Inverse Association of H_2O_2-Producing Lactobacilli and Vaginal *Escherichia coli* Colonization in Women with Recurrent Urinary Tract Infections

Kalpana Gupta, Ann E. Stapleton, Thomas M. Hooton, Pacita L. Roberts, Cynthia L. Fennell, and Walter E. Stamm

Department of Medicine, Division of Allergy and Infectious Diseases, University of Washington, Seattle

Women with recurrent urinary tract infection (UTI) often demonstrate persistent vaginal colonization with *Escherichia coli.* Since strains of lactobacilli that produce hydrogen peroxide inhibit the growth of *E. coli*, the absence of these strains may predispose to *E. coli* colonization and to UTI. To test this hypothesis, vaginal introital cultures were obtained from 140 women, 65 with recurrent UTI (case-patients) and 75 without (controls). Vaginal *E. coli* colonization was significantly more frequent in case-patients than controls (35% vs. 11%; $P < .001$) and in women without H_2O_2-positive lactobacilli than in women with (odds ratio [OR], 4.0; $P = .01$). Spermicide use was associated with greater risk of vaginal *E. coli* colonization (OR, 12.5; $P < .001$) and with absence of H_2O_2-positive lactobacilli (OR, 2.9; $P = .04$). The inverse association between H_2O_2-positive lactobacilli and vaginal *E. coli* colonization remained in case-patients after controlling for spermicide use (OR, 6.5; $P = .02$). Thus, absence of H_2O_2-positive lactobacilli may be important in the pathogenesis of recurrent UTI by facilitating *E. coli* introital colonization.

Colonization of the vaginal introitus with *Escherichia coli* appears to be a critical initial step in the pathogenesis of acute cystitis in young women, and women with recurrent urinary tract infections (UTIs) are often persistently colonized with *E. coli* [1–3]. While the pathogenic mechanisms underlying these events are not well understood, increasing evidence suggests that alterations of the normal, *Lactobacillus*-dominant vaginal flora may predispose to introital colonization with *E. coli* and to UTI. Diaphragm-spermicide use, for example, has been shown to markedly increase the prevalence of *E. coli* vaginal colonization [4–6] and the incidence of UTI, as well as to decrease the prevalence of *Lactobacillus* vaginal colonization [4, 6]. The potent in vitro microbicidal activity of the spermicide nonoxynol-9 against lactobacilli (especially H_2O_2-producing lactobacilli) but not *E. coli* may be a mechanism through which these associations arise [7–9].

While the inhibitory effects of lactobacilli against a variety of microorganisms, including *E. coli,* have long been recognized [10–12], the production of hydrogen peroxide by selected strains of lactobacilli has only recently been appreciated as an important factor in maintaining the normal vaginal flora [13–17]. To date, the relationships between hydrogen peroxide–producing lactobacilli, *E. coli* vaginal colonization, and recurrent UTI have been little studied. We hypothesized that the absence of hydrogen peroxide–producing strains of lactobacilli in the vagina may facilitate introital colonization with *E. coli,* which in turn may predispose to recurrent UTI. To examine this hypothesis, we compared vaginal introital cultures from 65 women having a history of recurrent UTI (case-patients) with cultures from 75 women who had no UTIs in the same time period (controls).

Methods

Study population. The study population was a subgroup of women who were enrolled in a larger case-control study of epidemiologic risk factors associated with recurrent UTI. Women enrolled at the University of Washington Student Health Service from February 1995 to November 1996 were eligible to participate in this additional study. Case-patients were defined as women with ⩾3 UTIs in the preceding 12 months or ⩾2 UTIs in the preceding 6 months. All UTIs had to be diagnosed and treated as such by a health care provider on the basis of laboratory or clinical criteria. Controls were attendees at the student health service for a non-UTI diagnosis in the same month as an age-matched case-patient but with no diagnosis of UTI in the preceding 12 months and no history of ⩾2 UTIs in any 12-month period in their lifetime. All subjects were ⩾18 years of age and nonpregnant and had not recently received antibiotics. None of the women had symptoms of UTI at the time of study enrollment.

Data collection and specimen processing. At enrollment, each subject provided information regarding history of UTI and current sexual activity and underwent a pelvic examination. A culture of the vaginal introitus was obtained with a swab and was transported to the laboratory in Amies transport medium. Semiquantitative cultures for aerobic gram-negative rods and lactobacilli were done

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Reprints or correspondence: Dr. Kalpana Gupta, 1959 NE Pacific St., University of Washington, HSB, BB1221, Box 356523, Seattle, WA 98195 (kalg@u.washington.edu).

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Table 1. Characteristics of women with a history of recurrent UTI (case-patients) compared with those of women without recurrent UTI (controls).

	Cases (n = 65)	Controls (n = 75)
Age, years, mean	22.3	22.3
No. of UTIs in lifetime, median (range)	5 (2–20)	0 (0–5)
No. sexually active*/total (%)	60/63 (95)	53/75 (71)
Frequency sexual activity,* median episodes/month	12	2.5
No. with spermicide use*/total (%)	17/63 (27)	6/75 (8)

* $P < .01$ for comparisons between case-patients and controls; sexual activity pertains to status at time of interview; frequency of sexual activity defined as episodes in the month preceding most recent UTI in case-patients and similar period in controls; spermicide use defined as use of diaphragm, spermicidal condom, foam and condom, or cervical cap as primary method of birth control at time of interview.

by use of previously published laboratory methods [16]. Aerobic gram-negative rods were identified by standard methods [18]. Lactobacilli were identified to the genus level by colony and Gram's stain morphology and lack of catalase production. Isolates were stored at −70°C in glycerol–trypticase soy broth. At a later date, lactobacilli were reisolated, and 3 colonies of each morphologically distinct colony type were transferred to tetramethylbenzidine medium for hydrogen peroxide testing as previously described [13]. After 48–72 h of anaerobic incubation, the isolates were exposed to ambient air for 25 min, and hydrogen peroxide–producing colonies turned blue via a previously described oxidation reaction involving hydrogen peroxide, horseradish peroxidase, and tetramethylbenzidine [13]. Women with any strain positive for H_2O_2 production were classified as H_2O_2-positive, even if they had H_2O_2-negative strains as well. Women with no lactobacilli were classified as H_2O_2-negative.

Statistical methods. Associations of vaginal flora with various risk factors were examined by using odds ratios (ORs) and 95% confidence intervals (CIs), and statistical significance was assessed by the χ^2 statistic or Fisher's exact test. Mantel-Haenszel weighted ORs were computed when combining case and control groups [19].

Results

Characteristics of the Study Groups

The subgroup of women studied included 65 with a history of recurrent UTI (case-patients) and 75 age-matched controls with no history of recurrent UTI. The mean age was 22 years in both groups. The median number of lifetime UTIs per subject was 5 (range, 2–20) for the case group and 0 (range, 0–5) for controls. Case-patients were more likely than controls to be sexually active (OR, 8.3; $P < .001$) and to be spermicide users (OR, 4.2; $P = .003$). In addition, the frequency of sexual activity in the month preceding the date of the most recent UTI in the case-patients was significantly greater than for the same time period in the controls (table 1).

Vaginal Microbial Flora in Women with and without Recurrent UTI

E. coli colonization. Introital colonization with *E. coli* was present in 30 (22%) of the 140 women studied, with quantities ranging from 1+ (<10 colonies in the primary zone of isolation) to 4.5+ (confluent growth in all three zones). *Citrobacter freundii* was isolated from 1 woman in whom *E. coli* was not isolated; she is grouped with the *E. coli*–positive group for further analyses. Case-patients demonstrated *E. coli* introital colonization significantly more often than did controls (OR, 4.6; 95% CI, 1.8–12.4; $P < .001$). In addition, semiquantitative cultures demonstrated heavy growth of *E. coli,* defined as confluence in the second zone of isolation, significantly more often in case-patients (OR, 3.72; 95% CI, 1.3–10.8; $P = .005$) (table 2).

Colonization with hydrogen peroxide (H_2O_2)–producing lactobacilli. Of the 140 study participants, 133 were evaluated for vaginal colonization with H_2O_2-producing lactobacilli. The remaining 7 were not evaluable because their organisms were not recoverable after stocking, and H_2O_2 production thus could not be assessed. Overall, 6 (4%) of the 133 women had no lactobacilli present on primary culture, 21 (16%) had only non–H_2O_2-producing lactobacilli, and 106 (80%) had at least 1 H_2O_2-producing strain present. To test the hypothesis that the absence of H_2O_2-producing lactobacilli was associated with *E. coli* colonization, we examined the prevalence of vaginal colonization with *E. coli* in women with and without H_2O_2-producing lactobacilli. Women without H_2O_2-producing lactobacilli were more likely than women with H_2O_2-producing lactobacilli to have *E. coli* introital colonization (44% vs. 16%; OR, 4.0; 95% CI, 1.3–11.6; $P = .01$). This relationship was true for case-patients, controls, and the study group as a whole (table 3), although the relationship was not statistically significant in the control group, in which there were few women colonized with *E. coli.*

Effects of Spermicide Use on Vaginal Flora

Since spermicide use has been shown to promote *E. coli* vaginal colonization, we also evaluated the effects of spermi-

Table 2. *E. coli* vaginal colonization in women with and without a history of recurrent UTI.

E. coli	Case-patients (n = 65)	Controls (n = 75)
Present*	23 (35%)	8 (11%)
Light	5 (8%)	1 (1%)
Heavy†	18 (28%)	7 (9%)
Absent	42 (65%)	67 (89%)

NOTE. Light growth is defined as ≤50 colonies in second streak zone; heavy growth as >50 colonies (confluence) in second streak zone.

* $P < .001$ for comparison of presence of *E. coli* among case-patients and controls.

† $P = .005$ for comparison of heavy growth of *E. coli* among cases and controls.

Table 3. Inverse association of hydrogen peroxide–producing lactobacilli with *E. coli* introital colonization in case-patients and controls.

	No. of women with vaginal *E. coli*			
	Lactobacilli H_2O_2-negative	Lactobacilli H_2O_2-positive	OR (95% CI)	*P*
Case-patients	10/16 (62%)	12/48 (25%)	5.0 (1.3–20.0)	.006
Controls	2/11 (18%)	5/58 (9%)	2.4 (0.3–17.6)	.309
All women	12/27 (44%)	17/106 (16%)	4.0* (1.3–11.6)	.010*

NOTE. OR, odds ratio; CI, confidence interval.
* Mantel-Haenszel statistic for stratified data.

cide use on H_2O_2-producing lactobacilli and vaginal *E. coli* colonization. Spermicide users were significantly more likely than non–spermicide users to have vaginal *E. coli* colonization ($P < .001$) and were also significantly less likely to have vaginal colonization with H_2O_2-producing lactobacilli ($P = .04$) (table 4). Since the association of spermicide use with changes in the vaginal microflora could confound the association of vaginal *E. coli* colonization with the absence of H_2O_2-producing lactobacilli, we also analyzed this relationship in non–spermicide users. In this subgroup analysis, *E. coli* colonization was present in 56% of case-patients without H_2O_2-producing lactobacilli, compared with 16% of case-patients with H_2O_2-producing lactobacilli (OR, 6.5; $P = .02$), demonstrating an even greater risk of vaginal *E. coli* colonization in the absence of H_2O_2-producing lactobacilli in non–spermicide users. There were only 3 non–spermicide-using control women colonized with *E. coli,* so little can be said about this group.

Discussion

More than 90% of the normal vaginal microflora consists of *Lactobacillus* species, the majority of which produce hydrogen peroxide [13]. These facultatively anaerobic gram-positive rods are present in quantities of 10^7–10^8 cfu/g of vaginal fluid [13]. The overwhelming predominance of these hydrogen peroxide–producing bacteria supports their suggested role as regulators of the vaginal ecosystem. This homeostatic function has also been inferred from clinical studies demonstrating an increased incidence of bacterial vaginosis and human immunodeficiency virus and *Neisseria gonorrhoeae* infections in women lacking vaginal lactobacilli [17, 20, 21].

Table 4. Effects of spermicide use on vaginal colonization with *E. coli* and H_2O_2-producing lactobacilli.

	No. colonized/total (%)		
Organism	Spermicide use	No spermicide use	OR (95% CI)
E. coli	15/23 (65)	15/115 (13)	12.5 (4.1–39.4)
H_2O_2-producing lactobacilli	14/22 (64)	91/109 (83)	0.35 (0.11–1.06)

NOTE. Spermicide use includes diaphragm, cervical cap, spermicidal condom, or foam and condom. OR, odds ratio (for presence of organism in spermicide users); CI, confidence interval.

There are several mechanisms through which lactobacilli may prevent colonization of the vagina by uropathogens [22, 23]. Competitive exclusion of uropathogens by adherence of *Lactobacillus* species to uroepithelial cells has been demonstrated in vitro and in animal models [11, 22, 23]. The production of lactic acid by lactobacilli contributes to the acidic pH of the vagina, an environment known to be inhibitory to several microorganisms, including *E. coli* [22, 24]. Lactobacilli also produce bacteriocins, substances with antimicrobial activity against bacteria as well as fungi [23]. Finally, although the production of hydrogen peroxide by lactobacilli has long been recognized, only recently has this been identified as an important means of bacterial antagonism in the vagina. Hydrogen peroxide alone is antimicrobial, and when combined with chloride anion and myeloperoxidase, both of which are found in the vagina, this activity is even further enhanced [14]. In vitro models have demonstrated that this vaginal antimicrobial defense system is active against *E. coli* as well as several other microorganisms [14]. While not all *Lactobacillus* species exhibit each of these properties, the net effect of lactobacilli in the vagina is clearly antimicrobial. It is possible in vivo that vaginal *Lactobacillus* strains possessing several of the previously mentioned characteristics are most inhibitory to invading exogenous bacteria and that H_2O_2 production is a marker for such strains.

The consequences of alterations in the *Lactobacillus*-dominant vaginal flora as they relate to UTI have been most clearly seen in studies of the effects of diaphragm and spermicide use on the vaginal microflora. Hooton and colleagues have shown in prospective clinical trials that diaphragm and spermicide use is associated with decreases in the prevalence of vaginal lactobacilli, increases in the prevalence of *E. coli* vaginal colonization [6], and increased rates of UTI [25]. Spermicide use alone has also been shown to increase *E. coli* vaginal colonization and UTI [6, 25]. Furthermore, in vitro studies by Klebanoff

[7] demonstrate that nonoxynol-9, the active ingredient in most spermicide preparations, inhibits hydrogen peroxide–producing lactobacilli but has little direct effect against *E. coli,* except in high concentrations. Thus, spermicide use clearly increases the risk of *E. coli* vaginal colonization and of UTI. Whether these changes are due primarily to alterations in H_2O_2-producing vaginal lactobacilli or to other factors is an important question under investigation.

Our findings confirm that women with recurrent UTI demonstrate a propensity for vaginal colonization with *E. coli,* even during asymptomatic periods. Previous studies have also demonstrated that *E. coli* attach in greater numbers to vaginal epithelial cells collected from women with recurrent UTI than to cells from normal women [26, 27]. These observations suggest that epithelial cells from susceptible women may possess specific types or greater numbers of receptors for *E. coli* binding, facilitating colonization. The current study suggests that the microbial ecology of the vaginal ecosystem also influences *E. coli* colonization in women with recurrent UTI, since these women demonstrated an inverse relationship between vaginal *E. coli* colonization and the presence of hydrogen peroxide–producing vaginal lactobacilli.

Our data suggest that maintenance or repletion of H_2O_2-producing lactobacilli could reduce the risk of recurrent UTI. Avoidance of factors that decrease the prevalence and concentration of H_2O_2-positive lactobacilli may thus reduce the propensity for *E. coli* vaginal colonization and subsequent UTI. Factors previously associated with a decrease in vaginal lactobacilli include spermicide use [6], β-lactam antimicrobial use [28, 29], and lack of estrogen [30]. Intercourse with a new sex partner and douching have also both been associated with alterations in vaginal flora [17]. However, the precise mechanisms through which each of these factors acts, their specific effects on H_2O_2-producing lactobacilli, and the temporal relationships of these exposures to loss of vaginal H_2O_2-positive lactobacilli remain to be elucidated.

In women with altered vaginal flora, recurrent UTI may be preventable by the repletion of H_2O_2-producing lactobacilli and inhibition of *E. coli* vaginal colonization through use of a *Lactobacillus* probiotic. Bruce and Reid [31] demonstrated a reduction in episodes of UTI in 5 women with recurrent UTI after intravaginal instillation of a specific *Lactobacillus* strain. However, variable results using *Lactobacillus* probiotics have been reported by other investigators [32]. It is likely that the development of an effective *Lactobacillus* probiotic will require further studies that carefully characterize potential *Lactobacillus* strains for properties such as adherence to vaginal epithelial cells and hydrogen peroxide production [33, 34].

In conclusion, these findings add to our understanding of the pathogenesis of recurrent UTI in young adult women and suggest new preventive strategies. However, the case-control study design and the availability of only a single introital culture do not allow us to make inferences about cause and effect or temporal associations between alterations in *Lactobacillus* colonization and UTI. Further prospective studies are needed to confirm the association between lack of H_2O_2-producing lactobacilli and vaginal *E. coli* colonization. Such studies should establish temporal relationships between these conditions, identify exposures that alter vaginal colonization with H_2O_2-producing lactobacilli, and define relationships between these alterations in vaginal flora and actual risk of UTI.

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