

## Preventing Urinary Tract Infections in Children—The Potential of Probiotics

a report by

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### Urinary Tract Infections in Children

Pediatric urinary tract infections (UTIs) occur in 2.6–3.4% of children each year and account for over 1.1 million office visits annually in the US.<sup>1</sup> Inpatient hospital costs alone for pediatric UTI are estimated to be over \$180 million each year and have steadily risen despite shorter hospital stays. Although no global data are available, it is likely that the societal morbidity of pediatric UTI is even greater in rural areas of developing nations due to limited diagnostic and therapeutic infrastructure, especially cross-sectional imaging and access to antibiotics. In several series, approximately 60% of children who were evaluated radiographically after an initial UTI did not have an anatomical basis for their propensity to infection.<sup>2,3</sup> The majority of the remaining children were found to have vesicoureteral reflux (VUR). In short, pediatric UTIs represent a clinical entity with significant socioeconomic and medical impact. This article will focus on children with and without VUR.

### Historical and Current Preventive Approaches to Pediatric Urinary Tract Infections

Traditional approaches to UTI prevention consist of antibiotic prophylaxis. Typically, this comprises a single daily low dose of oral antimicrobials such as amoxicillin, sulfonamides, or nitrofurantoin.<sup>4–6</sup> Purported benefits of this modified regimen include low rates of antibiotic resistance and adverse events, with preserved prophylactic effects. Despite this, many recent studies have questioned the routine use of antibiotic prophylaxis

in children with normal anatomy,<sup>7</sup> especially since in some children this therapy can extend to several years. For example, antibiotic resistance may be increased among children on long-term prophylaxis, and yet the rate of ‘breakthrough’ infections is often the same as the rate in children not on antibiotics.<sup>8,9</sup> This makes many parents and pediatricians hesitant to place children on chronic antibiotic prophylaxis.

### Alternatives for Preventing Pediatric Urinary Tract Infections

Since the 1970s, it has been known that most uropathogens in girls and women originate from the intestine, ascend along the perineum to the vagina, and then infect the bladder. This led to multiple studies into the pathogenesis and virulence properties of uropathogenic *Escherichia coli* in women.<sup>10–15</sup> Although this work has enhanced understanding of how uropathogens infect humans, it has had little impact on diagnosis, prevention, or treatment of UTIs. There have been recent efforts to develop vaccines to prevent pathogens from colonizing and infecting the bladder,<sup>16–18</sup> but their *in vivo* efficacy remains to be verified.

Cranberries, particularly in juice form, have been used for several decades with the goal of preventing UTIs. Part of the rationale is the ability of cranberry extracts to inhibit bacterial adhesion to the bladder urothelium.<sup>19,20</sup> However, a Cochrane review of the cranberry literature failed to identify appropriate randomized, controlled clinical trials that prove efficacy.<sup>21</sup> Furthermore, to the knowledge of the authors, there have been no trials of cranberries as prophylaxis for pediatric UTIs, and it remains to be determined what metabolic factor from cranberry intake is responsible for any anti-uropathogen effect.

Some investigators have sought to study the non-pathogenic lactobacilli found in high numbers in the vagina of women with no history of UTI but significantly depleted in patients with a history of recurrent UTI.<sup>22</sup> It has been hypothesized that the lactobacilli provide a bacterial barrier that interferes with the ability of pathogens to colonize the vagina and ascend into the bladder.<sup>23</sup> As yet, only one study has suggested how pathogens might displace or eradicate the lactobacilli, and this was the bacteriocin-producing *Enterococcus faecium*.<sup>24</sup> In addition, no studies have investigated how pathogens avoid killing by the indigenous microbiota, or whether continual seeding of the bladder from the vagina is an essential part of persistent or recurrent infections.<sup>25–27</sup> Thus, there have been a disproportionate number of studies into the virulence properties of *E. coli* and antibiotic use, without understanding the normal healthy microbes, their role in disease prevention, or the impact that chemotherapeutic agents have on them. The rapidly increasing resistance to and failure of antibiotics leaves care-givers with little to offer as alternatives.



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**Lactobacillus Probiotic Strains**

The concept of instilling *Lactobacillus* into the vagina is rooted in the hypothesis that its presence as the dominant bacterium may avert the ascension of uropathogens into the bladder through various mechanisms, including interference with pathogen adhesion, biofilm formation, invasion and growth, expression of virulence factors, and immunomodulation of the host's defenses.

In the future, other species commonly found in the urogenital area of children, or which have shown promise by interfering with pathogenesis (e.g. avirulent *E. coli*<sup>28</sup>), will be tested. Various approaches have been taken to test *Lactobacillus* for UTI prophylaxis. These include simply using a commercially available strain, a strategy that is doomed to failure given that almost none have scientific documentation suggesting they will function in the urogenital area.

Others have chosen to select a strain most commonly recovered from the vagina, a strategy that has not so far been based on the full picture. Twenty years ago such a selection would have been made using *L. acidophilus*, as culture methods tended to identify this species as the most ubiquitous. Several years ago, *L. crispatus* would have been selected because culture and molecular typing would have identified it as the most common. Today, *L. iners* would be chosen based on non-culture molecular studies, but as it is difficult to grow, no studies—apart from the authors' study of its *in vitro* anti-biofilm effects<sup>29</sup>—have investigated its potential for the urogenital tract.

In the 1980s, a range of *in vitro* adhesion, competitive exclusion, and growth inhibition assays were deemed the most appropriate for determining probiotic properties of strains.<sup>30</sup> Isolates from dairy, poultry, and human sources were speciated using biochemical methods and tested against a range of uropathogenic bacteria. The studies showed that there was a wide variation in anti-uropathogenic properties, even between strains of the same species.<sup>31</sup> It is still unknown why *L. rhamnosus* strain GR-1, originally isolated from the distal urethra of a healthy woman, is more capable of colonizing the vagina and reducing the risk for UTI than *L. rhamnosus* strain GG, which was originally isolated from feces.<sup>32,33</sup> These *in vitro* assay systems are still used by some researchers to select probiotic strains,<sup>34-36</sup> but very few studies have shown a correlation between *in vitro* properties and *in vivo* efficacy. In short, the ultimate proof that the best strains have been selected needs to be established through clinical trials.

**Clinical Evidence For and Against Probiotics to Prevent Urogenital Infection****Intra-vaginal Administration of Probiotics**

Clinical trials of intra-vaginal lactobacilli, some of which are unproven as probiotics, in adults have yielded mixed results.<sup>37-39</sup> To date, no studies have investigated this approach in children, in part because this route of administration is not ideal for pre-pubescent girls, and perhaps also because of ethical issues. One study reported a degree of success in a single subject with time to next infection delayed by several weeks compared with her regular chronic infected state.<sup>40</sup> Clearly, a large study is needed before any conclusions can be made as to the value of urogenital application of probiotics in girls.

**Oral Administration of Probiotics**

Similar to the literature on intra-vaginal probiotics, clinical trials and questionnaire-based studies of orally administered probiotics in adults have shown mixed outcomes.<sup>41-43</sup> One publication reported that oral *L. acidophilus* DDS-1 may have been associated with prevention of recurrent UTI in a single patient,<sup>44</sup> but clearly this is not sufficiently rigorous. In another study, 585 premature babies (<33 weeks or <1.5kg) were given milk supplemented with 6x10<sup>9</sup> colony-forming units of *L. rhamnosus* GG or placebo milk once a day from the first feed until discharge (mean 48 days).<sup>45</sup> The rate of UTI episodes was reduced (3.4 versus 5.8%), but the difference was not statistically significant. This study did show that the probiotic could be administered safely to neonates, although any use of live bacteria in newborns should be monitored in case of rare septic complications.

A recently published randomized clinical trial compared trimethoprim/sulfamethoxazole against oral *L. acidophilus* ATCC 4356 as prophylaxis for UTIs in 120 children with VUR.<sup>46</sup> The rate of UTIs was 18.3% in the probiotics group and 21.6% in the antibiotics group, which was not a statistically significant difference. This would strongly suggest that a probiotic could be used as an alternative to antibiotics. Additional studies are necessary to confirm these findings. Furthermore, it would be worthwhile having a group take both antimicrobials and probiotics, as a study by Anukam et al. has shown synergy of combined therapy in curing bacterial vaginosis.<sup>47</sup>

**Lactobacillus GR-1 and RC-14—Mechanisms of Action**

Significant basic research studies on urogenital-derived lactobacilli have shown that *Lactobacillus* strains GR-1 and RC-14 are multifunctional and affect the host in complementary ways. The *L. rhamnosus* GR-1 strain was originally not believed to produce hydrogen peroxide, but a recent assay using tetramethylbenzidine (TMB) agar showed that it does in fact secrete this antimicrobial. In addition, GR-1 has anticandidal activity and produces substances, such as auto-inducer 2 (AI-2) molecules, that influence the growth and biofilm development of uropathogens.<sup>29,48</sup> The organism can suppress inflammatory responses, using interleukin-10 (IL-10)-dependent and -independent pathways, as shown in macrophage-based studies.<sup>49</sup> Microarray experiments have shown that a single intra-vaginal dose of this GR-1 strain can activate host defense pathways (Kirjavainen et al., in press), suggesting that its probiotic properties work through the host as well as directly on pathogens.

For the RC-14 strain, hydrogen peroxide production is more pronounced, and the organism can upregulate production of mucin, a molecule that may act as a barrier to infection (unpublished data). It also produces biosurfactants that include a compound that interferes with adhesion of a wide range of uropathogens,<sup>50</sup> and one that downregulates virulence factor expression in pathogens such as *Staphylococcus aureus*.<sup>51</sup> Strain RC-14 also affects cell membrane components in *E. coli*, likely through its production of lactic acid. Of interest, this strain does not produce reuterin, the potent antimicrobial that is supposed to be the key to *L. reuteri* probiotic effects in the gut. This lack of production indicates that other antipathogen attributes are involved in RC-14's efficacy. Further studies on *Lactobacillus* GR-1 and RC-14 are needed to identify the key mechanisms involved in protecting the host, and to determine whether these function in children.

In conclusion, there are scarce data on probiotic use in children to prevent UTIs. The authors are completing a pilot clinical trial of *Lactobacillus* RC-14 and GR-1 to reduce bacteriuria in girls with spina bifida. Preliminary results indicate that probiotics may reduce bacterial colony counts in the bladders of some patients. However, final results are pending and a randomized, placebo-controlled, double-blind trial is being planned. We are also planning to test this strain combination in anatomically correct girls prone to UTIs.

It is difficult to predict with certainty that current probiotics will help all children, given the complexity of the condition and the variety of factors that can influence recurrence. Also, the probiotics being tested were not specifically designed for children. Nevertheless, if current probiotics

reduce infection by 10% (either fewer episodes or fewer children suffering an infection), and if given with antibiotics they help cure infection with fewer side effects and lower onset of bacterial resistance, they will have provided a new and useful addition to the armamentarium of pediatric physicians. ■

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### Conflict of Interest

Dr Reid holds patents to *Lactobacillus* strains GR-1 and RC-14.

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