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Identification of Urovirulence Traits in *Escherichia coli* by Comparison of Urinary and Rectal *E. coli* Isolates from Dogs with Urinary Tract Infection

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Spontaneously occurring urinary tract infection (UTI) in dogs was exploited as an experiment of nature to gain insights into UTI pathogenesis in humans. Concurrent urinary and rectal *Escherichia coli* isolates from 37 dogs with UTI were compared with respect to phylogenetic background, O antigens, and extended virulence genotype. In 54% of the UTI episodes, the dog's urinary and rectal isolates represented the same strain. Urinary isolates differed dramatically from rectal-only isolates in that they derived predominantly from *E. coli* phylogenetic group B2, expressed typical (human) UTI-associated O antigens, and possessed many virulence-associated genes, most notably *pap* elements (P fimbriae), *papG* (adhesin) allele III, *sfa*/*foc* and *sfaS* (S fimbriae), *hly* (hemolysin), *fyuA* (yersiniabactin), *iroN* (siderophore), and *ompT* (outer membrane protease T). The 20 urinary isolates that corresponded with the host's predominant rectal strain were no less virulent according to the markers analyzed than were the 17 urinary isolates that differed from the host's predominant rectal strain. These findings suggest that UTI pathogenesis is similar in dogs and humans, provide added support for the special-pathogenicity over the prevalence hypothesis of UTI pathogenesis, and identify numerous specific virulence-associated factors as significant correlates of urovirulence.

Dogs spontaneously develop urinary tract infection (UTI) due to strains of *Escherichia coli* similar to those that cause UTI in humans (4, 6, 7, 15, 18, 25, 30, 42, 45–47, 50, 51). In both host groups, the urinary pathogens typically derive from specific clonal groups within virulence-associated *E. coli* phylogenetic group B2, express one of several UTI-associated O antigens, and possess specialized virulence-associated factors (VFs) that directly contribute to, or are markers for, an enhanced ability to overcome host defenses and cause extraintestinal disease (15, 18, 25, 41). In both dogs (18, 30) and humans (8, 40, 49) at the time of a UTI episode, the infecting urinary strain is often the host's predominant rectal *E. coli* strain, consistent with the fecal reservoir as the proximate source from which pathogens enter the urinary tract. Even healthy dogs are frequently intestinally colonized with uropathogenic *E. coli* and shed these organisms in their feces (23, 50, 51).

These observations are potentially relevant to human health because they suggest that dogs may pose an infectious threat to humans by providing a reservoir of "human" uropathogenic *E. coli* for acquisition by susceptible human hosts (15, 18, 23, 25, 30, 45). In addition, they suggest that the pathogenesis of UTI is similar in dogs and humans, so that dogs might serve as a pathogenetically relevant model system for observational or experimental studies of UTI pathogenesis (15, 25).

Accordingly, in the present study we took advantage of spontaneously occurring UTI in dogs to address several questions regarding the pathogenesis of UTI in humans. In 37 dogs with UTI, which served as their own controls, we compared concurrent urinary and rectal *E. coli* isolates with respect to strain identity, phylogenetic background, O antigens, and VF profiles. We defined the proportion of UTI episodes in which the urinary strain represented the predominant rectal strain, determined whether rectal and UTI isolates constituted similar or different bacterial populations overall, and characterized in detail the differences encountered between the rectal and urinary *E. coli* populations. We also determined whether UTI isolates that correspond to the host's predominant rectal strain, for which simple prevalence might drive UTI pathogenesis, represent a different and possibly less virulent population than UTI isolates that differ from the host's predominant rectal strain, which instead presumably possess special pathogenicity as the basis for their ability to cause UTI (39).

MATERIALS AND METHODS

Strains. Specimens were collected from 37 canine patients of the Small Animal Medicine Service, Veterinary Medical Teaching Hospital, University of California from November 1975 through September 1985, prior to administration of antibacterial agents (29). Urine specimens were collected by antepubic cystocentesis (28) for analysis and bacterial culture as part of a laboratory database. The specimens were divided immediately after collection. Bacterial culture was conducted on a portion of the specimen if the urine specific gravity was found to be <1.013 (the typical canine range is 1.015 to 1.045) (38), if microscopic evaluation of the urine sediment revealed more than three white blood cells (WBC) per high-power field (hpf), and/or if bacteria were visualized on a Gram-stained preparation of the sediment. The specimens were streaked onto sheep blood agar and MacConkey agar plates using calibrated loops and quadrant streaking. Cultures were considered positive if they yielded $\geq 2 \times 10^2$ CFU of a particular species/ml. Rectal specimens were collected, after first cleansing and drying the anal area, by inserting a sterile cotton-tipped swab

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several centimeters into the rectum. The swabs were streaked onto sheep blood agar and MacConkey agar plates for colony isolation.

The agar plates were incubated in 5% CO_2 at 37°C and were observed for bacterial growth after 24, 48, and 72 h of incubation. For each dog, using the appropriate MacConkey agar plate, one lactose-positive colony each isolated from the urinary culture and the rectal culture was arbitrarily selected for species identification. In addition, arbitrarily selected hemolytic colonies from blood agar plates were subcultured to MacConkey's agar to assess lactose fermentation. A representative of each distinct lactose-positive colonial morphotype or hemolysin phenotype was picked from each culture if phenotypic diversity was noted, which occurred for four rectal cultures and two urinary cultures. From these six cultures, two isolates each were saved, and from four of the six cultures, the second isolate was viable when resuscitated for the present study. (In all four instances, the two isolates from the same culture yielded indistinguishable genomic profiles when analyzed as described below and so were considered to represent a single strain.)

Bacteria were presumptively identified as *E. coli* using standard methods (3, 34). Putative *E. coli* cells were lyophilized in 20% skim milk and stored at room temperature in heat-sealed glass vials. In preparation for this study, randomly selected rectal-urinary strain pairs from the database were resuscitated from lyophilized stocks, confirmed biochemically as *E. coli* using the API-20E system (bio-Merieux), and then stored in 20% glycerol at −70°C until they were used. The *E. coli* Reference (ECOR) collection has been described elsewhere (12, 14, 35). The human clinical *E. coli* isolates CA10, V32, 536, BOS02, and IA2 were described previously (2, 5, 17, 20, 24, 33).

Clinical data. The available clinical data for the canine patients included breed, age, gender, clinical diagnosis for the index visit, and degree of pyuria on microscopic urinalysis of cystocentesis-obtained urine. Clinical diagnoses were categorized as UTI related if they included a UTI syndrome or a UTI-associated clinical sign (e.g., incontinence, polakiuria, or stranguria). Pyuria results were analyzed using a semiquantitative score ranging from 1 to 9, with 1 corresponding to <3 WBC/hpf and 9 corresponding to >100 WBC/hpf.

Predominant and minor rectal strains. Published data indicate that, among canine fecal samples that yield *E. coli* when cultured, analysis of three arbitrarily selected *E. coli* colonies will reveal a single strain in approximately two-thirds of the samples, whereas in the remaining one-third of the samples two distinct strains will be detected (23). This permits the calculation that, on average, the predominant *E. coli* strain in canine feces accounts for 89% of the local *E. coli* population. This indicates that any arbitrarily selected canine fecal *E. coli* colony should have an ~89% probability of representing the predominant fecal *E. coli* strain, with any other strains present in the sample presumably representing minor strains. Similar calculations have been made for humans (27). Thus, the selected fecal isolates from the present canine subjects were regarded as putatively representing the host's predominant fecal strain at the time of the UTI episode, whereas different genotypes recovered from the same host were regarded as not representing the predominant fecal strain.

Phylogenetic analysis. The 74 canine rectal and urinary isolates, plus the four viable second isolates from four cultures with diverse colonial morphologies, were subjected to randomly amplified polymorphic DNA (RAPD) analysis, as described elsewhere (13, 17, 23, 25). Briefly, genomic profiles were generated for each isolate by using (separately) two arbitrary decamer primers (1281, 5′-AA CGCGCAAC-3′, and 1283, 5′-GCGATCCCCA-3′) (1), with boiled lysates as the template DNA. The amplification conditions were as described by Berg et al. (1), except that commercial Ready-To-Go PCR beads (Amersham Pharmacia Biotech, Piscataway, N.J.) were used.

After agarose gel electrophoresis, visual inspection of RAPD profiles was used to determine whether each subject's urinary and rectal isolate(s) and (when applicable) the two phenotypically distinct isolates from the same culture represented the same genotype (strain) or different strains. Next, both RAPD profiles for one representative of each unique genotype from each subject were digitally combined in series to create a virtual composite profile for computer-assisted phylogenetic analysis. Similar composite profiles were generated concurrently for 10 control strains from the ECOR collection (ECOR strains 4, 20, 39, 42, 48, 50, 52, 62, 69, and 71). The ECOR strains were selected to provide one (nonaligned strains), two (groups A, B1, and B2), or three (group D) representatives each from the five major ECOR phylogenetic groups, as defined by Herzer et al., based on the electrophoretic mobilities of 38 metabolic enzymes (9).

Phylogenetic analysis was done with the assistance of the application Molecular Analyst-Fingerprinting (Bio-Rad, Hercules, Calif.). Similarity values were generated for all pairwise comparisons among the composite RAPD profiles of the canine strains and the ECOR control strains. This was done using Pearson correlation coefficient analysis of analog densitometric scans of the composite profiles in the absence of subjective operator input. The canine strains were assigned to the phylogenetic group of the most similar ECOR strain according to these similarity coefficients. In addition, the resulting similarity matrix was used to create a dendrogram according to the unweighted pair group method with averaging (44) (Fig. 1). This dendrogram was inspected to identify major clusters and subclusters, which were then used to stratify the population for statistical analysis.

Based on their similarity to certain canine urinary or rectal isolates from the present study with respect to the O antigen and VF profile, the previously described human clinical isolates CA10 and BOS02 (diverse-source bacteremia) (17, 33), V32 (urosepsis) (21), and 536 and IA2 (pyelonephritis) (2, 20) were compared with selected canine isolates by RAPD analysis with primer 1290 (5′-GTGGATGCGA-3′) (1).

Virulence genotypes. The 74 canine urinary and rectal isolates were tested for 30 VFs of extraintestinal pathogenic *E. coli* (41) by using a multiplex PCR assay, as described elsewhere (12, 18, 21, 24). Strains that were positive for any *pap* element were tested for 12 alleles of *papA*, corresponding to the 11 established P-fimbrial F types (F7-1, F7-2, and F8 to F16) plus the recently defined F48 *papA* variant, by using another multiplex PCR assay as described elsewhere (18, 26). All virulence genotypes were determined at least in duplicate using boiled lysates prepared from two separate colonies of each strain. Discrepant results were investigated further with additional determinations as needed. Generation of an appropriate-size PCR product with gene-specific primers, which corresponds closely to the presence of the particular gene as assessed by probe hybridization (24), was interpreted as positivity for the particular VF, without phenotypic confirmation. The VFs were designated according to the gene targets used to detect them in the multiplex PCR assay. A VF score was calculated for each isolate as the sum of all tested VFs for which the isolate was positive, with proportional adjustment for multiple detection of the same operon in the cases of *pap*, *sfa/foc*, and *kpsMT* (12, 17).

Serotyping. O antigens were determined by the Gastroenteric Disease Center (formerly the *E. coli* Reference Center), University Park, Pa., using 180 O-specific antisera, according to standard methods (37). Nine O antigens that are associated with UTI in humans (O1, O2, O4, O6, O7, O16, O18, O25, and O5) were defined as O-UTI antigens (11).

Statistical methods. Comparisons involving the prevalences of a given trait in different populations were tested using Fisher's exact test. Comparisons involving the prevalences of different traits within the same population were tested using McNemar's test. Comparisons involving aggregate VF scores or pyuria scores were tested using the Mann-Whitney U test. The correlation of the pyuria score with the VF score was tested using the Spearman rank correlation coefficient. Independent bacterial predictors of the urine source were identified by multivariate logistic regression analysis. The criterion for statistical significance was a P value of <0.05.

RESULTS

Characteristics of the source population. The 37 canine subjects encompassed 27 different breeds. Twenty-one breeds were represented by a single subject each, whereas seven (boxer, dachshund, doberman pinscher, golden retriever, Labrador retriever, miniature poodle, and German shepherd) were represented by from two to four subjects each. The subjects ranged in age from <1 ($n = 1$) to 15 ($n = 1$) years, with a median age of 9 years and modes at 5 and 9 years (each $n = 4$), 11 years ($n = 6$), and 13 years ($n = 5$). Ten dogs (27%) were males (one castrated), and 27 were females (21 spayed). This gender distribution corresponds approximately to that observed at the same clinic among 8,354 UTI episodes diagnosed in canine patients from 1969 to 1995 (29). Ten dogs (27%) had a UTI-related diagnosis for the index clinic visit. Microscopic urinalysis of cystocentesis-obtained urine samples showed a median of 20 WBC/hpf (range, 0 to 2 to >100).

Correspondence of rectal and urinary isolates. According to RAPD analysis, for 20 (54%) of the 37 canine subjects, the paired concurrent rectal and urinary isolates were indistinguishable and hence putatively represented the same strain, whereas for 17 (46%), the rectal and urinary isolate(s) exhib-

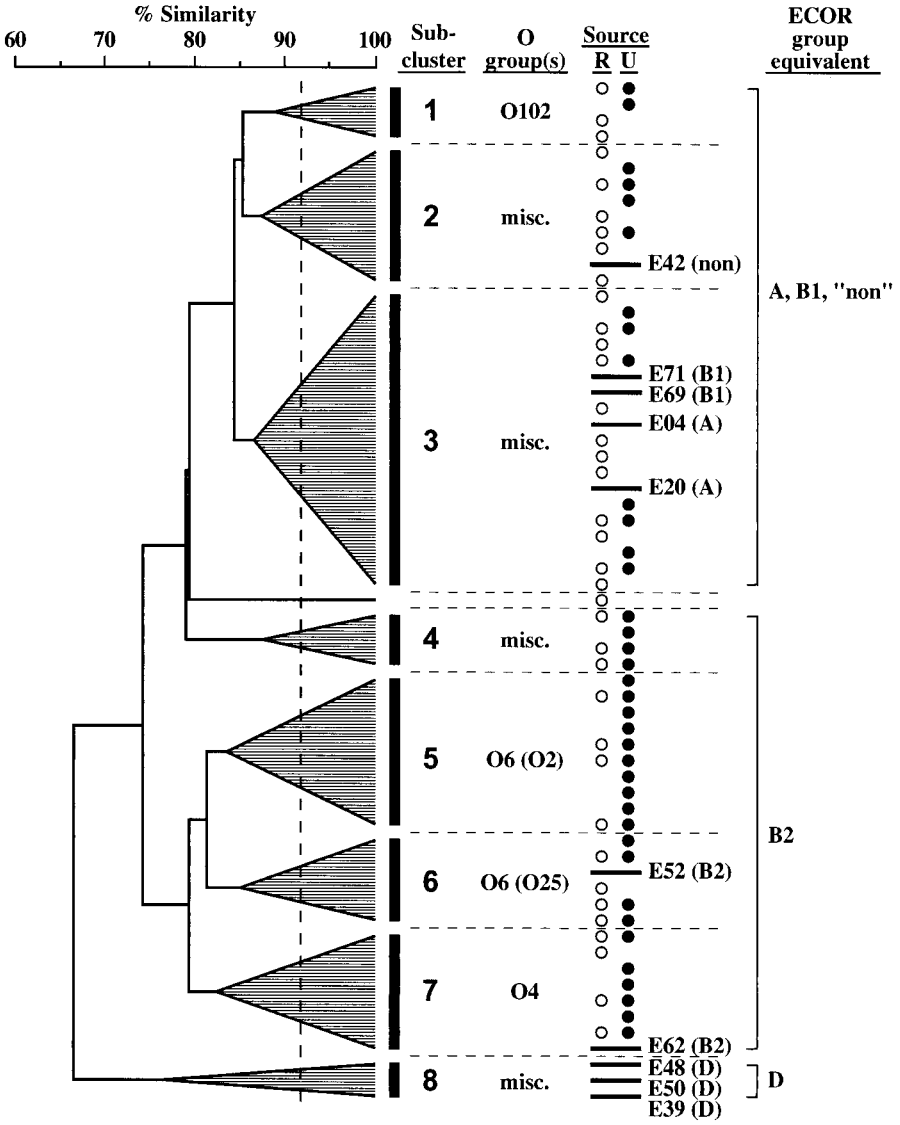

FIG. 1. Phylogenetic relationships among rectal and urinary isolates of *E. coli* from 37 dogs with UTI. The dendrogram is based on cluster analysis of genomic similarity as inferred from computer analysis of composite RAPD profiles for the 54 unique isolates. (The correspondence of 20 rectal isolates with the urinary isolates from the same 20 dogs was inferred from a separate RAPD analysis.) The shaded triangles represent arborizing clusters, which were arbitrarily designated 1 through 8. The dashed vertical line through the dendrogram marks the depth of the cluster that included 85% of the molecular size marker lanes (not shown), which indicates the theoretical limit of resolution and reproducibility of the assay. The predominant O antigens are shown for clusters 1, 5, 6, and 7. (Other clusters, listed as miscellaneous [misc.], had no predominant O antigen.) Dashed horizontal lines separate the individual clusters. For the column Source, each open (rectal [R]) or solid (urinary [U]) circle represents one canine isolate. Horizontally aligned urinary and rectal isolates are from the same dog. Reference strains from the ECOR collection (labeled E42, etc., with the phylogenetic group in parentheses) are shown as solid horizontal bars. Clonal groups corresponding to non-B2 phylogenetic groups (i.e., clonal groups 1, 2, and 3) or to phylogenetic group B2 (i.e., clonal groups 4, 5, 6, and 7) are grouped by brackets at the right. No canine isolates were placed in cluster 8 (phylogenetic group D ECOR strains).

ited distinct RAPD patterns and hence putatively represented different strains. For the 20 putative same-strain urinary-rectal pairs, the paired isolates were found to exhibit identical O antigens and extended virulence genotypes, except for three single-VF discrepancies (not shown). Consequently, the population was considered to include 54 unique isolates, i.e., 17 rectal-only isolates from 17 different dogs, 17 genotypically distinct urinary-only isolates from these same 17 dogs, and 20 unique urinary-rectal isolates from the remaining 20 dogs.

Phylogenetic analysis. Phylogenetic analysis (Fig. 1) of the 54 unique canine isolates according to composite RAPD profiles showed the population to consist of two major clusters of approximately equal size. One of these clusters corresponded to *E. coli* phylogenetic group B2, and the other corresponded to *E. coli* phylogenetic groups A and B1 and the nonaligned strains of the ECOR collection (Fig. 1). None of the 58 canine isolates was placed with the group D ECOR control strains (Fig. 1). The two major clusters in turn comprised seven sub-

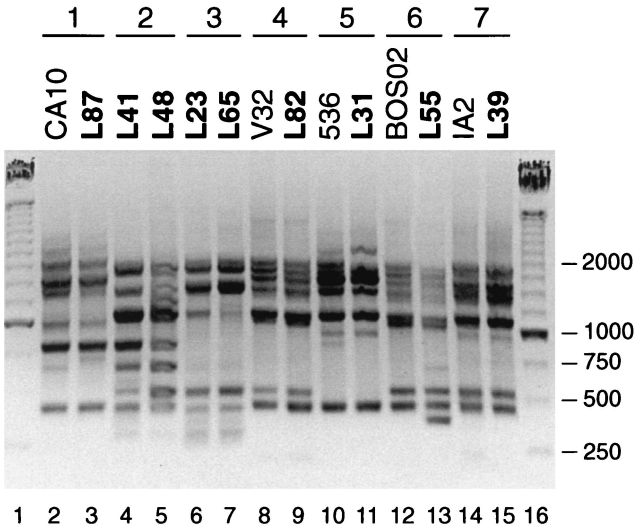

FIG. 2. Genomic profiles of canine and human *E. coli* isolates from diverse clonal groups. The profiles were generated by RAPD analysis using primer 1290. The boldface strain names (lanes 3 to 7, 9, 11, 13, and 15) indicate canine urinary or rectal isolates from the present study. The lightface strain names (lanes 1, 8, 10, 12, and 14) indicate human clinical isolates, which are paired with selected canine isolates based on anticipated similarity relationships. Clonal groups are listed above the strain names, with horizontal rules bracketing the included isolates. Lanes 1 and 16, 250-bp marker (Gibco).

clusters. These were presumed to represent separate clonal groups and were arbitrarily designated 1 through 7 (Fig. 1). Clonal groups 1 to 3 and a single ungrouped isolate (total, 27 isolates) corresponded to a non-group B2 phylogenetic background, whereas clonal groups 4 to 7 (26 isolates) corresponded to a group B2 phylogenetic background (Fig. 1).

Comparison of human and canine isolates. Figure 2 shows RAPD profiles for selected representative of each of these seven clonal groups and, for clonal groups 1, 4, 5, 6, and 7, also for human clinical isolates that were predicted to resemble the canine isolates based on similarities with respect to the O antigens and VF profiles. Within each of these five clonal groups, the paired human and canine isolates are as similar or more similar to one another than either isolate is to an isolate from a different clonal group, even from the same host species (Fig. 2). This illustrates the nature of the molecular data used to define the clonal groups (Fig. 1) and demonstrates genomic commonality among selected human and canine clinical isolates.

O antigens. Twenty-four unique O antigens were detected among the 54 unique canine isolates. Five O antigens were encountered in ≥3 isolates each: O6 (8 isolates), O8 and O4 (5 isolates each), O2 (4 isolates), and O102 (3 isolates). These five frequent O antigens, which accounted for only 21% of the unique O antigens overall, collectively accounted for 25 (46%) of the isolates. Three of the five frequent antigens (i.e., O2, O4, and O6) represented classic human UTI-associated O antigens (11). Additional human UTI-associated O antigens encountered in the population included O1 and O7 (one isolate each) and O25 (two isolates). Collectively, these six human UTI-associated O antigens, although representing only 25% of the unique O antigens overall, accounted for 21 (39%) of the 54 unique isolates.

Phylogenetic distribution of O-UTI antigens. The UTI-associated O antigens were significantly concentrated within phylogenetic group B2, occurring in 19 (73%) of the 26 unique B2 isolates but in only two (7%) of the 28 unique non-B2 isolates ($P < 0.001$). This trend was also evident within the individual constituent clonal groups (Fig. 3). As for specific O antigens within the individual clonal groups, clonal group 1 was characterized by the O102 antigen (40% versus 0% other strains; $P = 0.007$), clonal group 5 was characterized by the O2 antigen (30% versus 2% other strains; $P = 0.02$) and the O6 antigen (50% versus 7% other strains; $P = 0.003$), clonal group 6 was characterized by the O6 antigen (40% versus 12% other strains; $P > 0.10$), and clonal group 7 was characterized by the O4 antigen (71% versus 0% other strains; $P < 0.001$). This provided additional support for the phylogenetic validity of the inferred clonal groupings and specifically linked clonal groups 4 to 7 with known virulence-associated serogroups.

Phylogenetic background and O antigens according to clinical source. We next assessed the distribution of phylogenetic background and O antigen status according to clinical origin. Of the 54 unique isolates, the 17 rectal-only isolates exhibited a predominantly non-B2 phylogenetic background, with only two deriving from a B2-equivalent clonal group (Fig. 1 and Table 1). In contrast, the 37 urinary isolates were heavily concentrated within the B2-equivalent clonal groups, regardless of whether they corresponded with the host's rectal isolate (urinary-rectal subgroup; $n = 20$) or were distinct from it (urinary-only subgroup; $n = 19$) (Fig. 1 and Table 1). Differences with respect to phylogenetic group B2 status between the rectal-only isolates and either of the urinary subgroups alone, or all

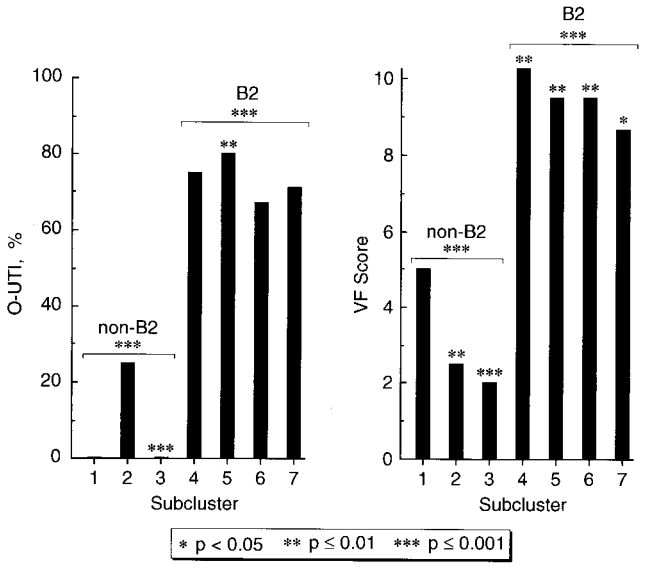

FIG. 3. Phylogenetic distributions of O antigens and VF scores. The prevalences (percent) of human UTI-associated O antigens (O-UTI) and mean VF scores are shown for the seven clonal groups, i.e., the subclusters from Fig. 1. Statistical comparisons (by the Mann-Whitney U test) are for each individual clonal group (subclusters 1 to 7) or phylogenetic group (phylogenetic group B2 or the non-B2 groups) versus the balance of the population.

TABLE 1. Phylogenetic distribution of 54 unique rectal and urinary isolates of *E. coli* from dogs with UTI

Bacterial trait	No. (%) of unique isolates ($n = 54$)[c]	Prevalence of bacterial trait by source [no. (%)]				*P* value[d] for rectal only versus:		
		Rectal only ($n = 17$[c])	Urinary					
			Urinary-rectal ($n = 20$)	Urinary only ($n = 17$)	All urinary ($n = 37$)	Urinary-rectal	Urinary only	All urinary
Phylogenetic group B2[a]	26 (48)	2 (12)	13 (65)	11 (65)	24 (65)	0.002	0.004	<0.001
UTI-associated O antigens[b]	9 (33)	2 (12)	10 (50)	9 (53)	19 (51)	0.017	0.026	0.007

[a] Phylogenetic group B2 (i.e., clonal groups 4 to 7) as defined by Herzer et al. (9) by multilocus enzyme electrophoresis.
[b] Human UTI-associated O antigens include O1, O2, O4, O6, O7, O16, O18, O25, and O75 (11). Of these, all but O16, O18, and O75 were encountered at least once in the population.
[c] Includes rectal, urinary-rectal, and urinary isolates.
[d] *P* values are from Fisher's exact test.

urinary isolates combined, were highly statistically significant (Table 1). In contrast, the two urinary subgroups did not differ from one another (Table 1).

Similar results were obtained with respect to the UTI-associated O antigens, which were significantly more prevalent among the urinary-rectal isolates, the urinary-only isolates, and all urinary isolates combined than among the rectal-only isolates (Table 1). In contrast, the two urinary subgroups again did not differ from one another (Table 1). These findings established that the urinary and rectal isolates represent distinct bacterial populations, with the urinary isolates exhibiting characteristics typical of human UTI isolates, i.e., enrichment for phylogenetic group B2 and virulence-associated O serogroups, irrespective of their same versus different status in comparison with the host's concurrent predominant rectal strain.

Prevalences and phylogenetic distributions of VFs (Table 2). According to PCR-based detection of 30 UTI-associated VFs, all of the VFs analyzed except *afa*/*dra*, *bmaE*, *gafD*, *nfaE*, and the K1 *kpsMT* variant were present in at least 1 of the 54 unique rectal and urinary isolates (Table 2). These VFs ranged in prevalence from 3% (*iha*) to 95% (*fimH*) (Table 2). Among the *pap*-positive isolates, the most frequently encountered *papA* alleles were F12 (nine isolates), F10 (eight isolates), and F14 and F48 (two isolates each). Other *papA* alleles each encountered in a single unique isolate included F7-2 and F15. Six strains had two different *papA* alleles: F10 plus F12 (four strains), F10 plus F14 (one strain), and F12 plus F15 (one strain).

The various VFs exhibited a significantly nonrandom phylogenetic distribution (Table 2). All VFs except *traT* and those with prevalence values of >95 or <12% were statistically associated with phylogenetic group B2 at the $P < 0.05$ level. For most VFs, similar associations were also observed with the serological analog of phylogenetic group B2, the UTI-associated O antigens (Table 2). The sole statistically significant exception to the B2 > non-B2 pattern was provided by *iutA*, which, consistent with trends previously noted among human source isolates (17, 24), was more prevalent among the non-B2 isolates (Table 2).

Consistent with the observed nonrandom phylogenetic and serogroup-specific distribution of VFs, aggregate VF scores differed significantly according to phylogenetic background and O antigen status. The median aggregate VF scores were higher among B2-derived isolates (median score, 9.25) and among isolates with UTI-associated O antigens (median score, 9.0) than among non-B2 or non-O-UTI isolates (median score,

TABLE 2. Distribution of VFs according to phylogenetic group and O antigen status among 54 unique *E. coli* isolates from dogs with UTI

VF[b]	Total prevalence [no. (%)] ($n = 54$)	Prevalence of VF within subgroup [no. (%)][a]	
		Phylogenetic group B2[c] ($n = 26$)	UTI-associated O antigens[d] ($n = 22$)
F10 *papA*	8 (15)	8 (31)**	7 (33)**
F12 *papA*	9 (17)	9 (35)***	7 (233)*
papC[e]	16 (30)[e]	16 (62)***	13 (62)***
papG allele I	1 (2)	1 (4)	1 (5)
papG allele II	1 (2)	1 (4)	0
papG allele III	15 (28)	15 (58)***	13 (62)***
sfa/*focDE*	21 (39)	21 (81)***	17 (81)***
sfaS	8 (15)	8 (31)***	8 (38)***
focG	5 (9)	5 (19)*	3 (14)
iha	2 (3)	2 (8)	1 (5)
fimH	53 (98)	26 (100)	21 (100)
hlyD	18 (33)	17 (65)***	15 (71)***
cnfI	23 (34)	15 (58)***	13 (62)***
cdtB	4 (7)	4 (15)*	2 (10)
fyuA	37 (69)	26 (100)***	21 (100)***
iroN	26 (48)	23 (88)***	19 (90)***
iutA	16 (27)	3 (12)(**)	2 (10)(**)
kpsMT II	21 (39)	19 (73)***	16 (76)***
kpsMT III	3 (6)	1 (4)	1 (5)
rfc	6 (11)	5 (19)	4 (19)
ibeA	8 (15)	8 (31)**	4 (19)
cvaC	3 (6)	1 (4)	1 (5)
traT	19 (35)	6 (23)	5 (24)
ompT	24 (44)	19 (73)***	14 (67)**
malX	27 (50)	24 (92)***	17 (81)***

[a] *P* values (from Fisher's exact test) for comparisons of indicated group versus all other isolates are coded as follows: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. The parentheses around *P* value codes indicate negative associations. *P* values are shown only where they are ≤ 0.05.
[b] VFs include *pap* (P-fimbrial) elements, i.e., *papA* alleles (structural-subunit variants), *papC* (pilus assembly), and *papG* alleles (adhesin variants), plus *sfa*/*foc* (S and F1C fimbriae), *sfaS* (S-fimbrial adhesin), *focG* (F1C fimbriae), *iha* (putative adhesin-siderophore), *fimH* (type 1 fimbrial adhesin), *hlyD* (hemolysin), *cnf1* (cytotoxic necrotizing factor 1), *cdtB* (cytolethal distending toxin), *fyuA* (yersiniabactin receptor), *iroN* (siderophore receptor), *iutA* (aerobactin receptor), *kpsMT* II and III (group II and III capsule synthesis), *rfc* (O4 lipopolysaccharide synthesis), *ibeA* (invasion of brain endothelium A), *cvaC* (colicin V), *traT* (serum resistance associated), *ompT* (outer membrane protease T), and *malX* (pathogenicity-associated island marker). Not detected were *afa*/*dra* (afimbrial and Dr-binding adhesins), *bmaE* (M fimbriae), *gafD* (G or F17 fimbriae), *nfaE* (nonfimbrial adhesins), and the K1 *kpsMT* (capsule) variant.
[c] Phylogenetic group B2 (i.e., clonal groups D to G) as defined by Herzer et al. (9) by multilocus enzyme electrophoresis.
[d] Human UTI-associated O antigens include O1, O2, O4, O6, O7, O16, O18, O25, and O75 (11).
[e] Results for *papA, papEF* (tip pilins), and *papG* were similar to those shown for *papC*.

TABLE 3. Distribution of VFs according to source among 54 *E. coli* isolates from dogs with UTI

VF	Prevalence of VF by source [no. (%)]				*P* value[a] for rectal only versus:		
	Rectal only (n = 17)	Urinary					
		Urinary rectal (n = 20)	Urinary only (n = 17)	All urinary (n = 37)	Urinary rectal	Urinary only	All urinary
papC[b]	0	9 (45)	7 (41)	16 (43)	0.002	0.007	0.001
papG allele I	0	1 (5)	0	1 (3)			
papG allele II	0	1 (5)	0	1 (3)			
papG allele III	1 (6)	7 (35)	7 (41)	14 (38)	0.048	0.04	0.02
sfa/focDE	1 (6)	12 (60)	8 (47)	20 (54)	0.001	0.02	0.001
sfaS	0	5 (25)	3 (18)	8 (22)	0.05		0.046
focG	0	5 (25)	0	5 (14)	0.05		
iha	0	1 (5)	1 (6)	2 (5)			
fimH	16 (94)	20 (100)	17 (100)	37 (100)			
hlyD	1 (6)	10 (50)	7 (41)	17 (46)	0.004	0.04	0.004
cnfI	2 (12)	9 (45)	6 (35)	15 (41)	0.04		0.06
cdtB	0	3 (15)	1 (6)	4 (11)			
fyuA	6 (35)	16 (80)	15 (88)	31 (84)	0.008	0.004	0.001
iroN	4 (24)	13 (65)	9 (53)	22 (59)	0.02		0.02
iutA	7 (44)	4 (20)	5 (29)	9 (24)			
kpsMT II	4 (24)	9 (45)	8 (47)	17 (46)			
kpsMT III	2 (12)	0	1 (4)	1 (3)			
rfc	1 (6)	2 (10)	3 (18)	5 (14)			
ibeA	1 (6)	2 (10)	5 (29)	7 (19)		0.096	
cvaC	0	2 (9)	1 (6)	3 (8)			
traT	7 (41)	9 (45)	3 (18)	12 (32)			
ompT	4 (24)	11 (55)	9 (53)	20 (54)	0.09		0.04
malX	5 (29)	12 (60)	9 (53)	22 (59)	0.099		0.08

[a] *P* values (from Fisher's exact test) are shown only where they are <0.10. All comparisons of urinary-rectal with urinary only yielded *P* values of >0.10 except for *focG* (P = 0.05).
[b] Results for *papA, papEF,* and *papG* were similar to those shown for *papC.*

3.0; $P < 0.001$ for both comparisons). The individual clonal groups also exhibited group-specific differences in aggregate VF scores, with the non-B2-derived groups all having lower scores and the B2-derived groups all having higher scores than the rest of the population (Fig. 3).

Distribution of VFs according to clinical source. Having established that among these canine-source isolates VFs are phylogenetically distributed much as has been observed among human-source isolates, we next assessed the distribution of VFs in relation to clinical source, in order to identify VFs specifically associated with urovirulence (Table 3). Consistent with their predominantly non-B2 phylogenetic background, the rectal-only isolates exhibited a markedly lower prevalence of most VFs than did the urinary-rectal isolates or the urinary-only isolates (Table 3). In contrast, the two urinary subgroups differed only for *focG* (25 versus 0%), and this difference was only marginally statistically significant (P = 0.05). Accordingly, the two urinary subgroups were combined as an all-urinary group, which also yielded multiple significant differences in comparison with the rectal-only group, all in a urinary > rectal pattern (Table 3).

Consistent with the concentration of most individual VFs among urinary isolates, aggregate VF scores were significantly higher among the urinary-rectal isolates (median, 9.0), the urinary-only isolates (median, 7.0), and the all-urinary isolates combined (median, 8.0) than among the rectal-only isolates (median, 3.0; P = 0.001 for all three comparisons). In contrast, the two urinary subgroups did not differ significantly with respect to the aggregate VF score ($P > 0.10$). Analysis of VF scores in relation to clinical source and phylogenetic background showed that the explanation for the higher overall VF scores among the urinary isolates as opposed to the rectal isolates was the different admixtures of phylogenetic group B2 versus non-B2 strains in the two source populations rather than possible source-specific differences in VF scores within a given phylogenetic group (Table 4).

Multivariate analysis. Stepwise multivariate logistic regression analysis was used to identify independent predictors of urovirulence among the multiple bacterial characteristics analyzed. When phylogenetic group B2 status and O-UTI status were included as predictor variables along with the individual VFs, the sole variable that was entered into the model was group B2 status (odds ratio [OR], 12.9 [range, 2.5 to 65.8]; P = 0.002). In a new model that was constructed after the removal of group B2 status from the predictor variable pool, the only trait that was entered was *fyuA* (OR, 8.6 [range, 2.3 to 32.8]; P = 0.002). In a third model that was constructed after the

TABLE 4. VF scores in relation to clinical sources and phylogenetic backgrounds among 54 unique urinary and rectal *E. coli* isolates from dogs with UTI

Clinical source (n)	Median aggregate VF score (n)		*P* value[a]
	Phylogenetic group B2	Non-group B2	
Rectal (17)	8.1 (2)	2.0 (15)	0.015
Urine (37)	9.5 (24)	3.0 (13)	<0.001

[a] *P* values by Mann-Whitney U test. For comparisons between rectal and urinary isolates within either phylogenetic category, *P* was >0.10.

further removal of *fyuA*, the only trait entered was *sfa*/*foc* (OR, 17.6 [range, 2.1 to 147.2]; $P = 0.008$).

Association of VFs with clinical variables. Among the 37 urinary isolates, the degree of associated pyuria was significantly greater in the presence of *traT* (median pyuria score, 7.0 versus 4.0; $P = 0.03$). Pyuria was not associated with other VFs or with the aggregate VF score, UTI-associated O antigens, or phylogenetic group B2 status (not shown). The presence of a UTI-associated diagnosis, gender, and age were not associated with any of these bacterial variables. The only observed association among the clinical variables was that the degree of pyuria was significantly greater in the presence than in the absence of a UTI-associated diagnosis (median pyuria score, 7.0 versus 3.0; $P = 0.02$). The urinary-only isolates (which corresponded by host to the rectal-only isolates) did not differ significantly from the urinary-rectal isolates with respect to any of the four associated clinical variables, i.e., diagnosis, gender, age, or degree of pyuria.

DISCUSSION

In this analysis of concurrent rectal and urinary *E. coli* isolates from 37 dogs with UTI, we found that, like human uropathogenic *E. coli*, canine UTI isolates characteristically derive from phylogenetic group B2, express UTI-associated O antigens, and possess numerous extraintestinal VFs, whereas commensal fecal strains from the same hosts lack these characteristics. These novel findings identify several specific VFs as significant predictors of urovirulence and provide support for the special-pathogenicity hypothesis over the prevalence hypothesis of UTI pathogenesis.

Consistent with previous reports, the canine UTI isolates in the present study were similar in many respects to human UTI isolates, including the predominance of phylogenetic group B2, certain O antigens (e.g., O1, O2, O4, O6, and O25), and many extraintestinal VFs (4, 6, 7, 15, 18, 25, 30, 42, 45–47, 50, 51). In particular, their distinctive O antigens and VF profiles suggested that clonal groups 5 and 6 correspond, respectively, with (serogroup O6) clonal groups 2 and 1 of Cherifi et al. (4), which include archetypal human pyelonephritis isolates 536 and CFT073 (15, 25), whereas clonal group 7 corresponds to the O4 serogroup, which includes the archetypal human pathogens J96, CP9, and IA2 (20, 22). In the present study, these epidemiological inferences were directly supported by comparative RAPD analysis (Fig. 2). These findings provide additional evidence that dogs spontaneously develop UTI due to *E. coli* strains that closely resemble human uropathogens, particularly those that cause cystitis (16).

The present study supersedes previous related work in several respects. It includes the largest population of canine UTI isolates to date that has been tested for this broad a range of VFs or for VF profile (regardless of extent) in relation to molecularly defined phylogenetic background (6, 15, 18, 25, 30, 42, 45, 50, 51). The larger sample size allowed us to define within the population individual clonal groups that were of sufficient size to support statistical comparisons regarding the phylogenetic distributions of VFs and O antigens (Fig. 3). It also allowed us to find numerous highly significant associations between VFs and urine source, including those for certain VFs not previously shown to be statistically associated with canine UTI, such as *sfaS*, *iroN*, *fyuA*, and *ompT*. The paired study design, in which each dog contributed its own rectal control isolate, provides a level of matching absent from previous comparisons of canine urinary and fecal isolates (50, 51), as well as from most such studies involving humans (11). This approach controls for confounding from possible unmeasured host-specific differences between the urinary and rectal populations, such as genetic, environmental, temporal (date of collection and age of host), and geographical factors. Such control greatly increases the probability that the observed associations are truly specific to the urinary versus fecal origin of the isolates and not extraneous factors.

This inclusion of paired rectal and urinary isolates from the same canine hosts allowed the canine subjects to serve as a "biofilter," functionally differentiating high-virulence from low-virulence *E. coli* strains according to whether the strains were able to overcome host defenses and cause UTI. A similar strategy was used in a previous comparison of urinary and fecal isolates from children with UTI, in which, however, the *pap* genotype was the only bacterial trait assessed (39). In the present study, the rectal-only canine strains, despite presumably representing the host's quantitatively predominant intestinal *E. coli* clone at the time of the UTI episode, failed to infect the urinary tract. However, in the same dogs, a genetically distinct strain presumably representing a minority component of the fecal flora that was missed by the sampling of a single colony from the rectal cultures out-competed the predominant rectal strain to cause UTI.

The rectal-only strains were almost all from non-B2 phylogenetic groups, lacked UTI-associated O antigens, and were largely devoid of extraintestinal VFs. These characteristics fit well conceptually with these strains' demonstrated nonvirulence and identify the rectal-only strains as predominantly commensals. The marked differences between the rectal and urinary isolates from the 17 dogs in which the rectal and urinary isolates represented different strains strongly support the special-pathogenicity hypothesis, according to which enhanced intrinsic virulence capability rather than simple quantitative predominance is required for extraintestinal infection to occur (39).

In addition, urinary isolates from those dogs in which the urinary strain instead was the predominant rectal strain, i.e., the urinary-rectal group, did not differ appreciably according to any of the traits analyzed from the urinary isolates from dogs in which a different strain was the predominant rectal strain, i.e., the urinary-only group. That is, these two groups of urinary isolates exhibited similarly high prevalences of phylogenetic group B2 origin, UTI-associated O antigens, and individual VFs (except for a marginally significant difference for *focG*, which actually favored the urinary-rectal group). They also had similarly high aggregate VF scores. This provides further support for the special-pathogenicity hypothesis over the prevalence hypothesis (39), since it indicates that a high level of intrinsic virulence is a prerequisite for a strain to be a successful uropathogen even if the strain has a quantitative advantage by virtue of its predominance in the rectal reservoir. The possibility that the dogs with matching urinary and rectal isolates represented a physiologically different host population from the dogs with distinct urinary and rectal isolates, i.e., were hosts in which prevalence might drive pathogenesis, was ar-

gued against by the similarity of these two host populations with respect to all clinical variables.

In view of the many parameters according to which canine UTI provides an analog of human UTI, valid insights presumably can be gleaned from the present study regarding the possible pathogenetic importance in both canine and human UTI of specific individual VFs. According to univariate analyses, the VFs most strongly predictive of a urinary source included *pap*, *papG* allele III, *sfa*/*foc*, *sfaS*, *hly*, *fyuA*, *iroN*, and *ompT*. Of these, *fyuA* and *sfa*/*foc* emerged as the most predictive in multivariate analyses. Of note, *papG* allele III is epidemiologically associated with cystitis in humans (10, 19) but has not been well evaluated for its contribution to bladder colonization (48) or explored as a vaccine target for the prevention of cystitis. S and F1C fimbriae (*sfa*/*foc*) have received little attention as possible contributors to urovirulence (31, 32), and S fimbriae in particular have been regarded as pathogenetically relevant principally in neonatal meningitis (43). The in vivo experimental evidence for hemolysin as a urovirulence factor is positive but quite limited (36). The "newer" VFs *iroN*, *fyuA*, and *ompT* also have not been evaluated experimentally as direct contributors to UTI pathogenesis. Consequently, further attention to all these traits as possible targets for future UTI-preventing interventions is warranted.

The diversity of breeds and ages represented among the present canine subjects suggests that the study's findings can be broadly generalized to the larger canine population. Although most subjects did not have a UTI-related diagnosis at the index clinic visit, we found that isolates associated with UTI-related diagnoses did not differ from other isolates according to any of the bacterial traits analyzed. This suggests that inclusion of the isolates from non-UTI-related diagnoses did not bias the study's results.

The use of multiple comparisons in this study increased the likelihood of a type I error, i.e., of falsely identifying a chance difference as significant. However, many of the differences identified yielded P values of ≤ 0.01, reducing the chances of a type I error, and were consistent with previous findings from other populations. Conversely, the study's power for correctly detecting between-group differences is limited by the sample size, with the attendant possibility of a type II error, i.e., of falsely concluding against a difference when one actually exists. Confirmation of these findings in a larger population would be desirable.

In summary, we found that *E. coli* urinary isolates from dogs with UTI, like human uropathogenic *E. coli*, characteristically derive from phylogenetic group B2, express UTI-associated O antigens, and possess numerous putative extraintestinal VFs, whereas fecal strains from the same hosts lack these characteristics. These findings provide added support for the special-pathogenicity hypothesis over the prevalence hypothesis of UTI pathogenesis and identify several specific VFs, including *papG* allele III, *sfa*/*foc*, *sfaS*, *hly*, *fyuA*, *iroN*, and *ompT*, as significant correlates of and hence as possible contributors to urovirulence.

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