ORIGINAL ARTICLE

Efficacy of Probiotic Prophylaxis After The First Febrile Urinary Tract Infection in Children With Normal Urinary Tracts

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Background. Growing antibiotic resistance and debates over their efficacy for urinary tract infection (UTI) recurrence warrants studying nonantibiotic prophylaxis for preventing UTI recurrences.

Methods. We randomly assigned 181 children, aged 4 months to 5 years, with a normal urinary tract after recovery from their first febrile UTI in a 1:1 ratio to receive a probiotic mixture of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis* (n = 91) or placebo (n = 90) for a total of 18 months of therapy. The primary objective was to show the superiority of probiotic prophylaxis to placebo. The primary end point was composite cure (UTI-free survival) at 18 months, and the secondary end point was the median time to first UTI recurrence.

Results. The probiotics were superior to placebo with respect to the primary efficacy end point. At 18 months, composite cure was observed in 96.7% (3 of 91) of the patients in the probiotic group and 83.3% (15 of 90) of those in the placebo group (*P* = .02). The median time to the first incidence of UTI recurrence was 3.5 months (range, 1–4 months) and 6.5 months (range, 2–14 months) in the probiotic and placebo groups, respectively (*P* = .04). The main microorganism that caused recurrent UTI was *Escherichia coli*, followed by *Klebsiella pneumoniae*, and these results were not significantly different between the 2 groups. We found no specific adverse events among the participants who received the probiotic mixture during the course of therapy.

Conclusions. The probiotics were more effective than placebo at reducing the risk of recurrent UTI in children with a normal urinary tract after their first episode of febrile UTI.

Keywords. Children; probiotic; prophylaxis; recurrent urinary tract infections.

Urinary tract infection (UTI) is a common infection in childhood that can lead to significant morbidity and death [1–3]. It has been a common practice for many years to use long-term antibiotics to prevent UTI recurrence. However, the routine use of prophylactic antibiotics has been a subject of debate over the past decade because of their associated adverse effects and because of the growth of antibiotic-resistant microorganisms [4–9]. The American Academy of Pediatrics (AAP) recently reaffirmed its 2011 UTI clinical practice guideline and recommended that antimicrobial prophylaxis not be given to children aged 2 to 24 months after their first febrile UTI if the results of renal and bladder ultrasonography are normal [10]. In support of the strong statement by the AAP that antimicrobial

prophylaxis seems to be ineffective in preventing the recurrence of febrile UTI is the fact that evidence regarding the value of prophylactic antibiotics for the prevention of recurrent UTI remains largely inconclusive [11–14].

The high UTI recurrent rates in infants and young children and increasing potential for the development of resistance have encouraged investigators to study alternative nonantibiotic measures, including drinking cranberry juice, applying topical estrogen, and taking probiotics for the prevention of recurrent infection [14–16].

When given in sufficient numbers, probiotic microorganisms are thought to establish a barrier against urogenital bacteria that ascend the urinary tract and cause infection. The protective effects of probiotics in preventing recurrent UTI is thought to be mediated through the restoration of normal vaginal and intestinal flora achieved by reducing the adherence, growth, and colonization of infectious pathogens and improving host defenses [17–19].

Results of recent studies have suggested that probiotics can offer advantages over placebo for the prevention of UTI, but these studies have been few and derived from studies with small

sample sizes and poor methodologic design and have not been compared in randomized controlled trial [20–22].

Because probiotics are safe products and are readily available without a prescription, we were interested in studying the role of probiotics in preventing UTI in otherwise healthy children in a randomized controlled trial to inform heath care providers about a potentially significant change in prophylactic therapy.

PATIENTS AND METHODS

This multicenter randomized controlled double-blind trial was conducted from January 2017 through November 2018 at 4 hospital clinics in Iran affiliated with the Kurdistan University of Medical Sciences, the Mashhad University of Medical Sciences, the Semnan University of Medical Sciences, and the Zahedan University of Medical Sciences. Participants consisted of 244 children aged 4 months to 5 years after recovery from their first episode of uncomplicated acute febrile UTI, documented by sterile urine culture results. Participants were randomly assigned to receive either a probiotic mixture or placebo during the study period and to undergo serial monitoring via urine culture.

Uncomplicated UTI was defined as UTI with normal renal and bladder ultrasound results. Children with an immunodeficiency syndrome, voiding dysfunction, abnormal renal function, or structural anomaly or history of vesicoureteral reflux (VUR), hypertension, or hepatic insufficiency were excluded. Also excluded were children who received concomitant antibiotic therapy or had used probiotics in the previous 3 months before enrolling in the study.

This study was conducted in accordance with the Declaration of Helsinki, revised in Brazil in 2013. All participating centers obtained approval from their health research ethics board before commencement of the study. Written parental informed consent was obtained for each participant before the study, and we followed the CONSORT 2010 checklist guidelines for reporting the results of the randomized trial.

Eligible patients were included in intention-to-treat and safety populations and randomly assigned in a 1:1 ratio to receive a probiotic mixture or placebo for a total of 18 months. The intention-to-treat and safety populations included all randomly assigned patients, including those who were noncompliant. Randomization was performed by the site pharmacist or designated medical staff according to a prespecified randomization schedule. The investigator, medical staff participating in patient care, the patients, and their families were unaware of the group assignments.

For this trial, the contents of a 500-mg probiotic capsule (Complete Probiotic Platinum [1MD, Sherman Oaks, California]) containing 11 diverse strains of *Lactobacillus* and *Bifidobacterium* spp, including *Lactobacillus acidophilus* (15×10^9 colony-forming units [CFU]), *Lactobacillus rhamnosus* (1.0×10^9 CFU), *Bifidobacterium bifidum* (4×10^9 CFU), and *Bifidobacterium lactis*

(15×10^9 CFU) was dissolved in 10 mL of 5% dextrose water (500 mg/10 mL), and it was given as 0.5 mL (25 mg)/kg 2 times per day (maximum daily dose, 20 mL [1.0 g]) with or without food during the course of the study. Participants in the placebo group received an equivalent volume (0.5 mL/kg) of drinking water twice daily during the course of study. A daily intake of 10^6 to 10^9 CFU of *Lactobacillus* and *Bifidobacterium* spp is reported as the minimum effective dose for therapeutic purposes in adults [17, 23].

The participants and the investigators were blinded to the content of the bottles, which contained liquid probiotics or placebo water. The hospital pharmacy at each study site was responsible for the preparation, assignment, dispensing, and distribution of liquid probiotics during the study period.

The liquid probiotic mixture was placed into plastic retail bottles, sealed, and refrigerated at 10°C. We performed bacterial counts on the liquid probiotics at the time of preparation and again 2 and 4 weeks later to determine the bacterial survival rate and found no significant reduction in total bacterial counts over the 4 weeks of storage time. The probiotics and placebo were delivered to the participants' parents every 2 to 4 weeks, and the parents were instructed to keep the product refrigerated after each use to retain viability during storage. Parents were contacted weekly during the trial by study staff to ensure adherence to the treatment protocol and to inquire about any clinical symptoms of UTI. Adherence was measured by the volume of leftover probiotic liquid returned to the investigator subtracted from the volume of liquid probiotic dispensed at the previous visits. Patients were excluded from the study if their rate of compliance to the study protocol was <80% during the monthly clinic visits. Participants were visited in the clinic at monthly intervals for routine physical examination.

UTI diagnosis was suspected for any febrile ill-looking child with no apparent source for the fever and confirmed by the presence of at least 50 000 CFU/mL of a single uropathogen cultured from a urine specimen obtained through transurethral catheterization or suprapubic aspiration [1–3, 10]. Pyuria was defined in any child with ≥10 leukocytes per μL in a centrifuged urine sample [2]. Children with a symptomatic UTI were treated with appropriate antibiotics according to the previously accepted guidelines [1–3, 10] and then excluded from the study. All laboratory tests were centralized at each participating center and performed in a blinded manner.

The primary end point of the study was composite cure (UTI-free survival) 18 months after the initiation of therapy. The secondary end point was the median time to first UTI recurrence.

The number of participants needed for this study that would permit a 2-sided significance level of 1% and 95% power was 70 patients in each group [24]. To anticipate the possibility of patients lost in follow-up, incomplete data collection, or poor compliance with taking probiotics (10%), the planned sample size was determined to be 83 patients per group. Continuous

variables were expressed as means and standard deviations, whereas categorical variables were expressed as absolute numbers and proportions of patients in a given category. Data were compared using the Fisher exact test (categorical variables) and the Student t test (continuous variables). The χ^2 test was used for comparing the probiotic and placebo groups. The Kaplan-Meier model was used to estimate the probability of UTI during prophylaxis in both treatment arms. From the Kaplan-Meier estimates, we computed the median time to the first UTI recurrence. Pearson regression models were used to obtain estimates of the mean rates of UTI recurrence at follow-up. Any P value of <.5 was considered significant. All statistical analyses were performed using SPSS 13.0 (SPSS, Inc, Chicago, Illinois).

RESULTS

Of the 244 patients enrolled, 181 (74.18%) were included in the safety intention-to-treat and safety populations and randomly assigned in a 1:1 ratio to receive probiotics (n = 91) or placebo

(n = 90) once daily for 18 months (Figure 1). Baseline characteristics did not differ significantly between the 2 treatment groups (Table 1).

Probiotics were superior to placebo with respect to the primary efficacy end point. At 18 months, composite cure was observed in 96.7% (3 of 91 patients) of the patients in the probiotic group and 83.3% (15 of 90) of the patients in the placebo group (difference, −13.3 percentage points [95% confidence interval, −8.9 to 4.1]; P = .02) (Figure 2).

The median time to the first incidence of UTI recurrence, the secondary end point, was 3.5 months (range, 1–4 months) and 6.5 months (range, 2–14 months) in the probiotic and placebo groups, respectively (P = .04) (Table 2). Girls had higher UTI recurrence rates than boys in both arms of the study (11.5% [6 of 52] vs 0% [0 of 39] in the probiotic group and 27.4% [14 of 51] vs 5% [2 of 40] in the placebo group, respectively; P = 0.02) (Table 2). The children younger than 12 months had a higher incidence of recurrent UTI than did older children (2.2% in the probiotic group vs 11.1% in the placebo group, respectively; P = .04).
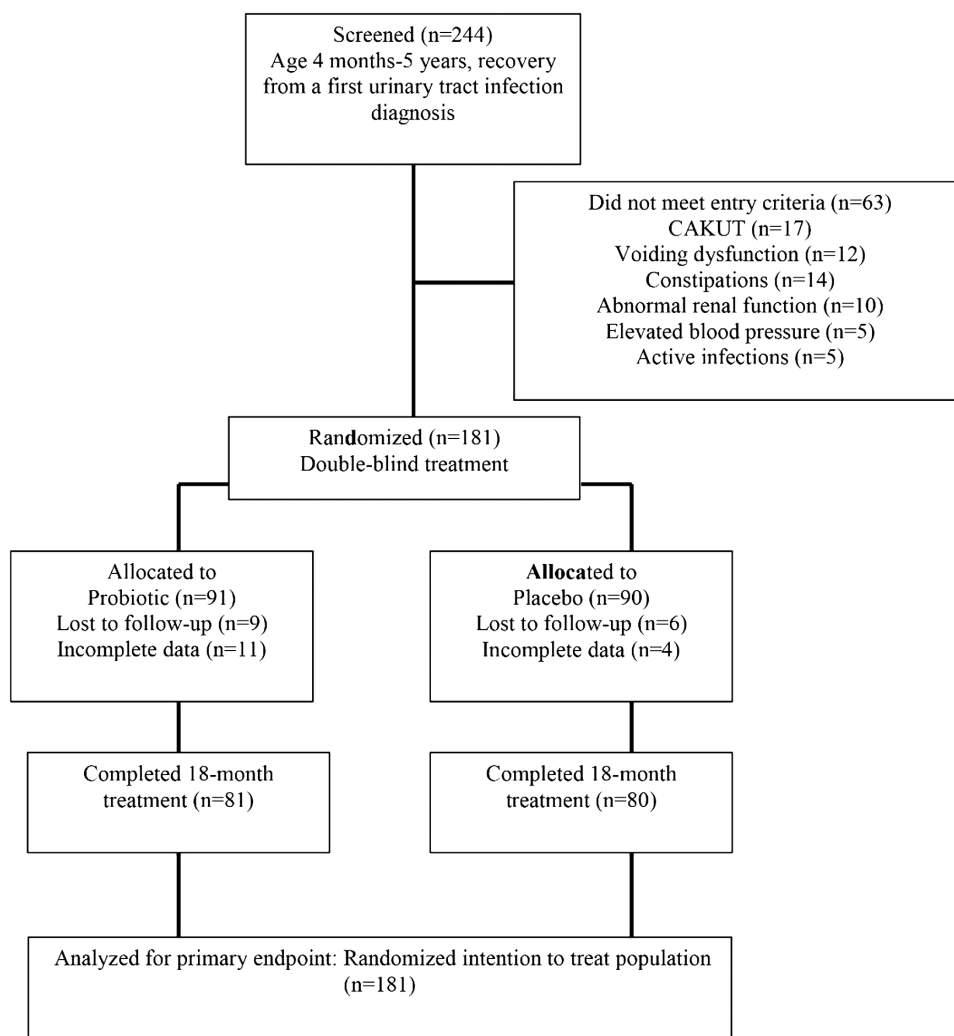

Figure 1. Enrollment, randomization, and retention-to-treat population.

Table 1. Characteristics of the Patients at Baseline (Intention-to-Treat Population)

Variable	Probiotic Group (n = 91)	Placebo Group (n = 90)	P
Age (mean ± SD) (years)	3.3 ± 1.5	3.6 ± 0.9	.89
Female/male (n [%])	52/39 (57/35)	50/40 (56/38)	.35
Weight (mean ± SD) (kg)	18.6 ± 4.0	17.9 ± 4.1	.63
Systolic BP (mean ± SD) (mm Hg)	84.3 ± 5.8	90.6 ± 4.8	.27
Diastolic BP (mean ± SD) (mm Hg)	49.5 ± 2.4	48.9 ± 2.6	.74
Serum creatinine (mean ± SD) (mg/dL)	0.5 ± 0.2	0.4 ± 0.2	.88
BUN (mean ± SD) (mg/dL)	10 ± 1	11 ± 3	.35
Urine culture	Sterile	Sterile	—
Kidney ultrasound result	Normal	Normal	—

Abbreviations: BP, blood pressure; BUN, blood urea nitrogen; SD, standard deviation.

The main microorganism that caused recurrent UTI was *Escherichia coli* (88%), followed by *Klebsiella pneumoniae* (12%); these results were not significantly different between the 2 groups.

No specific probiotic-related adverse events (including skin rash, nausea, vomiting, diarrhea, or leukopenia) among the participants emerged from a review of symptoms, physical examination, and laboratory tests during the study follow-up.

DISCUSSION

In this study, we compared the effect of probiotic and placebo prophylaxis in young children with uncomplicated UTI and found that probiotics, with a higher rate of composite cure observed after 18 months of therapy, were superior to placebo. The lower incidence of UTI recurrence in the probiotic group suggests that greater UTI-free survival with probiotics provides additional clinical benefit for children with a normal urinary tract.

To our knowledge this is the first randomized placebo-controlled clinical trial to be undertaken in a relatively large pediatric population over a length of time adequate enough to evaluate the role of probiotics in preventing future UTI in otherwise healthy children.

Previous studies that evaluated the effectiveness of probiotics on UTI prevention in children have produced discrepant results. The findings in our study are consistent with those reported by Lee et al [22]. In their retrospective study, these authors compared the efficacy of probiotic prophylaxis (n = 73) with that of placebo (n = 68) in infants aged between 1 and 24 months after a first UTI during 6 months of therapy. They found that compared to the infants who received placebo, the incidence of recurrent UTIs was significantly lower in infants treated with probiotics (8.2% vs 20.6%, respectively).

Unlike Lee et al, Hosseini et al [20], in a systematic review and meta-analysis on the efficacy of probiotics in the prevention of UTI in children, found that probiotic therapy did not have any beneficial effect in reducing the incidence or recurrence of UTI. The same conclusion came from a Cochrane Database systematic review by Schwenger et al [21]. These authors reviewed a total of 4 studies that compared probiotics with placebo in children and adult patients with complicated UTI and found no significant advantage for probiotics over placebo.

The discrepancy between the findings in the present study and the studies reported by Hosseini et al and Schwenger et al are likely the result of diversity in the study designs and methodologies used by different investigators.

A number of species and strains of probiotics are used in different formulations. Many factors can also influence the viability of probiotic microorganisms during production, storage, and delivery until the time of consumption, including temperature, pH, molecular oxygen, and additives such as sugar, sodium chloride, and antimicrobial preservative. In addition to storage, the selection of probiotic strains and differences in the dosing and duration of therapy influence the efficacy of probiotics.

Of the 200 probiotic strains studied, the 4 most common putative strains of human origin have been *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Bifidobacterium lactis* [18]. Recent studies have found that probiotic strains such as *Lactobacillus rhamnosus* and
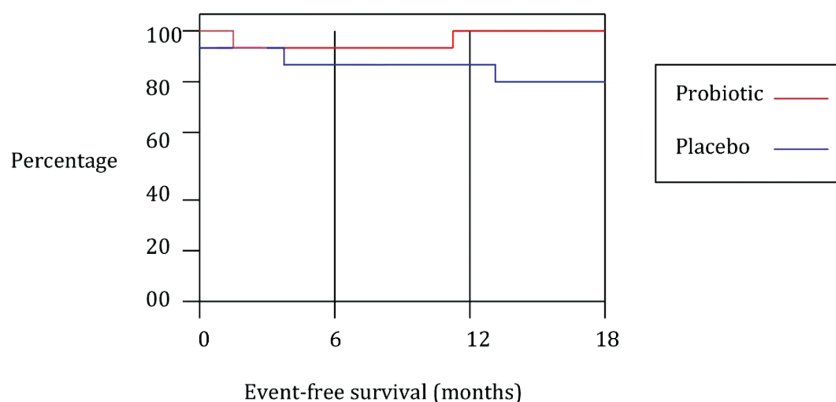
Figure 2. The Kaplan-Meier model estimating the probability of recurrent urinary tract infection during prophylaxis for both treatment arms.

Table 2. Mean Overall Rates of UTI After 18 Months of Therapy

Variable	Probiotic Group (n = 91)	Placebo Group (n = 90)	P
Participants with first UTI	3 (3.3)	15 (16.7)	.03
Female (n/N [%])	6/52 (11.5)	14/51 (27.4)	.02
Male (n/N [%])	0/39 (0)	3/40 (5.0)	.02
Time to first UTI recurrence (median [range]) (months)	3.5 (1–4)	6.5 (2–4)	.04

Abbreviation: UTI, urinary tract infection

Lactobacillus fermentum can prevent UTI recurrence by modulating the colonization of bacterial uropathogens [25–28].

All these discrepancies can introduce bias that affects the results and makes the interpretation of data between studies rather difficult. For probiotics to be efficacious in UTI prevention, it is essential to select the most effective strains, correct dosing, and appropriate duration of therapy.

To our knowledge, our study represents the first randomized controlled trial to evaluate the role of probiotic prophylaxis after a first febrile UTI in young children with a normal urinary tract. Unlike the studies reported previously, we used a mixture of *Lactobacillus* and *Bifidobacterium* probiotic strains (at a recommended daily dose of 1×10^9 CFU) rather than *Lactobacillus* species alone. In addition, according to previously published recommendations [23], we prepared the liquid probiotics by using sterile 5% dextrose water (pH 5.5), added no preservatives, sealed the preparation immediately, and stored it at 10°C until the time of delivery to achieve optimal bacterial survival.

The main cause of UTI in children is the ascension of bacteria from the periurethral area. The bacteria originate in the bowel. The gastrointestinal microflora contributes greatly to immune function and acts a physical barrier against uropathogenic organisms across the gut mucosa. Prolonged use of antibiotics can alter gut microflora and disrupt immune function between the host and gut mucosa to favor colonization and overgrowth of pathogens across intestinal mucosal barriers, which causes periurethral colonization. The normal urogenital microflora of a healthy woman comprises approximately 50 bacterial species, dominated by *Lactobacillus* species (10^7–10^8/mL) [29]. Indeed, maternal vaginal lactobacilli are the first source of lactobacilli for newborn infants, who acquire them while passing through the birth canal. After that time, lactobacilli in breast milk are a second important source of infant gut lactobacilli, which are known to prevent UTI during infancy [26].

Probiotics (live nonpathogenic microbes), when administered in an adequate amount, have the potential to restore immune function and reduce colonization by potentially pathogenic bacteria [17–19, 29].

Although most commercially available probiotic strains are widely regarded as safe, safety might be of concern in critically ill children and in children with a severe immunodeficiency syndrome, because probiotic strains could cause bacteremia or sepsis [30].

Our study has several limitations. First is the selection of the study patients. Our study was performed within the university hospitals' referral networks. We could have missed results from community and primary care settings, which limits the generalizability of the study. If the patients' heterogeneity, socioeconomic status, and ethnicity were different between the 2 groups, then ascertainment bias could have occurred. Second, AAP guidelines do not recommend routine screening with voiding cystourethrography [10], which prevented us from fully exploring the effect of VUR on recurrent UTI and the effectiveness of probiotic prophylaxis according to VUR grade. Third, we did not include uncircumcised boys in each study arm, which might have affected the validity of our analysis and introduced bias that affected the results. Last, we did not study whether probiotic therapy reduced colonization of the bowel by virulent infecting strains of *E coli*.

The major strength of this study is that it was a randomized controlled probiotic-versus-placebo clinical trial of a relatively large pediatric population. The length of follow-up was adequate for assessing the role of probiotics in the prevention of recurrent UTIs in children with a normal urinary tract after their first febrile UTI.

CONCLUSION

Compared with placebo, probiotic prophylaxis reduced the incidence of UTI recurrence significantly in children after a febrile UTI. This efficacy was more pronounced in girls than in boys.

Given the limitations of our study, additional investigation is needed to better understand the risks and benefits of probiotic prophylaxis. This type of study should be powered to examine the efficacy of prophylaxis in older patients and in those with and those without VUR. Selection of the best probiotic strains, optimal dosing, and appropriate duration of therapy are important issues that also must be addressed in future clinical trials.

References

1. Korbel L, Howell M, Spencer JD. The clinical diagnosis and management of urinary tract infections in children and adolescents. Paediatr Int Child Health **2017**; 37:273–9.
2. Paintsil E. Update on recent guidelines for the management of urinary tract infections in children: the shifting paradigm. Curr Opin Pediatr **2013**; 25:88–94.
3. Okarska-Napierała M, Wasilewska A, Kuchar E. Urinary tract infection in children: diagnosis, treatment, imaging—comparison of current guidelines. J Pediatr Urol **2017**; 13:567–73.
4. Mattoo TK. Evidence for and against urinary prophylaxis in vesicoureteral reflux. Pediatr Nephrol **2010**; 25:2379–82.
5. Williams G, Craig JC. Prevention of recurrent urinary tract infection in children. Curr Opin Infect Dis **2009**; 22:72–6.
6. Montini G, Tullus K, Hewitt I. Febrile urinary tract infections in children. N Engl J Med **2011**; 365:239–50.
7. Stein R, Dogan HS, Hoebeke P, et al; European Association of Urology; European Society for Pediatric Urology. Urinary tract infections in children: EAU/ESPU guidelines. Eur Urol **2015**; 67:546–58.
8. Nateghian AR, Robinson JL, Mohandessi S, Hooman N. Resistance pattern of breakthrough urinary tract infections in children on antibiotic prophylaxis. J Infect Public Health **2009**; 2:147–52.
9. Karpman E, Kurzrock EA. Adverse reactions of nitrofurantoin, trimethoprim and sulfamethoxazole in children. J Urol **2004**; 172:448–53.
10. American Academy of Pediatrics, Subcommittee on Urinary Tract Infection; Reaffirmation of AAP clinical practice guidelines: the diagnosis and management of the initial urinary tract infection in febrile infants and children 2 to 24 months of age. Pediatrics **2016**; 138:e20163026.
11. Craig JC, Simpson JM, Williams GJ, et al; Prevention of Recurrent Urinary Tract Infection in Children with Vesicoureteric Reflux and Normal Renal Tracts (PRIVENT) Investigators. Antibiotic prophylaxis and recurrent urinary tract infection in children. N Engl J Med **2009**; 361:1748–59.
12. Nordmann P, Poirel L, Toleman MA, Walsh TR. Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J Antimicrob Chemother **2011**; 66:689–92.
13. Delbet JD, Lorrot M, Ulinski T. An update on new antibiotic prophylaxis and treatment for urinary tract infections in children. Expert Opin Pharmacother **2017**; 18:1619–25.
14. Chua M, Ming J, Chang SJ, et al. A critical review of recent clinical practice guidelines for pediatric urinary tract infection. Can Urol Assoc J **2018**; 12:112–8.
15. Nelson CP, Hoberman A, Shaikh N, et al. Antibiotic resistance and urinary tract infection recurrence. Pediatrics **2016**; 137. pii:e20152490. doi:10.1542/peds.2015-2490
16. Jespen RG, Williams G, Craig JC. Cranberries for preventing urinary tract infections. Cochrane Database Syst Rev **2012**; 10:CD001321.
17. Libertucci J, Young VB. The role of the microbiota in infectious diseases. Nat Microbiol **2019**; 4:35–45.
18. Cadieux PA, Burton J, Devillard E, Reid G. *Lactobacillus* by-products inhibit the growth and virulence of uropathogenic *Escherichia coli*. J Physiol Pharmacol **2009**; 60(Suppl 6):13–8.
19. Matsuzaki T, Chin J. Modulating immune responses with probiotic bacteria. Immunol Cell Biol **2000**; 78:67–73.
20. Hosseini M, Yousefifard M, Ataey N, et al. The efficacy of probiotics in prevention of urinary tract infection in children: a systematic review and meta-analysis. J Pediatr Urol **2017**; 13:581–91.
21. Schwenger EM, Tejani AM, Loewen PS. Probiotics for preventing urinary tract infections in adults and children. Cochrane Database Syst Rev **2015**; 23:CD008772.
22. Lee SJ, Cha J, Lee JW. Probiotics prophylaxis in pyelonephritis infants with normal urinary tracts. World J Pediatr **2016**; 12:425–9.
23. Kailasapathy K, Chin J. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol **2000**; 78:80–8.
24. Sarao LK, Arora M. Probiotics, prebiotics, and microencapsulation: a review. Crit Rev Food Sci Nutr **2017**; 57:344–71.
25. Osset J, Bartolomé RM, García E, Andreu A. Assessment of the capacity of *Lactobacillus* to inhibit the growth of uropathogens and block their adhesion to vaginal epithelial cells. J Infect Dis **2001**; 183:485–91.
26. Zawistowska-Rojek A, Tyski S. Are probiotic really safe for humans? Pol J Microbiol **2018**; 67:251–8.
27. Vahabnezhad E, Mochon AB, Wozniak LJ, Ziring DA. *Lactobacillus* bacteremia associated with probiotic use in a pediatric patient with ulcerative colitis. J Clin Gastroenterol **2013**; 47:437–9.
28. Johnston BC, Goldenberg JZ, Vandvik PO, et al. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database Syst Rev **2011**; 11:CD004827.
29. Altman DG, Andersen PK. Calculating the number needed to treat for trials where the outcome is time to an event. BMJ **1999**; 319:1492–5.
30. Levy I, Comarsca J, Davidovits M, et al. Urinary tract infection in preterm infants: the protective role of breastfeeding. Pediatr Nephrol **2009**; 24:527–31.