Escherichia coli Colonization Patterns among Human Household Members and Pets, with Attention to Acute Urinary Tract Infection

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Background. Within-household transmission of Escherichia coli may promote urinary tract infection (UTI) but is poorly understood.

Methods. Fecal samples from 228 individuals (152 humans [5 with acute UTI] and 76 pets) in 63 households were extensively processed for unique E. coli clones, as defined by random-amplified polymorphic DNA analysis and pulsed-field gel electrophoresis. Patterns of strain sharing (presence of a clone in multiple individuals) were assessed.

Results. Of 335 E. coli clones, 90 (27%) were recovered from multiple hosts (up to 11 per clone). Within-household strain sharing (1) involved 68% of households, including 3 of 5 households in which a member had a UTI; (2) was more frequent than across-household strain sharing (27% vs. 0.8% of potential sharing pairs; P < .001); (3) increased with household size ($r^2 = 0.93; P < .001$); and (4) varied by host-pair type (pet-pet, 58%; human-human, 31%; human-pet, 17%). Sex partners shared strains more commonly than did other adults (31% vs. 7% of pairs; $P = .08$) but accounted for only 12% of within-household strain sharing.

Conclusions. Within-household sharing of E. coli, including in households in which a member has a UTI, is common and can involve any combination of humans and pets. Identification of the underlying mechanism(s) could lead to novel preventive measures against UTI.

Extraintestinal infections caused by Escherichia coli, including urinary tract infection (UTI), sepsis, and neonatal meningitis, cause significant morbidity, mortality, and increased health care costs [1]. Although the host’s own fecal flora is usually the immediate source of the responsible extraintestinal pathogenic E. coli (ExPEC) strain [2, 3], the external reservoir(s) from which the hosts initially acquire such strains are poorly understood, as are the relevant transmission mechanisms.

Accumulating evidence supports the occurrence of sexual transmission of ExPEC [4–14]. This may provide a mechanism for same-strain recurrent UTI because of the pathogen’s persistence in an external reservoir (the sex partner). However, 2 recent longitudinal studies, each involving a single household, suggested that within-household transmission of E. coli clones can also involve parents and children, siblings, and even pets [15, 16]. If this applies generally, if this strain sharing reflects within-household transmission, and if such transmission contributes significantly to the pathogenesis of UTI, efforts to prevent within-household transmission clearly would need to be targeted more broadly than only toward sexual transmission.

Accordingly, we conducted a cross-sectional point-prevalence survey of within-household E. coli colonization patterns. Specifically, we assessed the diversity of the fecal E. coli population among healthy humans and pets (or those with acute UTI) within multiple households. We used diverse selective culture methods to aid recovery of low-frequency clones and used random-amplified polymorphic DNA (RAPD) analysis and pulsed-field gel electrophoresis (PFGE) to resolve unique clones for across-host comparisons. We then compared the frequency of strain sharing (i.e., recovery
of a clone from multiple hosts) within versus across households, compared within-household strain sharing frequency with household size and host type, and examined strain sharing within households with acute UTI.

SUBJECTS, MATERIALS, AND METHODS

Subjects and samples. Index subjects were recruited as (1) healthy volunteer staff and visitors at the Minneapolis Veterans Affairs (VA) Medical Center or (2) patients with suspected acute uncomplicated cystitis (voiding symptoms; no fever, other constitutional symptoms, flank pain, or predisposing conditions) who presented to the VA medical center or a local private clinic. Recruitment was via flyers, word of mouth, and clinic personnel. After enrollment, index subjects were encouraged to refer all available household members, including pets, to the study and to facilitate specimen collection. All human subjects provided informed consent. The experimentation guidelines of the authors’ institutions were followed in the conduct of this clinical research.

Specimens included a fecal sample (collected as freshly passed feces, using a transport swab for human subjects and a plastic bag for pets) from all subjects and a pretherapy urine sample from subjects with UTIs. Specimens were refrigerated until processing. Index subjects identified participating household members as adult, child, or pet, provided the age and sex of human subjects, and indicated which adults were sex partners.

Sample processing. Feces were streaked to eosin–methylene blue (EMB) agar for overnight incubation at 37°C. The resulting bacterial growth was stored in 20% glycerol at −80°C. Later, a portion of this material was inoculated into 1 mL of nutrient broth. After overnight incubation at 37°C, broth cultures underwent serial dilution plating. The following day, volumes containing ~10^5 organisms were spread onto each of the following media (adapted from Gordon et al. [17]): EMB agar plus (individually, in appropriate concentrations) ampicillin, chloramphenicol, gentamicin, kanamycin, spectinomycin, streptomycin, tetracycline, nalidixic acid, ceftazidime, or ciprofloxacin; modified Mueller-Hinton agar plus trimethoprim-sulfamethoxazole; minimal medium agar plus (individually) 3 g/L rhamnose, raffinose, sucrose, sorbose, or dulcitol; or high-salt (4% NaCl) Luria-Bertani agar. Plates were incubated overnight at 37°C. Additionally, 10^5 organisms were plated onto EMB agar and incubated overnight at 50°C.

From the initial EMB plates, 7 E. coli colonies (lactose- and indole-positive, citrate-negative, gram-negative bacilli), as available, were saved for RAPD analysis, as described below. Likewise, from each selective plate that yielded E. coli, at least 1 E. coli colony was saved for RAPD analysis. Urine samples were plated quantitatively to EMB agar plates, and 1 E. coli colony was saved for further analysis. Identity was confirmed selectively by use of API 20E strips (bioMérieux).

RAPD analysis. The multiple E. coli isolates saved from a given fecal sample (and, if applicable, the corresponding urine sample) were compared for clonality by RAPD analysis [18]. All RAPD profiles for a given subject were generated using the same polymerase chain reaction run and were compared visually in the same gel. If the initial 7 colonies from the primary EMB plate yielded ≥4 different RAPD profiles, an additional 13 E. coli colonies (20 total) from this plate were similarly screened. Pilot studies indicated that additional diversity of RAPD profiles was unlikely among an additional 13 unselected fecal colonies if the first 7 colonies yielded ≤3 unique profiles (authors’ unpublished data).

PFGE analysis. One representative of each unique RAPD profile per subject and each E. coli urine isolate underwent PFGE analysis with XbaI and, selectively, AvrII [19]. Profiles were captured and analyzed digitally using BioNumerics software (Applied Maths). Any newly generated profile that exhibited a Dice similarity coefficient of <94% (corresponding approximately to a 3-band difference) in comparison with any previously encountered profile was defined as representing a new pulstype (unique clone) [20]. Profiles exhibiting ≥94% similarity to an existing profile were assigned to the corresponding pulstype (clone).

Dendrograms based on PFGE profiles were inferred according to the unweighted pair group method with averaging (UPGMA) based on Dice similarity coefficients. Isolates that consistently sheared during PFGE analysis were compared side by side by RAPD analysis, to allow presumptive clonal assignments. Pilot experiments showed that RAPD analysis was ~80% accurate in identifying unique clones among multiple isolates from a fecal sample. That is, ~80% of colonies from a given sample with the same RAPD profile yielded indistinguishable PFGE profiles, whereas ~80% of those with distinct RAPD profiles yielded distinct PFGE profiles.

UTI households. For 5 households (designated UTI households), an acute E. coli UTI episode occurring in 1 household member was captured by the study. An additional round of samples was sought from all UTI household members ~2 weeks after the UTI episode. In 1 UTI household, the UTI episode occurred during ongoing biweekly culture surveillance [15], so samples were available at ~2-week intervals before, during, and after the UTI episode. In a nested exploratory study, for each of the 5 UTI households an additional 1 (4 households) or 3 (1 household) sets of fecal and urine samples were screened for unique clones by sequential RAPD and PFGE analysis of multiple colonies from the primary EMB plates, as for the main study.

Colonization definitions. Presence of a specific clone in multiple hosts was defined as clone sharing, regardless of the underlying mechanism. Clones isolated from multiple hosts were defined as shared clones. Clone sharing was designated as within household when it involved members of the same household and as across household when it involved individuals in
different households. Households that exhibited within-household clone sharing were defined as clone-sharing households. The total number of potential clone-sharing pairs was calculated as the overall number of pairwise combinations of subjects, that is, \( n(n-1)/2 \), where \( n \) is the total number of subjects. A similar statistic was calculated for each household separately to give the number of potential within-household clone-sharing pairs in that household. The sum of these by-household values across all households gave the total number of potential within-household clone-sharing pairs. The difference between this value and the total number of potential clone-sharing pairs gave the number of potential across-household clone-sharing pairs.

**Statistical analysis.** Comparisons of proportions were tested using Fisher’s exact test. Comparisons involving continuous variables were tested using the Mann-Whitney U test. Linearity was assessed using simple regression. The threshold for statistical significance was \( P < .05 \).

**RESULTS**

**Subjects and households.** Overall, 351 subjects were enrolled. Of these, 286 provided fecal samples, 228 (80%) of which yielded *E. coli*. The 228 subjects with fecal *E. coli* constituted the study population. They included 152 humans (44 men, 60 women, 29 boys, and 19 girls) and 76 pets (48 dogs, 26 cats, and 2 other). They represented 63 households, which contained 2–8 (median, 3) subjects each. Forty-eight (76%) of the households included \( \geq 1 \) pet.

**Distribution of clones by subject and household.** Sequential RAPD and PFGE analysis of multiple fecal and urine *E. coli* colonies per subject yielded a total of 500 unique (by subject and clone) isolates. These represented 335 unique clones, of which 328 were defined by PFGE and 7 by RAPD (because of shearing during PFGE). Of the 335 unique clones, 245 (73%) were isolated from only 1 host each, whereas 90 (27%) were recovered from 2–11 (median, 2) hosts each; that is, they represented shared clones. Conversely, individual hosts yielded 1–6 (median, 2) clones each (figure 1).

At the household level, 291 (87%) of the 335 clones were isolated from only 1 household each, whereas 44 (13%) were identified in 2–9 (median, 2) households each. Individual households yielded 1–18 (median, 6) unique clones each (figure 2).

**Clone sharing within and among households.** Clone sharing was analyzed in 3 ways: by individual subjects, potential sharing pairs, and households. Of the 228 individual subjects, 52% shared \( \geq 1 \) clone with another subject (range, 1–4 shared clones each). Strain sharing was similarly frequent for pets and humans (52%–53%). Among humans, strain sharing was slightly more frequent for children (60%) than for adults (49%) (\( P > .10 \)) but did not differ in frequency by sex (53% for both male and female subjects).

As for strain-sharing pairs, overall the study population included 25,878 potential sharing pairs, including 371 (1.4%) within-household pairs and 25,507 (98.6%) across-household pairs. Strain sharing was observed in 313 (1.2%) of the total potential sharing pairs but was much more prevalent among within-household pairs (100/371 [27%]) than among across-household pairs (213/25,507 [0.8%]) (\( P < .001 \)), evidence that it was a household-specific phenomenon.

By sharing-pair composition, within-household strain sharing involved all combinations of subject groups (i.e., adult, child, and pet), in descending order of prevalence as follows: pet-pet (22/38 [58%]), adult-child (29/85 [34%]), child-child (5/15 [33%]), adult-adult (13/54 [24%]), adult-pet (22/120 [18%], and child-pet (9/59 [15%]). Strain sharing was more common among pet-pet pairs (58%) than among human-human pairs (47/154 [31%]) (\( P = .007 \)) and was more common among human-human pairs (31%) than among pet-human pairs (31/179 [17%]) (\( P = .004 \)). Pet-pet sharing also was more common within a given species (dog-dog, 11/14 [79%]; cat-cat, 9/11 [82%]) than across species (dog-cat, 4/18 [22%]) (\( P < .001 \)). Pet-human sharing was somewhat more common overall with cats (cat-human, 12/47 [26%]) than with dogs (dog-human, 19/115 [17%]) but varied between adults and children and according to pet species (cat-adult, 12/36 [33%]; cat-child, 0/11 [0%]; dog-adult, 11/75 [15%]; dog-child, 8/40 [20%]). Although adult sex-partner pairs shared strains somewhat more commonly (12/39 [31%]) than other adult-adult pairs (1/15 [7%]) (\( P = .08 \)), they accounted for only 12% of all within-household strain sharing.

By household, 42 (67%) of the 63 study households provided at least 1 instance of within-household strain sharing. The by-
household prevalence of strain sharing increased linearly with household size, from 33% for 2-member households to 100% for households with ≥6 members ($r^2 = 0.93; P < .001$) (figure 3). Among strain-sharing households, the number of shared clones ranged from 1 to 7 (median, 1) per household, with 15 households exhibiting ≥2 shared clones each. The extreme was an 8-member household (2 adults, 2 children, and 4 pets) in which 7 of the total 16 E. coli clones were shared among 2–3 hosts each, involving diverse combinations of adults, children, and pets (figure 4). Of the 18 households with ≥1 pet, 13 (72%) yielded evidence of pet-pet sharing.

Interestingly, within-household strain sharing among 2 humans and a pet, a scenario in which the pet might represent an intermediary vector among the humans, was documented in 8 instances (i.e., 4% of 183 potential “triple-sharing” combinations), each involving a separate household. However, overall strain sharing among humans (whether by individual, potential sharing pair, or household) was no different between households with and without pets (data not shown). Conversely, certain shared clones were recovered only from subjects representing a particular host category, suggesting possible host specificity. This phenomenon varied in frequency and extent by host type, from cat-only clones (2 clones with 2–3 feline hosts each) to human-only clones (17 clones with 2–7 human hosts each).

To validate the XbaI-based clone assignments, representatives of 4 XbaI clones that exhibited both within- and across-household strain sharing underwent secondary PFGE analysis using AvrII. For each clone, 2 same-household and 2 different-household isolates were analyzed. For 3 clones, AvrII results corresponded precisely with XbaI results; that is, profiles were ho-

**Figure 2.** No. of fecal Escherichia coli clones per household among 63 households. Clones (as defined by pulsed-field gel electrophoresis, after an initial screen by random-amplified polymorphic DNA analysis) were counted only once per household, regardless of the no. of subjects in which they were encountered.

**Figure 3.** Probability of any within-household sharing of fecal Escherichia coli strains in relation to household size (63 households in total). Strain sharing was defined as detection of the same E. coli clone (as defined by pulsed-field gel electrophoresis, after an initial screen by random-amplified polymorphic DNA analysis) in 2 or more household members. Households were classified as exhibiting strain sharing if any strain sharing was observed among household members, regardless of the no. of shared clones or the no. of affected hosts. The probability of within-household clone sharing varied linearly with the no. of individuals per household, from 2 to ≥6 ($r^2 = 0.93; P < .001$).
mogenous both within and across households. In contrast, for the fourth clone, although the same-household isolates exhibited indistinguishable AvrII profiles, each different-household isolate exhibited a unique AvrII profile (<92% similarity to others in the group), evidence of greater homogeneity within than across households.

**UTI households.** For 5 (8%) of the 63 households, a human household member experienced an acute *E. coli* UTI episode around the time of fecal sampling. These 5 UTI households, which accounted for 19 subjects (14 humans and 5 pets), ranged in size from 3 to 5 (median, 4) members each. Their primary samples yielded 51 (15%) of the study’s 334 total unique *E. coli* clones, with 8 of these clones also being encountered in non-UTI households. Three (60%) of the 5 UTI households exhibited within-household strain sharing; conversely, 10 (20%) of their 51 clones were shared among multiple subjects (range, 2–4 subjects each). Within 1 household the acute UTI clone was shared among multiple household members.

In a nested exploratory study, for each UTI household an additional 1–3 sets of fecal and urine samples (as available) from several weeks before (1 household) and/or after (all 5 households) the UTI episode were processed using RAPD and PFGE analysis. Up to 20 arbitrarily selected colonies from the EMB plate were analyzed per sample, as in the main study. Within each household, 1–4 clones not previously encountered in that household were identified (total, 13 total new clones). In 2 households, new within-household strain sharing was discovered, involving 3 clones and 4 strain-sharing pairs. Specifically, (1) a newly encountered fecal clone was shared between a patient with UTI and her sex partner; (2) this patient’s urine clone, already known to be shared with the sex partner, was newly found in the pet; and (3) in a different household, a previously encountered clone was recovered from a new household member. Thus, longitudinal surveillance identified more extensive within-household strain sharing than did cross-sectional analysis, some involving urine and fecal clones from the subject with UTI.

**DISCUSSION**

In this predominantly cross-sectional point-prevalence survey, we used diverse selective culture conditions and state-of-the-art genomic profiling methods to intensively assess the *E. coli* flora of 228 individuals from 63 households. We documented extensive within-household sharing of fecal *E. coli*, including within 3 of 5 UTI households. Our findings both confirm the results of previous smaller studies [15, 16] and extend them by demonstrating that within-household *E. coli* strain sharing is widely prevalent (being essentially a normal condition) and commonly occurs among pets, humans, and non–sex partners, as well as between sex partners. Moreover, because of sampling limitations, our findings provide only a minimum estimate of the extent of within-household strain sharing. Consequently, these data suggest a new paradigm for *E. coli* traffic patterns within households, one that could importantly influence the design of preventive measures against the diffusion of extraintestinal pathogenic and antimicrobial-resistant *E. coli*.

The considerable documented extent of within-household sharing of *E. coli*, although similar to that found in 2 recent longitudinal single-household studies [15, 16], was greater than that observed by Gordon et al. [17] or Caugant et al. [21] in their multiple-household longitudinal studies that used multilocus enzyme electrophoresis to resolve *E. coli* clones. This difference may reflect our use of a more-discriminating typing method (i.e., PFGE) and, in contrast to Caugant et al. [21], our use of diverse selective culture conditions to isolate low-prevalence clones from within the fecal microflora.

We found that, despite the greater absolute number of across-household strain-sharing pairs, when the data were adjusted for the number of potential sharing pairs, within-household sharing was proportionally much more common than across-household sharing (i.e., 27% vs. 0.8% of pairs). This indicates that within-household strain sharing truly is a household-specific phenom-
sequently, efforts to block within-household sharing of a small fraction (here, possibly 12%) of total strain sharing. Data indicate that sexual contact is by no means necessary for strain sharing by humans (especially between siblings and between children and sex partners) 

It is noteworthy, particularly because recent studies of this phenomenon have focused primarily on sex partners [4–14], that most strain sharing appeared to be nonsexual. That is, strain sharing was most common among pets, followed by humans (especially between siblings and between children and parents), followed by pet-human pairs. Thus, overall the data indicate that sexual contact is by no means necessary for within-household transmission and likely accounts for only a small fraction (here, possibly 12%) of total strain sharing. Consequently, efforts to block within-household sharing of _E. coli_ clearly would need to address routes other than sexual transmission and hosts other than adult sex partners.

Our findings leave uncertainty regarding the possible contribution of pets to human-human strain sharing [15, 16]. Notably, we documented strain sharing within 17% of potential pet-human sharing pairs, thereby establishing unambiguously that humans and their pets commonly carry the same _E. coli_ clones. This suggests that pets and humans may exchange _E. coli_ in either direction. Moreover, in 5 instances multiple human household members shared a strain with a household pet, which conceivably could have transmitted the strain among the humans, thereby creating human-human strain sharing. However, overall we found no association of pet ownership and within-household strain sharing among humans. This suggests that, although pets may participate in within-household strain transmission among humans, they may not do so to any greater extent than do human household members. Nonetheless, because upward of 50% of fecal _E. coli_ from dogs and cats exhibit virulence characteristics suggesting human pathogenic potential [23–27], it is possible that strain sharing involving pets actually poses a greater health threat to humans, on balance, than does direct human-human strain sharing.

The small number of acute UTI households limited power for assessing the contribution to UTI pathogenesis of within-household strain sharing. Nonetheless, strain sharing was observed with similar frequency in UTI and non-UTI households (60% vs. 67%), and it involved 1 (20%) of 5 _E. coli_ clones versus 27% of clones overall. Taken together with previous data from our laboratory [11, 14–16], the present findings suggest that when an acute UTI episode occurs in 1 household member, in ≥50% of instances the causative _E. coli_ clone is detectable in at least 1 additional household member. Future observational studies focusing more closely on acute UTI episodes, ideally with longitudinal surveillance (which increases sensitivity for detecting both preexisting clones and new acquisitions), could help define how frequently acute UTI episodes involve _E. coli_ clones that concurrently or sequentially colonize other household members. Likewise, an intervention that reduces within-household strain sharing conceivably could be used to determine whether prevention of strain sharing also reduces UTI incidence.

A limitation of our study is the uncertain representativeness of the study population. In this regard, according to the 2000 census [28] the average US household size is 2.59 persons, which compares closely with our mean of 2.4 persons. Additionally, based on a 1998–1999 study [29], 39% of US households have at least 1 dog and 32% have at least 1 cat, which can be compared with our corresponding values of 59% (for dogs) and 27% (for cats).

In summary, our assessment of the fecal _E. coli_ population of 228 individuals from 63 households documented extensive within-household sharing of fecal _E. coli_ clones, including from individuals with acute UTI. Our findings demonstrate that within-household _E. coli_ strain sharing occurs commonly among pets, humans, and non–sex partners, as well as between sex partners. This suggests a new paradigm for within-household _E. coli_ traffic patterns that could influence the design of preventive measures against the diffusion of pathogenic and antimicrobial resistant _E. coli_ through the population.

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**References**


