Long-Term *Escherichia coli* Asymptomatic Bacteriuria among Women with Diabetes Mellitus

**Shona Dalal,1 Lindsay Nicolle,2,3 Carl F. Marrs,1 Lixin Zhang,1 Godfrey Harding,2,3 and Betsy Foxman1**

1Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor; and Departments of 2Internal Medicine and 3Medical Microbiology, University of Manitoba, Manitoba, Canada

**Background.** Persistent *Escherichia coli* asymptomatic bacteriuria (ASB) is common among persons with diabetes mellitus, but the duration of colonization and the rates of recolonization are unknown. We estimated the duration of colonization and the rate of recolonization among successively isolated *E. coli* from diabetic women with ASB and compared the virulence profiles with uropathogenic and commensal *E. coli*.

**Methods.** A total of 105 women with diabetes were enrolled in a randomized, controlled clinical trial for treatment of ASB in Manitoba, Canada, and were observed at least every 3 months for up to 3 years. We analyzed 517 isolates from 70 women with repeated *E. coli* ASB for genetic similarity using enterobacterial repetitive intergenic consensus polymerase chain reaction. Unique strains were screened for uropathogenic virulence characteristics using dot blot hybridization and compared with different collections of *E. coli* isolates.

**Results.** On average, differences were found among women assigned to treatment for ASB, those treated only for symptomatic infections, and untreated women in (1) follow-up time with bacteriuria (29%, 31%, and 66%, respectively; P < .001), (2) duration of bacteriuria (2.2, 2.5, and 3.7 months, respectively; P = .04), and (3) carriage of unique isolates (2.4, 2.8, and 4 months, respectively; P = .03). Women assigned to antibiotic treatment usually had recurrent infection (76%), 64% of the time with a genetically new *E. coli* strain. Virulence characteristics of these isolates were comparable to those of fecal isolates from healthy women.

**Conclusions.** Treatment may reduce the overall proportion of time infected in the long term and carriage of a unique strain, but most treatment regimens were followed by subsequent recolonization. Infecting strains did not have virulence factors characteristic of uropathogenic *E. coli*.

Urinary tract infections (UTIs) occur in women with diabetes mellitus more frequently than in women without diabetes, are more severe (with pyelonephritis occurring at a 5-fold higher rate), and often result in complications that are otherwise rare (such as emphysematous cystitis and fungal infections) [1, 2]. Asymptomatic bacteriuria (ASB) occurs 3 times more often among women with diabetes than among otherwise healthy women; ASB is associated with an increased risk of symptomatic infection but is not causative [2–5]. The presence of ASB in diabetic women is not associated with a faster decrease in renal function [6] or a greater risk of diabetic complications or mortality [4]. The most common infecting organism in diabetic women with ASB is *Escherichia coli*; other organisms include *Klebsiella* species, *Enterococcus* species, and group B *Streptococcus* species (*Streptococcus agalactiae*) [2, 7]. Uropathogenic *E. coli* (UPEC) have a variety of virulence traits that enable them to successfully invade the normally sterile urinary tract [8, 9]. These traits include a number of adhesins, iron sequestration systems, and toxins that distinguish them from normal bowel flora *E. coli* [8].

Risk factors for ASB in diabetic women include sexual intercourse, degree of metabolic control, duration of diabetes, presence of diabetic complications, and insulin use [10–12]. In a study of symptomatic UTI among 589 women with diabetes, sexual intercourse in the preceding week was the most significant risk factor for women with type 1 diabetes, whereas ASB was most significant for women with type 2 diabetes [10]. Stringent control of blood glucose decreases risk of complications, such as neuropathy and nephropathy, but a...
direct effect on bacteriuria has not been observed [1]. Neuropathy, however, may affect underlying bladder dysfunction and thus contribute indirectly to a predisposition for UTI [1].

Although persistent E. coli ASB is more common in diabetic patients than in nondiabetic patients, it is unknown whether the bacteriuria is caused by the same E. coli strain or what the effect of treatment is on the carriage of genetically similar or different E. coli strains. We conducted the present study to characterize urinary E. coli isolated from diabetic women in Manitoba, Canada, to determine whether successive isolates from the same individual were genetically similar and whether infecting organisms have a distribution of virulence genes similar to that of UPEC or to normal bowel flora E. coli.

METHODS

Patient population. Diabetic women aged >16 years were identified from 1991 to 1997 through ambulatory endocrinology clinics in Manitoba, Canada. A total of 105 asymptomatic women with bacteriuria in 2 consecutive urine samples, obtained within a 2-week period, were enrolled in a prospective, randomized trial of antimicrobial or no antimicrobial treatment for ASB. Subsequently, urine specimens were obtained at least every 3 months or more frequently after treatment or if symptoms occurred. Women were followed up to a maximum of 36 months. The original study was undertaken to determine whether there were any benefits of screening for and treating ASB in diabetic women.

Women randomized to treatment received antimicrobial therapy for initial ASB, any subsequent ASB identified on 3 monthly screenings, and any symptomatic infections. Women randomized to no treatment received treatment only for symptomatic UTI. All women received antimicrobials as ordered by their physicians for other indications. The trial design and patient population have been described in detail elsewhere [3].

We provide further observations on E. coli isolates from a subgroup of 70 women who had at least 2 positive E. coli culture results during the study period. All E. coli isolates (n = 517) from asymptomatic and symptomatic women in both study arms were typed for genetic similarity using enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR). Genetically unique strains (n = 238) from each individual were then analyzed for known UTI-associated virulence genes. We weighted data analyses for the length of follow-up time and interval between visits by assuming that ASB extended up to the midpoint between visits on either side of each sample. We compared means using the Student t test and analysis of variance, and we used χ² tests to compare the proportions of virulence genes between diabetic women and isolates from otherwise healthy women. This study was approved by the University of Manitoba Conjoint Ethics Committee for Human Subjects.

Definitions. Asymptomatic E. coli bacteriuria was defined as urine specimens with an E. coli culture of ≥10⁵ CFU/mL and no symptoms in the genitourinary tract. When symptoms consistent with infection were present, a urine specimen yielding an E. coli culture of ≥10⁵ CFU/mL was sufficient to diagnose a UTI.

Recurrent infection was defined as a urine specimen yielding E. coli after a patient received antimicrobial therapy (for ASB, symptomatic UTI, or other indications) or after spontaneous resolution of ASB. Recurrent infection was considered relapse when the strain of E. coli isolated after antimicrobial therapy was similar to the pretherapy strain by ERIC-PCR typing and reinfection when a new strain was isolated. Infection was considered persistent when additional urine specimens yielded E. coli cultures of ≥10⁵ CFU/mL among patients who did not receive any antimicrobials for the duration of their follow-up period.

Molecular typing. ERIC sequences are highly conserved sequences found in intergenic regions of the genome in Enterobacteriaceae but whose chromosomal location differs among species [13]. They are short (~126 base pairs) and contain a central core inverted repeat. Although the function of these sequences is not fully known, amplification of these sequences by PCR allows clear distinction among different bacterial species and strains that contain these elements [13].

In brief, cultures were grown overnight and lysed and the crude DNA lysate used for PCR under the following conditions: 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 4.5 min, with a 1-min final extension at 72°C, using the ERIC primer AAGTAAGTGACTGGGGTG-AGCG in a thermal cycler (DNA Engine PTC-200; MJ Research). UPEC sequenced strain E. coli CFT073 was used as a positive control. Samples were resolved on a 2% agarose gel (Figure 1).

The presence of a same-size band of a similar intensity, identified using BioNumerics software (Applied Maths), was used to compare isolates and create a dendogram using the unweighted pair group method with arithmetic averages (Figure 2). Strains were considered identical if they had >=90% similarity. Laboratory analyses were completed before clinical linkages between isolates were considered.

In brief, dot blot hybridization involves fixing crude genomic DNA from each isolate on a nylon membrane using a Bio-Dot Microfiltration apparatus (Bio-Rad Laboratories). Fixed DNA was hybridized with a fluorescent-labeled probe, the presence of which was detected using a fluorescein-based system using the Amersham prime labeling and ECF detection system (Amersham). A STORM 860 Phosphor Imager (Molecular Dynamics) was used to scan the membranes to capture signal intensity of the image. The image was then analyzed using ImageQuant software, version 5.2 (Molecular Dynamics). The
signal of each isolate on the blot was expressed as a percentage of its respective positive control for each probe, after correcting for background signal [14]. Each isolate was tested in duplicate on independent membranes, and any discrepancy found in a particular isolate was retested using either another dot blot or Southern blot hybridization.

We tested 238 unique E. coli isolates from 70 women for the presence of the following virulence genes associated with UTI using dot blot hybridization: the P pili family (papE) and its subclasses papGJ96 and papGAD; S fimbrae (sfa); the Dr family of adhesins drb; cytotoxic necrotizing factor 1 (cnf1); hemolysin (hly); aerobactin (iucD); group 2 capsule (kpsMT); and outer membrane protease T (ompT). Virulence genes in the isolates from diabetic women were compared with previously published distributions among isolates collected from otherwise healthy women from Michigan with a first UTI and recurring UTI and with periurethral and fecal isolates from healthy women from Michigan [15].

RESULTS

The median age of the 70 women with E. coli ASB included in this analysis was 54 years (interquartile range, 43–64 years). Twelve women (17%) had bladder neuropathy, and 11 (16%) of the 70 women had undergone prior genitourinary surgery. Overall, women had ASB for 36% of their follow-up time, with a mean duration of bacteriuria of 2.6 months, and carried a unique single strain for an average of 2.8 months (range, 0.6–13 months). Seventeen (25%) of the 68 women followed up for 6 months or longer remained continuously colonized with a single strain for at least a 6-month period.

Of the 70 women, 36 were originally randomized to receive treatment and 34 to no treatment. Among the 34 women originally assigned to no treatment, 22 received treatment for a symptomatic UTI or other indications at least once during the study period and are referred to as women who received symptomatic treatment. Twelve women received no treatment for symptomatic UTI or other infections for the duration of their follow-up. Three of these 12 women had spontaneous resolution of E. coli ASB, 1 of whom was subsequently reinfected.

Women with bladder neuropathy, prior genitourinary surgery, or both (n = 20) had bacteriuria for 43% of their follow-up time, compared with 26% for women without those conditions, although the difference was not statistically significant (P = .10). They also did not carry a single strain for longer (2.5 vs. 2.4 months; P = .90) or have a different mean duration of E. coli bacteriuria than women without these conditions (2.2 and 2.0 months, respectively; P = .80).

No statistically significant differences were found in the mean follow-up time among women who received treatment for ASB, women who received symptomatic treatment only, and women who received no treatment (Table 1). However, treatment groups varied significantly in the mean proportion of follow-up time with E. coli bacteriuria (P < .001), the mean duration of bacteriuria (P = .04), and the mean duration of carriage of a single strain (P = .03) (Table 1). Specifically, women in the treatment group had bacteriuria for a lower proportion of their follow-up time, had shorter durations of bacteriuria, and carried a single unique isolate for less time compared with women who did not receive treatment. Women who received symptomatic treatment had a statistically lower proportion of their follow-up time with bacteriuria than women without these conditions (2.2 and 2.0 months, respectively; P = .80).

On average, women assigned to treatment for ASB received more antimicrobial courses than did women who received only symptomatic treatment (Table 1). Among the 57 treated women with complete data, most treatment regimens among women in the treatment group (76%) were followed by recurrent E. coli bacteriuria, most (64%) with a new strain of E. coli. Women who received treatment only for asymptomatic infections also had frequent recurrent bacteriuria (65%). However, most (57%) were relapses with a strain genetically identical to the previous infecting strain. The differences between the 2 groups were not statistically significant, possibly because of the small sample size.

The frequency of uropathogenic virulence characteristics among isolates causing ASB in diabetic women was not statistically different from the frequency found among fecal E. coli in healthy women (Table 2), except for the frequency of cnf,
which was higher. Three virulence factors, *pff*, *kps*MT, and *omp*T, were found at a significantly lower frequency than that seen in fecal strains.

**DISCUSSION**

This study demonstrates that untreated diabetic women with ASB may carry a genetically unique *E. coli* strain for up to 13 months, whereas treated women had more frequent acquisition of new strains. Women who received treatment for ASB had bacteriuria for a shorter duration and carried a single strain of *E. coli* for a shorter period compared with women who did not receive treatment. However, treatment was followed by recurrent infections for most women, usually with a new strain of *E. coli*. The ASB-causing *E. coli* from diabetic women did not have virulence characteristics typical of UTI-causing strains. Women in the treatment group received antimicrobial therapy an average of 3 times, but some received treatment up to 15 times during the trial for ASB, symptomatic infections, or other indications. The high proportion of recurrent infections indicates that repeated treatment does not resolve asymptomatic bladder infection in the long term for most diabetic women who have frequent *E. coli* ASB. These findings are consistent with the results of the clinical trial from which patients in this analysis were selected, showing a much higher frequency of recurrent ASB in women who received treatment for ASB [3].

Whereas treated women had a shorter time with bacteriuria but frequent additional infections, untreated diabetic women with ASB carried a single strain for longer periods, with eventual clearance in a few patients. In a study of otherwise healthy women with ASB, <1% of women had ASB lasting longer than 2 consecutive monthly cultures, 26% were colonized with the same strain, and persistent infection with the same strain over

### Table 1. *Escherichia coli* Bacteriuria, Strain Carriage, and Recurrent Infection among Diabetic Women with Asymptomatic Bacteriuria by Treatment Received

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment (n = 36)</th>
<th>Symptomatic treatment (n = 22)</th>
<th>No treatment (n = 12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean person-months of follow-up (IQR)</td>
<td>26 (18–36)</td>
<td>29 (20–36)</td>
<td>27 (17–36)</td>
<td>.74</td>
</tr>
<tr>
<td>Mean proportion of follow-up time with bacteriuria (IQR)</td>
<td>0.29 (0.09–0.40)</td>
<td>0.31 (0.16–0.40)</td>
<td>0.66 (0.46–0.91)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Duration of bacteriuria, mean months (IQR)</td>
<td>2.2 (1.6–2.9)</td>
<td>2.5 (1.8–3.1)</td>
<td>3.7 (1.3–5.4)</td>
<td>.04</td>
</tr>
<tr>
<td>Duration of carriage of a single strain, mean months (IQR)</td>
<td>2.4 (1.6–2.8)</td>
<td>2.8 (2.1–3.2)</td>
<td>4 (1.6–6.6)</td>
<td>.03</td>
</tr>
<tr>
<td>Mean no. of times that treatment was received (IQR)</td>
<td>3.2 (1–4)</td>
<td>2.0 (1–3)</td>
<td>…</td>
<td>.05</td>
</tr>
<tr>
<td>Mean proportion of treatment courses followed by recurrent <em>E. coli</em> infection (IQR)</td>
<td>0.76 (0.67–1)</td>
<td>0.65 (0.5–1)</td>
<td>…</td>
<td>.18</td>
</tr>
<tr>
<td>Mean proportion of recurrence caused by reinfection (IQR)</td>
<td>0.64 (0.35–1)</td>
<td>0.43 (0.1–1)</td>
<td>…</td>
<td>.16</td>
</tr>
</tbody>
</table>

**NOTE.** IQR, interquartile range.

* Treatment indicates women originally randomized to treatment for asymptomatic bacteriuria.

* Symptomatic treatment indicates women originally randomized to no treatment for asymptomatic bacteriuria but who received antimicrobials for symptomatic urinary infections or other indications during their follow-up.

* No treatment indicates women originally randomized to no treatment for asymptomatic bacteriuria who did not receive any antimicrobials for the duration of their follow-up.

* Reinfection is defined as recurrent *E. coli* infection that is genetically different from the pretreatment strain.
Table 2. Comparison of the Frequency of *Escherichia coli* Virulence Genes Seen in Diabetic Women with Asymptomatic Bacteriuria with *E. coli* Isolates from Various Other Collections

<table>
<thead>
<tr>
<th>Virulence factor (gene name)</th>
<th>Diabetic women with ASB &gt;16 years ((n = 238))</th>
<th>Healthy women aged 18–39 years with fecal (no UTI) isolates ((n = 269))</th>
<th>Women aged 18–39 years with first UTI ((n = 237))</th>
<th>Women aged 18–39 years with recurring UTI ((n = 27))</th>
<th>Women aged 40–65 years with UTI ((n = 87))</th>
<th>Women aged 18–39 years with periurethral isolates ((n = 53))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr family of adhesins ((drb))</td>
<td>9.7</td>
<td>5.6</td>
<td>15.2</td>
<td>7.4</td>
<td>10.3</td>
<td>3.8</td>
</tr>
<tr>
<td>P pili family ((pff))</td>
<td>24.1</td>
<td>34.2</td>
<td>49.8</td>
<td>59.3</td>
<td>57.5</td>
<td>34.0</td>
</tr>
<tr>
<td>Class I, P pili ((papG_{ya}))</td>
<td>0.0</td>
<td>0.0</td>
<td>2.1</td>
<td>3.7</td>
<td>2.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Class II, P pili ((papG_{ab}))</td>
<td>18.5</td>
<td>23.1</td>
<td>27.0</td>
<td>37.0</td>
<td>31.0</td>
<td>11.3</td>
</tr>
<tr>
<td>Class III, P pili ((prsG_{yu}))</td>
<td>11.1</td>
<td>8.6</td>
<td>21.1</td>
<td>29.6</td>
<td>24.1</td>
<td>20.8</td>
</tr>
<tr>
<td>S fimbriae ((sfa))</td>
<td>18.5</td>
<td>12.6</td>
<td>27.9</td>
<td>44.4</td>
<td>41.4</td>
<td>24.5</td>
</tr>
<tr>
<td>Cytotoxic necrotizing factor 1 ((cnf 1))</td>
<td>18.3</td>
<td>10.0</td>
<td>26.6</td>
<td>40.7</td>
<td>29.9</td>
<td>24.5</td>
</tr>
<tr>
<td>Hemolysin ((hly))</td>
<td>18.3</td>
<td>14.9</td>
<td>37.6</td>
<td>48.2</td>
<td>44.8</td>
<td>26.4</td>
</tr>
<tr>
<td>Aerobactin ((aer))</td>
<td>40.6</td>
<td>41.3</td>
<td>46.0</td>
<td>40.7</td>
<td>37.9</td>
<td>26.4</td>
</tr>
<tr>
<td>Outer membrane protease T ((ompT))</td>
<td>31.4</td>
<td>67.7</td>
<td>83.1</td>
<td>85.2</td>
<td>88.5</td>
<td>75.5</td>
</tr>
<tr>
<td>Capsule, group 2 ((kpsMT))</td>
<td>38.4</td>
<td>63.6</td>
<td>81.9</td>
<td>74.1</td>
<td>75.9</td>
<td>73.6</td>
</tr>
</tbody>
</table>

NOTE. UTI, urinary tract infection.
time was uncommon [16]. In contrast, our data from diabetic women with ASB showed long-term carriage of the same strain of *E. coli* over time (25% of women carried the same strain for at least a 6-month period), and whether they received treatment or not, most had recurrent asymptomatic infections, symptomatic infections, or both. Interestingly, diabetic women with ASB who had conditions predisposing them to UTI (bladder neuropathy or prior genitourinary surgery) did not differ from diabetic women with ASB without those conditions in the proportion of time that they were infected, the length of carriage of a single strain, or the average duration of a bacteriuric episode. The reason for this is unclear and may be a result of the small number of women in these groups but warrants further research.

In uncomplicated UTI, infecting *E. coli* have a number of virulence factors that assist in their colonization of the urinary tract, including a variety of adhesins, iron sequestration systems, and toxins [8]. Most of the published literature on ASB causing *E. coli* indicates that these strains are less virulent [17–19]. Recent molecular studies demonstrate that some ASB causing *E. coli* strains are nonviral commensal strains, whereas others were originally virulent strains that have evolved to commensalism [20, 21]. We show that virulence characteristics of isolates from diabetic women with ASB were different from those seen in fecal isolates. This low prevalence of virulence characteristics is consistent with previous reports among otherwise healthy individuals [22] and among diabetic women with ASB compared with diabetic women with symptomatic UTI [23]. Only *cnf* was found more frequently than in fecal *E. coli*, the presence of which has been associated with a decrease in renal function in diabetic women [23]. Moreover, 3 virulence genes, *pff, ompT*, and *kpsMT*, occurred at a significantly lower frequency than observed in our collection of fecal *E. coli* from healthy young women. These data, combined with the strain carriage patterns that were observed, indicate that virulence characteristics typically found in UPEC are uncommon among isolates that infect the urinary tract in diabetic women with ASB. Thus, normal bowel inhabitants that do not invade the urinary tract under normal circumstances may be capable of doing so in diabetic women and can persist for long periods.

There are several limitations in this analysis. The ERIC-PCR technique has a degree of variability in the banding pattern intensity seen in strains. By running a variable control in each run and by running large batches of isolates together, we attempted to minimize this variation. Most isolates from an individual were run in the same batch. In addition, individuals may carry multiple *E. coli* strains, but the strain collection techniques only allowed for the collection of the predominant, morphologically distinct strain for genetic analysis. This could have resulted in an understimation of strain turnover. Molecular typing and dot blot hybridization may not account for minor mutations arising in isolates in the long term. However, the small proportion of isolates in which this could have occurred is unlikely to have influenced the current analyses.

Our analyses of diabetic women with long-term ASB show that a diverse group of *E. coli* strains is capable of long-term urinary colonization in diabetic women. Recurrent infections were common after treatment, frequently with a new *E. coli* strain. The proportion of strains with UTI virulence characteristics was not significantly different from that seen in fecal strains from healthy women, indicating that in a predisposed host additional bacterial aids for initiating infection are not a necessity.

**Acknowledgments**

We thank Patricia Tallman and Joanna Brunka for the isolation and maintenance of *E. coli* strains.

**Financial support.** This work was supported by an award from the National Institutes of Health (RO1 DK54946 to C.F.M.) and, in Canada, National Health Research and Development Program (6607-1618-502).

**Potential conflicts of interest.** All authors: no conflicts.

**References**

2. Zhang L, Foxman B. Molecular epidemiology of *Escherichia coli* me-
6. Meiland R, Geerlings SE, Stolk RP , Netten PM, Schneeberger PM, Hoepelman AI. Asymptomatic bacteriuria in women with diabetes mel-
8. Zhang L, Foxman B. Molecular epidemiology of *Escherichia coli* me-
    diated urinary tract infections. Front Biosci 2003; 8:e235–44.
11. Geerlings SE, Meiland R, van Lith EC, Brouwer EC, Gaaster W, Hoepelman AI. Adherence of type 1-fimbriated *Escherichia coli* to uroepi-
    theelial cells: more in diabetic women than in control subjects. Diabe-
12. Boyko EJ, Fihn SD, Scholes D, Abraham L, Monsey B. Risk of urinary tract infection and asymptomatic bacteriuria among diabetic and non-