Urinary tract infections (UTIs) are one of the most common infections in feline medicine. As a group, UTIs also account for a significant proportion of overall antimicrobial usage in veterinary medicine. Cats are often prescribed antimicrobial therapy based solely on clinical signs of lower urinary tract disease without performing a urinalysis or culture and susceptibility, leading to inappropriate antimicrobial therapy and development of antimicrobial resistance. Multidrug resistant (MDR) uropathogens can result in recurrent and persistent UTIs, and conventional preventative management approaches for these clinical scenarios rely on antimicrobial prophylaxis. Recent studies have brought this approach into question due to concerns with antimicrobial stewardship. Similarly, multiple studies have shown a significant temporal increase in resistance patterns of uropathogens, and collectively, these findings underscore the need for novel approaches to UTI management and improved antimicrobial stewardship practices.

OBJECTIVE
To characterize uropathogenic *Escherichia coli* (UPEC) in cases of clinical feline urinary tract infection (UTI) and subclinical bacteriuria and investigate the in vitro effects of *E coli* strain Nissle 1917 on isolate growth.

ANIMALS
40 cats with positive *E coli* culture results for urine collected during routine evaluation.

PROCEDURES
Characterization of UPEC isolates was performed by PCR-based phylotype analysis and serotyping. Nissle 1917 effects on growth inhibition and competitive overgrowth against UPEC isolates were evaluated in vitro using a plate-based competition assay.

RESULTS
Feline phylogroups were similar to previous human and feline UPEC studies, with most of the isolates belonging to phylogroup A (42.5%), B2 (37.5%), and D (15.0%). Fifty-two percent of isolates were found to be resistant to antimicrobials, with 19% of these being multidrug resistant (MDR). Nissle 1917 adversely affected the growth of 82.5% of all isolates and 100% of MDR isolates in vitro. The median zone of inhibition was 3.33 mm (range, 1.67 to 10.67 mm). Thirteen isolates were affected via competitive overgrowth and 20 via growth inhibition.

CLINICAL RELEVANCE
UPEC isolates from cats were similar in phylogroup analysis to human and dog isolates. The in vitro effects of Nissle 1917 on UPEC warrant additional studies to determine if similar results can be duplicated in vivo.
E coli strain Nissle 1917 (EcN) is a commercially available probiotic containing a single strain of E coli (serotype O6:K5:H1). Using a number of phenotypic, biochemical, and genomic criteria, EcN is classified as a nonpathogenic probiotic. More recently, EcN has emerged as a potential biotherapeutic to prevent UTIs after demonstrating in vitro efficacy against 79% of pediatric and 68% of canine UPEC isolates. This benefit is attributed to its ability to produce bacterial microcins against closely related bacterial species as well as outcompeting uropathogens through its inherent growth rate characteristics.

The objectives of this study were to classify feline UPEC isolates of cats presenting to The Ohio State University Veterinary Medicine Center for aerobic bacterial culture or subclinical bacteriuria based on phylotype and serotype analysis and to assess the in vitro effects of EcN on UPEC isolates. The hypothesis of this study was that feline UPEC isolates will have a similar genetic background to previous reports of human and veterinary UPEC isolates as well as EcN will adversely affect the growth of UPEC isolates in vitro similar to what is shown in humans and dogs.

Materials and Methods

Cats presenting to The Ohio State University Veterinary Medicine Center with positive E coli urine cultures between 2014 and 2020 were eligible for inclusion. Samples were obtained from surplus urine cultures collected for routine evaluation of urinary tract infections. Diagnosis of a UTI required the presence of overt clinical signs, supporting evidence on the urinalysis (active urinary sediment), and a positive aerobic bacterial culture (> 10^4 CFU/mL for free catch, > 10^3 for urinary catheter, and > 10^2 for cystocentesis). Diagnosis of subclinical bacteriuria was based on an active urinary sediment and positive aerobic bacterial culture in the absence of overt clinical signs.

The study protocol was approved by the Institutional Animal Care and Use Committee. Informed consent was acquired prior to banking of residual UPEC isolates. Clinical case data for each cat included in the study were obtained and included clinical history, physical exam findings, urinalysis, and urine culture and susceptibility results.

All clinical samples were submitted to the Clinical Microbiology Laboratory of the The Ohio State University Veterinary Medicine Center for aerobic culture and antimicrobial susceptibility testing (AST). In brief, 10 μL of each urine sample was plated on 3 separate commercial culture plates: a Columbia Agar plate with 5% sheep blood was streaked for isolation and incubated at 35°C under CO2 atmospheric conditions, and another plate was streaked for quantification and incubated with an inoculated MacConkey plate agar at 35°C under regular air conditions. Standard microbiological techniques for enumeration and isolation were performed following laboratory procedures. Bacterial isolate identification was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry following the manufacturer recommendations. AST was performed by microbroth dilution using a commercial antimicrobial panel COMPGN1F (Sensititre; TREK/Thermo Fisher Scientific), following the manufacturer recommendations. Standards of the Clinical and Laboratory Standard Institute (CLSI) were used for interpretation of AST results. Isolates were considered to be resistant to multiple drugs if the isolate was not susceptible to greater than or equal to 3 different antimicrobial categories. For amoxicillin-clavulanic acid and amoxicillin, urinary tract breakpoints were used unless the history provided to the laboratory indicated a potential complicated UTI and/or the patient had already failed to respond to these medications. In these circumstances, the skin and soft tissue CLSI breakpoints were used for these antimicrobials. Although the CLSI states these breakpoints are for skin and soft tissue, these breakpoints are routinely used for in veterinary diagnostic laboratories for the interpretation of E coli isolated from other body sites such as in the case of complicated UTIs. Samples identified as E coli were subcultured onto fresh blood agar plates and then cryobanked 24 hours later in 75% glycerol and 1X phosphate-buffered saline. Banked samples were then stored at −80°C until analysis.

Effects on UPEC growth by EcN were assessed using a competition plate assay. Each individual feline UPEC strain and the EcN strain were reanimated separately in Luria broth (200 rpm, 37°C, 24 h) to a concentration of 10^8 CFU/mL. Each feline UPEC isolate was spread evenly across a Luria broth agar plate. The antagonist, EcN, was then stab inoculated into the background, and the plates were incubated at 37°C and evaluated at 24 hours. A positive result was defined as an area of clearing (zone of growth inhibition) around the EcN strain stab location or overgrowth of the Nissle bacteria into the background, and the plates were incubated at 37°C and evaluated at 24 hours. A positive result was defined as absence of both a zone of clearing and overgrowth of the EcN bacteria (Figure 1). The area of clearing or overgrowth was measured at the maximal diameter using a dissecting microscope and reported in millimeters. Assays were completed in triplicate on each plate for each feline UPEC strain. The maximal diameter of inhibition or overgrowth was averaged among the total number of tests.

Genomic DNA was extracted from each UPEC isolate using a commercially available kit (Invitrogen Easy DNA). Purified genomic DNA was stored at −20°C until further analysis. Phylootyping analysis was performed using the modified Clermont system based on the evaluation of the presence or absence of specific genes: trpA, arpA, chua, yjaA, and TspE4. This approach allows each UPEC isolate to be assigned into one of the recognized E coli phylogenetic groups (A, B1, B2, C, D, E, F, and I; Appendix 1). The primer sets used are shown in Appendix 2.

The PCR mix for each primer was composed of 1 μL of forward primer at 20 mM, 1 μM of reverse
primer at 20 mM, 1 μm of 10 mM dNTPs, 5 μm of DreamTaq Buffer, 0.5 μm of DreamTaq polymerase, 40.5 μm of ultrapure distilled water, and 1 μm of each UPEC isolate DNA. The PCR reaction was carried out as follows for the evaluation of trpA, chuA, yjaA, and TspE4.C2: step 1, 95°C for 2 minutes; followed by step 2, 25 cycles of 95°C for 60 seconds, 48°C for 60 seconds, and 72°C for 2 minutes; and then step 3, 72°C for 10 minutes prior to cooling to 4°C in a standard PCR thermocycler. The PCR reaction for arpA was as follows: step 1, 95°C for 2 minutes; followed by step 2, 25 cycles of 95°C for 30 seconds, 48°C for 30 seconds, and 72°C for 2 minutes; and then step 3, 72°C for 10 minutes.

Each PCR reaction product was then stained by adding 2 μm of 6X loading dye (Thermo Fisher Scientific). Ten microliters of each product were then evaluated on a 1% agarose Tris-acetate-EDTA gel with ethidium bromide. Gel electrophoresis was performed at 100 W for 40 minutes. Gels were visualized utilizing a UV imaging system (PhotoDoc-It Imaging) to evaluate for DNA band size consistent with the amplified target genes. All isolates were run in triplicate. PCR amplification products were purified prior to confirmation sequencing using a commercially available kit (QIAquick PCR Purification Kit; Qiagen Sciences). PCR products were sequenced through a commercial laboratory (EuroFins Genomics), and results were compared to known E coli genome sequences through the National Center for Biotechnology Information BLAST database and RefSeq database for confirmation. A subset of 17 samples were also serotyped through the E coli Reference Center at Pennsylvania State University.

Descriptive statistics were performed for clinical, phylogtype, serotype, and probiotic effect data. Normality was evaluated via Shapiro Wilk and visual inspection. Statistical analysis was performed with commercial software (Prism version 8.0.0; GraphPad Software) and the Fisher exact or χ²-test where applicable to assess for associations between categorical variables. The level of significance for all tests was set at P < 0.05.

Results

Uropathogenic E coli isolates collected from 40 cats were available for inclusion. Only 37 of the 40 cats had urinalyses performed as some patients were submitted for urine culture only based on the discretion of the attending clinician. All samples were collected via cystocentesis except for 4 obtained via indwelling urinary catheter and 1 obtained via free catch.

Breeds represented in the study included 27 domestic shorthair cats, 7 domestic medium hair cats, 3 domestic longhair cats, 2 Siamese, and 1 Himalayan. Twenty-eight cats were spayed females, and 12 were castrated males. Median age at time of diagnosis was 14 years (range, 8 mo to 21 y).

Clinical signs among the included cats were variable and included 6 cats that were not reported to be clinical (subclinical bacteriuria), 19 cats that exhibited only lower urinary tract signs (stranguria, hematuria, pollakiuria, periria), 7 cats with only signs of systemic illness (vomiting, anorexia, lethargy), and 8 cats that exhibited both signs of lower urinary tract disease and systemic illness. Known comorbidities were present in 35 of 40 cats and included chronic kidney disease (n = 18), history of urethral obstruction (8), hyperthyroidism (6), cardiomyopathy (5), diabetes mellitus (4), prior history of feline idiopathic cystitis (4), inflammatory bowel disease (4), gastrointestinal lymphoma (4), previous placement of a subcutaneous ureteral bypass system (2), seizures (2), renal abscess (1), nasal neoplasia (1), chronic pancreatitis (1), hepatopathy (1), and asthma (1). One cat each was diagnosed with suspected heat stroke and urosepsis. One cat was found to have a positive E coli culture at the time of urinary catheter placement for deobstruction, and 6 cats were subsequently diagnosed with positive E coli cultures within 10 days of initial urinary catheter placement. For 3 of these 6 cats, samples were obtained via the indwelling catheter and 3 via a cystocentesis after the urinary catheter had been removed. One cat had a history of a urethral obstruction 1 month prior with an indwelling catheter and was found to have
suspected urosepsis upon presentation. This sample was collected via cystocentesis. Urinalyses results were summarized (Supplementary Table S1).

Urine culture and susceptibility results were available for all 40 samples (Figure 2). In total, 21 (52%) of all 40 isolates were found to be resistant to at least 1 specific antimicrobial. The most commonly resistant antimicrobial classes were nonpotentiated and potentiated penicillins with 21 (52%) and 19 (48%) of the 40 isolates being resistant, respectively, to at least 1 drug within those classes. Resistance was also noted in other antimicrobial classes: cephalosporins

![Figure 2](image_url)

**Figure 2**—Culture and susceptibility profiles of the uropathogenic *Escherichia coli* isolates. The Clinical and Laboratory Standard Institute standards were used for interpretation of antimicrobial susceptibility test results. Breakpoints used for interpretation are categorized by color: green (susceptible), orange (intermediate), and red (resistant). For amoxicillin–clavulanic acid, amoxicillin urinary tract breakpoints were used unless the history provided to the laboratory indicated a potential complicated urinary tract infection and/or the patient had already failed to respond to these medications. In these circumstances, skin/soft tissue breakpoints were used for these antimicrobials. N/A = Not applicable.
(4/40 [10%]), fluoroquinolones (3/40 [7.5%]), and tetracyclines (2/40 [5%]). Of the 21 isolates resistant to at least 1 specific antimicrobial class, 4 (19%) were found to be MDR. Of these 4, 1 each was resistant to 3, 4, 5, and 6 classes of antimicrobials. Fifteen of the 21 (71%) resistant isolates were identified in patients without a previous history of UTIs. Additionally, 4 of the 8 (50%) cats that had been recently catheterized in hospital due to urethral obstruction were found to have resistant infections. There was no significant (P = 0.99) difference between the frequency of non-resistant, resistant, and MDR isolates between cats with subclinical bacteriuria and those with clinical UTIs.

Of the 40 isolates analyzed, the most common phylotypes observed were A (n = 17), B2 (15), and D (6). Other phylotypes noted were E (n = 1) and B1 (1). Seventeen samples were submitted to the E coli Reference Center at Pennsylvania State University for analysis of O-serotype. The isolates were found to be O25 (n = 4), O6 (2), O4 (2), O15 (2), O82 (1), O120 (1), O83 (1), O54 (1), and O127 (1). Two samples were unidentifiable. The remaining samples were not able to be submitted as serotyping as the reference laboratory closed due to the COVID-19 pandemic.

Discussion

Our study evaluated the phylotype, O-serogroup, antimicrobial resistance pattern, and effect of EcN on the growth of UPEC isolates in cats. The UPEC isolates in this study were consistent with previously reported UPEC isolate phylotype, serotype, and resistance profiles. Furthermore, when cultured alongside EcN, 82.5% of isolates experienced adverse effects on their growth in vitro. If EcN is shown to be a successful probiotic in cats and the results of this study translatable to in vivo studies, EcN has the potential to become a novel therapeutic for the prevention of UPEC UTIs including MDR strains through exclusion of UPEC from the gastrointestinal reservoir. As increasing levels of data are published on the detrimental effects of antimicrobials on the microbiome and microbial resistance patterns, the critical need for novel, probiotic approaches like EcN to manage these diseases is essential.4,6,8,13-15

Our study demonstrated that EcN effects on UPEC growth were primarily inhibitory and less commonly resulting from overgrowth. Previous work has shown this is primarily mediated by microcin production; however, this study did not specifically investigate the mechanism of inhibition or overgrowth. Previous human studies have shown a similar inhibition pattern; however, canine studies showed a predominance of overgrowth. The reason for the difference in suspected microcin production by EcN in dogs, cats, and humans is unknown at this time but may be secondary to perceived differences in stress signals leading to activation of microcin mRNA expression. Microcins are small antimicrobial peptides produced by Enterobacteriaceae during times of stress, such as nutrient competition, to locally limit the growth of nearby bacteria.61 EcN has been shown to produce several different microcins including H47 and M.62,63 These specific microcins have been shown to target bacteria, such as UPEC, which produce siderophores to enable colonization in low iron environments including the gastrointestinal tract.62,63 As the gastrointestinal tract is the reservoir location of UPEC, EcN may offer a targeted oral probiotic approach to limit the proliferation of UPEC. Further studies are warranted to investigate, confirm, and determine the role of microcin production for EcN exposed to feline UPEC isolates as well as investigate other potential mechanisms for the observed effects on UPEC growth.

Known E coli phylotypes include A, B1, B2, C, D, E, F, I, and II. Previous studies in dogs and humans have shown that the most common clades associated with UTIs are B2, D, and E; however, significant regional variability exists with some studies showing significant proportions of clades A, B1, and C. Our study showed that feline urine samples follow a similar pattern with the majority of samples identifying as A, B2, and D.4,5,24,25,39,41 Our study revealed a similar distribution pattern of O-serotypes as previously documented in UPECs found in both people and dogs.4,22,43 The most commonly associated O-serotypes in people are O1, O2, O4, O6, O7, O8, O14, O15, O16, O18, O21, O22, O25, O75, and O83.43-45 A recent study from our group found that the most common O-serogroups found in a subset of UPEC canine isolates were O1, O4, O6, O15, O22, O25, and O83. Our study showed that of the 15 typeable samples 73.3% of isolates showed similar O-serogroups as previously documented in human and companion animal UPEC isolates.4,5,24,25,39,41 Concerningly, our data also showed a concerning percentage (10%) of MDR isolates. While some of the resistant isolates were from a patient being treated for either recurrent or relapsing UTIs, over three-quarters of the resistant
isolates were from animals without a recent history of a UTI or recent antimicrobial exposure. Additionally, our study showed that of the 8 cats who had been recently catheterized for a urethral obstruction, 4 were found to have a resistant UTI. This finding is consistent with the well-known increasing rate of nosocomial obtained MDR organisms in both human and veterinary medicine. 54–56 This high proportion of antimicrobial resistance in both companion animals as well as people highlights the need for a targeted nonantimicrobial therapy for the treatment of urinary tract infections. Our study has several limitations that should be considered. Clinical data were obtained retrospectively after confirmation of a UTI caused by UPEC leading to some data not being available for all patients. However, as the 3 missing urinalyses were from patients with lower urinary tract signs and the attending clinician had a high clinical suspicion of a UTI, the missing data are unlikely to impact the findings of this paper. Additionally, clinical signs of a urinary tract infection may be missed by owners leading to a falsely elevated proportion of patients without reported clinical signs. 57 Furthermore, for 5 cats the urine collected for culture was obtained either via free catch (n = 1) or via an indwelling urinary catheter (4). However, as all of these cats had signs consistent with a urinary tract infection, active pyuria, and a clinical response to antimicrobials, contamination is unlikely in these cases. Finally, the clinical breakpoints used to determine the susceptibility of isolates to amoxicillin and amoxicillin-clavulanic acid could vary based on the clinical history provided to the clinical microbiology laboratory. In cases where the laboratory was provided with a clinical history consistent with a complicated UTI (renal azotemia, concurrent anatomic/functional abnormality or comorbidity that predispose to the development of a UTI, or prior treatment failure), the use of skin/soft tissue for a systemic breakpoint was utilized. 27,30,31 As a complete clinical history may not have been provided for all clinical samples, some isolates may have been evaluated using the UTI instead of skin/soft tissue breakpoints leading to a reduction in reported resistant isolates. There were also limitations regarding interpretation of the EcN probiotic data. Traditionally, the benefit of the EcN probiotic has been attributed to its ability to produce bactericidal microcins against closely related bacterial species as well as outcompeting uropathogens through its inherent growth rate characteristics. 24,26 This study did not specifically look at the mechanisms behind which overgrowth and growth inhibition occurred against these isolates. This is an important area for future study. Until then, it is unknown whether the effects are mediated by similar mechanisms as those that are seen in people and dogs or a separate mechanism. The results were also reported in absolute (positive or negative effect) as is consistent with the competition plate assay. Further evaluation of this assay, including specific zone of effect size cut-offs for clinical relevance and effectiveness, is an important area of need for future study. Lastly, as mentioned previously, it is vital to note that this is a preliminary in vitro study on the effects of the EcN probiotic against feline UPEC isolates. For clinical benefit to be determined, further study on the viability of the EcN probiotic in cats gastrointestinal tracts, effectiveness of EcN against UPEC residing in the gastrointestinal tract reservoir, and the ability of EcN to prevent urinary tract infection from UPEC exclusion in the gastrointestinal tract needs to be documented. In conclusion, UPEC constitutes a major reason for antimicrobial usage in veterinary medicine. Our study corroborated the growing concern of antimicrobial resistant UPEC isolates. As antimicrobial resistance increases, the development of novel nonantimicrobial therapeutics is paramount. The results of this study showed that EcN inhibits the in vitro growth of feline UPEC either through competitive overgrowth and growth inhibition. As the gastrointestinal tract is the major source of UPEC, additional studies are needed to further investigate the viability of EcN at reducing the feline UPEC gastrointestinal load in vivo. If successful, routine probiotic supplementation with EcN could offer a nonantimicrobial approach to UTI prevention in at risk cats.

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Supplementary Materials
Supplementary materials are posted online at the journal website: avmajournals.avma.org

Appendix 1

Protocol for assigning phylotype based on the modified Clermont system.

### Quadruplex genotype

| Name  | Sequence                        |
|-------|---------------------------------|
| TspE4.C2 | ATGCCCTTCAATCGTGAAGTGCAG        |
| chuA   | TCTCGTTAGTGAACCGAGAAATTG       |
| yjaA   | GGAATCCGAGAAGTCTGCA            |
| arpa   | TGGCGCCGCTTCAATCGTGAAGTGCAG   |

The primary assignment was based on the presence of 4 specific genes (arpa, chuA, yjaA, and TspE4.C2). Phylotype C-specific primers can be used to differentiate A or C phylotypes. Phylotype specific primers can be used to differentiate D, E, or clade I phylotypes. Gene assignments not listed require multilocus sequence typing or cryptic clade primers for definitive assignment.

Appendix 2

The primers used for identification of target genes (arpa, arpa, chuA, yjaA, and Tsp.E4.C2) for phylotyping.

| Name  | Sequence                          |
|-------|-----------------------------------|
| arpa_F | AAAAGTTTCGCGCAGCTTGC             |
| arpa_R | TCTCGTTAGTGAACCGAGAAATTG         |
| chuA_F  | GTACCAGCAGGAACAAACAG              |
| chuA_R  | TGCCCGCCGCTTCAATCGTGAAGTGCAG    |
| yjaA_F  | GAAATCCGAGAAGTCTGCA              |
| yjaA_R  | ATGCCCTTCAATCGTGAAGTGCAG         |
| TspE4.C2_F | GCTGTCTTTGCGCGGTTGAG          |
| TspE4.C2_R | CATTTTGATAGCCTTCCCTCC          |
| arpa2_F | GATTTCTACCGCCTGAAATA            |
| arpa2_R | GAAAAGTTTCGCGCAGCTTGC           |
| trpa_F  | AGTGTCTTTGCGCGGTTGAG            |
| trpa_R  | TCTCGCGGGCGCTAGCCG             |