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**Draft Report - ER-2023-18**

# A Randomized, Double-Blind, Placebo-Controlled Study to Assess the Effects of Consumption of *Limosilactobacillus reuteri* 3613-1 Supplement on Recurrence of Urinary Tract Infections (UTI) in Healthy Women During a 6-Month Period

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# A randomized, double-blind, placebo-controlled study to assess the effects of consumption of *Limosilactobacillus reuteri* 3613-1 supplement on recurrence of urinary tract infections (UTI) in healthy women during a 6-month period – Draft Report

Amy Wescott — 08-03-2023

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## Background

Urinary tract infections are a common, often recurring health problem that primarily affects women, accounting for 10-20% of all infections treated in primary care facilities (Wawrysiuk et al., 2019). Urogenital tract infections can be acute or chronic, symptomatic, or asymptomatic. Various microbial pathogens can cause urogenital tract infections, including Gram-negative bacteria (e.g., *Escherichia coli*), Gram-positive bacteria (e.g., *Staphylococcus*), Gram-variable bacteria (e.g., *Gardnerella vaginalis*, and yeasts (e.g., *Candida albicans*).

The healthy human vagina is populated by a variety of lactic acid bacteria, which play an essential role in protecting women from urogenital infections. When a shift occurs in the normal vaginal microbiome and lactic acid bacteria populations decrease, an abnormal growth of pathogens can occur leading to a urinary tract infection (UTI). The most common treatment for a UTI are antibiotic or anti-fungal medications. However, the use of an antibiotic or anti-fungal can lead to a reduction of the normal microbiota in the urogenital tract resulting in recurrent infections as well as drug-resistant pathogens. Therefore, alternative methods for treating and preventing these infections are needed.

Recently, probiotics have emerged as a promising alternative for UTI prevention and for the promotion of urogenital health. Certain strains of probiotics have been used to promote urinary and vaginal health (Yefet et al., 2020; Yang et al., 2020; Ho et al., 2016; Stapleton et al., 2011). While women's health products do exist on the market, the majority of products on the shelves include the same bacterial strain, leaving the female consumer with few choices. In addition, the response to these probiotics is not always consistent. Two-hundred and fifty lactic acid bacteria from the Arm & Hammer Animal and Food Production library were screened *in vitro* for their ability to produce lactic acid and hydrogen peroxide, prevent the growth of urinary tract pathogens, and ability to clump together for survival and colonization in the vaginal tract. *Limosilactobacillus reuteri* 3613-1 was chosen for its multiple modes of action for prevention of pathogen growth, maintenance of vaginal pH, and potential for urogenital health benefits. A clinical trial was conducted to evaluate UTI occurrence, UTI symptom severity, maintenance of vaginal pH, time until first UTI, changes in the vaginal microbiome, and quality of life in participants orally administered *L. reuteri* 3613-1 to demonstrate efficacy of this novel probiotic.

## Methods

### PARTICIPANTS

One hundred and thirty healthy women (18-65 years old) with a confirmed medical history of recurrent uncomplicated UTIs were enrolled in Cork, Ireland by Atlantia Clinical Trials. Exclusion criteria included women who were nursing or pregnant, use of tobacco products, individuals not willing to adhere to the dietary supplement restrictions, and those who had taken oral or topical antibiotics within the previous 30 days prior to their baseline visit. Exclusions and demographic information were determined at visit 1 (screening).

## INTERVENTION PHASE

The trial consisted of 4 subsequent visits over a 26-week intervention period; visit 2 (baseline), visit 3 (week 4), visit 4 (week 12), and visit 5 (week 24). At visit 2, participants were randomized and provided a 4-week supply of *L. reuteri* 3613-1 (1 x$10^9$ CFU/day) or placebo (carrier) with daily dose instructions. All participants were provided with a list of foods and supplements (e.g. cranberry and other probiotics) to avoid. At each additional visit, subjects were provided an additional 4-week supply of *L. reuteri* 3613-1 or placebo.

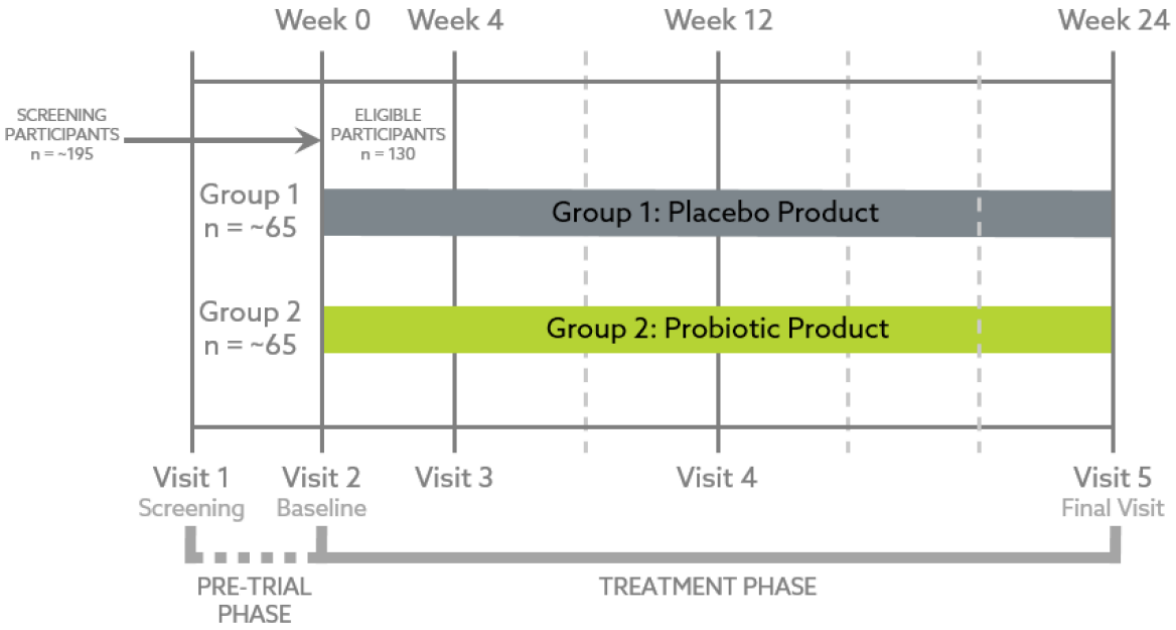


FIGURE 1. Schematic representation of the randomized, double-blind, placebo-controlled intervention study with probiotic strain *Limosilactobacillus reuteri* 3613-1.

Anthropometric measurements (height and weight), medication history, and vitals were measured for safety and tolerability at visits 1-5. Participants were instructed to collect information regarding UTI symptoms, adverse events, compliance, and medication use daily using the eDiary App on their personal devices.

A urine sample was collected at each visit for urinalysis and urine culture was performed. If a urine sample returned a positive result for the presence of bacteria but the participant was not reporting a symptomatic UTI, the participant was informed but not recommended to seek treatment. If the participant returned a symptomatic positive UTI, they were directed to seek treatment from their general practitioner.

Vaginal samples were collected by the trial doctor at visits 2 and 5 by inserting a vaginal swab 1-2 inches in the vagina, twisting the swab in several full circles for 20 seconds. Samples were logged and sent to SeqBiome for microbiome analysis and probiotic detection. Vaginal pH was assessed at visits 2, 3, 4, and 5 using a vaginal pH measurement test stick.

TABLE 1. Identification of biological measurements and assessments by visit.

| | Urine | | | Blood | Vaginal | | |
|---|---|---|---|---|---|---|---|
| | Pregnancy | UTI Culture | Urinalysis | Safety | pH | Probiotic Detection | Microbiome |
| Visit 1 - Screening | x | x | x | x | | | |
| Visit 2 – Week 0 | x | x | x | | x | x | x |
| Visit 3 – Week 4 | | x | x | | x | | |
| Visit 4 – Week 12 | | x | x | | x | | |
| Visit 5 – Week 24 | x | x | x | | x | x | x |
| UTI Visit | | x | x | | | | |
| Early Termination Visit | x | x | x | | x | x | x |

If the participant began experiencing symptoms of a UTI at any point during the trial, they were asked to provide a urine sample to the clinic. The urine sample was used for urinalysis and a urine culture test prior to starting treatment for a UTI.

A Statistical Analysis Plan (SAP) was written by a biostatistician from Atlantia Clinical Trials. The SAP was approved and signed by Church and Dwight prior to database lock.

## RESULTS

The trial was successfully completed by 90 subjects: 40 in the active *L. reuteri* 3613-1 group and 50 in the placebo group. The *L. reuteri* 3613-1 active group had a significant delay (p=0.036) in the onset of their first symptomatic UTI (66 days) compared to the placebo group (33 days). Similarly, participants supplemented with *L. reuteri* 3613-1 tended (p=0.095) toward an increase in the number of days until their first culture confirmed UTI (90.5 days) compared to the placebo group (30 days).

The *L. reuteri* 3613-1 active group had fewer incidences of culture-confirmed UTI (4/40; 10%) compared to the placebo group (7/50; 14%) with less severe symptoms, (10.75 vs 7.29). Higher average symptom severity scores indicate less severe symptoms.

Normal vaginal pH was maintained through the 24-week study in participants supplemented with *L. reuteri* 3613-1 and no adverse events or clinically relevant changes in safety parameters were reported, confirming the safety of *L. reuteri* 3613-1 for human consumption.

## CONCLUSION

In this trial, we demonstrate the beneficial health effects of *L. reuteri* 3613-1 upon oral ingestion. Addition of *L. reuteri* 3613-1 delayed the onset of both culture-confirmed and symptomatic UTIs, a clinically relevant finding for women with chronic recurring UTIs. A reduction in symptom severity at the time of a culture-confirmed UTI was also recorded in the *L. reuteri* 3613-1 active group. In addition, *L. reuteri* 3613-1 supported the maintenance of vaginal pH over a 24-week period, likely through the production of lactic acid, which helps to support the balance of the vaginal microbiome. The data demonstrates *Limosilactobacillus reuteri* 3613-1 is a safe and effective women's health probiotic.

## REFERENCES

Ho, M., Chang, Y.-Y., Chang, W.C., Lin, H.C., Wang, M.H., Lin, W.C, and Chiu, T.H. (2016). Oral *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 to reduce Group B *Streptococcus* colonization in pregnant women: A randomized controlled trial. Taiwanese Journal of Obstetrics and Gynecology 55, 515-518.

Stapleton, A., Au-Yeung, M., Hooton, T.M., Fredricks, D.N., Roberts, P.L., Czzaja, C.A., Yarova-Yarovaya, Y., Fiedler, T., Cox, M., and Stamm, W.E. (2011). Randomized, placebo-controlled phase 2 trial of a *Lactobacillus crispatus* probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis 52, 1212-1217.

Wawrysiuk, S., Naber, K., Rechberger, T., and Miotla, P. (2019). Prevention and treatment of uncomplicated lower urinary tract infections in the era of increasing antimicrobial resistance- non-antibiotic approaches: a systemic review. Arch Gynecol Obstet 300, 821-828.

Yang, S., Reid, G., Challis, J.R.G., Glood, G.B., Asztalos, E., Money, D., Seney, S., and Bock, A.D. (2020). Effect of oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 on the vaginal microbiota, cytokines, and chemokines on pregnant women. Nutrients 12.

Yefet, E., Colodner, R., Strauss, M., Gam Ze Letova, Y., and Nachum, Z. (2020). A randomized controlled open label crossover trial to study vaginal colonization of orally administered *Lactobacillus reuteri* RC-14 and *Lactobacillus rhamnosus* GR-1 in pregnant women at high risk for preterm labor. Nutrients 12.