ \$1050 (\$332-\$1833) |     | 4 <sup>B</sup> $\pm$ (0-8)        |     | 3/3                       |
| $S_{ENT}$ | 12   | \$987 $\pm$ \$768 (\$499-\$1475)   |     | 3 <sup>B</sup> $\pm$ (1-5)        |     | 3/4                       |

Note: <sup>A,B</sup> Means within a column with no common superscripts differ significantly ( $P \leq .05$ ).

<sup>a</sup>The total dollar cost for all UTI-related hospital visits was adjusted for inflation by converting US dollar values to October 2019 dollar values using data from the US Bureau of Labor Statistics.<sup>18</sup> Means were compared with the nonparametric Kruskal-Wallis test by ranks.

<sup>b</sup>Total visits included all billable UTI-related hospital visits that accrued after an initial UTI diagnosis by aerobic culture for a given dog. Means were compared with the nonparametric Kruskal-Wallis test by ranks.

<sup>c</sup>Resolved UTIs were determined for dogs with follow up cultures that resulted in no growth vs those having positive culture results of either *E. coli* or *Enterococcus* spp.

n = 59/94, 95% CI: 53%-72%; gross bacteriuria: 74%, n = 70/94, 95% CI: 65%-82%). In general, urinalyses of dogs with PB more closely resembled those with  $S_{ENT}$ , with no significant differences noted in PB and  $S_{ENT}$  groups.

### 3.4 | Clinical outcomes of dogs with recurrent UTI

Suspected recurrent UTI defined as having at least 2 UTIs in a 6-month period or recurrent UTI noted in the history was associated with polymicrobial bacteriuria (Table 1). Thereafter, nonhospitalized dogs with confirmed recurrent UTI (presenting with overt lower urinary tract signs and no comorbidities) from each group were identified to compare treatment costs and clinical outcomes. Dogs that were hospitalized or diagnosed with comorbidities were excluded resulting in 9 dogs with recurrent polymicrobial UTI without comorbidities that were compared to 10 dogs from the *E. coli* and 12 dogs from the *Enterococcus* spp. monomicrobial cohort groups (Table 4).

Dogs with polymicrobial UTI had 6 hospital visits to the NCSU-VH on average (95% CI: 1.7-9.8), which was significantly greater ( $P = .05$ ) than the average number of visits for dogs with monomicrobial recurrent UTI (mean = 4 and 3 visits, 95% CI: 1.0 to 4.4 and -0.7 to 7.7 for *E. coli* and *Enterococcal* monomicrobial bacteriuria, respectively). Of the 6 dogs with urine cultures posttreatment, 4 were treated with either enrofloxacin or meropenem but had positive follow-up urine cultures over the study period. In 3 of 4 cases of unresolved UTI, follow-up urine cultures that were negative for *E. coli* but had either new or persisting growth of *Enterococcus faecalis* (2) or *E. faecium* (1). In monomicrobial recurrent UTI groups, recurrent UTI resolved in all 3 dogs with *E. coli* monomicrobial UTI and 3 out of 4 dogs with *Enterococcus* spp. monomicrobial UTI. One case of *Enterococcus* spp. monomicrobial UTI was unresolved due to new growth of *E. coli* in a follow-up urine culture. This was designated as a monomicrobial case because *E. coli* was isolated over 1 week after isolation of *Enterococcus* per the inclusion criteria for dogs with polymicrobial UTI.

## 4 | DISCUSSION

Although many studies have investigated the epidemiology and causative agents of UTI in dogs, this study specifically compares clinical outcomes of dogs with polymicrobial bacteriuria and UTI due to *E. coli* and *Enterococcus* spp. to dogs with monomicrobial bacteriuria and UTI using a retrospective cohort study. Reported interactions between these pathogens<sup>12,13,20</sup> led us to hypothesize that polymicrobial UTI would have different outcomes in a clinical setting when compared to monomicrobial UTI. We report that in 44 dogs over a 5-year period, polymicrobial bacteriuria was associated with adverse clinical outcomes including greater prevalence of suspected recurrent UTI, hospitalization, and hospital visits when compared to dogs with monomicrobial bacteriuria regardless of signalment (Tables 1 and 4). *Escherichia coli* isolates from dogs with PB exhibited a greater prevalence of drug resistance (Table 2); however, the urinalysis of these dogs had significantly reduced pyuria and bacteriuria (Table 3). These were findings in dogs with bacteriuria regardless of clinical signs, and reduced pyuria and gross bacteriuria likely coincided with less obvious signs of lower urinary tract infection. Comorbidities in the original groups further confounded interpretations of lower urinary tract signs, cost of treatment, and UTI resolution. Therefore, we assessed a sample of dogs with diagnosed recurrent UTI and found that polymicrobial *E. coli* and *Enterococcus* spp. UTI had more adverse clinical outcomes for dogs. These results add important clinical context to experimental studies that demonstrated interactions between these species that enhance severity of disease.<sup>12,13,21</sup>

One such interaction is suppression of macrophage signaling by *Enterococcus* in the urinary bladder allowing for *E. coli* colonization of the urinary tract.<sup>13</sup> In our study, this phenomenon could be related to decreased prevalence of pyuria and bacteriuria in dogs with PB as compared to those with *S<sub>EC</sub>* (Table 3). Urinary tract infections due to Enterococci are associated with decreased or absent pyuria when compared to *E. coli* UTI in human medicine,<sup>22</sup> presumably by reducing the degree of proinflammatory immune responses to infection or catheterization of the urinary tract.<sup>13</sup> Our study suggests this is also true for polymicrobial UTI of dogs where *Enterococcus* spp. are present. These urinalysis characteristics of PB could interfere with empirical therapy decisions for clinicians who base empirical treatment on urinalysis while awaiting culture and antimicrobial sensitivity results.<sup>1</sup> The association of PB with reduced pyuria and bacteriuria was observed independently of clinical signs in the present study, and we acknowledge the decision to treat PB should be based on the presence of clinical signs of UTI in addition to urinalysis results. Nevertheless, decreased inflammation combined with greater prevalence of drug-resistant *E. coli* isolated from dogs with PB could contribute to UTI development and increased prevalence of recurrent UTI and observed treatment difficulties in these dogs.

In a sample of dogs with recurrent UTI, those with polymicrobial UTI required more follow up visits and accrued more overall treatment costs (Table 4). An apparent risk factor for polymicrobial UTI was hospitalization (Table 1). This result highlights the risk of hospital-acquired infections of either *E. coli* or *Enterococcus* spp. causing

polymicrobial UTI, which has been documented in catheterized dogs.<sup>9</sup> Thus, cases of polymicrobial UTI in dogs were distinguished from monomicrobial UTI by more adverse clinical outcomes.

Limitations of this study included a low number of cases of culture-confirmed polymicrobial UTI. While the limited number of cases restricted our ability to perform multinomial regression analysis and required us to consolidate several variables, we employed inferential and descriptive statistical methods to gain insight into clinical features of polymicrobial UTI in dogs.

Another potential limitation is the inclusion of contaminated samples in the study groups. However, we mitigated this last limitation with strict inclusion criteria for urine samples that were sterilely collected and exhibited levels of bacterial growth consistent with infection. Further, the standard urine culture methods did not include selection for *Enterococcus* spp., which might be present in low numbers and difficult to detect in mixed culture on nonselective media with heavy *E. coli* growth. Because of this, we suspect the overall prevalence of *Enterococcus* spp. in PB could have been underrepresented in our study.

In addition, our study population originated in a referral hospital which could introduce confounding factors from biased selection of cases. To address confounding variables that might have impacted outcomes of dogs with recurrent UTI in each study group, we selected a subpopulation of dogs that presented to the NCSU-VH primarily for treatment of recurrent UTI. The only dogs that were successfully treated for polymicrobial UTI were dogs that were administered enrofloxacin and meropenem, which are not first-line drugs for treatment of dogs with UTI.<sup>1,23</sup> The treatment difficulties could be due to combined acquired or intrinsic antimicrobial resistance of each agent, host factors that interfere with antimicrobial efficacy, or unknown mechanisms allowing for antimicrobial tolerance in polymicrobial communities.<sup>24</sup> The sample size of dogs in this sample was small and not all dogs had follow-up cultures, at least at the NCSU-VH, to determine the impact of treatment. Additional prospective studies with a greater number of cases are warranted to more accurately determine the relevance of polymicrobial UTI due to *E. coli* and *Enterococcus* spp. in dogs.

Taken together, these results demonstrate how polymicrobial UTI caused by 2 of the most common uropathogens in veterinary medicine is associated with more adverse clinical outcomes for dogs. Further investigations of the pathophysiological mechanisms involved in polymicrobial UTI due to *E. coli* and *Enterococcus* spp. are warranted. The in vivo antimicrobial tolerance, evasion of host immunity, and mechanisms of host colonization by these 2 pathogens are important yet unknown features of polymicrobial UTI in dogs. It is also clear that polymicrobial infections are not limited to UTI in dogs and are involved in other veterinary species and diseases such as wound infections in horses and mastitis in cattle.<sup>25,26</sup> Nevertheless, the development of treatment strategies that target both pathogens and drivers of their interactions during UTI are needed to better manage polymicrobial *E. coli* and *Enterococcus* spp. UTI in dogs.

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### CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

### 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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