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# Lactobacilli for prevention of urogenital infections: a review

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**Abstract. – Urogenital infections are a worldwide shared problem that represent the most common reason for a woman to decide to visit to gynaecologist or urologist.**

**The origin of the uropathogens in uncomplicated urinary tract infection and bacterial vaginosis is the fecal flora.**

**Key element of pathogenesis namely the ability of the pathogens to survive exposure to the microflora that exists on the external urogenitalia, in which lactobacilli predominate.**

**Some health food appear to contain ≥ 1 common *Lactobacillus* strain; *L. rhamnosus* GR-1 was found to be the best of a group of 34 *Lactobacillus* strains isolated from dairy, poultry, health food.**

**Recently has been reported the first clinical evidence that probiotic lactobacilli can be delivered to the vagina following oral intake.**

**These *L.* strains possess the ability to adhere to and colonize tissues and the capacity to inhibit the pathogenesis of disease-causing organisms that make them effective probiotic agents.**

**In particularly, two strains, *Lactobacillus GG* and *Lactobacillus rhamnosus* GR-1 appear to be effective at colonizing and protecting the intestine and urogenital tract, respectively, against microbial infection.**

**Treating and preventing urogenital infection by instillating probiotic organisms has great appeal to patients and caregivers. The ability to administer orally *L. rhamnosus* GR-1 and *L. fermentum* RC-14, which colonize the intestine and vagina, provides a major step in the right direction for patients as it potentially allows for the self administration of therapy.**

*Key Words:*

Urinary tract infection, Bacterial vaginosis, Lactobacilli.

## Introduction

Urogenital infections is a worldwide shared problem that affects the bladder, kidneys, vagina, urethra, periurethra, and cervix. These infections make-up the most common reasons for a woman to decide to visit to gynaecologist or urologist[1]. The main clinical outcome amongst a large percentage of the female population is morbidity and discomfort and enormous costs for health care treatment. Many patients will experience a recurrence of symptoms, especially within the first year of the original infection. Serious complications can arise during pregnancy, sometimes leading to premature birth[2].

### Urinary Tract Infection

Historical data indicates that the vast majority (70%) of urinary tract infection (UTI) in a suburban, nonhospitalized community is caused by *Escherichia coli*, followed by other *enterobacteriaciae* and *Staphylococcus saprophyticus*. Furthermore, the latest 7 years study indicated a different result that demonstrated *E. coli* infections were less common and *Enterococcus faecalis* was the second leading uropathogen[3]. The latter result was also found in hospitalized patients[4]. Most uncomplicated UTI cases are resolved between 1 and 7 days of antibiotic therapy. Nevertheless, drug resistance to commonly used antibiotics (e.g., trimethoprim/sulfamethoxazole) is increasing among uropathogens[3] and patients are trying continuous alternative natural remedies such as cranberry juice, which appears to contain antiadhesive compounds that are active against uropathogens and can help prevent UTI[5-8]. Preventive therapies for UTI currently almost completely depend on the use of antibiotics. In real terms, no true prophylaxis exists: current therapy involves long-term, low-dose antibiotic treatment, which involves the active killing of bacteria that enter the bladder.

### Bacterial Vaginosis

The major cause of urogenital disease in females is *bacterial vaginosis (BV)*, formerly known as nonspecific vaginitis[9]. It has been defined as a mild infection of the lower female genital tract, characterized by the presence of 3 of the 4 of the following criteria defined as urogenital disease:

1. Release of an amine (putrescine, cadaverine, and trimethylamine) or fishy odor after the addition of 10% potassium hydroxide,
2. A vaginal pH > 4.5,
3. Clue cells in the vaginal fluid, and
4. A milky homogeneous vaginal discharge[10,11].

The clue-cell scoring of the cell population is called either normal (0-3; dominated by lactobacilli rods), intermediate (4-6; colonization by small gram-negative or gram-variable rods, e.g., *Bacteroides* or *Gardnerella*, and curved gram-variable rods, e.g., *Mobiluncus*), or BV (7-10; dominated by pathogens)[12]. Diagnosing BV with the use of DNA probes has its defects[11,13] and future improvements which are fast and accurate detection of amines, and short-chain volatile acids and enzymes that should make diagnosis easier and more reliable for BV and other urogenital pathogens, e.g., *Chlamydia trachomatis, Neisseria gonorrhoeae,* herpes simplex virus, HIV, papillomavirus, *Treponema pallidum,* and *Trichomonas vaginalis*[14-18]. BV may lead to complications in pregnancies, causing premature rupture of the membranes, premature birth, or the death of the fetus or newborn[19-24]. Premature rupture of the membranes can also be associated with BV, urinary tract infections, group B streptococcal infections, and the presence of organisms such as ureaplasma and mycoplasma in the urogenital tract[25-32].

### Pathogenesis of Urogenital Infections

The origin of the uropathogens in uncomplicated UTI and BV is the fecal flora. For > 20 y, the key factor in pathogenesis has been regarded as the ability of the pathogens to attach to epithelial cells, thereby allowing them a niche in which to establish, multiply, spread, and avoid host defenses[33,34]. Many studies have examined and documented the adhesins and receptor sites involving the attachment process. Factors such as hemolysins, aerobactin, capsular antigens, and others play a role in *E. coli* pathogenesis in UTI[35-42]. Furthermore, there are host susceptibility factors, including genetic determinants, age-related changes, and mucosal differences that influence the infection process[43]. Although the adhesion phenomenon is unquestionably important, there appears to be another key element of pathogenesis that is less understood, namely the ability of the pathogens to survive exposure to the microflora that exists on the external urogenitalia. It has been reported that > 50 species colonize the healthy vagina[44]. Studies have shown that urogenital cells are covered by dense bacterial biofilms[45,46] whose composition constantly changes in which lactobacilli predominate, at least until menopause. Uropathogenic organisms emerge from the intestine[47] and come into contact with these biofilms on vaginal and urethral cells, yet little is known about what happens thereafter. It is presumed that uropathogens can either bypass the microflora or successfully enter the biofilms, survive, and continue their ascension into the bladder. Biofilms have been described in the human host for > 20 y[48,49] but their role in health has not been studied as well as their association with disease. Current understanding indicates that the structure and dynamics of biofilms vary with the organisms, the surface to which they attach, the nutritional environment, and shear forces present in any given case. The organisms appear to benefit from biofilm formation by gaining access to nutrients, escaping host immune cells and antimicrobial attack, and having an ability to better control their multiplication. A recurrent theme is that various factors, including nutrients, antimicrobials, and arriving bacteria, change the properties and the composition of the biofilms.

In women, other factors such as hormonal concentrations, particularly estrogen, as well as changes induced by oral contraception, glycogen content, vaginal pH, steroid therapy, immunosuppression, and diseases (e.g., diabetes mellitus) all influence the composition of the bacterial biofilms[50]. The menstrual cycle appears to affect the adherence of lactobacilli to epithelial cells in healthy women in that days corresponding to high circulating

concentrations of estrogens result in a higher adherence in vitro[51] and restored colonization postmenopause[52]. Studies have shown that antibiotic or spermicide exposure can cause disruption of the urogenital microflora and increase a woman's risk of infection[53,54]. Indeed, in vitro studies showed that most lactobacilli are eradicated by exposure to a low dose of nonoxyno[1-9], whereas uropathogens grow and prosper in high concentrations of this compound[55]. Thus, protection of the host by her vaginal *Lactobacillus* flora is likely reduced upon exposure to spermicides. The fact that antimicrobials do not always completely wipe out the urogenital flora is due not only to resistance of individual strains, but also likely to the existence of biofilms, which themselves confer resistance to attack[56]. Scanning confocal laser microscopy has shown that biofilms are mushroom shaped and form water channels through which the organisms are nourished[57-59]. With the use of such techniques, it should be possible to follow the entry of a uropathogen into the urogenital biofilm system, from whence it infects the host. The dominant presence of lactobacilli in the urogenital microflora of healthy women and the obliteration of lactobacilli in patients who develop UTI[60-62], BV, and many other genital infections[30,63] [except candidiasis[64]] has led to a focus on these bacteria.

### The Role of Lactobacilli

*Lactobacilli* are gram-positive rods, primarily facultative or strict anaerobes that generally have a fastidious growth requirement. They prefer an acidic environment and help create one by producing lactic and other acids. In general, lactobacilli have not been associated with disease and for > 100 years have been regarded as nonpathogenic members of the intestinal and urogenital floras[65]. Lactobacilli have long been of interest to the dairy and agriculture industries[66,67], although over the past century, studies in relation to human health were sporadic and often inconclusive. Some examples can be found in which lactic acid bacteria have been used to treat or prevent infections of the intestinal and genital tracts with different degrees of success[68-71]. However, there has been failure in identifying the properties of lactobacilli required to prevent and treat disease; in determining the optimal dosage, duration, and mode of lactobacilli delivery; and including a placebo in the study. Studies have failed to recover and document lactobacilli properly and show their mechanisms of action. A further problem with probiotic therapeutics is their reliability. In one study, this was highlighted by the fact that the contents of some health food products were inconsistent and different from those stated on the labels[72]. More recently, has been discovered that some health food appear to contain 1 common *Lactobacillus* strain, apparently identical to ATCC strain 21052, some products contain *Lactobacillus plantarum*, although its presence is not stated on the food label[73]. In more recent years, the use of probiotics per se and lactobacilli specifically has received greater attention as an alternative, inexpensive, and natural remedy to restore and maintain health[74-79]. Two strains, *Lactobacillus GG* (ATCC 53103) and *Lactobacillus rhamnosus* GR-1 appear to be effective at colonizing and protecting the intestine[80-86] and urogenital tract[87-91], respectively, against microbial infection. Recently[118] has been reported the first clinical evidence that probiotic lactobacilli can be delivered to the vagina following oral intake. In 10 women with a history of recurrent yeast vaginitis, bacterial vaginosis (BV) and urinary tract infections, strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 given twice daily for 14 days, were recovered from the vagina and identified by morphology and molecular typing within 1 week of commencement of therapy. In all cases of asymptomatic BV or intermediate BV (based upon Nugent scoring) was resolved within 1 week of therapy.

There is one obvious question: what properties do these strains possess that make them effective probiotic agents? The answer is not fully known, but some common denominators appear to exist, namely the ability to adhere to and colonize tissues and the capacity to inhibit the pathogenesis of disease-causing organisms. Another question can be raised: do we expect an exogenous probiotic strain to colonize the gastrointestinal and urogenital tracts of a given person for a long time and even become part of the normal flora, replacing or coexisting with the endogenous lactobacilli organisms? Or is the aim to substitute the endogenous bacteria only while the nor-

mal flora is repressed (e.g., as a result of antibiotic therapy)[92]? Again, no clear answers are available. To more fully ascertain which properties are required by lactobacilli to protect the host, we recommend a series of extensive microbiological, physico-chemical, and molecular biology methodologies[89,93-97]. It appears that a given strain of *Lactobacillus* can express several, but not necessarily all, of the known key factors and be able to compete in the urogenital microenvironment. For example, lactobacilli can use many mechanisms to adhere to surfaces, such as electrostatic, hydrophobic, hydrophilic, capsular, and fimbrial mechanisms[95,98-102]: in the urogenital tract, hydrophilic *L. rhamnosus* GR-1 and hydrophobic *Lactobacillus fermentum* B-54 both colonize[91]. Some strains can bind better to intestinal cells and inhibit pathogen adhesion[103,104], but they may not be able to effectively inhibit growth of uropathogens[94]. Before commencing more human studies, *L. rhamnosus* GR-1 was found to be the best of a group of 34 *Lactobacillus* strains isolated from dairy, poultry, health food, and human sources, with respect to adhesion to squamous and transitional uroepithelial cells, competitive exclusion of pathogens, and production of inhibitors of uropathogen growth[94]. In 1995, another characteristic of lactobacilli was discovered that appeared to be important in conferring probiotic action against uropathogens. Fifteen strains were found to produce biosurfactant[10]. The substance or substances adsorbed to surfaces and inhibited the initial adhesion of *E. faecalis* by 70%. The crude substance was analyzed and was found to contain proteins and carbohydrates. Amino acid analysis of hydrolyzed material from strain *Lactobacillus acidophilus* RC-14 showed a high alanine content compared with less active strains. The activity is not due to lipoteichoic acid or glycosyldiglycerides or to factors such as acid or bacteriocins, which inhibit bacterial growth. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis showed a variety of proteins with molecular masses of from 14.4 to 140 kDa[106,107].

The biosurfactant activity is resistant to trypsin and pepsin, and sensitive to -amylase and lysozyme, and resistant to 75° C degree heating. The antiadhesive molecules produced by certain lactobacilli hold promise for application to many human sites where pathogens attach, colonize, and confer disease. More recently, the activity was shown to affect a broad range of pathogens[108] and an active component was found to be a collagen binding protein[109]. The discovery of biosurfactants in lactobacilli and several antiadhesion components is an exciting development. Hydrogen peroxide-producing strains are believed to be important in vaginal colonization[110,55,111,112]. Lactic acid was shown to inhibit growth of *Gardnerella vaginalis* more so than hydrogen peroxide, unless the latter is in the presence of myeloperoxidase[113]. Strains with identical molecular profiles, e.g., poultry isolate *L. rhamnosus* A-60 and vaginal isolate *L. rhamnosus* RC-17[73], may actually utilize quite different mechanisms for colonization (namely high adhesiveness or production of inhibitory substances for *L. rhamnosus* RC-17 and high capacity to exclude pathogens in the case of *L. rhamnosus* A-60). *L. rhamnosus* GR-1 and strain 36 have similar molecular profiles, yet the former produces a biosurfactant active in preventing enterococci from adhering to surfaces[105], whereas the latter has a biosurfactant that is inactive. Presumably, lactobacilli continually enter the intestine from food sources. There are studies that indicate that certain species, e.g., *L. acidophilus*, are better able to survive stomach pH and bile salt exposure so that they may pass through into the intestine[114]. In other studies of biopsied intestinal mucosa, strains of *L. plantarum, Lactobacillus casei* subsp. *rhamnosus, Lactobacillus reuteri,* and *Lactobacillus agilis* were recovered postimplantation[115]. However, without the use of molecular typing and specific probes, one cannot be certain that these strains are transmitted to, or subsequently colonize, the female urogenital tract. One could argue that the strains that appear in stool and in vaginal mucosa throughout life are those that colonized the urogenital tract shortly after birth, and that subsequent events, e.g., nutritional and hormonal changes, led to the emergence or suppression of certain strains. A lengthy and large epidemiologic study might be needed to better understand the succession of the normal flora during different phases of life. There is an indication that probiotic strains can undergo genetic changes during colonization: a tetracycline-sensitive *Bifidobacterium* strain administered to antibiotic-decontaminated mice maintained in isolators was found

to produce 2 undistinguished intestinal populations according to restriction endonuclease patterns of total DNA, but one variant was highly resistant to tetracycline (Smeianov V et al, unpublished observations, 1998). Both variants coexisted in the mouse intestine and there was a strong advantage of the newly appeared variant. This possibility of genetic adaptation or in vivo selection of probiotic bacteria is an area of interest for our group. We assume that artificial colonization is possible and that in vitro analyses will allow us to construct methods for selecting and delivering these strains. However, an adjunct to such studies must be an examination of the strains already colonizing healthy humans and a clarification of how these organisms are altered by food intake. On the basis of our knowledge, strains with different origins and probiotic properties seem to exist in the vagina of healthy women[73,116]. The challenge will be to identify which strains are the most beneficial for health and why. Further proof of urogenital colonization and protection from infection was obtained from a clinical trial in which 55 premenopausal women were given weekly either one suppository of *L. rhamnosus* GR-1 and B-54 (0.5 g) or one suppository of a lactobacillus growth factor for 1 y. The patients were followed up after 2 wk and then monthly[91]. Six patients were excluded in the first month for noncompliance, moving, or becoming pregnant, and 11 patients did not complete the study in full; however, their data were included to examine infection rates. Again, there were no side effects in the study. The UTI infection rate decreased from 6.0 per previous year to 1.6 (73% decrease) for those given lactobacilli and to 1.3 (79% decrease) for those given *Lactobacillus* growth factor. Time to first infection was the same in both groups, namely 21-22 wk. Vaginal pH was the same for both groups, namely 4.6-5.0. The viable lactobacillus counts recovered from vaginal swabs increased with therapy, especially for months 7-12 for lactobacilli-treated patients, during which time lower UTI rates were seen. It had been hypothesized by some researchers that a low vaginal pH (< 5) was sufficient to prevent infection, including UTI, sexually transmitted diseases, and AIDS. An analysis of 10 patients showed that lactobacillus therapy maintained the vaginal pH at acidic levels (4.8 ± 0.5) during the study[117]. The UTI rates fell by 79.4% (94) but UTI did occur despite a vaginal pH of 4.8 and the causative agents were common uropathogens (6 *E. coli*, 3 streptococci, and 3 staphylococci). Thus, from these studies[91,117], it was concluded that a vaginal acid pH < 5 was not sufficient alone to prevent UTI.

In conclusion, there is now growing evidence that certain species and strains present in the healthy urogenital tract protect the host against infection by pathogenic microorganisms. Many properties required to confer this protection have been identified, but evidence of their expression in vivo is scant and the relative significance of each is unknown. The concept of treating and preventing urogenital infection by instillating probiotic organisms has great appeal to patients and caregivers. The ability to administer orally *L. rhamnosus* GR-1 and *L. fermentum* RC-14, which colonize the intestine and vagina, provides a major step in the right direction for patients as it potentially allows for the self administration of therapy.

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