Research Article

Protective Effect of Vaginal Lactobacillus paracasei CRL 1289 against Urogenital Infection Produced by Staphylococcus aureus in a Mouse Animal Model

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Urogenital infections of bacterial origin have a high incidence among the world female population at reproductive age. Lactobacilli, the predominant microorganisms of the healthy vaginal microbiota, have shown a protective effect against the colonization and overgrowth of urogenital pathogens that increased the interest for including them into probiotics products assigned to restore the urogenital balance. In the present work, we determined in a mouse animal model the capability of Lactobacillus paracasei CRL 1289, a human vaginal strain with probiotic properties, to prevent the vaginal colonization of a uropathogenic strain of Staphylococcus aureus. Six-week-old female BALB/c mice, synchronized in their estral cycle, were intravaginally inoculated with two doses of $10^9$ lactobacilli before challenging them with a single dose of $10^5$ or $10^7$ CFU of S. aureus. The vaginal colonization of both microorganisms and the effect on the vaginal structure were determined at 2, 5, and 7 days after pathogen inoculation. Control mice and those challenged only with the pathogen showed an insignificant lactobacilli population, whereas $10^5$ lactobacilli/mL of vaginal homogenate were recovered at 2 days after challenge from the L. paracasei and the probiotic + pathogen groups, decreasing this number on the following days. The treatment with L. paracasei CRL 1289 decreased significantly the number of staphylococci recovered at 2 and 5 days when mice were challenged only with $10^5$ CFU of pathogen. The inoculation of S. aureus produced a remarkable inflammatory response and structural alterations in the vaginal mucosa that decreases in a significant manner when the mice were protected with L. paracasei CRL 1289. The results obtained suggest that this particular Lactobacillus strain could prevent the onset of urogenital infections by interfering with the epithelial colonization by uropathogenic S. aureus.

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1. INTRODUCTION

Urogenital tract infections of bacterial origin have a high incidence among the world female population at reproductive age. A great proportion of these diseases, such as vaginosis and urinary tract infections are often caused by pathogens that emerge from the intestinal microbiota and ascend along perineum to the vagina and then to the urethra and bladder [1]. While antibiotics have been extensively used as a quite effective therapy for the treatment of these bacterial infections, the increasing drug resistance of urogenital pathogens makes imperative the development of alternative therapeutics.

In healthy women, the vaginal microflora is dominated by Lactobacillus species, at a level of $10^7-10^8$ CFU g$^{-1}$ of fluid, which exert a significant influence on the microbiology of the ecosystem [2]. It has been observed that indigenous lactobacilli prevent the overgrowth and invasion of pathogenic bacteria [3] by a combination of competitive exclusion, competition for nutrients, and release of antimicrobial substances such as hydrogen peroxide, organic acids, bacteriocins, and biosurfactants [3–6]. In consequence, a depletion of vaginal lactobacilli has been directly associated with an increase in the incidence of genital and urinary infections [7–9]. For this reason, there is a growing interest in the use of human lactobacilli as probiotics that restore and maintain a normal vaginal flora and prevent disease recurrence by forming a pellicle on the vaginal epithelium as a biological barrier against colonization of pathogenic bacteria. In this sense, previous studies have reported that adhesive lactobacilli can inhibit in vitro the attachment of pathogens such as Escherichia coli, Gardnerella vaginalis, Candida albicans, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Streptococcus agalactiae to urogenital epithelial cells [5, 10–13].
Having in mind the objective of developing a probiotic formulation for the prevention and therapy of urogenital tract infections, our research group has previously isolated and identified vaginal lactobacilli from healthy women of Tucumán city in Argentina [14]. The strains were extensively characterized for their probiotic and technological features and some promising properties such as adhesion, auto and coaggregation abilities, hydrogen peroxide, bacteriocin-like substances, and organic acids production were reported [15–18]. Relevant technological properties, for instance, the optimal conditions for the production of antimicrobial substances and the viability and biological properties after processing, were also determined for selected strains [19–24].

*Lactobacillus paracasei* CRL 1289 is a selected human vaginal strain selected by its probiotic potential, since it is able to inhibit the growth of uropathogenic *Staphylococcus aureus* in vitro by release of H₂O₂ [20], and its adhesion to *aureus* able to inhibit the growth of uropathogenic *S. aureus*. The strains were extensively characterized for their probiotic and technological features and some promising properties such as adhesion, auto and coaggregation abilities, hydrogen peroxide, bacteriocin-like substances, and organic acids production were reported [15–18]. Relevant technological properties, for instance, the optimal conditions for the production of antimicrobial substances and the viability and biological properties after processing, were also determined for selected strains [19–24].

*Lactobacillus paracasei* CRL 1289 is a selected human vaginal strain selected by its probiotic potential, since it is able to inhibit the growth of uropathogenic *Staphylococcus aureus* in vitro by release of H₂O₂ [20], and its adhesion to vaginal epithelial cells by exclusion and competence for specific receptors [24]. *Staphylococcus aureus* is a major opportunistic pathogen that can cause a variety of local and systemic infections ranging from skin abscesses, bone and soft tissue surgical infections, sepsis, invasive endocarditis, and toxic shock syndrome (TSS) [25]. TSS is a geographically widespread disease affecting mainly young healthy menstruating women, especially those using tampons [26].

The aim of the present work was to determine, in a mouse animal model, the capability of *Lactobacillus paracasei* CRL 1289 to prevent the vaginal colonization of uropathogenic *Staphylococcus aureus*.

### 2. MATERIALS AND METHODS

#### 2.1. Microorganisms and growth conditions

*Lactobacillus paracasei* CRL 1289 was originally isolated from vaginal smears of healthy women [14] and was previously characterized by their probiotic and technological properties [15, 20, 22–24]. The human uropathogenic strain of *Staphylococcus aureus* used in this study was kindly provided by the Institute of Microbiology “Luis Verna” of the University of Tucumán, Argentina, and was isolated from pathological urine. Before experimental use, each strain stored in milk-yeast extract at −20°C was propagated in LAPtG broth (1.5% peptone, 1% tryptone, 1% glucose, 1% yeast extract, and 0.1% Tween 80, pH 6.8) [27] at 37°C and subcultured at least twice in this media every 12 hours. Lactobacilli were cultivated under static conditions in order to avoid the detrimental effects of oxygen whereas staphylococci were incubated with shaking at 100 rpm.

#### 2.2. Animals

Six-week-old female BALB/c mice from the inbreed colony of CERELA (Centro de Referencia para Lacobacilos), each weighing from 25 to 30 g, were used throughout the investigation. Animals were housed in plastic cages and fed ad libitum with a conventional balanced diet, keeping their environmental conditions constant. All the animals were synchronized in their estrous cycle with an intramuscular single dose of 0.5 mg of estradiol valerate (Progynon Depot. Schering Laboratories) and randomly assigned to the following experimental groups: (1) lactobacilli treated group, (2) lactobacilli + pathogen treated group, and (3) pathogen treated group. Five animals were used as control synchronized non-treated group. The CERELA Committee of Ethics approved the protocol used for animal studies.

#### 2.3. Microorganisms inoculation procedure

A spontaneous rifampicin resistant strain obtained by plating *L. paracasei* CRL 1289 on Rogosa agar (Merck) supplemented with 150 μg/mL of rifampicin was used to inoculate mice. The resistant strain showed exactly the same properties of the original strain. Overnight cultures of lactobacilli and staphylococci grown on LaptG broth (12 hours, 37°C) were centrifuged (10,000 g, 10 minutes, 4°C), washed twice with sterile saline solution, and incorporated into glyco-gelatin ovules at a concentration of 10⁶ CFU of lactobacilli and 10⁵ and 10⁷ CFU of *S. aureus* per each ovule. The base preparation of the ovules was obtained by mixing 21% gelatin and 58% glycerol in distilled water. This matrix was sterilized at 121°C for 15 minutes, and supplemented with 0.5% ascorbic acid and the suspensions of microorganisms in a proportion 1 : 5. Forty-eight hours after estradiol synchronization, animals of groups 1 and 2 were intravaginally inoculated with two doses of 10⁶ lactobacilli (with a 24-hour interval between each other). On the third day, the animals of group 2 and those belonging to group 3 were challenged with a single dose of 10⁵ or 10⁷ CFU of uropathogenic *S. aureus*. Figure 1 shows the inoculation protocol used.

#### 2.4. Bacterial counts in vaginal homogenates

At 2, 5, and 7 days after pathogen inoculation, the animals were sacrificed by cervical dislocation. The vagina of each animal was removed aseptically, placed in 0.5% peptone-water, and homogenized with a Teflon pestle. Serial ten-fold dilutions from this homogenate were plated on Rogosa agar (LBS, Merck); Rogosa agar supplemented with 150 μg/mL of rifampicin and Manitol Salt Agar (MSA, Britannia) for counts of lactic acid bacteria, *L. paracasei* CRL 1289, and *S. aureus*, respectively. The LBS plates were incubated 72 hours under microaerophilic conditions whereas MSA plates were aerobically incubated.

| DAY | −3 | −2 | −1 | 0 | 2 | 5 | 7 |
|-----|----|----|----|---|---|---|---|
| H   | +  | −  | −  | − | − | − | − |
| L   | —  | 10⁶ CFU | 10⁶ CFU | Gg | — | — | — |
| P   | —  | Gg | Gg | 10⁵/10⁷ CFU | — | — | — |
| L + P | —  | 10⁶ CFU | 10⁶ CFU | 10⁵/10⁷ CFU | — | — | — |
| S   | —  | —  | —  | — | — | — | — |

**Figure 1:** Scheme of inoculation of *L. paracasei* CRL 1289 and *Staphylococcus aureus* on Balb/c mice. H: hormone (estradiol valerate), L: lactobacilli inoculation, P: pathogen inoculation, L + P: lactobacilli plus pathogen inoculation, S: sacrifice of animals, and Gg: glyco-gelatin ovules without microorganisms.
2.5. **Cytological and histological studies**

The cytological and histological evaluations were carried out by light microscopy. For cytological studies, 50 µL of vaginal exudates were collected with a micropipette tip, spread onto glass slides, and stained with Giemsa. For the study of histological structures, the vaginas were aseptically removed, fixed with 10% paraformaldehyde for 24 hours at room temperature, and then embedded in paraffin according to standard histological methods [28]. Serial paraffin sections of 4 µm were stained with hematoxylin-eosin and observed at 40X.

2.6. **Statistical analysis**

The results are expressed as the mean value ± standard deviation of the data obtained from three animals at each sample time of two independent experiments. Significant differences between means were determined by Tukey’s test after analysis of variance (ANOVA) with Minitab Statistic Program, release 12 for Windows. A *P* value of <.05 was considered statistically significant.

### 3. RESULTS

Figure 2(a) shows the viable counts of lactobacilli and Figure 2(b) those of staphylococci on the vaginal homogenates of Balb/c mice at different sampling times after inoculation of probiotic *L. paracasei* CRL 1289 and uropathogenic *S. aureus*. Synchronized nontreated mice and those challenged only with the pathogen (Group 3) showed an insignificant lactobacilli population. On the other side, 10^5 lactobacilli/mL of vaginal homogenate were recovered from the group inoculated only with probiotic *L. paracasei* CRL 1289 (Group 1) and the probiotic + pathogen group (Group 2) at 2 days after challenge. However, this number decreased progressively on the following days (see Figure 2(a)).

Referred to the number of pathogens recovered from mice, control group and the lactobacilli treated group (Group 1) were almost depleted of staphylococci population (Figure 2(b)). The inoculation of 10^7 CFU of *S. aureus* produced a high and constant colonization of the pathogen that was not prevented by the pretreatment with lactobacilli (results not shown). However, the treatment with *L. paracasei* CRL 1289 previous to the pathogen infection decreased significantly the number of staphylococci recovered at 2 and 5 days when mice were challenged with 10^5 CFU of the pathogen (Figure 2(b)).

Figure 3 shows the vaginal smears stained with Giemsa of mice inoculated with lactobacilli and staphylococci. No cytological modifications were observed after the administration of *L. paracasei* CRL 1289. By the contrary, the inoculation of *S. aureus* produced a remarkable inflammatory response (Group 3) with a high infiltration of polymorphonuclear cells in the vaginal secretions. This effect decreased in a significant manner when mice were previously protected with *L. paracasei* CRL 1289.

No histological alterations were produced by the lactobacilli inoculation (Figure 4), whereas significant structural modifications of the vaginal mucosa, with disappearance of the keratin layer and neutrophiles infiltration in the epithelium, were observed in the group inoculated solely with *S. aureus* (Figure 4). An intermediate effect was observed in mice protected with *L. paracasei* CRL 1289.

### 4. DISCUSSION

The potential use of human lactobacilli as probiotics assigned to restore and maintain a healthy urogenital tract represents a
promising alternative to conventional chemotherapy [6, 29]. At present, a lot of scientific evidence supports, by in vitro and in vivo studies, the effectiveness of probiotics to prevent the attachment or stimulate the removal of enteropathogens from intestinal cells [30, 31]. Probiotics have been successfully used to prevent and treat gastrointestinal diseases caused by antibiotics treatments, rotavirus, enterobacteria and clostridia infections [32]. However, there are much lesser antecedents on the preventive and therapeutic effects of probiotics against diseases of the urogenital tract. Some in vitro studies have reported the inhibition of pathogens growth and adherence to uroepithelial cells by lactobacilli [10, 12, 13, 21, 24, 33]. This “anti-infective” mechanism involves the release of antimicrobials and the blockage of uropathogens.
adherence by both steric hindrance and competition for receptors [10, 34]. With respect to in vivo studies, it has been reported that vaginal lactobacilli prevented urinary tract colonization of mice by *E. coli* but were not able to exert significant therapeutic effects [35]. In humans, clinical efficacy for urogenital health maintenance and disease prevention has been demonstrated only for *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 [1]. Both probiotic lactobacilli strains colonized the urogenital tract by vaginal instillation and oral consumption, and were able to reduce the risk of UTI and cure bacterial vaginosis [36, 37].

In the healthy urogenital tract of adult females, it is supposed that the indigenous lactobacilli exclude the colonization of pathogenic bacteria by antagonistic compounds and/or by occupying/masking their potential binding sites in the mucosa [10, 38]. However, in a depleted lactobacilli environment such as an infected urogenital tract, it should be expected that exogenous probiotic lactobacilli have the capacity to compete for the same receptors and displace previously attached pathogens [11]. In a previous study, we observed that selected vaginal lactobacilli interfered to different extents with the growth and adherence to vaginal epithelial cells of some genitourinary pathogens [21, 24]. Among these strains, *Lactobacillus paracasei* CRL 1289 was able to decrease, in a significant level, the adhesion of *S. aureus* by exclusion and competition mechanisms [24], as well as to inhibit its growth by H$_2$O$_2$ production [15, 20]. Based on these findings, we evaluated in the present work the ability of these lactobacilli to prevent the vaginal colonization of *S. aureus* in an animal model, and the protection achieved.

The results obtained showed that lactobacilli were not a dominant population of the vaginal microbiota of the Balb/c mice used in this study and that human *Lactobacillus paracasei* CRL 1289 was able to colonize transiently the murine vaginal tract, since $10^5$ lactobacilli/mL of vaginal homogenate were recovered after 2 days of inoculation but decreased progressively on the following days. However, the human *S. aureus* uropathogenic strain was able to produce a very strong infection when inoculated at $10^5$ or $10^7$ CFU levels, producing significant morphological alterations of the mucosal structure, mainly the infiltration of polymorphonuclear cells that appeared as capsulated groups of cells in the vaginal epithelium and lamina propria; and the complete disappearance of the keratin layer. *Lactobacillus paracasei* CRL 1289 was not able to protect mice challenged with $10^7$ CFU of *S. aureus* but effectively decreased the number of staphylococci in the vagina and the damage caused, when the infecting dose of the pathogen was $10^6$ CFU.

In conclusion, the preliminary results obtained in this work suggest that *L. paracasei* CRL 1289 could prevent the onset of urogenital infections caused by uropathogenic *S. aureus* interfering with the epithelial colonization (possibly through barrier/interference mechanisms) and encourage further in vivo studies, such as clinical trials designed to test their capacity to prevent and manage urogenital tract infections in females.

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### REFERENCES

[1] G. Reid and A. W. Bruce, "Probiotics to prevent urinary tract infections: the rationale and evidence," *World Journal of Urology*, vol. 24, no. 1, pp. 28–32, 2006.
[2] S. I. Pavlova, A. O. Kilic, S. S. Kilic, et al., "Genetic diversity of vaginal lactobacilli from women in different countries based on 16S rRNA gene sequences," *Journal of Applied Microbiology*, vol. 92, no. 3, pp. 451–459, 2002.
[3] J.-P. Leparpagne and V. Rousseau, "Protective role of the *Doderlein flora*," *Journal de Gynécologie Obstétrique et Biologie de la Reproduction*, vol. 31, no. 5, pp. 485–494, 2002.
[4] M. M. C. Velraeds, B. Van De Belt-Gritter, H. C. Van Der Mei, G. Reid, and H. J. Busscher, "Infection in initial adhesion of uropathogenic bacteria and yeasts to silicone rubber by a *Lactobacillus acidophilus* biosurfactant," *Journal of Medical Microbiology*, vol. 47, no. 12, pp. 1081–1085, 1998.
[5] S. Boris and C. Barbès, "Role played by lactobacilli in controlling the population of vaginal pathogens," *Microbes and Infection*, vol. 2, no. 5, pp. 543–546, 2000.
[6] G. Reid and J. Burton, "Use of *Lactobacillus* to prevent infection by pathogenic bacteria," *Microbes and Infection*, vol. 4, no. 3, pp. 319–324, 2002.
[7] S. E. Hawes, S. L. Hillier, J. Benedetti, et al., "Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections," *Journal of Infectious Diseases*, vol. 174, no. 5, pp. 1058–1063, 1996.
[8] K. Gupta, A. E. Stapleton, T. M. Hooton, P. L. Roberts, C. L. Fennell, and W. E. Stamm, "Inverse association of H$_2$O$_2$-producing lactobacilli and vaginal *Escherichia coli* colonization in women with recurrent urinary tract infections," *Journal of Infectious Diseases*, vol. 178, no. 2, pp. 446–450, 1998.
[9] H. L. Martin Jr., B. A. Richardson, P. M. Nyange, et al., "Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition," *Journal of Infectious Diseases*, vol. 180, no. 6, pp. 1863–1868, 1999.
[10] R. C. Y. Chan, G. Reid, R. T. Irvin, A. W. Bruce, and J. W. Costerton, "Competitive exclusion of uropathogens from human uroepithelial cells by *Lactobacillus* whole cells and cell wall fragments," *Infection and Immunity*, vol. 67, no. 1, pp. 84–89, 1985.
[11] G. Reid, "In vitro testing of *Lactobacillus acidophilus* NCFMTM as a possible probiotic for the urogenital tract," *International Dairy Journal*, vol. 10, no. 5–6, pp. 415–419, 2000.
[12] J. Osset, R. M. Bartolomé, E. García, and A. Andreu, "Assessment of the capacity of *Lactobacillus* to inhibit the growth of uropathogens and block their adhesion to vaginal epithelial cells," *Journal of Infectious Diseases*, vol. 183, no. 3, pp. 485–491, 2001.
[13] Y. Lee, "Characterization of *Weissella kimchii* PL9023 as a potential probiotic for women," *FEMS Microbiology Letters*, vol. 250, no. 1, pp. 157–162, 2005.
[14] V. S. Ocaña, E. Bru, A. A. P. de Ruiz Holgado, and M. E. Nader-Macias, "Surface characteristics of lactobacilli isolated from human vagina," Journal of General and Applied Microbiology, vol. 45, no. 5, pp. 203–212, 1999.

[15] V. S. Ocaña, A. A. P. de Ruiz Holgado, and M. E. Nader-Macias, "Selection of vaginal H2O2-generating Lactobacillus species for probiotic use," Current Microbiology, vol. 38, no. 5, pp. 279–284, 1999.

[16] V. S. Ocaña, A. A. P. de Ruiz Holgado, and M. E. Nader-Macias, "Characterization of a bacteriocin-like substance produced by a vaginal Lactobacillus salivarius strain," Applied and Environmental Microbiology, vol. 65, no. 12, pp. 5631–5635, 1999.

[17] V. S. Ocaña and M. E. Nader-Macias, "Adhesion of Lactobacillus vaginal strains with probiotic properties to vaginal epithelial cells," Biocell, vol. 25, no. 3, pp. 265–273, 2001.

[18] V. S. Ocaña and M. E. Nader-Macias, "Vaginal lactobacilli: self-and co-aggregating ability," British Journal of Biomedical Science, vol. 59, no. 4, pp. 183–190, 2002.

[19] M. S. Juárez Tomás, E. Bru, B. Wiese, A. A. P. de Ruiz Holgado, and M. E. Nader-Macias, "Influence of pH, temperature and culture media on the growth and bacteriocin production by vaginal Lactobacillus salivarius CRL 1328," Journal of Applied Microbiology, vol. 93, no. 4, pp. 714–724, 2002.

[20] M. S. Juárez Tomás, E. Bru, and M. E. Nader-Macias, "Comparison of the growth and hydrogen peroxide production by vaginal probiotic lactobacilli under different culture conditions," American Journal of Obstetrics and Gynecology, vol. 188, no. 1, pp. 35–44, 2003.

[21] M. S. Juárez Tomás, V. S. Ocaña, B. Wiese, and M. E. Nader-Macias, "Growth and lactic acid production by vaginal Lactobacillus acidophilus CRL 1259, and inhibition of uropathogenic Escherichia coli," Journal of Medical Microbiology, vol. 52, no. 12, pp. 1117–1124, 2003.

[22] M. S. Juárez Tomás, V. S. Ocaña, and M. E. Nader-Macias, "Viability of vaginal probiotic lactobacilli during refrigerated and frozen storage," Anaerobe, vol. 10, no. 1, pp. 1–5, 2004.

[23] G. Zárate, M. S. Juárez Tomás, and M. E. Nader-Macias, "Effect of some pharmaceutical excipients on the survival of probiotic vaginal lactobacilli," Canadian Journal of Microbiology, vol. 51, no. 6, pp. 483–489, 2005.

[24] G. Zárate and M. E. Nader-Macias, "Influence of probiotic vaginal lactobacilli on in vitro adhesion of urogenital pathogens to vaginal epithelial cells," Letters in Applied Microbiology, vol. 43, no. 2, pp. 174–180, 2006.

[25] F. D. Lowy, "Staphylococcus aureus infections," New England Journal of Medicine, vol. 339, no. 8, pp. 520–532, 1998.

[26] J. M. Musser, P. M. Schlievert, A. W. Chow, et al., "A single clone of Staphylococcus aureus causes the majority of cases of toxic shock syndrome," Proceedings of the National Academy of Sciences of the United States of America, vol. 87, no. 1, pp. 225–229, 1990.

[27] P. Raibaud, J. V. Galpin, R. Ducluzeau, F. Mocquot, and G. Olivier, "Le genre Lactobacillus dans le tube digestif du rat. II. Caractères des souches hétérofermentaires isolées de rats "hola-" et "gnotoxéniques"," Annales de Microbiologie, vol. 124, no. 2, pp. 223–235, 1973.

[28] G. Sainte-Marie, "A paraffin embedding technique for studies employing immunofluorescence," Journal of Histochemistry & Cytocchemistry, vol. 10, no. 3, pp. 250–256, 1962.

[29] G. Reid and A. W. Bruce, "Selection of Lactobacillus strains for urogenital probiotic applications," Journal of Infectious Diseases, vol. 183, supplement 1, pp. 577–580, 2001.

[30] M.-H. Coconnier, M.-F. Bernet, S. Kerneis, G. Chauviere, J. Fourniat, and A. L. Servin, "Inhibition of adhesion of enteroinvasive pathogens to human intestinal Caco-2 cells by Lactobacillus acidophilus strain LB decreases bacterial invasion," FEMS Microbiology Letters, vol. 110, no. 3, pp. 299–305, 1993.

[31] Y.-K. Lee, K.-Y. Puong, A. C. Ouwehand, and S. Salminen, "Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli," Journal of Medical Microbiology, vol. 52, no. 10, pp. 925–930, 2003.

[32] D. Goossens, D. Jonkers, E. Stobberingh, A. Van Den Bogaard, M. Russel, and R. Stockbrügger, "Probiotics in gastroenterology: indications and future perspectives," Scandinavian Journal of Gastroenterology, Supplement, vol. 38, no. 239, pp. 15–23, 2003.

[33] S. Boris, J. E. Suárez, F. Vázquez, and C. Barbés, "Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens," Infection and Immunity, vol. 66, no. 5, pp. 1985–1989, 1998.

[34] G. Reid, R. L. Cook, and A. W. Bruce, "Examination of strains of lactobacilli for properties that may influence bacterial interference in the urinary tract," Journal of Urology, vol. 138, no. 2, pp. 330–335, 1987.

[35] M. E. Nader-Macias, C. S. De Ruiz, M. E. L. DeBocanera, and A. A. P. de Ruiz Holgado, "Preventive and therapeutic effects of Lactobacilli on urinary tract infections in mice," Anaerobe, vol. 2, no. 2, pp. 85–93, 1996.

[36] G. Reid, A. W. Bruce, and M. Taylor, "Instillation of Lactobacillus and stimulation of indigenous organisms to prevent recurrence of urinary tract infections," Microecology and Therapy, vol. 23, pp. 32–45, 1995.

[37] K. C. Anukam, E. Osazuwa, G. I. Osemen, F. Ehigbeghe, A. W. Bruce, and G. Reid, "Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis," Microbes and Infection, vol. 23, no. 12, pp. 2772–2776, 2006.

[38] R. J. Spencer and A. Chesson, "The effect of Lactobacillus spp. on the attachment of enterotoxigenic Escherichia coli to isolated porcine enterocytes," Journal of Applied Bacteriology, vol. 77, no. 2, pp. 215–220, 1994.

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