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Phylogroup and virulence gene association with clinical characteristics of *Escherichia coli* urinary tract infections from dogs and cats

Tabitha A. Hutton, Gabriel K. Innes, Josée Harel, Philippe Garneau, Andrew Cucchiara, Dieter M. Schifferli, Shelley C. Rankin[1]

Abstract. *Escherichia coli* isolates from infections outside the gastrointestinal tract are termed extra-intestinal pathogenic *E. coli* (ExPEC) and can be divided into different subpathotypes; one of these is uropathogenic *E. coli* (UPEC). The frequency with which UPEC strains cause urinary tract infections in dogs and cats is not well documented. We used an oligonucleotide microarray to characterize 60 *E. coli* isolates associated with the urinary tract of dogs ($n = 45$) and cats ($n = 15$), collected from 2004 to 2007, into ExPEC and UPEC and to correlate results with patient clinical characteristics. Microarray analysis was performed, and phylogroup was determined by a quadruplex PCR assay. Isolates that were missing 1 or 2 of the gene determinants representative of a function (capsule, iron uptake related genes, or specific adhesins) were designated as "non-classifiable" by microarray. Phylogroup B2 was positively associated with the UPEC subpathotype ($p < 0.0005$) and negatively associated with "non-classifiable" isolates ($p < 0.0005$). Phylogroup D was positively associated with ExPEC pathotype ($p = 0.025$) and negatively associated with UPEC subpathotype ($p = 0.014$). The ExPEC pathotype was positively associated with hospitalization for one or more days ($p = 0.031$). The UPEC subpathotype was negatively associated with previous antimicrobial therapy ($p = 0.045$) and previous hospitalization within the 3 mo prior to the positive culture ($p = 0.041$). The UPEC subpathotype was positively associated with prostatitis ($p = 0.073$) and negatively associated with current immunosuppressive therapy ($p = 0.090$). Our results indicate that the case history observations may be critically important during the interpretation of laboratory results to encourage judicious use of antimicrobials.

Key words: Canine; *Escherichia coli*; feline.

Introduction

The bacterial species *Escherichia coli* contains a wide array of genetic diversity, most notably among genes that confer virulence.[3] A reliable discriminatory typing scheme to differentiate commensal nonvirulent strains from specific pathotypes of *E. coli* does not exist.[13] Extraintestinal pathogenic *E. coli* (ExPEC) have been shown to cause a wide range of infections such as urinary tract infection (UTI), sepsis, pneumonia, meningitis, skin and soft tissue infection, and osteomyelitis. The ExPEC designation defines a pathotype that has been shown to contain subpathotypes of *E. coli* known as uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), and avian pathogenic *E. coli*, among others.[17] Each subpathotype causes specific lesions aligned with the pathotype name; for example, UPEC strains possess virulence factors that enhance the ability to cause disease within the host's urinary tract.[19]

UTIs are widely studied in humans and animals. Several studies have shown relationships between animal and human isolates, but it is not known how frequently organisms categorized as ExPEC and/or UPEC cause urinary tract disease in companion animals compared to less virulent opportunistic strains.[5] There is no single definition to categorically define an organism as ExPEC or UPEC, and numerous virulence factor profiles are possible.[13]

An oligonucleotide microarray has been developed, and expanded, for *E. coli* that includes known virulence and putative virulence genes.[2,8] The oligonucleotide microarray can assign *E. coli* isolates to pathotypes and subpathotypes and also to 1 of 4 defined phylogroups (A, B1, B2, and D).[4] A quadruplex PCR assay has been described that more accurately differentiates *E. coli* sensu stricto into 3 additional phylogroups (C, E, and F) and also recognizes an *Escherichia*

School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA (Hutton, Innes, Schifferli, Rankin); The Research Group on Infectious Diseases in Animal Production, Faculty of Veterinary Medicine, University of Montreal St-Hyacinthe, Quebec, Canada (Harel, Garneau); Center for Translational and Clinical Research and Center for Clinical Epidemiology and Biostatistics, School of Medicine, University of Pennsylvania, Philadelphia, PA (Cucchiara).

[1]Corresponding author: Shelley C. Rankin, University of Pennsylvania, School of Veterinary Medicine, 3900 Delancey Street, Philadelphia, PA 19104. srankin@vet.upenn.edu

cryptic clade 1.[6] Phylogroup A, which contains primarily commensal *E. coli* strains, can now be divided further with the addition of the *trpA* primer to distinguish between phylogroups A and C (which is closely related to, but distinct from, phylogroup B1). Similarly, phylogroup D can be further divided with the *arpA* primer to distinguish between phylogroup D and E, and the newly described phylogroup F is a sister group to phylogroup B2.[6]

We used the microarray to determine which virulence genes were present in a group of 60 *E. coli* isolates obtained from canine and feline UTIs. The quadruplex PCR assay[6] was also applied to this group of isolates to determine which phylogroups were the most common. The phylogroup and pathotype data were correlated with clinical information obtained from the medical record to determine phylogroup and pathotype associations with clinical characteristics.

To our knowledge, no previous studies have evaluated canine and feline patients with UTIs for potential risk factors or clinical syndromes that may be associated with infection caused by ExPEC, and more specifically UPEC. The recognition of patient epidemiologic factors that are associated with the UPEC subpathotype may allow for more rapid identification of high-risk animals or populations (e.g., specific breeds) that may benefit from more aggressive treatment and monitoring strategies as soon as a UTI is diagnosed. Some risk factors, such as antimicrobial use practices, may be diminished or even eliminated completely, with new therapeutic recommendations. If UPEC infections are shown to be associated with increased morbidity (such as pyelonephritis, persistent infection, or septicemia, for example) then testing with a rapid molecular assay specific to UPEC may be an important step for clinical laboratories to consider.

Materials and methods

Bacterial isolates

E. coli isolates of urinary origin (n = 146) were available from canine (n = 107) and feline (n = 39) patients at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania (Philadelphia, PA). Specimens were collected as part of the routine diagnostic evaluation for UTI at the hospital and included 1 prostatic wash, 1 renal pelvis sample, 2 catheter tips, 4 bladder mucosa samples, 81 cystocentesis samples, and 57 urine samples, some or all of which could have been free-catch as this was not documented on the laboratory submission form. Aerobic culture was performed by plating 1 μL of urine to 1 plate each of MacConkey agar (Remel, Lenexa, KS); trypticase soy agar with 5% sheep blood (Remel); and Columbia CNA with 5% sheep blood, colistin, and nalidixic acid (Remel). The plates were incubated overnight at 37°C. Isolates were collected between September 1, 2004, and December 31, 2007 and stored in a cryopreservation tube (Microbank tubes, Pro-Lab Diagnostics, Ontario, Canada) at −80°C.

Pulsed-field gel electrophoresis and comparison of profiles

All 146 isolates had pulsed-field gel electrophoresis (PFGE) performed according to the standard CDC PulseNet USA protocol[16] with *Salmonella enterica* serovar Braenderup H9812 as the control strain, with the following modifications: 6 μL of the *Xba*I enzyme was used per sample, and thiourea solution (10 mg/mL) was added to the 0.5× Tris–borate–EDTA (TBE) buffer in the electrophoresis chamber at a dose of 836 μL of thiourea stock solution per 2.2 L of 0.5× TBE.

Gel image files were imported into a database (BioNumerics v.5.0, Applied Maths, Austin, TX), and a dendrogram was created using all 146 strains. Percent similarities were derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients, in accordance with standard CDC PulseNet USA protocol.[16]

Microarray experiments

A subset of 60 isolates (45 from dogs, 15 from cats) was selected for microarray analysis. The subset was chosen following review of the dendrogram created from the PFGE profiles of all 146 isolates (Supplementary Fig. 1). Isolates that represented the major clonal clusters and the outliers were randomly selected. Genetically distinct representatives within each cluster were selected to allow for the best representation of genetic diversity. The 60 isolates were then reviewed to ensure that each isolate was from a unique patient. Microarray analysis was performed in 2 batches. For the first 30 strains, microarray hybridizations were performed with an oligonucleotide microarray that detected 264 *E. coli* virulence genes and their variants.[2] The *E. coli* DNA microarray was updated prior to testing the second 30 isolates; the updated assay detected 313 *E. coli*–specific virulence genes.[8]

DNA extraction, DNA labeling, hybridizations, and data acquisition

Extraction and labeling of DNA was performed as described previously with the modification of an increased starting culture volume (1 mL instead of 200 μL).[2] Hybridizations on the *E. coli* virulence microarrays were performed as described previously.[2] After hybridization, arrays were scanned with a microarray analysis system (ScanArray Lite, Canberra-Packard Canada, Montreal, Canada), and acquisition and quantification of fluorescent spot intensities were performed using analysis software (ScanArray Express v.2.1, Perkin-Elmer, Foster City, CA). For all of the microarray data, the local background was subtracted from the recorded spot intensities. The median value of each set of duplicate spotted oligonucleotides was then compared to the median value of the negative control spots present on the array. Oligonucleotides

that had a signal-to-noise fluorescence ratio >3.0 were considered positive.

Pathotype assignment

Published criteria[9] were used to classify *E. coli* isolates as potential ExPEC. In brief, isolates must possess a group I or group II capsule synthesis gene and some of the iron uptake related genes (such as *irp*, *fyuA*, or *iutA*), as well as at least one of the following adhesin related genes: *pap* (P fimbriae), *sfa* or *foc* (S/F1C fimbriae), *afa* or *dra* (Dr binding adhesins).[7] If an isolate was missing 1 or 2 of the gene determinants representative of a function (i.e., capsule, iron uptake related genes, or specific adhesins), it was put into a "non-classifiable" category. *E. coli* isolates that possessed the ExPEC determinants and in addition contained the hemolysin gene *hlyA*, cytotoxic necrotizing factor *cnf1*, uropathogenic-specific protein *usp*, and iron uptake related genes were classified as UPEC. ExPEC isolates that possessed the invasion encoding gene *ibeA*, and also K1 capsular related genes *neuA* and *neuC*, were classified as NMEC.[7]

The quadruplex PCR[6] was used to determine the phylogroup of each of the 60 isolates that were analyzed with the microarray. The quadruplex assay was slightly modified and used 10 µL of master mix (AmpliTaq Gold 360, Applied Biosystems, Foster City, CA), 1 µL of each primer (8 µL total), and 2 µL of target DNA. The assay conditions were: 1 min at 95°C; 35 cycles of 15 s at 95°C, 30 s at 55°C, and 60 s at 72°C; followed by 7 min at 72°C. The tubes were then cooled to 4°C. A 5-µL aliquot of loading dye was added to each reaction tube, and electrophoresis was performed in 2% agarose gel at 100 V for 2 h. The gel was stained in ethidium bromide and photographed under ultraviolet light. The quadruplex method is more complex and requires an iterative approach to assign a phylogroup. All isolates assigned to phylogroup A were screened using C-specific primers. Similarly, all D phylogroup isolates were screened using E-specific primers. The addition of the *arpA* gene to create the quadruplex assay allows phylogroup F isolates to be differentiated from phylogroup D because phylogroup F does not contain this gene.

Medical record review

The medical records of the 60 patients were reviewed to gather information about the hospital visit at which the culture sample was collected, as well as pertinent historical and follow-up information using a standardized form (available on request). The following clinical characteristics were then summarized as a dichotomous result (yes/no/blank) in tabular format: 1) whether the patient had the following preexisting medical diagnoses: a) diabetes mellitus, b) kidney disease, c) hyperadrenocorticism, or d) physical abnormalities of the urinary tract (defined as tumors, congenital malformations, or urolithiasis) or neurologic abnormalities (defined as spinal cord disease or urethral sphincter incompetence) that impaired normal voiding of urine; 2) whether the patient was currently receiving the following classes of medications: a) immunosuppressives, b) antimicrobials; 3) whether the patient had received antimicrobial therapy within the 3 mo prior to the positive culture; 4) whether the patient had been hospitalized within the 3 mo prior to the positive culture; 5) whether the patient had a previous history of UTI; 6) whether the patient had a previous history of at least 1 UTI known to be caused by *E. coli* (based on a positive culture result); 7) whether the patient was hospitalized for 1 (or more) d at the visit when the positive culture was collected; 8) whether the patient had a urinary catheter placed at the visit when the positive culture was collected; 9) whether the patient had the following parameters during the hospital visit when the positive culture was collected: a) fever (defined as a rectal temperature ≥39.4°C), b) leukocytosis, c) leukopenia, d) left shift or toxic changes to neutrophils, e) azotemia, f) diagnosis of pyelonephritis, g) diagnosis of prostatitis; 10) whether the UTI associated with the positive culture was suspected to have been acquired while in the hospital; 11) whether the patient had 1 or more subsequent UTIs following the positive culture.

Statistical analysis

Contingency tables were constructed for the subset of 60 isolates for which microarray analysis was performed, classified by presence (yes/no) of the specific genotype with that phenotype of interest. Pearson χ^2 statistic was obtained to test the null hypothesis of no association between specific genotype and specific phenotype. Statistical significance was achieved when the associated 2-tailed p value was less than the type I error rate of $\alpha = 0.05$; additionally, contingency tables with associated p values $> \alpha$ but $< \alpha^* = 0.10$ were characterized as statistically suggestive of an association that borders on being statistically significant.

The presence (yes/no) of the pathotype/subpathotype ExPEC/UPEC, and isolates that were non-classifiable, were each compared to the clinical characteristics listed as items 1–11 in the aforementioned Medical record review section. Likewise, the presence (yes/no) of each of the individual virulence genes that were found to be positive for at least 1 analyzed isolate was compared with the presence (yes/no) of each clinical characteristic listed as items 1–11 in the previous section (Medical record review).

Membership (yes/no) in a given phylogroup was compared with membership (yes/no) in a specific PFGE cluster. Similarly, membership (yes/no) in a given phylogroup was compared with classification (yes/no) as ExPEC, UPEC, NMEC, or non-classifiable. Finally, membership (yes/no) in a specific PFGE cluster was compared with classification (yes/no) as ExPEC, UPEC, NMEC, or non-classifiable.

To reduce the probability of compounding the type I error rate associated with making multiple comparisons of the

Table 1. Pathogroup and pathotype assignments for 60 *Escherichia coli* isolates.

Isolate	Pathogroup	Pathotype
4679-04; 56-05; 1713-05; 5828-05	A	ExPEC
2807-05; 3571-06; 4886-07	A	Non-classifiable
5374-04	B1	ExPEC
5695-05; 6012-05	B1	Non-classifiable
4121-04; 782-05; 1162-07; 2895-07; 3138-07	B2	ExPEC
878-05; 2467-05; 2906-05; 462-07; 1170-07	B2	UPEC
1057-07; 854-07; 909-07; 786-07; 1299-07		
1382-07; 1331-07; 1303-07; 1706-07; 1924-07		
1965-07; 2925-07; 5636-07; 5525-07; 4133-05		
4057-05; 914-07; 1498-07; 4860-07; 4086-07	B2	Non-classifiable
5700-07; 5615-07; 6224-07; 6542-07		
3475-07; 2906-07	B2	NMEC
431-05; 6461-07	C	Non-classifiable
5824-04; 13-05; 1395-05; 2695-05; 3424-05	D	ExPEC
4312-07; 4556-07; 6407-07	D	Non-classifiable
4270-04; 5406-04; 117OP-05; 2423-05	F	ExPEC

ExPEC = extra-intestinal pathogenic *E. coli*; UPEC = uropathogenic *E. coli*; NMEC = neonatal meningitis–associated *E. coli*.

individual virulence genes with patient clinical characteristics, the following steps were taken when evaluating the data: 1) we decided that 5% of the total number of comparisons could potentially be found to be statistically significant simply by chance, and therefore this number of associations with the highest p values that were <0.05 were discarded, and 2) an approximate Bonferroni correction was applied; specifically, each p value was inflated by a multiple of the k, the number of genes being compared (restricted to $k \times p < 0.9999$).

Results

E. coli isolates of urinary origin (n = 146) had PFGE performed: 107 from dogs (73%) and 39 from cats (27%). This represented 90 unique canine patients and 36 unique feline patients. Five groups of organisms were identified by PFGE with percent similarities of 63.6–69.7%; 7 isolates were outliers with ≤60% similarity to the other 5 main groups (Supplementary Fig. 1). Cluster 1 contained 28 isolates, cluster 2 contained 44 isolates, cluster 3 contained 16 isolates, cluster 4 contained 18 isolates, and cluster 5 contained 33 isolates. A total of 60 isolates were chosen for microarray analysis, including 14 from cluster 1, 16 from cluster 2, 7 each from clusters 3 and 4, 13 from cluster 5, and 3 from the outliers.

Using the microarray, 108 virulence genes were positive for at least one of the isolates analyzed (Supplementary Table 1). All 60 isolates were positive for the following 6 virulence genes: *artJ*, *csgE*, *hlyE*, *mviM*, *mviN*, and *ompA*. Most isolates were positive for genes *b1121* (58 of 60) and *ibeB* (56 of 60). Seven isolates were members of phylogroup A (13%), 3 were members of phylogroup B1 (5%), 36 were members of phylogroup B2 (60%), 2 were members of phylogroup C (3%), 8 were members of phylogenetic group D (13%), and 4 were members of phylogroup F (7%).

Nineteen isolates were characterized as ExPEC (32%), and phylogroups A, B1, B2, D, and F were represented. Twenty isolates were subpathotype UPEC (33%), and all of these isolates belonged to phylogroup B2. There were 2 NMEC, both B2 (3%), and 19 non-classifiable (32%) isolates that contained representatives from phylogroups A, B1, B2, C, and D (Table 1).

Two patients had diabetes mellitus (3%), 14 had kidney disease (23%), and 9 had hyperadrenocorticism (15%). Thirty patients (50%) had one or more physical abnormality of the urinary tract or a neurologic abnormality, including 2 tumors, 8 urinary congenital malformations, 9 cases of urolithiasis, and 9 neurologic abnormalities. Eight patients were receiving immunosuppressives (13%), and 24 patients were currently receiving antimicrobials (40%). Thirty-two patients had received antimicrobials within the 3 mo prior to the positive culture (53%). Thirty-eight patients had been hospitalized within the 3 mo prior to the positive culture (63%). Thirty-four patients had a history of a previous UTI (57%), and, of those, 25 had at least 1 known positive *E. coli* (42%) culture. Thirty-one patients were hospitalized for one or more days during the visit when the positive culture was collected (52%). Fifteen patients had a urinary catheter placed during the hospital stay when the positive culture was collected (25%). Ten patients had a fever (6%), 8 had leukocytosis (13%), 5 had leukopenia (8%), 16 had a left shift or toxic changes to neutrophils (27%), 18 were azotemic (30%), 14 had pyelonephritis (23%), and 3 had prostatitis (5%). Seven patients had an infection that may have been acquired while in the hospital (12%). Eleven patients were known to have at least one subsequent UTI following the positive culture (18%).

ExPEC, UPEC, and isolates that were non-classifiable were compared to the clinical characteristics studied. The

following statistically significant associations were identified: the ExPEC pathotype was positively associated with hospitalization for one or more days during the visit when the positive culture was collected ($p = 0.031$); the UPEC subpathotype was negatively associated with previous antimicrobial therapy within the 3 mo prior to the positive culture ($p = 0.045$), and was negatively associated with previous hospitalization within the 3 mo prior to the positive culture ($p = 0.041$). The UPEC subpathotype was positively associated with prostatitis ($p = 0.073$) and negatively associated with current immunosuppressive therapy ($p = 0.090$). A lack of pathotype identification (non-classifiable) was positively associated with current antimicrobial therapy ($p = 0.070$). A lack of pathotype identification was positively associated with current immunosuppressive therapy ($p = 0.045$), positively associated with previous antimicrobial therapy within the 3 mo prior to the positive culture ($p = 0.025$), and negatively associated with hospitalization for one or more days during the visit when the positive culture was collected ($p = 0.029$).

Phylogenetic groups were compared to the PFGE clusters. Phylogenetic group A was positively associated with PFGE cluster 5 ($p = 0.005$). Phylogenetic group B1 was positively associated with PFGE cluster 5 ($p = 0.039$). Phylogenetic group B2 was positively associated with PFGE cluster 3 ($p = 0.008$), and negatively associated with PFGE cluster 2 ($p = 0.010$). Phylogenetic group D was positively associated with PFGE cluster 2 ($p < 0.0005$), and negatively associated with PFGE cluster 5 ($p = 0.016$). The UPEC subpathotype was positively associated with PFGE cluster 3 ($p = 0.001$), and negatively associated with PFGE cluster 2 ($p = 0.043$). A lack of pathotype identification was negatively associated with PFGE cluster 3 ($p = 0.017$).

Phylogroup was compared to the ExPEC, UPEC, and isolates that were non-classifiable. Phylogroup A was positively associated with isolates that were non-classifiable ($p < 0.0005$) and negatively associated with the UPEC pathotype ($p = 0.094$). Phylogroup B1 was positively associated with isolates that were non-classifiable ($p = 0.006$). Phylogroup B2 was positively associated with the UPEC pathotype ($p < 0.0005$) and negatively associated with isolates that were non-classifiable ($p < 0.0005$). Phylogroup D was positively associated with ExPEC ($p = 0.025$) and negatively associated with the UPEC subpathotype ($p = 0.014$).

Discussion

The "special pathogenicity" hypothesis proposes that *E. coli* isolates that infect the urinary system possess particular virulence attributes that enhance the ability to cause disease in this organ system.[12] We showed that *E. coli* strains that were classified as UPEC were isolated at the same frequency as ExPEC strains that did not have significant uropathogenic potential and also strains that were non-classifiable. There were 19 ExPEC isolates, 20 subpathotype UPEC isolates, 2 subpathotype NMEC isolates, and 19 isolates that were "non-classifiable" and had no pathotype association. The majority of isolates in our study were not classified as UPEC, and this was somewhat unexpected, as it does not correlate with the special pathogenicity hypothesis. This may be attributed to a number of variables such as a previous history of UTI, a history of either previous and/or current antimicrobial therapy at the time the culture was obtained, and/or a recent history of hospitalization. Given that the University of Pennsylvania is a tertiary referral hospital, it is unclear what influence the previous medical history may have had on the population of *E. coli* isolates that were obtained from these patients, and whether this could have influenced the pathotypes identified.

It is not unexpected that phylogroups were associated with specific PFGE clusters, given that organisms within a particular PFGE group will have genetic relatedness.[20] The finding that phylogenetic group D was associated with the ExPEC pathotype and phylogenetic group B2 with the UPEC subpathotype has been reported previously.[9,17] The data presented herein support the idea that UPEC organisms, in particular, appear to have relatively close genetic relatedness, as they were found associated with one particular PFGE cluster in our study.

Two of the most common phylogenetic groups in our study (B2, D) comprised 73% of the sample pool, an observation that is consistent with the fact that the most urovirulent phylogenetic groups in humans are B2 and D.[19] Such overlap may be the result of transmission events between humans and their companion animals. A similar observation was recorded when *E. coli* isolates from cats in the United States were characterized; that study showed that 72% of the isolates belonged to phylogroup B2 and 6.8% were phylogroup D.[14] The B2 phylogroup has been associated with food animals[8] and, as such, the presence of B2 isolates from dogs and cats was not unexpected. The *E. coli* phylogroup B2 is a possible zoonotic agent that may cross species and cause UTI in humans.[11,15] The B2 phylogroup is highly associated with UTI in humans and this has consistently been reported in the literature.[10,18] However, because not all phylogroup B2 strains are UPEC,[3,13] it is tempting to speculate that B2 UPEC strains are more commonly isolated from "animals that live with humans" because there is an anthropozoonotic association. This requires further investigation.

The UPEC subpathotype was also suggestively positively associated with the diagnosis of prostatitis. This makes intrinsic sense, as by definition a UPEC organism should be more specifically adapted to the urinary tract, and therefore the prostate. Further investigations are needed to identify specific factors and/or genes that promote prostatic virulence in dogs (prostatitis is not a recognized clinical syndrome in cats as they do not have a discrete prostate gland), given that no individual virulence genes were significantly associated with prostatitis in our study. There were only 3 dogs with prostatitis in our study and that is a limitation of this finding. In humans, the hemolysin phenotype, *cnf1* gene, and P fimbriae have been found to be associated with prostatitis.[1]

The ExPEC organisms were positively associated with a hospital stay for one or more days at the time of infection. One might infer that these patients were more severely ill and that accounted for the need for hospitalization, although ExPEC did not correlate with other characteristics that might connote a more severe illness, such as fever, leukocytosis, leukopenia, left shift, and azotemia. However, this lack of correlation may in part be attributed to missing data. Of the 60 patients included in our study, initial body temperatures were not known or recorded for 12 patients, 23 patients did not have hematology performed (and thus no white blood cell count or differential), and 20 did not have renal analytes checked. Whether ExPEC strains cause more severe clinical illness needs to be investigated further. If further study confirms these associations, then performing testing that identifies whether an *E. coli* isolate is an ExPEC might have prognostic significance in the clinical setting and might result in a different set of clinical recommendations for that patient, such as treating the infection with higher antimicrobial doses or for a longer period of time, or monitoring the success of treatment with follow-up urine cultures, for example.

Individual virulence genes were evaluated in groups according to their class of function to investigate possible trends of association. The presence of adhesin-associated genes (*fimA*, *fimH*, *papC*, and *sfaD*) was positively associated with a hospital stay for one or more days at the time of infection. This may explain more specifically why the ExPEC pathotype, as discussed earlier, was also associated with this finding, and may suggest that host cell adhesion and/or invasion may be a critical determinant of illness severity. This is an area that may warrant further study in companion animal medicine.

There are limitations to our study, as is common with retrospective observational studies. Not all of the *E. coli* isolates that could have qualified for our study during the time period were collected and saved for further evaluation. In addition, not all of the strains in our collection received microarray analysis, thus further limiting our data pool. Many of the clinical characteristics, as mentioned previously, suffered from missing data points because of incomplete medical record keeping or simply given the fact that patients were not evaluated in a standardized fashion (i.e., laboratory testing to obtain blood counts and chemistry profiles was not performed in all cases, as might occur with a prospective study). Given the overall small patient sample size, the presence or absence of any particular clinical characteristic was not controlled for, meaning that some characteristics were likely overrepresented whereas others were underrepresented.

Our investigation can be viewed as a pilot study meant to generate plausible hypotheses that can become the basis for future work, ideally prospective in nature. Although limited generalizable conclusions can be drawn from such a study, several areas of interest have been identified. Some of the initial areas for consideration include an examination of pathotype trends in larger collections of *E. coli* from canine and feline urinary tracts, ideally including isolates from primary-care veterinarians and patients without the complicating histories of previous or current medications (particularly antimicrobials), other concurrent diseases, and preferably, without a history of previous UTIs. The ExPEC pathotype also should be investigated to determine whether it is associated with more severe clinical illness than other pathotypes in the urinary tract. Examination of the role of the adhesin genes in clinical disease severity may need to be undertaken. Future studies should consider evaluating the isolates obtained from cats separately from dogs, given that these 2 species were not examined independently in our study. Finally, identification and further characterization of the ExPEC non-classifiable *E. coli* strains may give new insight into the origins of sporadic UTI in both animals and people. The presence of "non-pathogenic" isolates from animals that have clinical signs of UTI may suggest alternative reservoirs.

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Declaration of conflicting interests

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