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Lactobacillus colonization status in infants with urinary tract infection

Jung Won Lee · Yoon Hee Shim · Seung Joo Lee

Abstract To explore the potential protective role of urogenital lactobacilli against urinary tract infection (UTI), lactobacillus cultures were performed on stool and urine specimens and periurethral/vaginal swabs of febrile infants who were suspected of having UTI. Those infants diagnosed with UTI based on the results of the suprapubic urine cultures were allocated to the UTI group (n=60), and those who had a simple viral illness with negative urine cultures were allocated to the control group (n=31). Lactobacilli were anaerobically cultured in lactobacillus-specific DifcoTM Rogosa SL agar for 48 h at 37°C and then counted. The lactobacillus colony counts for the stool and urine specimens and periurethral swabs from the UTI group were significantly lower than those for the control group ($P<0.05$). The geometric means of stool, periurethra, and urine lactobacilli in the UTI group were significantly lower than those in the control group ($P<0.05$). The colony count of the vaginal lactobacillus demonstrated an equivocal difference between the UTI and control group. In conclusion, this is the first prospective case–control study to demonstrate reduced lactobacillus urogenital colonization in infants with UTI. Our results support the view that less urogenital lactobacillus colonization may be a risk factor for UTI in infants even though there is an unclear possibility that the UTI itself could be the cause of the lower lactobacillus colonies.

Keywords Infants · Lactobacilli · Urinary tract infection

J. W. Lee
Department of Pediatrics,
Hallym University, Kangnam Sacred Heart Hospital,
94-195 Yeongdeungpo-dong,
Yeongdeungpo-gu, Seoul 150-030, Korea

Y. H. Shim · S. J. Lee (✉)
Department of Pediatrics,
Ewha Womans University Mokdong Hospital,
911-1 Mokdong,
YangCheon-Ku, Seoul 158-710, Korea
e-mail: sjoolee@ewha.ac.kr

Introduction

Urinary tract infection (UTI) is known as an ascending type of infection by fecal uropathogens, as documented by a genomic profile study [1]. The imbalance between uropathogens and normal flora has been suggested to be a risk factor for UTI [2].

A 1915 study reported that cystitis could be successfully treated by intravesical injection of a lactobacillus strain [3], but this finding was ignored during the following era of widespread antibiotic use. Subsequent evidence on the role of lactobacilli against urogenital infection came from a 1973 study which showed significantly depleted vaginal lactobacilli in women with recurrent urethritis relative to healthy women [4]. An inverse association between hydrogen peroxide (H_2O_2)-producing lactobacilli and *Escherichia coli* colonization was demonstrated in the vaginal discharge of the women with recurrent UTI [5]. The inhibitory effects of vaginal lactobacilli against *E. coli* has also been recognized [6]. Aerobic and anaerobic urethral flora of healthy women were found to be quite different from those of UTI women [7]. Intraurethrally administered indigenous lactobacilli were also found to prevent the development of UTI ostensibly in animal models [6, 8–10]. Human breast milk, which was demonstrated to be a natural source of lactobacilli, was found to have a protective effect against UTI in infants [11, 12]. Several clinical trials in adults reported that a number of well-characterized lactobacillus strains improved vaginal

ecology and prevented urogenital infection [13–15]. Among the pediatric population, there has been one case report of successful lactobacillus prophylaxis in a girl with recurrent UTI [16], and there has been a recent prospective study in which oral lactobacillus prophylaxis was effective in preventing persistent primary vesicoureteral reflux (VUR) [17]. However, to date, there has been no information published on the lactobacillus status of children.

The object of this study was to evaluate the genitourinary and fecal lactobacillus status of infants with UTI.

Subjects and methods

Three specimens (stool, urine, and periurethral/vaginal swab) were obtained for lactobacillus culture from 91 febrile infants admitted to Ewha Womans University Medical Center between March 2005 and March 2006. Febrile infants with a past history of UTI and antibiotic treatment before the time of the urine sample were excluded from the study. Inclusion criteria of the UTI group were infants with unexplained fever who were suspected of having UTI. The exclusion criteria were genitourinary tract anomaly/malformation and obstructive uropathy. The UTIs were confirmed by significant bacteriuria on the suprapubic aspirated urine culture (single uropathogen >10^3 CFU/mL). During the same period, control samples were obtained in infants who were suspected of having simple viral illness [acute bronchiolitis (18 infants), exanthem subitum (three infants), upper respiratory infection (ten infants)]. Each sample for lactobacillus was adequately prepared for anaerobic culture. All UTI-suspicious infants were initially treated with ceftriaxone after three samples had been taken for lactobacillus culture. Urine cultures were collected by suprapubic aspiration in febrile infants who were suspected of having UTI and by sterile bag urine culture in control infants. The imaging studies [renal ultrasound; technetium-99m dimercatosuccinic acid scintigraphy (^{99m}TC DMSA) and voiding cystourethrography (VCUG)] were performed on infants with definite UTI. Of the infants with UTI, 41.6% (25/60) had ^{99m}TC DMSA (+) pyelonephritis and 18.3% (11/60) had VUR.

Lactobacillus was anaerobically cultured in lactobacillus-selective DifcoTM Rogosa agar (Becton, Dickinson and Co, Franklin Lakes, NJ) at 37°C for 48 h, at which time the colonies were counted and the colony forming units (CFU)/mL determined. The study protocol was approved by the ethical committee of the hospital and informed consent was obtained from each parent. The calculated sample size to detect a 50% difference between the two groups at a value of 0.05 (one-sided) and a power of 0.80 was 91 cases.

When 100 samples of lactobacillus were obtained, the study was terminated. Infants (n=3) with negative urine

cultures or less than significant bacteriuria (single uropathogen <10^3 CFU/mL) and infants (n=6) with positive urine culture in the control group were excluded from further study. A total of 60 infants were ultimately allocated to the UTI group and 31 infants to the control group. The age (5.6±3.9 vs. 5.3±3.4 months, respectively), male-to-female ratio (41:19 vs. 25:6, respectively), duration of fever, and feeding type were not significantly different between the two groups (Table 1).

For statistical analysis, the Mantel-Haenszel κ^2 was used to compare the distribution of lactobacillus CFU from the stool and urine specimens and the periurethral/vaginal swabs. The Spearman correlation and Wilcoxon rank sum test were used to compare the CFUs of lactobacilli between the two groups. All data were expressed as mean ± standard deviation. A P value <0.05 was considered to be statistically significant.

Results

Lactobacillus colonies in the control group were most abundant in the stool cultures, less abundant in the periurethra/vagina cultures, and least abundant in the urine cultures. The numbers of stool lactobacilli were over 10^3 CFU/mL in all infants, whereas there was less than 10^3 CFU/mL in the majority of infants (Fig. 1).

There were significantly lower levels of lactobacilli colonies in the urine cultures of the UTI group than in those of the control group (P<0.05). In the UTI group, 95.0% (57/60) of the infants had less than 10^3 CFU/mL of urine lactobacilli, which was significantly higher than the 67.7% (21/31) in the control group (P<0.01). Among the male infants, there were significantly lower levels of periurethral lactobacilli colonies in the UTI group than in the control group (P<0.01). In the UTI group, 43.9% (18/41) of the infants had less than 10^3 CFU/mL of periurethral

Table 1 Clinical characteristics of the study infants

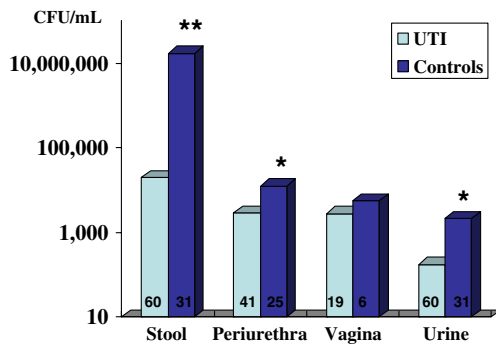| Clinical characteristics	UTI group (n=60)	Control group (n=31)
Age (months)	5.6±3.9	5.3±3.4
Sex (male/female)	41/19	25/6
Duration of fever (h)		
<24	36 (60.0%)	14 (45.2%)
≥24, < 48	15 (25.0%)	8 (25.8%)
≥48	9 (15.0%)	9 (29.0%)
Nutrition type		
Breast feeding (BF)	19 (31.7%)	7 (22.6%)
Milk feeding (MF)	35 (58.3%)	20 (64.5%)
BF + MF	6 (10.0%)	4 (12.9%)

P>0.05,
UTI Urinary tract infection

Fig. 1 Comparison of the geometric mean of lactobacillus colony forming units (*CFU*). *P<0.05, **P<0.01. *UTI* Urinary tract infection

lactobacilli, which was significantly higher than the 8% (2/25) in the control group ($P<0.01$). Among the female infants, the level of vaginal lactobacilli colonies in the UTI group was not significantly different from those of the control group ($P=0.074$), but 26.4% (5/19) of the UTI group had less than 10^3 CFU/mL of vaginal lactobacilli, which was significantly higher than the 0% (0/6) in the control group ($P=0.03$). There were also significantly lower levels of lactobacillus colonies in the stool cultures of the UTI group than in those in the control group ($P<0.01$). In the UTI group, 11.6% (7/60) had less than 10^3 CFU/mL of stool lactobacilli, which was significantly higher than the 0% (0/31) in the control group ($P<0.01$) (Table 2). In the UTI group, we analyzed the number of each lactobacilli colony according to fever duration, urine collection time, and the response time to antibiotics; none of these parameters were significant between the two groups.

The geometric means of lactobacillus CFUs in the UTI group were significantly lower than those of the control group in the stool cultures ($19,286\pm30,920$ vs. $16,969,129\pm 89,717,956$, respectively) ($P<0.01$), the male periurethras ($2,814\pm5,672$ vs. $12,448\pm25,443$) ($P<0.05$), and the urine cultures (173 ± 469 vs. $2,087\pm5,681$) ($P<0.05$). However, the geometric mean of vaginal lactobacilli in the UTI group was not significantly different from that of the control group ($2,731\pm4,781$ vs. $5,500\pm4,394$) ($P=0.16$) (Fig. 1).

This statistical insignificance may be due to the small number of cases.

Discussion

In this first prospective study, there were clearly fewer lactobacillus colonies in the stool, periurethra, and urine cultures and a not as distinct lower number in the vagina swabs of UTI infants than in those of the control infants. In adults, a significant depletion of vaginal lactobacilli in women with recurrent urethritis has been reported: vaginal lactobacilli were cultured only in 61% of women with recurrent urethritis, compared with 97% of healthy control women [4]. A more recent study also showed an inverse association between H_2O_2-producing lactobacilli and *E. coli* colonization in the vagina of women with recurrent UTI. The women without H_2O_2-producing lactobacilli were more likely to have introital *E. coli* colonization than those with H_2O_2-producing lactobacilli (Odds ratio 4.0; 95% confidence interval 1.3–11.6; $P=0.01$). The absence of H_2O_2-producing lactobacilli has been suggested to be an important factor in the pathogenesis of recurrent UTI [5]. That the intravaginal administration of indigenous lactobacilli can eliminate vaginal *E. coli* colonization and inhibit *E. coli* has long been recognized [6]. In a study of urethral flora (first 10 mL of voided urine), lactobacilli were also dominant organisms, accounting for 14.6–28.3% of the total aerobic and anaerobic flora. Lactobacilli were cultured in 80% of healthy reproductive-age women compared with 50% of UTI women [7]. These results differ from our data, which showed that vaginal and periurethral lactobacilli were cultured in 94.8% of UTI infants and 87.8% of male UTI infants, respectively; these values are lower but not significantly different to the 100% figure in control infants. This discrepancy may be related to the difference in the study population (infant with first UTI in our study vs. women with recurrent urethritis or UTI) and a small sample size.

There are several mechanisms through which lactobacilli prevent the colonization of uropathogens. In in vitro tests,

Table 2 Distribution of lactobacillus colony forming units (CFU)

Lactobacilli (CFU/mL)	Stool		Periurethral		Vaginal		Urine	
	UTI**	Control	UTI**	Control	UTI***	Control	UTI*	Control
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
No lactobacillus colony	5 (8.3)	0 (0)	5 (12.2)	0 (0)	1 (5.3)	0 (0)	27 (45.0)	11 (35.5)
$<10^3$	2 (3.3)	0 (0)	13 (31.7)	2 (8.0)	4 (21.1)	0 (0)	30 (50.0)	10 (32.3)
10^3 to approx. 10^5	51 (85.0)	15 (48.4)	23 (56.1)	22 (88.0)	14 (73.7)	5 (83.3)	3 (5.0)	10 (32.3)
$\geq 10^5$	2 (3.3)	16 (51.6)	0 (0)	1 (4.0)	0 (0)	1 (16.7)	0 (0)	0 (0)
Total	60 (100)	31 (100)	41 (100)	25 (100)	19 (100)	6 (100)	60 (100)	31 (100)

*P<0.05 vs. control group, **P<0.01 vs. control group, ***P=0.074 vs. control group

lactobacilli were proven to adhere to the mucus and form a biosurfactant barrier that disrupts the adherence of uropathogens to the uroepithelial receptors [18, 19] and produces antimicrobial compounds, such as H_2O_2, lactic acid, and bacteriocin, that in turn interferes with the growth of uropathogens [20]. It has also been proven that lactobacilli modulates the host immune system by stimulating dendritic cell, T-cell, and phagocytic activity, by up-regulating Th-2 anti-inflammatory cytokines [interleukin (IL)-10, IL-12] and proinflammatory cytokines (IL-6), and by down-regulating inflammatory cytokines (IL-8, cyclooxygenase) or virulence factor expressions [21, 22].

In an animal model, indigenous *L. casei* strains that were instilled into the bladders before challenge with uropathogens prevented UTIs in 84% of the tested rats [8], and vaginal flush with indigenous lactobacilli eliminated vaginal *E. coli* colonization in four adult monkeys with pyelonephritogenic *E. coli* infection [6]. A single intraurethral administration of *L. casei* Shirota strain before the challenge of *E. coli* dramatically inhibited *E. coli* growth and the subsequent inflammatory response in the mouse urinary tracts [9].

In human clinical trials, the intravaginal instillation of *L. rhamnosus* GR-1 and *L. fermentum* RC-14 stimulated indigenous vaginal lactobacilli and reduced the UTI recurrence rate from 6 to 1.6 episodes per year in women [13, 14] and was also effective in reducing the recurrence rate of UTI in 41 UTI women—from 47 to 21%—even after antimicrobial therapy [15]. In premature infants, breast milk supplemented with *L. rhamnosus* GG reduced the number of UTI episodes, although the difference was not statistically significant [23]. In children, there has been a single case report showing successful probiotic prophylaxis in a 6-year-old girl with frequently recurrent UTI, who was given *L. acidophilus* DDS-1 (2×10^9 CFU) twice daily for a month and then once daily for 5 months; she had no recurrent UTI, which is in contrast to three consecutive relapses contracted for the preceding 3 months [16]. The role of oral probiotics in the resolution of the urogenital infection including UTI has been the subject of recent reports [24, 25]. The importance of urogenital probiotics could be accentuated by the avoidance of long-term use of antibiotics and the subsequent emergence of resistant strains [26]. Another advantage would be that it is the natural approach that replenishes the depleted normal flora to create a better environment to fight off uropathogens. There are still many questions to be resolved, but promising evidence on urogenital lactobacilli suggest the possible benefits and may provide the rationale for studying lactobacilli as prophylaxis to prevent UTI, even in children. In order to develop the ideal urogenital probiotics, it is important to identify the best lactobacillus strains that are detrimental to uropathogens in further studies [27].

In conclusion, this is the first prospective case–control study to demonstrate reduced lactobacillus urogenital colonization in infants with UTI. Our results supports the view that lower urogenital lactobacillus colonization may be a risk factor for UTI in infants even though there is an unclear possibility that the UTI itself may actually have caused the low lactobacillus colony count. The limitation of our study is in not being able to clarify whether the difference in the number of lactobacilli colonies originated simply from the difference in bacterial or viral infections. Further clinical studies are needed to clarify the effect of lactobacilli in preventing UTI in infants.

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