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# Intestinal Population Dynamics of UTI-Causing *Escherichia coli* within Heterosexual Couples

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## Abstract

From October 1999 to July 2001, a prospective cohort study was conducted to assess the intestinal *Escherichia coli* population dynamics of 23 sexually active couples. We tested the hypothesis that intestinal persistence and predominance of specific *E. coli* strains, co-colonization of sex partners with the same *E. coli* strain, and the intestinal diversity of fecal *E. coli*, contribute to recurrent urinary tract infection (UTI). *E. coli* isolates causing UTI, asymptomatic bacteriuria (ABU), or intestinal co-colonization were evaluated by ERIC2 PCR and compared with strains recovered exclusively from stool samples with respect to intestinal persistence, predominance, and diversity. Contrary to our hypothesis, UTI-causing strains exhibited similar levels of intestinal persistence and predominance as did fecal strains, and UTI episodes were not associated with shifts in fecal *E. coli* diversity. In contrast, intestinal co-colonization strains exhibited greater persistence and predominance than did fecal strains and were more likely to cause ABU, and co-colonization episodes were associated with significantly increased fecal *E. coli* diversity. Nonetheless, intestinal co-colonization strains were not associated with UTI. These findings suggest that *E. coli* strains involved in co-colonization may be more important contributors to intestinal *E. coli* dynamics than to UTI pathogenesis.

## Introduction

Uncomplicated, community-acquired urinary tract infection (UTI) occurs frequently in young, healthy women and is most often caused by *Escherichia coli* found in the human intestinal tract. Genotypic and phenotypic analyses have shown that UTI-causing *E. coli* belong to specific closely related lineages (Bettelheim, 1997; Caugant et al., 1983; Cooke, 1974a; Cooke, 1974b; Cooper et al., 1980; Elkins and Cox, 1974; Gruneberg, 1969; Pfau et al., 1983; Stamey et al., 1971; Stapleton et al., 1995; Yamamoto et al., 1997).

The bulk of recent research on the pathogenesis of uncomplicated UTI has focused on the role of putative virulence genes or the "special pathogenicity" of UTI-causing *E. coli* and on host-specific and epidemiological factors (Donnenberg and Welch, 1996; Fihn et al., 1996; Foxman et al., 1995a; Foxman et al., 1995b; Hooton et al., 1996; Ikaheimo et al., 1996; Johnson, 1991; Johnson et al., 1998b; Russo et al., 1995; Stamm et al., 1991). A competing hypothesis to the "special pathogenicity" hypothesis is the "prevalence hypothesis", i.e. that certain strains of *E. coli* have the ability to persist and predominate in the human intestine, making them more likely than other strains to cause UTI (Cooke, 1974a). Previous research has demonstrated that intestinal colonization with a uropathogen is critical to the development of a UTI (Caugant et al., 1983; Cooke, 1974a; Johnson et al., 1998a; Stamey et al., 1971; Yamamoto et al., 1997). However, little attention has been paid to understanding the intestinal population dynamics of UTI-causing *E. coli*, specifically the roles that persistence, predominance, and diversity of intestinal *E. coli* play in the development of recurrent UTI. Increased intestinal persistence and predominance, as the result of competitive bacterial exclusion or colonization-enhancing bacterial factors, may result in a greater opportunity for specific strains to cause disease. Likewise, lack of intestinal diversity may indicate an expansion of a particular clone in the gut, which could predispose a woman to a UTI caused by that strain. Intestinal persistence, predominance and diversity of UTI-causing *E. coli* in the male partner and intestinal co-colonization of sexual partners may also modify the risk of UTI in the female partner. A recent cross-sectional study observed that 32% of enrolled couples shared identical *E. coli* strains isolated from their rectum (co-colonization) and couples in which the woman developed UTI were also more likely to be rectally co-colonized than non-UTI couples (Foxman et al., 2002).

The specific aim of this prospective study was to describe the intestinal *E. coli* population dynamics in a cohort of young, healthy women predisposed to recurrent UTI and their male sexual partners. The study examined the hypothesis that increased intestinal persistence and predominance and decreased diversity of the intestinal *E. coli* flora contribute to an increased risk of UTI (Wold et al., 1992). The persistence, predominance and diversity of *E. coli* causing episodes of UTI, ABU and co-colonization was evaluated in the male partner to determine whether these factors lead to the re-infection of the female partner (Foxman et al., 2002; Foxman et al., 1997; Johnson et al., 1998a). Although recurrence of UTI in the female partner was the primary outcome of interest in this study, asymptomatic bacteriuria (ABU) was also examined because it can precede symptomatic UTI (Hooton et al., 2000).

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Table 1. Study outcome summary.

| Outcome                            | Women   | Episodes | Incidence per visit* | 95% CI     |
|------------------------------------|---------|----------|----------------------|------------|
|                                    | N (%)   | N        |                      |            |
| Baseline and recurrent UTIs        | 12 (52) | 18       | 0.20                 | 0.11, 0.28 |
| Recurrent UTIs                     | 6 (26)  | 8        | 0.09                 | 0.03, 0.15 |
| Any asymptomatic bacteriuria (ABU) | 14 (61) | 33       | 0.38                 | 0.27, 0.48 |
| HCABU                              | 8 (35)  | 12       | 0.14                 | 0.06, 0.21 |
| LCABU                              | 11 (48) | 21       | 0.24                 | 0.15, 0.33 |
| Intestinal co-colonization         | 14 (61) | 38       | 0.42                 | 0.32, 0.53 |

\*Incidence was estimated using the number of study visits for each woman as the denominator; total number of study visits varied from 88 for asymptomatic bacteriuria (ABU) to 93 for UTI. Women subjects were required to have a baseline UTI for entry in the study; therefore the incidence of all UTI is overestimated. Binomial, exact 95% confidence intervals (CI) are presented for the incidence measure.  
 UTI was defined by the report of two or more UTI symptoms and by medical record review. Recurrent UTI was defined as any UTI episode occurring at least two weeks after a previously treated episode.  
 High-count asymptomatic bacteriuria (HCABU) was defined as  $\geq 10^3$  cfu of *E. coli* per mL of urine.  
 Low-count asymptomatic bacteriuria (LCABU) was defined as between  $< 10^3$  and  $\geq 10$  cfu of *E. coli* per mL of urine. HCABU and LCABU in some cases involved more than a single unique *E. coli* strain and some women experienced more than one HCABU or LCABU episode caused by the same *E. coli* strain.

## Results

### Study subjects

From October 1999 to July 2001, 23 couples were enrolled and were followed for between 1 and 6 months (mean number of visits per couple = 3.96). Two couples had unequal numbers of visits. The median age of the women participants was 20 years (range 18–28) and of the male partners was 21 years (range 18–40). Eighty-eight percent of the women had experienced more than one UTI in the past 12 months (mean = 2.6 UTI episodes, range 1 – 5) and 91% had a lifetime history of  $\geq$  one prior UTI (mean = 4.4 UTI episodes, range 1 – 10).

### Bacterial strains

During the study period, 942 bacterial isolates were collected; 765 (81%) were *E. coli*. Of these, 686 (90%) were from stool samples (337 from the female and 349 from the male partners), 64 (8%) were from routine urine samples (60 from the female and 4 from the male partners), and 15 (2%) were from acute UTI samples (all females). Many of these isolates represented replicates of the same strain due to the selection of multiple colonies from each culture.

### ERIC2 PCR Fingerprinting

A total of 156 unique *E. coli* strains were resolved by ERIC2 PCR from among the 756 stool, urine, and

UTI *E. coli* isolates. One strain was identified only in a man's routine urine sample and so was excluded from subsequent analyses. The number of unique strains identified per couple varied by couple, from two strains over three visits to 13 strains over six visits.

### Incidence of study outcomes

A summary of the study outcomes is presented in Table 1. During the study 12 women experienced 18 clinically confirmed UTI episodes including, 10 baseline episodes and eight recurrent episodes. Three isolates causing baseline UTI episodes were not collected. The incidence of recurrent UTI during the follow-up period was 9 episodes per woman per 100 months. None of the male partners developed a UTI during the study. The strains causing recurrent UTI were isolated from the woman's stool sample prior to the UTI in five (63%) of the eight episodes.

The incidence of ABU was approximately four-fold greater than the incidence of UTI, with the incidence of low-count asymptomatic bacteriuria (LCABU) (0.24, 95% CI 0.15, 0.33) being almost twice that of high-count asymptomatic bacteriuria (HCABU) (0.14, 95% CI 0.06, 0.21) (Table 1). There were only three cases of LCABU among the male partners.

Thirty-eight episodes of intestinal co-colonization occurred among 14 (61%) of the couples, for a mean

Table 2. Joint distribution of study outcomes associated with unique *Escherichia coli* strains.

| Outcome (no. of strains) | No. (%) of strains with associated outcome |                   |                   |
|--------------------------|--|-------------------|-------------------|
|                          | Baseline and recurrent UTI<br>(N = 12)     | HCABU<br>(N = 13) | LCABU<br>(N = 21) |
| HCABU (N = 13)           | 3 (25)                                     |                   |                   |
| LCABU (N = 21)           | 5 (42)                                     | 3 (23)            |                   |
| Co-colonization (N = 22) | 4 (33)                                     | 5 (38)            | 11 (52)           |

Urinary tract infection (UTI) was defined by the report of two or more UTI symptoms and by medical record review. Recurrent UTI was defined as any UTI episode occurring at least two weeks after a previously treated episode.

High-count asymptomatic bacteriuria (HCABU) was defined as  $\geq 10^3$  cfu of *E. coli* per mL of urine. Low-count asymptomatic bacteriuria (LCABU) was defined as between  $< 10^3$  and  $\geq 10$  cfu of *E. coli* per mL of urine.

Table 3. Comparison of the median intestinal persistence of UTI, HCABU and LCABU-causing *Escherichia coli* strains to *E. coli* strains recovered from the stool only.

| Strain source              | N   | Women                    |                  | Men                      |                  |
|----------------------------|-----|--------------------------|------------------|--------------------------|------------------|
|                            |     | Median persistence (IQR) | <i>p</i> -value* | Median persistence (IQR) | <i>p</i> -value* |
| Baseline and recurrent UTI | 12  | 0.25 (0.21, 0.63)        | 0.63             | 0 (0, 0.50)              | 0.16             |
| HCABU                      | 13  | 0.20 (0, 0.60)           | 0.33             | 0 (0, 0.40)              | 0.01             |
| LCABU                      | 21  | 0.33 (0.17, 0.50)        | 0.38             | 0.17 (0, 0.60)           | 0.14             |
| Co-colonization episodes   | 22  | 0.50 (0.25, 0.75)        | 0.003            | 0.55 (0.25, 0.75)        | < 0.001          |
| Stool only                 | 109 | 0.25 (0.20, 0.33)        | --               | 0.25 (0.20, 0.33)        | --               |

Intestinal persistence was defined as the proportion of visits at which a specific *E. coli* strain was isolated from a stool sample. IQR = interquartile range.

Urinary tract infection (UTI) was defined by the report of two or more UTI symptoms and by medical record review. Recurrent UTI was defined as any UTI episode occurring at least two weeks after a previously treated episode. High-count asymptomatic bacteriuria (HCABU) was defined as  $\geq 10^3$  cfu of *E. coli* per mL of urine. Low-count asymptomatic bacteriuria (LCABU) was defined as between  $<10^3$  and  $\geq 10$  cfu of *E. coli* per mL of urine.

\*Wilcoxon rank-sum tests were performed to compare the persistence of each unique *E. coli* strain causing UTI, HCABU, LCABU and intestinal co-colonization to a comparison group of all *E. coli* strains recovered exclusively from the stool samples of male and female participants. Single-sided *p*-values are presented.

of 1.17 episodes per couple. There did not appear to be a relationship between recurrent UTI and intestinal co-colonization (OR = 1.71, 95% CI 0.25, 11.58). A preceding co-colonization episode involving the subsequent UTI-causing strain was documented in only two (25%) of the eight couples that experienced recurrent UTI. There was a significantly increased risk of LCABU associated with intestinal co-colonization (OR = 4.79, 95% CI 1.51, 15.25), while there was no relationship between HCABU and intestinal co-colonization (OR = 2.64, 95% CI 0.36, 19.54).

Many of the strains were involved in multiple outcomes, as summarized in Table 2. Strains that caused both LCABU and co-colonization episodes ( $n = 11$ ) were the most common.

#### *Persistence of E. coli strains in the intestinal tract*

The median intestinal persistence of *E. coli* strains causing UTI, HCABU, and LCABU was calculated separately for men and women and was compared to

the median intestinal persistence of strains recovered exclusively from stool samples (Table 3). In women, the intestinal persistence of strains causing UTI, HCABU, and LCABU did not differ significantly from that of the comparison stool-only strains. However, in men the intestinal persistence of these same strains was considerably lower than the intestinal persistence of the strains identified exclusively in the stool samples collected from all participants. There was a statistically significant difference in the intestinal persistence of HCABU strains versus stool-only strains (Table 3).

The intestinal persistence of strains involved in co-colonization episodes was also compared to the persistence of non-co-colonizing stool-only strains (Table 3). In both the male and female partners, median intestinal persistence was significantly higher among strains causing co-colonization than among the stool-only strains ( $p < 0.003$  and  $p < 0.001$  for women and men, respectively).

Table 4. Comparison of the median intestinal predominance of UTI, HCABU and LCABU-causing *Escherichia coli* strains to *E. coli* strains recovered from the stool only.

| Strain source              | N   | Women                     |                  | Men                       |                  |
|----------------------------|-----|---------------------------|------------------|---------------------------|------------------|
|                            |     | Median predominance (IQR) | <i>p</i> -value* | Median predominance (IQR) | <i>p</i> -value* |
| Baseline and recurrent UTI | 12  | 0.25 (0.06, 0.47)         | 0.27             | 0 (0, 0.39)               | 0.13             |
| HCABU                      | 13  | 0.06 (0, 0.50)            | 0.37             | 0 (0, 0.22)               | 0.02             |
| LCABU                      | 21  | 0.22 (0.09, 0.44)         | 0.19             | 0.10 (0, 0.33)            | 0.21             |
| Co-colonization episodes   | 22  | 0.40 (0.17, 0.53)         | < 0.001          | 0.38 (0.22, 0.57)         | < 0.001          |
| Stool only                 | 109 | 0.13 (0.07, 0.25)         | --               | 0.13 (0.07, 0.25)         | --               |

Intestinal predominance was defined as the proportion of all *E. coli* colonies selected from stool samples that were accounted for by a given strain. IQR = interquartile range.

Urinary tract infection (UTI) was defined by the report of two or more UTI symptoms and by medical record review. Recurrent UTI was defined as any UTI episode occurring at least two weeks after a previously treated episode. High-count asymptomatic bacteriuria (HCABU) was defined as  $\geq 10^3$  cfu of *E. coli* per mL of urine. Low-count asymptomatic bacteriuria (LCABU) was defined as between  $<10^3$  and  $\geq 10$  cfu of *E. coli* per mL of urine.

\*Wilcoxon rank-sum tests were performed to compare the predominance of each unique *E. coli* strain causing UTI, HCABU, LCABU and intestinal co-colonization to a comparison group of *E. coli* strains recovered exclusively from the stool samples of male and female participants. Single-sided *p*-values are presented.

Table 5. Relationship between KL diversity\* of intestinal *Escherichia coli* by study visit and risk of recurrent UTI, HCABU, LCABU and intestinal co-colonization in women.

| Study outcomes             | Episodes of outcome | Low Diversity at visit | OR (95% CI)           |
|----------------------------|---------------------|------------------------|-----------------------|
|                            | N                   | N (%)                  | Low v. high diversity |
| Recurrent UTI              | 8                   | 6 (75)                 | 1.99 (0.33, 12.03)    |
| HCABU                      | 12                  | 7 (58)                 | 0.47 (0.08, 2.93)     |
| LCABU                      | 21                  | 12 (57)                | 0.90 (0.27, 3.05)     |
| Intestinal co-colonization | 38                  | 20 (53)                | 0.20 (0.04, 0.92)†    |

\* Kullback-Leibler (KL) divergence or diversity measures the intestinal *E. coli* strain distribution by visit. KL diversity is categorized as low (KL = 0) and high (< -0.217). A low KL diversity was considered the exposure.  
† Statistically significant.

#### Predominance of *E. coli* strains in the intestinal tract

The median intestinal predominance of strains causing UTI, HCABU and LCABU was compared to the median intestinal predominance of strains recovered exclusively from stool samples (Table 4). In women, strains causing UTI and LCABU were somewhat more predominant in the intestine than were the stool-only strains. Although the predominance of strains causing HCABU was lower than that of the strains causing UTI, it was not significantly lower than that of the stool strains. In men, the median intestinal predominance of strains causing UTI, HCABU and LCABU was lower than in the women, but the predominance of these strains did not differ significantly from that of the stool-only strains, with the exception of strains causing HCABU, which exhibited lower predominance ( $p = 0.02$ ).

The median intestinal predominance of strains causing co-colonization episodes was compared to that of non-co-colonizing stool-only strains (Table 4). The median predominance of the strains causing co-colonization was significantly higher than the predominance of strains isolated from the stool-only ( $p < 0.001$  and  $p < 0.001$  for women and men, respectively).

#### *E. coli* population diversity in the intestinal tract

Intestinal *E. coli* population diversity was estimated for each study visit by using the KL diversity measure. The association between lack of intestinal diversity (with KL diversity = 0 defined as “exposed” and KL diversity < 0 defined as “unexposed”) and the risk of recurrent UTI, HCABU, LCABU, and intestinal co-colonization at each visit was evaluated. Results from the logistic regression model for repeated measurements are presented in Table 5.

There did not appear to be an association between low intestinal diversity and the risk of recurrent UTI,

LCABU or HCABU (Table 5). However, women with a low level of intestinal *E. coli* diversity were significantly less likely to experience intestinal co-colonization (OR = 0.20, 95% CI 0.04, 0.92). There were no observed associations between low intestinal diversity in the male intestine and the risk of recurrent UTI, HCABU or LCABU in the female partner (data not shown).

#### Epidemiologic characteristics and intestinal dynamics

Selected epidemiologic characteristics of the couples enrolled in the study are summarized by study outcome in Table 6. There was no difference in the median persistence or predominance of strains recovered from women ( $n = 97$ ) who lived together with their partners, who had frequent sex ( $\geq 4$  times per week) or who used male condoms as their primary contraceptive method.

Similarly, there was no difference in the median persistence or predominance of strains recovered from men ( $n = 59$ ) who lived together with their partners, who had frequent sex or who used male condoms as their primary contraceptive method. There was also no difference in the KL diversity of *E. coli* in the female partners’ intestine among those couples that lived together, had frequent sex or used male condoms as their primary contraceptive method.

#### Discussion

This study sought to evaluate whether intestinal persistence, predominance, and diversity modified the risk of recurrent UTI in young, sexually active women. Initially, we hypothesized that persistent and/or predominant strains might either be protective against the acquisition and proliferation of transient urovirulent strains or, alternatively, might represent urovirulent *E. coli* that could persist or predominate in the intestine, thereby putting some women at increased risk for UTI

Table 6. Summary of epidemiological characteristics by study outcome.

| Risk Factor                                | Couples<br>( $n = 23$ ) | Recurrent UTI<br>( $n = 6$ ) | HCABU<br>( $n = 8$ ) | LCABU<br>( $n = 11$ ) | Intestinal co-colonization<br>( $n = 14$ ) |
|--|-------------------------|------------------------------|----------------------|-----------------------|--|
|  | N (%)                   | N (%)                        | N (%)                | N (%)                 | N (%)                                      |
| Live together                              | 12 (52)                 | 2 (33)                       | 4 (50)               | 5 (45)                | 7 (50)                                     |
| Frequent sex<br>( $\geq 4$ times per week) | 11 (48)                 | 4 (66)                       | 2 (25)               | 5 (45)                | 7 (50)                                     |
| Primary condom users                       | 4 (17)                  | 0 (0)                        | 2 (25)               | 2 (18)                | 2 (14)                                     |
| Primary hormonal method<br>users           | 19 (83)                 | 5 (83)                       | 6 (75)               | 9 (82)                | 11 (79)                                    |

(“prevalence hypothesis”). However, we found that strains causing UTI, HCABU and LCABU actually persist and predominate in the intestine to a similar degree as strains found exclusively in the stool. We also did not observe a relationship between low intestinal diversity and development of UTI, HCABU or LCABU.

The low levels of intestinal persistence and predominance of UTI, HCABU and LCABU causing *E. coli* strains in the male partner suggest that the male partner may not be serving as a reservoir for these strains or contributing meaningfully to the development of recurrent UTI in the female partner. Although the relationship between LCABU and co-colonization suggests that transmission of *E. coli* may be occurring between sexual partners; this transmission appears to lead infrequently to symptomatic UTI.

The most important findings in this study involved intestinal co-colonization. Sexual partners were frequently co-colonized with the same *E. coli* strains (61% of couples) and sometimes shared UTI-causing strains. The incidence of co-colonization was approximately the same for couples that experienced recurrent UTIs and those that did not. Therefore, even though couples frequently shared the same *E. coli* strain, this did not appear to lead to an increased risk of UTI in the female partner. However, *E. coli* strains that were identified in intestinal co-colonization episodes were more likely to persist, and significantly more likely to predominate, in the intestinal tracts of the couples. This suggests that certain subgroups of *E. coli* may possess factors that contribute to successful colonization of the intestinal tract (Ikaheimo et al., 1996; Wold et al., 1992). Interestingly, intestinal co-colonization was significantly associated with increased or high intestinal diversity, which may indicate that co-colonization occurs during transitional periods in the intestinal tract. This observation may reflect the introduction of new *E. coli* strains into the intestinal tracts of the couple through shared environmental (i.e., food) or other exposures (i.e., household or sexual contact). However, when we evaluated selected epidemiologic factors including, living together, frequent sex and contraceptive method we did not observe any impact on persistence, predominance and diversity of *E. coli* in the intestines of participating couples.

There are several limitations to the study and analyses presented. First, the sample was small, resulting in imprecise risk estimates, limiting the statistical power to adequately evaluate specific epidemiological variables, such as sexual activity and living arrangements. Second, some women participating in the study had histories of numerous recurrent UTI whereas others did not. This likely produced a sample that was either heterogeneous in terms of underlying UTI risk, or possibly at higher risk for recurrences than in previous studies. Thus, the results are not necessarily generalizable to the larger population of young women. Third, due to the complex nature of the intestinal flora, it is inevitable that some strains were missed. However, sampling five colonies from stool cultures yield a 99% probability of capturing the predominant intestinal strain at a given visit (Bettelheim et al., 1972; Gruneberg, 1969; Lidin-Janson et al., 1978); we also selected representatives of all morphologically

distinct stool colonies in order to capture as many minor strains as possible.

The evaluation of intestinal strain dynamics using molecular biologic methods represents a novel approach to investigating the “prevalence hypothesis” that intestinal persistence, predominance, and diversity are related to the pathogenesis of UTI. We speculate that the sharing of intestinal *E. coli* between sexual partners may not be as important in UTI pathogenesis as are the attributes of the shared *E. coli* that promote successful intestinal colonization. The strains causing intestinal co-colonization seem to be associated with a diverse intestinal *E. coli* population and are frequently present in the female partner’s urine. These characteristics, in addition to their superior levels of intestinal persistence and predominance, suggest that these strains are important contributors to intestinal *E. coli* dynamics, but may not be primarily responsible for causing symptomatic UTI. Therefore, given the limitations of this study, we did not find evidence to support the “prevalence hypothesis” in UTI pathogenesis. However, larger prospective epidemiological studies are needed to explore the acquisition, transmission, and intestinal dynamics of UTI-causing *E. coli*. Virulence factor profiling and phylogenetic studies of predominant, persistent, co-colonizing and UTI-causing strains from this study will be conducted and may reveal mechanisms for their ecological behavior.

## Experimental Procedures

### Study site and subjects

The study was conducted in collaboration with the University Health Services at the University of California at Berkeley campus from October 1999 to July 2001. The Committee for the Protection of Human Subjects, University of California at Berkeley, approved the human subject’s protocol and all participants gave written informed consent. Women with acute, uncomplicated UTI, whose male partner was also willing to participate, were passively recruited from among students presenting to the University Health Services with a UTI. Women were excluded based on the following criteria: (i) no sexual activity; (ii) antibiotic use or prophylaxis, hospitalization, or urinary catheterization in the past month; (iii) urinary tract abnormality; (iv) pregnancy or intention to become pregnant; and (v) diabetes mellitus.

### Definitions

**UTI** was defined as (i) the report by the subject of more than two UTI symptoms, including, painful urination, frequent and/or urgent urination, and hematuria, and (ii) the isolation of  $> 10^2$  colony-forming units (cfu) of *E. coli* per mL of urine from a clean-catch urine specimen (Gupta et al., 1999; Hooton and Stamm, 1997). Confirmation of the initial and recurrent UTI diagnoses was made by medical record review. **Recurrent UTI** was defined as any UTI episode occurring at least two weeks after a previous UTI episode that had been treated with antimicrobial agents. **Asymptomatic bacteriuria (ABU)** was evaluated as two categories. **High-count ABU (HCABU)** was defined as  $\geq 10^3$  colony-forming units (cfu) of *E. coli* per mL of urine in a routine urine sample (urine collected in the absence of UTI symptoms), whereas **low-count ABU (LCABU)**

was defined as the detection of  $\geq 10$  but  $< 10^3$  cfu of *E. coli* per mL from a routine urine sample (Hooton et al., 2000). The LCABU category is likely to include strains involved in asymptomatic bacteriuria as well as vaginal and periurethral colonization. **Intestinal co-colonization** was defined as the detection of the same *E. coli* strain in the stool or urine culture(s) of both members of the couple at a given visit. The term “**isolate**” refers to a bacterial colony isolated from the stool or urine, whereas the term “**strain**” refers to isolates that were indistinguishable by ERIC2 PCR fingerprinting.

#### *Procedures*

Aliquots of urine samples (“acute UTI sample”) from all women seen for a possible UTI episode during the study period (October 1999 to July 2001) were collected from the University Health Services clinical laboratory and stored in 15% glycerol at  $-80^{\circ}\text{C}$ . These urine samples were archived according to clinic visit date, date of birth, and patient medical record number. When a woman expressed interest in the study, her archived UTI urine samples were located by medical record number and were thawed, cultured, and included in the study. This approach allowed us to include the uropathogens responsible for UTI episodes occurring at or around the baseline study visit. Acute UTIs that occurred during the follow-up period were either reported directly by participants or were detected through the ongoing collection of all UTI samples from the University Health Services laboratory. Subjects were also provided with a sterile tube containing bacteriologic media to collect and store a urine sample in the event they developed a UTI outside of the local area.

Stool and urine samples were collected monthly from each partner. Monthly sampling was chosen because there is evidence that changes in intestinal flora can occur in 14–30 day intervals (Caugant et al., 1981). Clean-catch urine samples (“routine urine samples”) were collected in sterile containers from both partners immediately prior to study visits. Freshly voided stool samples (“routine stool samples”) were collected, using sterile swabs containing Cary-Blair transport media (Difco, BBL, Sparks, MD), by the subjects within the 24 hours prior to each couple’s monthly study visit. Interviews were administered to all participants at their baseline visit. Self-administered, follow-up questionnaires were also completed after a recurrent UTI episode. Initially, couples were followed for six months (10 couples). In the latter part of the study, the observation period was shortened to four months to improve recruitment (13 couples).

#### *Bacterial strains*

Urine samples collected at each study visit and at the time of an acute UTI episode were cultured by serial dilution on LB and MacConkey agar plates (Difco, BBL, Sparks, MD). From one to five morphologically distinct colonies (as available) from the urine cultures performed at each study visit were selected for fingerprinting. One colony from each acute UTI sample culture was arbitrarily selected for further analyses. Routine stool samples were streaked on MacConkey agar plates. Five colonies per stool culture plate were selected for fingerprinting. Colonies were

selected to represent morphologically unique isolates in proportion to their prevalence on the culture plate. Isolates were stored in 15% glycerol at  $-80^{\circ}\text{C}$ . Lactose and indole positive colonies with characteristic colony morphology were presumptively identified as *E. coli*. Non-*E. coli* isolates were not further identified.

#### *ERIC2 PCR fingerprinting*

ERIC2 PCR fingerprinting was performed on all selected *E. coli* recovered from stool, routine urine, and acute UTI samples, as previously described (Johnson and O’Byrne, 2000; Manges et al., 2001). Reference strain CFT073 was used as an internal control strain for each PCR run and gel electrophoresis (Mobley et al., 1990). ERIC2 PCR patterns were only compared within couples because of the limited reproducibility of this method (Manges, 2002).

#### *Electrophoretic pattern analysis*

Electrophoretic gel images were captured by using the digital GelDoc System and Quantity One software (Bio-Rad, Hercules, CA) and were imported into the GelCompar software, version 2.50 (Applied Maths, Kortrijk, Belgium), for fingerprint analysis. To define identity among fingerprints, previously described scaling error and Dice similarity coefficient thresholds were used (Manges, 2002). Dendrograms were inferred from the Dice similarity coefficient matrix generated in GelCompar by using the unweighted pair group method with arithmetic averages (UPGMA).

Unique strains recovered from each couple were defined based on a consensus among (i) phenotypic characteristics (colonial morphology, lactose and indole status), (ii) visual inspection of the ERIC2 PCR fingerprints, and (iii) GelCompar cluster analysis of the ERIC2 PCR fingerprints. The unique strains were assigned arbitrary alphabetical designations. Discrepancies regarding strain designations were resolved by visual inspection of repeat ERIC2 PCR fingerprints.

#### *Intestinal population dynamics*

Three components of intestinal population dynamics were examined in relation to the risk of UTI, ABU, and co-colonization: intestinal persistence, predominance, and diversity. **Intestinal persistence** was defined as the proportion of visits at which a specific *E. coli* strain was isolated from a particular subject’s stool sample. This reflected a given strain’s average duration of colonization in a study subject. **Intestinal predominance** was defined as the proportion of colonies from all stool samples from an individual that were represented by a particular *E. coli* strain. This reflected how much of the intestinal *E. coli* flora was accounted for by a unique strain in a given study subject over time. Intestinal persistence and predominance values were calculated based on stool isolates only.

Each unique *E. coli* strain was categorized as to whether it caused UTI, HCABU, LCABU, and/or intestinal co-colonization, or was recovered exclusively from stool samples. Certain strains caused more than one outcome (UTI, HCABU, and LCABU), such that these groups overlapped somewhat with respect to the constituent strains. Median persistence and predominance values,

Table 7. Kullback-Leibler diversity values: measurement of intestinal diversity.

| Combinations of fingerprint types |   |   |   |   | Diversity |
|-----------------------------------|---|---|---|---|-----------|
| 5                                 |   |   |   |   | 0*        |
| 4                                 | 1 |   |   |   | -0.217    |
| 3                                 | 2 |   |   |   | -0.292    |
| 3                                 | 1 | 1 |   |   | -0.413    |
| 2                                 | 2 | 1 |   |   | -0.458    |
| 2                                 | 1 | 1 | 1 |   | -0.579    |
| 1                                 | 1 | 1 | 1 | 1 | -0.699    |

\* When five isolates at a given visit had the indistinguishable fingerprints the diversity was zero.

and interquartile ranges (IQR), were calculated using the persistence and predominance proportions (described above) estimated for each *E. coli* strain.

The **Kullback-Leibler divergence or diversity measure (KL)** was used to summarize the ERIC2 PCR fingerprint data by visit (Kullback and Leibler, 1951). When the ERIC2 PCR fingerprints of all five stool isolates from a given visit were the same the diversity estimate was zero. As the number of unique fingerprints increased, the diversity estimate became an increasingly large negative number:

$$\text{Kullback-Leibler divergence} = \sum_{i=1}^k p_i \cdot \log(p_i)$$

where  $p_i$  is defined as the proportion of the  $i$ th unique fingerprint at a given visit (Table 7). The distribution of diversity values was evaluated separately by visit. The KL diversity values were evaluated using both the discrete KL values and dichotomous KL values. In this analysis, absence of intestinal diversity (KL diversity = 0) was considered the exposure.

#### Statistical Analyses

Three different units of analysis were used: (i) study outcomes (UTI, ABU, co-colonization) were summarized by using the woman as the primary unit of analysis; (ii) intestinal persistence and predominance were evaluated by using the set of unique *E. coli* strains, as defined by ERIC2 PCR, as the unit of analysis, and; (iii) intestinal diversity was evaluated by using the visit as the unit of analysis.

Since intestinal persistence and predominance proportions were not symmetrically distributed (not shown), medians were used to summarize the data. The Mann-Whitney rank sum test was used to compare medians.

As this study required repeated outcome measurements on couples, some analyses required adjustment for correlated measurements within subjects. Selected bivariate analyses were performed by using logistic regression analysis for repeated measurements in STATA Version 7.0 (STATA Corporation, College Station, TX). A random effects model was used, assuming an exchangeable correlation structure (Fahrmeir and Tutz, 2001; StataCorp, 2001). This model was chosen in order

to capture subject-specific effects and to allow for the estimation of odds ratios and 95% confidence intervals.

#### Epidemiologic characteristics and intestinal dynamics

Epidemiologic factors such as frequent sex and contraceptive method are associated with an increased risk of UTI. Other shared environmental exposures may also contribute to intestinal changes and intestinal co-colonization. Epidemiologic factors, including frequent sex ( $\geq 4$  times per week), living together and contraceptive method were evaluated to determine if these factors might impact our measures of intestinal dynamics. Differences in median intestinal persistence and predominance of intestinal *E. coli* in couples according to epidemiologic characteristic were evaluated using the Mann-Whitney rank sum test. A logistic regression model (see above) was used to evaluate the relationship between intestinal diversity and the same epidemiologic factors.

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