Hindawi Publishing Corporation
Interdisciplinary Perspectives on Infectious Diseases
Volume 2008, Article ID 256490, 9 pages
doi:10.1155/2008/256490

Review Article

Vaginal Microbiota and the Use of Probiotics

Sarah Cribby,[1,2] Michelle Taylor,[1,2] and Gregor Reid[1,2]

[1] *Canadian Research and Development Centre for Probiotics, Lawson Health Research Institute, 268 Grosvenor Street, London, ON, Canada N6A4V2*
[2] *Department of Microbiology and Immunology, University of Western Ontario, London, ON, Canada N6A4V2*

Correspondence should be addressed to Gregor Reid, gregor@uwo.ca

Received 10 July 2008; Revised 31 October 2008; Accepted 18 November 2008

Recommended by Robert A. Britton

The human vagina is inhabited by a range of microbes from a pool of over 50 species. Lactobacilli are the most common, particularly in healthy women. The microbiota can change composition rapidly, for reasons that are not fully clear. This can lead to infection or to a state in which organisms with pathogenic potential coexist with other commensals. The most common urogenital infection in premenopausal women is bacterial vaginosis (BV), a condition characterized by a depletion of lactobacilli population and the presence of Gram-negative anaerobes, or in some cases Gram-positive cocci, and aerobic pathogens. Treatment of BV traditionally involves the antibiotics metronidazole or clindamycin, however, the recurrence rate remains high, and this treatment is not designed to restore the lactobacilli. In vitro studies have shown that *Lactobacillus* strains can disrupt BV and yeast biofilms and inhibit the growth of urogenital pathogens. The use of probiotics to populate the vagina and prevent or treat infection has been considered for some time, but only quite recently have data emerged to show efficacy, including supplementation of antimicrobial treatment to improve cure rates and prevent recurrences.

1. THE MICROBIOTA OF THE VAGINA

The microbial species that inhabit the vaginal tract play an important role in the maintenance of health, and prevention of infection. Over 50 microbial species have been recovered from the vaginal tract [1–3]. These species do not exist independently, and studies in vitro and in humans have shown that a multispecies microbiota, usually associated with bacterial vaginosis (BV), are present in dense biofilms [4–7], while a lactobacilli dominant microbiota can be sparsely distributed on the epithelium [4, 5, 8]. In comparison, the gut is populated with more than 800 species of microbes, the majority of which are excreted in feces, and a number of which are well equipped to be pathogenic. Despite the close proximity of the vagina to the anus, the diversity of microbes present in the vagina is much lower than in the gut. The reason for this lower diversity is still unclear, but may involve poor receptivity of the vagina, different nutrient availability compared to the gut, and competition with indigenous organisms. Some species found in the gut, such as *E. coli* and *Streptococcus,* can also be found in the vagina, indicating the proper receptors, nutrients, and oxygen tension are present for these organisms to grow.

Different methodologies are being used to identify the composition of the vaginal microbiota. Each has its strengths and weaknesses. Culture-based methods allow strains to be identified and used for further experimentation. However, as there remains a major defect in our ability to grow many bacterial species, we must rely on nonculture methods to identify the breadth of vaginal microbiota. This has been achieved by analyzing their ribosomal DNA sequences [3, 9], using a combination of PCR and denaturing gel gradient electrophoresis (DGGE) [2, 5, 10–12], and by using degenerate, universal polymerase chain reaction primers to amplify an approximately 555 base-pair regions of the universal chaperonin-60 gene [13].

The species that are present in the vaginal mucosa vary between premenopausal woman and those who have gone through menopause. The microbiota of healthy premenopausal woman is generally dominated by *Lactobacillus* species, the most common of which are *L. iners*, *L. crispatus*, *L. gasseri*, *L. jenesenii,* followed by *L. acidophilus, L. fermentum, L. plantarum, L. brevis, L. casei, L. vaginalis, L. delbrueckii, L. salivarius, L. reuteri,* and *L. rhamnosus* [2, 5, 9–16]. As more studies are performed on the vaginal organisms in healthy women, it is possible that some women

will be identified, who do not have a lactobacilli-dominated microbiota [17]. However, until we know more about the dynamics of such a population, and are sure that it does not increase the risk of the disease, lactobacilli will remain the organisms of most importance to vaginal health.

Factors such as hormonal changes (particularly estrogen), vaginal pH, and glycogen content can all affect the ability of lactobacilli to adhere to epithelial cells and colonize the vagina [16]. The menstrual cycle can also cause changes in the vaginal microbiota, with high concentrations of estrogen increasing adherence of lactobacilli to vaginal epithelial cells [18]. With the decrease in estrogen levels associated with menopause, there is also a decrease in lactobacilli present in the vaginal tract of postmenopausal women [5, 11, 12, 19]. Postmenopausal women are also more susceptible to urogenital infections, supporting the theory that colonization of the vagina by commensal lactobacilli serves as a protection from these pathogens [19, 20]. Although the methods by which these organisms do this are still unclear, it appears to involve an ability to adhere to and to populate the vaginal epithelium and mucin layer, to inhibit pathogens from taking over [21–24], to reduce pathogen virulence [25, 26], and to modulate host defenses [27].

Hormone replacement therapy (HRT) alters the bacterial profile of the vaginal tract of postmenopausal women, and restores a lactobacilli-dominated state, as well as reduces the incidence of urinary tract infections (UTI) [19]. In a study of women taking combination conjugated equine estrogen and progesterone HRT, only 1 to 3 species of bacteria, mainly *Lactobacillus*, were detected in the vaginal mucosa of 87% of the women [5]. In postmenopausal women not receiving HRT, almost all subjects had vaginal mucosa populated with more than 1 organism, many of which had pathogenic potential such as *Bacteroides, Prevotella,* and *Gardnerella*, associated with bacterial vaginosis (BV), and *E. coli* and *Enterococcus,* associated with UTI [5].

While a vaginal tract dominated by lactobacilli appears to protect the host against some vaginal infections, it does not fully prevent colonization by other species. Pathogens are still able to coexist with these commensal organisms, as shown by Burton and Reid [10], where *G. vaginalis*, a pathogen associated with BV, was detected in a vaginal sample which also contained a species of *Lactobacillus*. Interestingly, *G. vaginalis* was displaced beyond detectable limits for 21 days, following a single intravaginal instillation of probiotic lactobacilli [11]. As more and more studies are uncovering the diversity microbiota of the vagina, it seems apparent that the balance between a healthy and diseased state involves some sort of equilibrium or see-saw effect, which can swing in either direction depending on a number of factors, such as hormone levels, douching, sexual practices, as well bacterial interactions and host defenses [20, 21].

Witkin et al. [28] have proposed that innate immunity plays an important role in the switch to BV from a healthy state. The mechanism they propose is through microbial-induced inhibition of Toll-like receptor expression and/or activity blocking proinflammatory immunity, as well as a lack of 70-kDa heat-shock protein production, and a deficit in vaginal mannose-binding lectin concentrations decreasing the capacity for microbial killing. Three recent studies have provided further insight into the host's role. In a study of women susceptible to UTI, it was discovered that immunological defects in peripheral blood coexisted with a persistently aberrant microbiota (Kirjavainen et al. [29]). In postmenopausal women, BV was associated with apparent reduced expression of host antimicrobial factors [30]. When probiotic *L. rhamnosus* GR-1 was administered to the vagina of premenopausal women, it resulted in 3 536 gene expression changes and increased expression levels of some antimicrobial defenses [31].

2. NONSEXUALLY TRANSMITTED INFECTIONS OF THE VAGINAL TRACT AND INTERFERENCE BY LACTOBACILLI

Pathogenic organisms are able to infect the vagina, with BV, yeast vaginitis, and UTIs causing an estimated one billion or more cases per year [32–35]. While there is some evidence that the causative organisms can be transmitted by sexual partners, these conditions will be discussed here as nonsexually transmitted. Other reviews adequately cover sexually transmitted infections [36, 37].

Yeast vaginitis is characterized by white discharge, local itching, and irritation. The majority of cases are caused by *Candida albicans*, but *C. glabrata*, *C. krusei*, and *C. tropicalis* can be problematic [35]. It is diagnosed by microscopic detection of dense numbers of yeast cells on a vaginal smear, and by physical examination and the presence of a white, mucous-like yeast discharge. Of note, lactobacilli are often found in patients with yeast vaginitis, therefore, the induction of infection does not appear to require the yeast displacing or killing off the lactobacilli.

Urinary tract infections occur when pathogenic bacteria ascend from the vagina and replicate on, and sometimes within, the bladder urothelium [32, 38, 39]. These infections are frequent among women, with an estimated 50% suffering at some time in their life. Symptoms and signs include suprapubic pain, dysuria, pyuria, frequency and painful micturition, and occasionally hematuria. Asymptomatic bacteriuria is also a common occurrence, particularly amongst the elderly. The most frequent pathogen is *E. coli*, followed by *Enterococcus faecalis,* and *Staphylococcus saprophyticus* [39]. Diagnosis can be achieved by presence of symptoms and signs, and urine samples containing over 10^3 organisms/mL of the pathogens. In a portion of patients, the *E. coli* invade the bladder epithelium and form dense biofilms that are recalcitrant to antibiotics [40]. In women with no history of UTI, their vagina and perineum is most commonly colonized by lactobacilli [20], while in women with recurrent UTI there is an inverse association between lactobacilli and *E. coli* [41], suggesting that lactobacilli play a role in preventing infection.

The most common urogenital disorder in women of reproductive age is BV, a condition discussed above. The vaginal microbiota of BV patients typically contains a broader range of species than found under healthy conditions, with *Atopobium vaginae*, *Bacteroides* spp., *Gardnerella vaginalis*, *Mobiluncus*, *Megasphera*, *Mycoplasma hominis*, *Peptostreptococcus*, and *Prevotella* being particularly

prevalent [3, 42–46]. BV is associated with multiple species of bacteria that occur in 90% of the cases, and essentially consists of an elevated vaginal pH (>4.5) and depletion of lactobacilli. It affects women of all age groups, and is often asymptomatic [47]. When symptoms and signs do occur, they include fishy odor, discharge, and vaginal pH above 4.5 [48]. Indeed, this formed the basis of the often-used Amsel criteria for BV diagnosis: presence of at least 3 of the following criteria: (1) release of an amine or fishy odor upon addition of 10% potassium hydroxide, (2) a vaginal pH higher than 4.5, (3) detection of at least 20% of clue cells (which are vaginal cells colonized by Gram-negative rods), and (4) a milky homogeneous vaginal discharge [48]. A Gram-staining method called the Nugent score has also been used [8]. It comprises a scoring system based on the morphology of bacteria present in vaginal swab samples. A normal score is given to samples showing predominantly Gram-positive rods indicative of lactobacilli, while the presence of predominantly small and curved shaped Gram-negative rods and Gram-positive cocci, along with the absence of lactobacilli, is indicative of BV. The BVBlue test is another kit used to diagnose BV, and works by detecting sialidase produced by pathogens associated with the condition [49, 50]. Of note, aerobic vaginitis has also been described in which the vagina is colonized by organisms such as *E. coli* and enterococci [51]. During pregnancy, BV can increase the risk of preterm labor and low birth weight [52, 53]. Other problems associated with BV include pelvic inflammatory disease, UTI, and increased susceptibility to sexually transmitted diseases, including HIV [54–57].

The organisms associated with BV form dense biofilms on the vaginal epithelium, and these are associated with increased resistance to lactobacilli-produced lactic acid and hydrogen peroxide (H_2O_2) which are normally antagonistic to planktonic organisms [58]. The biofilms are also able to induce host expression of certain inflammatory factors, such as IL-1 and IL-8 [59]. It is not currently known whether the production of H_2O_2 by lactobacilli has a clinically protective role against BV. The increased prevalence of H_2O_2-peroxide producing vaginal lactobacilli in healthy women has been given as a reason to believe that it is a protective factor [60], however, those studies used culture to recover the lactobacilli, and arguably had they used nonculture methods, *L. iners* would have been the most commonly isolated and it does not appear to produce H_2O_2. It is possible to isolate *L. iners* by culture, but it requires selective media and extensive incubation. The same group found that women with the H_2O_2-producing vaginal *L. crispatus* or *L. jensenii* had a significantly lower incidence of BV than women with a different vaginal flora [14]. However, Alvarez-Olmos et al. [61] and Rosenstein et al. [62] found H_2O_2-producing lactobacilli in 85% and 91.7%, respectively, of women with BV. It could be argued that the high prevalence of H_2O_2-producing lactobacilli shows that this compound is not protective [32]. Either way, it is difficult to make a definitive conclusion.

McLean and McGroarty [63] conducted an in vitro study showing that increasing culture pH reduced the bacteriostatic effects of *L. acidophilus* on *G. vaginalis* NCTC 11292 by 60%; a 30% reduction in bacteriostatic effects was seen when catalase was introduced to degrade H_2O_2. Klebanoff et al. [64] found that the toxicity of H_2O_2-producing lactobacilli was inhibited by the presence of catalase but lactobacilli that do not produce H_2O_2 were not affected. High concentration of H_2O_2-producing lactobacilli inhibits the growth of both *G. vaginalis* and *Bacteroides bivius*. However, low concentrations of H_2O_2-producing lactobacilli must be combined with myeloperoxidase and chloride in vaginal mucus, to be toxic toward *G. vaginalis*, with a maximum toxicity in a pH range of 5 to 6. A pH of $\leq$4.5 inhibited the growth of *G. vaginalis* on its own and this effect increased with the addition of the above combination. Suffice to say, H_2O_2 is likely one of several factors involved in competition with other organisms in the vagina.

3. PROBIOTICS TO PREVENT AND TREAT UROGENITAL INFECTIONS

As antimicrobial treatment of urogenital infections is not always effective, and problems remain due to bacterial and yeast resistance, recurrent infections [65, 66], as well as side effects, it is no surprise that alternative remedies are of interest to patients and their caregivers. It is assumed that recurrences are due to antimicrobials failing to eradicate the pathogens, perhaps because of biofilm resistance, or that the virulent organisms come back from their source (the person's gut, or a sex partner) and attack a host whose defenses are suboptimal. Young girls who suffer from UTI are more likely to have repeated episodes in adulthood, and overall many UTI, BV, and yeast vaginitis patients will have a recurrence [21, 67]. Recurrent infection may also be due to the elimination of the commensal organisms in the vagina by the antimicrobial, thereby increasing susceptibility to recolonization by pathogens [68, 69]. This is one of the main reasons for considering the use of probiotics, to replenish the commensal microbes as a way to lower the risk of reinfection. In a study of 120 children with persistent primary vesicoureteral reflux, *L. acidophilus* treatment daily was as effective as trimethoprim/sulfamethoxazole in reducing the rate of UTI ($P = .926$), suggesting that probiotics could provide a prophylactic option [70].

The route of delivery of probiotic lactobacilli has intuitively been via direct instillation into the vagina. For example, the weekly application of *L. rhamnosus* GR-1 and *L. fermentum* B-54 was shown to reduce UTI recurrences from an average of 6 to 1.6 per year [71]. The ability of a given strain of lactobacilli to adhere to vaginal cells was considered an advantage in temporarily populating the vaginal [71, 72] and creating an environment conducive to the restoration of the host's indigenous lactobacilli rather than a return of pathogens. The adhesion of lactobacilli to the uroepithelium varies among species and strains, as shown by in vitro studies [72], and may be mediated by glycoprotein and carbohydrate adhesins binding to glycolipid receptors [73]. Still, it is unclear the extent to which a difference in in vitro adhesion, say of 10 per cell, means that an organism will succeed or fail to protect the host if instilled into the vagina. Thus, adhesion per se is not the definitive criteria to predict success. Once

administered in a viable count of one billion or more, *L. rhamnosus* GR-1 and *L. reuteri* (formerly *fermentum)* RC-14 have been found to be detectable for three weeks or more, depending on the host [74, 75]. This implies a correlation between in vitro adherence and in vivo presence.

The concept of delivering lactobacilli orally to repopulate the vagina was first reported in 2001 [76], and based upon the question "if urogenital pathogens can do this, why cannot lactobacilli"? The organisms were delivered in a milk base and shown to be recovered from the rectum [77]; therefore supporting the concept that ingested strains could pass through the intestine, reach the rectum, and potentially ascend to the vagina. This was confirmed independently by others [78].

In order to conduct clinical studies with the view of providing more women with access to these strains, a two-year shelf life capsule formulation was then developed and used successfully in a number of studies. An oral dose of over one billion organisms per day was found to maintain a lactobacilli-dominated vaginal presence [79]. The time for this intervention to affect the vaginal tract is obviously longer than direct vaginal instillation, and will depend on viability of the strains as they pass through the stomach and gut [78]. In addition, the load of lactobacilli that can be delivered this way is clearly lower than via vaginal administration. However, an advantage of the oral approach may be the ability of the lactobacilli to reduce the transfer of yeast and pathogenic bacteria from the rectum to the vagina [80], which could potentially lower the risk of infection. In that randomized, placebo-controlled trial of 64 healthy women, 37% of the patients in the *L. rhamnosus* GR-1 and *L. reuteri* RC-14 probiotic group had a lactobacilli-dominated normal vaginal microbiota restored from a BV vaginal flora compared to 13% in the placebo group ($P = .02$). At both the 28-day and 60-day test points, women in the lactobacilli treatment group had a greater number of vaginal lactobacilli than women in the control group ($P = .08$ and $P = .05$, resp.) as shown by microscopy and culture. The ability of this oral probiotic therapy to create a lactobacilli normal flora and convert some subjects from a BV status to normal [79] goes beyond the proof-of-concept stage and provides a method for women to help maintain vaginal health. Failure of *L. rhamnosus* GG to be effective, at least in one small study [79], emphasizes the strain-specific aspects of probiotic use. Thus, one cannot and should not utilize the data from one strain to infer that another untested strain will provide the same benefits.

The mechanisms whereby lactobacilli function as anti-infective defenses are still not fully understood. As discussed above, this may involve production of antimicrobial factors [81], and maintenance of a vaginal pH of ≤4.5. It could also be due to biosurfactants which alter the surrounding surface tension and reduce the ability of a wide range of pathogens to adhere [82, 83]. This might explain the relatively sparse coverage of epithelial cells noted in healthy women [8]. In addition, lactobacilli have been shown to bind (coaggregate) some pathogens and this may be a means to block their adhesion, kill them through production of antimicrobials, and prevent their spread to other areas of the vagina and bladder [84]. Among 10 strains of lactobacilli being evaluated for use in a probiotics tablet, Mastromarino et al. [85] found, in vitro, that *Lactobacillus gasseri* 335 and *Lactobacillus salivarius* FV2 were able to coaggregate with *G. vaginalis*. When these strains of lactobacilli were combined with *Lactobacillus brevis* CD2 in a vaginal tablet, adhesion of *G. vaginalis* was reduced by 57.7%, and 60.8% of adherent cells were displaced. Boris et al.found that the adherent properties *G. vaginalis* were similarly affected by *Lactobacillus acidophilus* [73].

It has been known for some time that *Lactobacilllus* produce bacteriocins that can inhibit the growth of pathogens, including some associated with BV, such as *G. vaginalis* [86]. Only relatively recently has a study shown in animals that bacteriocin production might have an effect in vivo. A stable mutant of *Lactobacillus salivarius* UCC118 that did not produce a specific bacteriocin was unable to protect mice against *Listeria* intestinal infection, while the wild type did, thereby leading the authors to conclude that bacteriocin production can be a primary mediator of anti-infective defense [87].

Relatively few studies have attempted to prevent urogenital infection using probiotics. Shalev et al. [88] assessed 46 premenopausal women with ≥4 episodes of BV and/or vaginal candidiasis in the previous year, to compare the recurrence of BV using a probiotic yoghurt versus one that was pasteurized. Patients were not receiving long-term antibiotics or immunosuppressive therapy and had not consumed yoghurt prior to the commencement of the study. They were randomly assigned to one of two treatment groups and ingested 150 mL of either pasteurized yoghurt ($n = 23$) or yoghurt containing *L. acidophilus* at $> 1.0 \times 10^8$ colony-forming units ($n = 23$). Yoghurt was consumed daily for two months followed by two months of no yoghurt. There was a 60% reduction in BV episodes among patients consuming probiotic yoghurt after one month while only a 25% reduction occurred in subjects who received pasteurized yoghurt ($P = .004$). After two months of yoghurt consumption, the results were similar; however, 25% of patients from both groups had left the study. Product integrity was only assessed prior to the study and no adverse effects were reported.

Neri et al. [89] studied 84 women in the first trimester of pregnancy to observe the effects of probiotic-containing yoghurt on BV. The subjects were randomized to one of three treatment groups: inserting a tampon containing 5% acetic acid ($n = 32$), a 10 to 15 mL vaginal douche containing $> 1.0 \times 10^8$ colony-forming units/mL of *L. acidophilus* ($n = 32$), or no treatment ($n = 20$). Both active treatments were administered twice a day for one week. Amsel criteria (three of five findings: release of an amine fishy odor; release of amine odor after the addition of 10% potassium hydroxide; vaginal pH greater than 4.5; clue cells in the vaginal fluid; milky homogenous vaginal discharge) were absent in 88%, 38%, and 15% of subjects who received intravaginal lactobacilli, acetic acid tampons, and placebo, respectively, after 30 days. There was a significant difference in the cure rate between probiotic and control groups ($P < .005$), and lactobacilli and acetic acid groups ($P = .004$).

Fredricsson et al. [90] conducted an open-label trial to compare the cure rates of 61 women with BV given one of four intravaginal products. Patients were diagnosed with BV if ≥3 Amsel criteria were present. Each of the four treatments that patients were randomized to receive was administered twice a day for seven days: 5 mL of fermented milk containing between $5.0 \times 10^8$and 2.0×10^9 colony-forming units/mL of *L. acidophilus* NCDO 1748 ($n = 13$), 5 mL of acetic jelly ($n = 15$), 5 mL of estrogen cream ($n = 16$), or 500 mg metronidazole vaginal tablets ($n = 15$). BV was considered to have been cured if ≤1 Amsel criterion was present at 4 and 8 weeks. After both 4 and 8 weeks from the initiation of treatment, the cure rates in the metronidazole, acetic acid, probiotic, and estrogen groups were 93%, 18%, 7%, and 6%, respectively; no statistical analysis was reported. In this case, the so-called probiotic was not effective. No information about the strain was provided.

The cure rates of BV in 57 women with a mean age of 24 were studied following treatment with either "probiotics" or placebo in a double-blind trial [91]. Subjects were randomized to receive either a vaginal suppository containing $1.0 \times 10^{8\text{-}9}$ colony-forming units of *L. acidophilus* ($n = 28$) or placebo ($n = 29$). The vaginal suppositories were administered twice a day for 6 days. Symptom resolution, which was not clearly defined, was used to evaluate the cure of BV. At 7–10 days after the commencement of treatment, BV symptoms were absent in 57% of women in the probiotic group and 0% of women in the placebo group ($P < .005$). After 20 to 40 from the initiation of treatment, the cure rate in the probiotic group fell to 21% and remained at 0% in the placebo group (p = NS). This poorly conceived study is hard to interpret and is insufficient to verify efficacy of the product.

Eriksson et al. [92] studied how lactobacilli augmented antibiotics in curing BV through a double-blind, placebo-controlled trial including 187 women with a median age of 32 over two menstrual periods. Open-label treatment with 100 mg/d of clindamycin was administered to all patients for 3 days. The subjects were then randomized to one of two treatment groups which required at least five tampons to be inserted during the next menstrual period. The treatment groups were placebo tampons ($n = 96$) and tampons impregnated with *L. fermentum, L. gasseri*, and *L. rhamnosus* at 1.0×10^8 colony-forming units per tampon ($n = 91$). Cure rates of BV were assessed by the absence of Amsel criteria after the second menstrual period in both the probiotic and placebo groups, and found to be 56% and 62%, respectively, (p = NS). Infection with *Candida* was reported in 14.3% of subjects in the probiotic group and 13.5% of patients in the placebo group. The viable number of bacteria per tampon diminished to 10^6 colony-forming units by the end of the study. In short, this product was not successful. The rationale for administering lactobacilli during menses could be questioned, as it exposes the users' blood stream directly to the organisms, and the flushing effect of menstruation may be nonconducive to lactobacilli repopulating the vagina.

A comparison of intravaginal probiotics and metronidazole gel in treating 40 women (ages 18 to 50) with BV was conducted by a single-blind study by Anukam et al. [93]. The presence of ≥3 Amsel criteria, a Nugent score of ≥7, and a positive sialidase test led to a diagnosis of BV. Patients were randomized to one of two treatment groups for five days. They either inserted an intravaginal capsule with *L. rhamnosus* GR-1 and *L. reuteri* RC-14 at 1.0×10^9 colony-forming units nightly ($n = 20$) or applied a 0.75% metronidazole gel twice daily ($n = 20$). A Nugent score of ≤3 at 30 days indicated a cure of BV. A BV cure rate of 88% in the probiotic group and 50% in the metronidazole group was found (p = NS). Treatment was prematurely discontinued by patients in both the metronidazole and probiotic groups at 10% and 15%, respectively. This study, albeit small in size, showed the potential of probiotics to cure BV.

The efficacy of combining probiotics or placebo with oral metronidazole was assessed in 125 women aged 18 to 44 [94]. Oral metronidazole was administered at 500 mg twice daily to all patients for 7 days, and they were randomized to receive twice-daily oral capsules containing either a placebo ($n = 60$) or *L. rhamnosus* GR-1 and *L. reuteri* RC-14 at 1.0×10^9 colony-forming units ($n = 65$) for a total treatment duration of 30 days. At the end of 30 days, BV was considered absent if the patient had a negative sialidase test and a Nugent score of <3. This was the case in 40% of placebo and 88% of probiotic subjects ($P < .001$). If an intermediate Nugent score was regarded as "cure of BV", the cure rate was 100% with metronidazole and probiotics versus 70% with metronidazole and placebo. This study is important as it implies that probiotics can augment the effects of antibiotics in treatment of disease. Further studies have confirmed this effect, but are awaiting publication.

4. POSSIBLE NEGATIVE EFFECTS OF PROBIOTIC USE

Annually, over one billion doses of probiotics are administered worldwide, and those administered for urogenital health have been well tolerated [11, 75, 93–96]. In addition, the mouth, gastrointestinal tract, and female genitourinary tract are inhabited by *Lactobacillus* [96]. Yet, endocarditis and bacteremia caused by lactobacilli are extremely rare. Most cases occur in patients with chronic diseases or debilitating conditions that provide direct access to the bloodstream from a leaky gut. Only 1.7% of 241 cases of bacteremia, endocarditis, and localized infections associated with *Lactobacillus* that were investigated by Cannon et al. were considered to have a possible link with heavy consumption of dairy products [97]. Only one case had a *Lactobacillus* isolate that was indistinguishable from a probiotic strain. There was no connection between the species of *Lactobacillus* isolated and the type of infection or mortality. A recent study that directly instilled a six-strain bacterial product into the intestine of patients with severe, potentially fatal pancreatitis portrayed probiotics as being dangerous [97]. However, the product had never been proven to be probiotic, it was administered as a drug unlike 99.9% of probiotics, the randomization process led to patients with multiorgan failure being given large doses of live bacteria, and the authors failed to provide a rationale for the study in an appropriate animal model. All this led to unwarranted adverse publicity for the field of probiotics [98].

Nevertheless, safety of probiotic use must continually be monitored and considered when doing clinical studies. The potential for transfer of antibiotic resistance is one factor to consider, although it remains to be proven that probiotics have contributed in any way to drug resistance, or disease. Rather, the overuse of antibiotics, especially in livestock feed and long-term prevention of infection, remains a root cause of the increasing concerns over drug resistance. Efforts to substitute prophylactic antibiotics with probiotics, especially in children with recurrent UTI [70] and perhaps some patients preparing to undergo surgery [99], are worthy of pursuit.

5. CONCLUSION

Molecular methodologies are providing a greater understanding of the dynamic microbial presence, both short and long term, in the vagina. The defenses of the host which include some of these microbes perform a remarkable function given the opportunity of pathogens to cause infection. The use of probiotic lactobacilli to prevent infection has a good rationale, and an excellent safety record, but so far only a few strains have been clinically proven to be effective, in particular to prevent BV. It is critically important that strains be characterized and tested clinically using the delivery system of choice (oral, vaginal, dried powder, or in suspension). An advantage for women is that they can self-administer the probiotics. Many more studies are needed to optimize the defensive properties of the vaginal microbiota, but the potential remains that the health of many women can be improved by probiotic intervention.

ACKNOWLEDGMENT

This work is supported by grants from NSERC and AFMnet.

REFERENCES

[1] V. Redondo-Lopez, R. L. Cook, and J. D. Sobel, "Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora," *Reviews of Infectious Diseases*, vol. 12, no. 5, pp. 856–872, 1990.

[2] K. C. Anukam, E. O. Osazuwa, I. Ahonkhai, and G. Reid, "16S rRNA gene sequence and phylogenetic tree of *Lactobacillus* species from the vagina of healthy Nigerian women," *African Journal of Biotechnology*, vol. 4, no. 11, pp. 1222–1227, 2005.

[3] B. B. Oakley, T. L. Fiedler, J. M. Marrazzo, and D. N. Fredricks, "Diversity of human vaginal bacterial communities and associations with clinically defined bacterial vaginosis," *Applied and Environmental Microbiology*, vol. 74, no. 15, pp. 4898–4909, 2008.

[4] G. Reid, J. A. McGroarty, P. A. G. Domingue, et al., "Coaggregation of urogenital bacteria in vitro and in vivo," *Current Microbiology*, vol. 20, no. 1, pp. 47–52, 1990.

[5] C. Heinemann and G. Reid, "Vaginal microbial diversity among postmenopausal women with and without hormone replacement therapy," *Canadian Journal of Microbiology*, vol. 51, no. 9, pp. 777–781, 2005.

[6] A. Swidsinski, W. Mendling, V. Loening-Baucke, et al., "Adherent biofilms in bacterial vaginosis," *Obstetrics & Gynecology*, vol. 106, no. 5, part 1, pp. 1013–1023, 2005.

[7] S. G. Saunders, A. Bocking, J. Challis, and G. Reid, "Effect of *Lactobacillus* challenge on *Gardnerella vaginalis* biofilms," *Colloids and Surfaces B*, vol. 55, no. 2, pp. 138–142, 2007.

[8] R. P. Nugent, M. A. Krohn, and S. L. Hillier, "Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation," *Journal of Clinical Microbiology*, vol. 29, no. 2, pp. 297–301, 1991.

[9] D. N. Fredricks, T. L. Fiedler, and J. M. Marrazzo, "Molecular identification of bacteria associated with bacterial vaginosis," *The New England Journal of Medicine*, vol. 353, no. 18, pp. 1899–1911, 2005.

[10] J. P. Burton and G. Reid, "Evaluation of the bacterial vaginal flora of 20 postmenopausal women by direct (Nugent score) and molecular (polymerase chain reaction and denaturing gradient gel electrophoresis) techniques," *The Journal of Infectious Diseases*, vol. 186, no. 12, pp. 1770–1780, 2002.

[11] J. P. Burton, P. A. Cadieux, and G. Reid, "Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation," *Applied and Environmental Microbiology*, vol. 69, no. 1, pp. 97–101, 2003.

[12] E. Devillard, J. P. Burton, J.-A. Hammond, D. Lam, and G. Reid, "Novel insight into the vaginal microflora in postmenopausal women under hormone replacement therapy as analyzed by PCR-denaturing gradient gel electrophoresis," *European Journal of Obstetrics Gynecology & Reproductive Biology*, vol. 117, no. 1, pp. 76–81, 2004.

[13] J. E. Hill, S. H. Goh, D. M. Money, et al., "Characterization of vaginal microflora of healthy, nonpregnant women by chaperonin-60 sequence-based methods," *American Journal of Obstetrics & Gynecology*, vol. 193, no. 3, pp. 682–692, 2005.

[14] M. A. D. Antonio, S. E. Hawes, and S. L. Hillier, "The identification of vaginal *Lactobacillus* species and the demographic and microbiologic characteristics of women colonized by these species," *The Journal of Infectious Diseases*, vol. 180, no. 6, pp. 1950–1956, 1999.

[15] A. Vásquez, T. Jakobsson, S. Ahrné, U. Forsum, and G. Molin, "Vaginal *Lactobacillus* flora of healthy Swedish women," *Journal of Clinical Microbiology*, vol. 40, no. 8, pp. 2746–2749, 2002.

[16] R. P. Galask, "Vaginal colonization by bacteria and yeast," *American Journal of Obstetrics & Gynecology*, vol. 158, no. 4, pp. 993–995, 1988.

[17] X. Zhou, C. J. Brown, Z. Abdo, et al., "Differences in the composition of vaginal microbial communities found in healthy Caucasian and black women," *ISME Journal*, vol. 1, no. 2, pp. 121–133, 2007.

[18] R. C. Y. Chan, A. W. Bruce, and G. Reid, "Adherence of cervical, vaginal and distal urethral normal microbial flora to human uroepithelial cells and the inhibition of adherence of gram-negative uropathogens by competitive exclusion," *The Journal of Urology*, vol. 131, no. 3, pp. 596–601, 1984.

[19] R. Raz, R. Colodner, Y. Rohana, et al., "Effectiveness of estriol-containing vaginal pessaries and nitrofurantoin macrocrystal therapy in the prevention of recurrent urinary tract infection in postmenopausal women," *Clinical Infectious Diseases*, vol. 36, no. 11, pp. 1362–1368, 2003.

[20] A. W. Bruce, P. Chadwick, A. Hassan, and G. F. VanCott, "Recurrent urethritis in women," *Canadian Medical Association Journal*, vol. 108, no. 8, pp. 973–976, 1973.

[21] G. Reid, "Probiotic agents to protect the urogenital tract against infection," *The American Journal of Clinical Nutrition*, vol. 73, no. 2, supplement, pp. 437S–443S, 2001.

[22] 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," *The Journal of Infectious Diseases*, vol. 183, no. 3, pp. 485–491, 2001.

[23] C. Heinemann, J. E. T. van Hylckama Vlieg, D. B. Janssen, H. J. Busscher, H. C. van der Mei, and G. Reid, "Purification and characterization of a surface-binding protein from *Lactobacillusfermentum* RC-14 that inhibits adhesion of *Enterococcus faecalis* 1131," *FEMS Microbiology Letters*, vol. 190, no. 1, pp. 177–180, 2000.

[24] P. Hütt, J. Shchepetova, K. Lõivukene, T. Kullisaar, and M. Mikelsaar, "Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens," *Journal of Applied Microbiology*, vol. 100, no. 6, pp. 1324–1332, 2006.

[25] J. M. Laughton, E. Devillard, D. E. Heinrichs, G. Reid, and J. K. McCormick, "Inhibition of expression of a staphylococcal superantigen-like protein by a soluble factor from *Lactobacillus reuteri*," *Microbiology*, vol. 152, part 4, pp. 1155–1167, 2006.

[26] M. J. Medellin-Peña, H. Wang, R. Johnson, S. Anand, and M. W. Griffiths, "Probiotics affect virulence-related gene expression in *Escherichia coli* O157:H7," *Applied and Environmental Microbiology*, vol. 73, no. 13, pp. 4259–4267, 2007.

[27] S. O. Kim, H. I. Sheikh, S.-D. Ha, A. Martins, and G. Reid, "G-CSF-mediated inhibition of JNK is a key mechanism for *Lactobacillus rhamnosus*-induced suppression of TNF production in macrophages," *Cellular Microbiology*, vol. 8, no. 12, pp. 1958–1971, 2006.

[28] S. S. Witkin, I. M. Linhares, P. Giraldo, and W. J. Ledger, "An altered immunity hypothesis for the development of symptomatic bacterial vaginosis," *Clinical Infectious Diseases*, vol. 44, no. 4, pp. 554–557, 2007.

[29] PV. Kirjavainen, S. Pautler, ML. Baroja, et al., "Abnormal Immunological Profile and Vaginal Microbiota in Women Prone to Urinary Tract Infections," *Clinical Vaccine Immunology*, vol. 16, no. 1, pp. 29–36, 2009.

[30] A. Dahn, S. Saunders, J.-A. Hammond, et al., "Effect of bacterial vaginosis, *Lactobacillus* and Premarin estrogen replacement therapy on vaginal gene expression changes," *Microbes and Infection*, vol. 10, no. 6, pp. 620–627, 2008.

[31] P. V. Kirjavainen, R. M. Laine, D. E. Carter, J.-A. Hammond, and G. Reid, "Expression of anti-microbial defense factors in vaginal mucosa following exposure to *Lactobacillus rhamnosus* GR-1," *International Journal of Probiotics and Prebiotics*, vol. 3, pp. 99–106, 2008.

[32] 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.

[33] B. Foxman, R. Barlow, H. D'Arcy, B. Gillespie, and J. D. Sobel, "Urinary tract infection: self-reported incidence and associated costs," *Annals of Epidemiology*, vol. 10, no. 8, pp. 509–515, 2000.

[34] J. E. Allsworth and J. F. Peipert, "Prevalence of bacterial vaginosis: 2001–2004 National Health and Nutrition Examination Survey data," *Obstetrics & Gynecology*, vol. 109, no. 1, pp. 114–120, 2007.

[35] J. D. Sobel, "Vulvovaginal candidosis," *The Lancet*, vol. 369, no. 9577, pp. 1961–1971, 2007.

[36] M. E. Tarr and M. L. Gilliam, "Sexually transmitted infections in adolescent women," *Clinical Obstetrics & Gynecology*, vol. 51, no. 2, pp. 306–318, 2008.

[37] R. Y. Kropp, C. Latham-Carmanico, M. Steben, T. Wong, and E. Duarte-Franco, "What's new in management of sexually transmitted infections? Canadian Guidelines on Sexually Transmitted Infections, 2006 Edition," *Canadian Family Physician*, vol. 53, no. 10, pp. 1739–1741, 2007.

[38] T. M. Hooton, D. Scholes, J. P. Hughes, et al., "A prospective study of risk factors for symptomatic urinary tract infection in young women," *The New England Journal of Medicine*, vol. 335, no. 7, pp. 468–474, 1996.

[39] C. Imirzalioglu, T. Hain, T. Chakraborty, and E. Domann, "Hidden pathogens uncovered: metagenomic analysis of urinary tract infections," *Andrologia*, vol. 40, no. 2, pp. 66–71, 2008.

[40] D. A. Rosen, T. M. Hooton, W. E. Stamm, P. A. Humphrey, and S. J. Hultgren, "Detection of intracellular bacterial communities in human urinary tract infection," *PLoS Medicine*, vol. 4, no. 12, p. e329, 2007.

[41] K. Gupta, A. E. Stapleton, T. M. Hooton, P. L. Roberts, C. L. Fennell, and W. E. Stamm, "Inverse association of H_2O_2-producing lactobacilli and vaginal *Escherichia coli* colonization in women with recurrent urinary tract infections," *The Journal of Infectious Diseases*, vol. 178, no. 2, pp. 446–450, 1998.

[42] G. Reid and E. Devillard, "Probiotics for mother and child," *Journal of Clinical Gastroenterology*, vol. 38, no. 6, pp. S94–S101, 2004.

[43] G. B. Hill, "The microbiology of bacterial vaginosis," *American Journal of Obstetrics & Gynecology*, vol. 169, no. 2, part 2, pp. 450–454, 1993.

[44] J. P. Burton, E. Devillard, P. A. Cadieux, J.-A. Hammond, and G. Reid, "Detection of *Atopobium vaginae* in postmenopausal women by cultivation-independent methods warrants further investigation," *Journal of Clinical Microbiology*, vol. 42, no. 4, pp. 1829–1831, 2004.

[45] R. C. R. Martinez, S. A. Franceschini, M. C. Patta, et al., "Analysis of vaginal lactobacilli from healthy and infected Brazilian women," *Applied and Environmental Microbiology*, vol. 74, no. 14, pp. 4539–4542, 2008.

[46] K. C. Anukam and G. Reid, "Organisms associated with bacterial vaginosis in Nigerian women as determined by PCR-DGGE and 16S rRNA gene sequence," *African Health Sciences*, vol. 7, no. 2, pp. 68–72, 2007.

[47] M. A. Klebanoff, J. R. Schwebke, J. Zhang, T. R. Nansel, K.-F. Yu, and W. W. Andrews, "Vulvovaginal symptoms in women with bacterial vaginosis," *Obstetrics & Gynecology*, vol. 104, no. 2, pp. 267–272, 2004.

[48] R. Amsel, P. A. Totten, C. A. Spiegel, K. C. S. Chen, D. Eschenbach, and K. K. Holmes, "Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations," *The American Journal of Medicine*, vol. 74, no. 1, pp. 14–22, 1983.

[49] L. Myziuk, B. Romanowski, and S. C. Johnson, "BVBlue test for diagnosis of bacterial vaginosis," *Journal of Clinical Microbiology*, vol. 41, no. 5, pp. 1925–1928, 2003.

[50] M. Milani, E. Barcellona, and A. Agnello, "Efficacy of the combination of 2 g oral tinidazole and acidic buffering vaginal gel in comparison with vaginal clindamycin alone in bacterial vaginosis: a randomized, investigator-blinded, controlled trial," *European Journal of Obstetrics Gynecology & Reproductive Biology*, vol. 109, no. 1, pp. 67–71, 2003.

[51] G. G. Donders, A. Vereecken, E. Bosmans, A. Dekeersmaecker, G. Salembier, and B. Spitz, "Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis," *An International Journal of Obstetrics & Gynaecology*, vol. 109, no. 1, pp. 34–43, 2002.

[52] M. G. Gravett, D. Hummel, D. A. Eschenbach, and K. K. Holmes, "Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis," *Obstetrics & Gynecology*, vol. 67, no. 2, pp. 229–237, 1986.

[53] B. Jacobsson, P. Pernevi, L. Chidekel, and J. J. Platz-Christensen, "Bacterial vaginosis in early pregnancy may

predispose for preterm birth and postpartum endometritis," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 81, no. 11, pp. 1006–1010, 2002.

[54] N. Sewankambo, R. H. Gray, M. J. Wawer, et al., "HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis," *The Lancet*, vol. 350, no. 9077, pp. 546–550, 1997.

[55] T. L. Cherpes, L. A. Meyn, M. A. Krohn, and S. L. Hillier, "Risk factors for infection with herpes simplex virus type 2: role of smoking, douching, uncircumcised males, and vaginal flora," *Sexually Transmitted Diseases*, vol. 30, no. 5, pp. 405–410, 2003.

[56] S. H. Sharami, M. Afrakhteh, and M. Shakiba, "Urinary tract infections in pregnant women with bacterial vaginosis," *Journal of Obstetrics and Gynaecology*, vol. 27, no. 3, pp. 252–254, 2007.

[57] M. F. Gallo, L. Warner, M. Macaluso, et al., "Risk factors for incident herpes simplex type 2 virus infection among women attending a sexually transmitted disease clinic," *Sexually transmitted diseases*, vol. 35, no. 7, pp. 679–685, 2008.

[58] J. L. Patterson, P. H. Girerd, N. W. Karjane, and K. K. Jefferson, "Effect of biofilm phenotype on resistance of *Gardnerella vaginalis* to hydrogen peroxide and lactic acid," *American Journal of Obstetrics and Gynecology*, vol. 197, no. 2, pp. 170.e1–170.e7, 2007.

[59] H. N. Simhan, S. N. Caritis, M. A. Krohn, and S. L. Hillier, "The vaginal inflammatory milieu and the risk of early premature preterm rupture of membranes," *American Journal of Obstetrics and Gynecology*, vol. 192, no. 1, pp. 213–218, 2005.

[60] S. L. Hillier, M. E. Krohn, S. J. Klebanoff, and D. A. Eschenbach, "The relationship of hydrogen peroxide-producing lactobacilli to bacterial vaginosis and genital microflora in pregnant women," *Obstetrics and Gynecology*, vol. 79, no. 3, pp. 369–373, 1992.

[61] M. I. Alvarez-Olmos, M. M. Barousse, L. Rajan, et al., "Vaginal lactobacilli in adolescents: presence and relationship to local and systemic immunity, and to bacterial vaginosis," *Sexually Transmitted Diseases*, vol. 31, no. 7, pp. 393–400, 2004.

[62] I. J. Rosenstein, E. A. Fontaine, D. J. Morgan, M. Sheehan, R. F. Lamont, and D. Taylor-Robinson, "Relationship between hydrogen peroxide-producing strains of lactobacilli and vaginosis-associated bacterial species in pregnant women," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 16, no. 7, pp. 517–522, 1997.

[63] N. W. McLean and J. A. McGroarty, "Growth inhibition of metronidazole-susceptible and metronidazole-resistant strains of *Gardnerella vaginalis* by lactobacilli in vitro," *Applied and Environmental Microbiology*, vol. 62, no. 3, pp. 1089–1092, 1996.

[64] S. J. Klebanoff, S. L. Hillier, D. A. Eschenbach, and A. M. Waltersdorph, "Control of the microbial flora of the vagina by H_2O_2-generating lactobacilli," *Journal of Infectious Diseases*, vol. 164, no. 1, pp. 94–100, 1991.

[65] C. Schmitt, J. D. Sobel, and C. Meriwether, "Bacterial vaginosis: treatment with clindamycin cream versus oral metronidazole," *Obstetrics and Gynecology*, vol. 79, no. 6, pp. 1020–1023, 1992.

[66] G. Reid and A. Seidenfeld, "Drug resistance amongst uropathogens isolated from women in a suburban population: laboratory findings over 7 years," *The Canadian Journal of Urology*, vol. 4, no. 4, pp. 432–437, 1997.

[67] W. E. Stamm and R. Raz, "Factors contributing to susceptibility of postmenopausal women to recurrent urinary tract infections," *Clinical Infectious Diseases*, vol. 28, no. 4, pp. 723–725, 1999.

[68] G. Reid, A. W. Bruce, R. L. Cook, and M. Llano, "Effect on the urogenital flora of antibiotic therapy for urinary tract infection," *Scandinavian Journal of Infectious Diseases*, vol. 22, pp. 43–47, 1990.

[69] T. M. Hooton, S. Hillier, C. Johnson, P. L. Roberts, and W. E. Stamm, "*Escherichia coli* bacteriuria and contraceptive method," *The Journal of the American Medical Association*, vol. 265, no. 1, pp. 64–69, 1991.

[70] S. J. Lee, Y. H. Shim, S. J. Cho, and J. W. Lee, "Probiotics prophylaxis in children with persistent primary vesicoureteral reflux," *Pediatric Nephrology*, vol. 22, no. 9, pp. 1315–1320, 2007.

[71] 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.

[72] 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," *The Journal of Urology*, vol. 138, no. 2, pp. 330–335, 1987.

[73] 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.

[74] G. Reid, K. Millsap, and A. W. Bruce, "Implantation of *Lactobacillus casei var rhamnosus* into vagina," *The Lancet*, vol. 344, no. 8931, p. 1229, 1994.

[75] P. Cadieux, J. Burton, G. Gardiner, et al., "*Lactobacillus* strains and vaginal ecology," *The Journal of the American Medical Association*, vol. 287, no. 15, pp. 1940–1941, 2002.

[76] G. Reid, A. W. Bruce, N. Fraser, C. Heinemann, J. Owen, and B. Henning, "Oral probiotics can resolve urogenital infections," *FEMS Immunology & Medical Microbiology*, vol. 30, no. 1, pp. 49–52, 2001.

[77] G. E. Gardiner, C. Heinemann, M. L. Baroja, et al., "Oral administration of the probiotic combination *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 for human intestinal applications," *International Dairy Journal*, vol. 12, no. 2-3, pp. 191–196, 2002.

[78] L. Morelli, D. Zonenenschain, M. Del Piano, and P. Cognein, "Utilization of the intestinal tract as a delivery system for urogenital probiotics," *Journal of Clinical Gastroenterology*, vol. 38, supplement 2, pp. S107–S110, 2004.

[79] G. Reid, D. Beuerman, C. Heinemann, and A. W. Bruce, "Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora," *FEMS Immunology & Medical Microbiology*, vol. 32, no. 1, pp. 37–41, 2001.

[80] G. Reid, D. Charbonneau, J. Erb, et al., "Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women," *FEMS Immunology and Medical Microbiology*, vol. 35, no. 2, pp. 131–134, 2003.

[81] A. Aroutcheva, D. Gariti, M. Simon, et al., "Defense factors of vaginal lactobacilli," *American Journal of Obstetrics and Gynecology*, vol. 185, no. 2, pp. 375–379, 2001.

[82] M. M. C. Velraeds, H. C. van der Mei, G. Reid, and H. J. Busscher, "Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* by biosurfactants from *Lactobacillus* isolates," *Applied and Environmental Microbiology*, vol. 62, no. 6, pp. 1958–1963, 1996.

[83] M. M. C. Velraeds, B. van der Belt, H. C. van der Mei, G. Reid, and H. J. Busscher, "Interference in initial adhesion

of uropathogenic bacteria and yeasts silicone rubber by a *Lactobacillus acidophilus* biosurfactant," *Journal of Medical Microbiology*, vol. 49, pp. 790–794, 1998.

[84] G. Reid, J. A. McGroarty, P. A. Gil Domingue, et al., "Coaggregation of urogenital bacteria in vitro and in vivo," *Current Microbiology*, vol. 20, no. 1, pp. 47–52, 1990.

[85] P. Mastromarino, P. Brigidi, S. Macchia, et al., "Characterization and selection of vaginal *Lactobacillus* strains for the preparation of vaginal tablets," *Journal of Applied Microbiology*, vol. 93, no. 5, pp. 884–893, 2002.

[86] J. A. Simoes, A. Aroutcheva, I. Heimler, S. Shott, and S. Faro, "Bacteriocin susceptibility of *Gardnerella vaginalis* and its relationship to biotype, genotype, and metronidazole susceptibility," *American Journal of Obstetrics and Gynecology*, vol. 185, no. 5, pp. 1186–1190, 2001.

[87] S. C. Corr, Y. Li, C. U. Riedel, P. W. O'Toole, C. Hill, and C. G. M. Gahan, "Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 18, pp. 7617–7621, 2007.

[88] E. Shalev, S. Battino, E. Weiner, R. Colodner, and Y. Keness, "Ingestion of yogurt containing *Lactobacillus acidophilus* compared with pasteurized yogurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis," *Archives of Family Medicine*, vol. 5, no. 10, pp. 593–596, 1996.

[89] A. Neri, G. Sabah, and Z. Samra, "Bacterial vaginosis in pregnancy treated with yoghurt," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 72, no. 1, pp. 17–19, 1993.

[90] B. Fredricsson, K. Englund, L. Weintraub, A. Olund, and C.-E. Nord, "Bacterial vaginosis is not a simple ecological disorder," *Gynecologic and Obstetric Investigation*, vol. 28, no. 3, pp. 156–160, 1989.

[91] A. Hallén, C. Jarstrand, and C. Påhlson, "Treatment of bacterial vaginosis with *Lactobacilli*," *Sexually Transmitted Diseases*, vol. 19, no. 3, pp. 146–148, 1992.

[92] K. Eriksson, B. Carlsson, U. Forsum, and P.-G. Larsson, "A double-blind treatment study of bacterial vaginosis with normal vaginal lactobacilli after an open treatment with vaginal clindamycin ovules," *Acta Dermato-Venereologica*, vol. 85, no. 1, pp. 42–46, 2005.

[93] K. C. Anukam, E. Osazuwa, G. I. Osemene, F. Ehigiagbe, 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. 8, no. 12-13, pp. 2772–2776, 2006.

[94] K. Anukam, E. Osazuwa, I. Ahonkhai, et al., "Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14: randomized, double-blind, placebo controlled trial," *Microbes and Infection*, vol. 8, no. 6, pp. 1450–1454, 2006.

[95] J. M. Marrazzo, R. L. Cook, H. C. Wiesenfeld, et al., "Women's satisfaction with an intravaginal *Lactobacillus* capsule for the treatment of bacterial vaginosis," *Journal of Women's Health*, vol. 15, no. 9, pp. 1053–1060, 2006.

[96] S. P. Borriello, W. P. Hammes, W. Holzapfel, et al., "Safety of probiotics that contain lactobacilli or bifidobacteria," *Clinical Infectious Diseases*, vol. 36, no. 6, pp. 775–780, 2003.

[97] J. P. Cannon, T. A. Lee, J. T. Bolanos, and L. H. Danziger, "Pathogenic relevance of *Lactobacillus*: a retrospective review of over 200 cases," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 24, no. 1, pp. 31–40, 2005.

[98] G. Reid, G. Gibson, M. E. Sanders, F. Guarner, and J. Versalovic, "Concerns over the Utrecht pancreatitis study," *The Lancet*, vol. 372, pp. 112–113, 2009.

[99] N. Rayes, D. Seehofer, S. Hansen, et al., "Early enteral supply of lactobacillus and fiber versus selective bowel decontamination: a controlled trial in liver transplant recipients," *Transplantation*, vol. 74, no. 1, pp. 123–128, 2002.

Journal of Mid-Life Health
Official publication of the Indian Menopause Society
Home Current issue Instructions Submit article

J Midlife Health. 2011 Jan-Jun; 2(1): 5–10. PMCID: PMC3156505
doi: 10.4103/0976-7800.83253: 10.4103/0976-7800.83253 PMID: 21897732

Role of probiotics in urogenital healthcare

Santosh S. Waigankar and Vimal Patel

Department of Urology, Jaslok Hospital and Research Center, Mumbai, India

Address for correspondence: Dr. Santosh S. Waigankar, Senior Resident in Urology, Jai Sudharma Nester D'souza Compound, Kalina Santacruz (E), Mumbai - 400 029, India. E-mail: sandoc2005@yahoo.com

Abstract

Urogenital infections are one of the most common causes for a woman to visit a gynecologist or a urologist. The well-known association between abnormal vaginal microbial flora and its formidable risk in the increased incidence of urinary tract infection underscores the importance of understanding the microbial flora and the efforts needed to maintain it, for ensuring urogenital health. Surprisingly in spite of the increased incidence urogenital infections receive very less attention from the medical fraternity. Growing awareness among people and newer advances in the medical field has brought them into the limelight. The importance of replenishing these depleting commensals with 'probiotics' has resurfaced in a big way. As the days go by science and medicines will touch new milestones, which will include probiotics. The value of a probiotics cannot be taken at face value. Probiotics must not be considered a panacea for treating urogenital infections. However, the available data promises that it will be a strong option in improving and maintaining urogenital health.

Keywords: Probiotics, urinary tract infections, urogenital infections

INTRODUCTION

Urogenital infections are one of the most common causes for a woman to visit a gynecologist or a urologist. P. B. Carter *et al*., estimated that one billion women around the world suffer from infections such as nonsexually transmitted urogenital infections, which include bacterial vaginosis (BV), urinary tract infection (UTI), and yeast vaginitis.[1] The well-known association between abnormal vaginal microbial flora and its formidable risk in the increased incidence of UTI underscores the importance of understanding the microbial flora and the efforts needed to maintain it, for ensuring urogenital health. Surprisingly in spite of the increased incidence, urogenital infections receive very little attention from the medical fraternity. Growing awareness among people and newer advances in the medical field has brought them into the limelight. The importance of replenishing these depleting commensals with 'probiotics' has resurfaced in a big way. Here we give a brief account of the health implications of probiotics in urogynecology.

VAGINAL MICROBIOTA

The microbial species that inhabit the vaginal tract play an important role in the maintenance of health and prevention of infection. The number of microbial species inhabiting the vagina amount to 50 as compared to the 800 species inhabiting the gut. Despite the close proximity of the vagina to the anus, the different microbes present in the vagina is much lower than in the gut. The reason is still unclear. The species that are present in the vaginal mucosa vary between premenopausal woman and those who have gone through menopause. Microbial flora of a healthy premenopausal woman is generally dominated by the *Lactobacillus* species, the most common of which are *L. iners, L. crispatus, L. gasseri*, and *L. jenesenii*, followed by *L. acidophilus, L. fermentum, L. plantarum, L. brevis, L. casei, L. vaginalis, L. delbrueckii, L. salivarius, L. reuteri*, and *L. rhamnosus*. All the factors such as hormonal changes (particularly estrogen), vaginal pH, and glycogen content can affect the colonization of the *Lactobacilli* in the vagina. Menstrual cycle can also cause hormonal changes.[2–11]

ROLE OF COMMENSAL MICROBIAL FLORA IN PREVENTING URINARY TRACT INFECTIONS

L. iners is the most common habitant found primarily in the white population.[9] After attaining menopause, some 25 to 30% of the women still have *Lactobacilli* present, and this number rises to between 60 and 100% with the use of vaginal or oral estrogen-replacement therapy.[4,6,7,12,13] The interest in the potential role of the 'normal' vaginal flora began almost 30 years ago with the finding of low lactobacilli counts in the vagina and urethra, in women suffering from recurrent UTIs.[14] J M Seddon *et al*., in their study of normal volunteers showed a marked variation in introital organisms mediated by changes in urinary frequency.[15] The defensive role of *Lactobacillus* also depends on multiple factors,[16–19] namely:

1. Their symbiosis with potential pathogens.
2. Their capability of producing antibacterial materials, such as hydrogen peroxide, to limit pathogen growth.
3. Their production of biosurfactants that inhibit pathogen adherence.
4. Their ability to prime macrophages, leukocytes, cytokines, and other host defenses.

REVISITING 'PROBIOTICS'

In our daily practice as private practitioners, specialists, and super specialists we come across many instances where the patients complain of loose motions after or during a course of antibiotics. We prescribe *'Sporolac,'* which alleviates the symptoms. Once again the *Lactobacillus* does the trick. Even the food industry has begun exploiting this 'bug' through a variety of their products, for example, Probiotic-curd/yogurt, which is nothing but usual *dahi* fortified with the 'probiotic bug'. When a new concept is introduced in the field of medicine, efforts are made to apply the same to various subspecialties like urogynecology. Defined as, "live microorganisms, which when administered in adequate amounts confer a health benefit on the host,"[20] probiotic strains have already been shown to effectively prevent diarrhea and hold a potential in preventing and treating tonsillitis, caries, renal calculi, and respiratory infections. The concept of probiotics came from the belief that a 'dismantled' microflora in the host could be restored by the exogenous application of bacteria commonly found in that area. Probiotic therapy was probably practiced many hundreds of years ago via fermented milk products such as those used by Nobel Laureate Elie Metchnikoff in the early part of the twentieth century. On account of the association with milk fermentation, most probiotic organisms have been ingested as dairy products, to confer benefits to the gut. Food products containing probiotics are almost exclusively dairy products — fluid milk and yogurt — due to the historical association of lactic acid bacteria with fermented milk. The most frequently used bacteria in these products include the *Lactobacillus* and *Bifidobacterium* species.

Commercially used probiotic species

Lactobacillus species

L. acidophilus, L. casei, L. fermentum, L. gasseri, L. johnsonii, L. lactis, L. paracasei, L. plantarum, L. reuteri, L. rhamnosus, L. salivarius

Bifidobacterium species

B. bifidum, B. breve, B. lactis, B. longum.

Streptococcus species

S. thermophilus

SCOPE OF THE PROBLEM

Urinary tract infection

All around the world, it is estimated that several hundred million women suffer from UTIs annually. This figure may even be an underestimate, given that the incidence of uncomplicated UTI in women is 0.5 episodes per person per year, with a recurrence rate of between 27 and 48%.[21] The annual cost of healthcare services is staggering, reaching $2 billion in the United States alone and over $6 billion worldwide.[22]

Multiple risk factors predisposed to UTI include, sexual intercourse with multiple partners and exposure to spermicidal agents.[23,24] Spermicides lead to loss of *Lactobacilli* and an increase in pH, which stimulates the growth of gram-negative organisms and subsequent UTI. McGroarty[25,26] has clearly demonstrated the impact of nonoxynal-9 on the growth and adherence of urogenital bacteria and Candida. Additional risk factors found in postmenopausal women include a history of previous genitourinary surgery, altered bladder function and loss of estrogen.[27,28]

Stamm and Hooton[29] reported *Escherichia coli* as the agent responsible in most cases (up to 85%), followed by *Staphylococcus saprophyticus*[29] and *Enterococci*.[30] The incidence of asymptomatic bacteriuria increases with age. Krieger *et al*., found that among school-aged girls, 1 to 2% are afflicted, compared to 2 to 5% of premenopausal women and 10 to 15% of postmenopausal women.[31] An estimated 13 to 27% of pregnant women with asymptomatic bacteriuria will develop acute pyelonephritis,[32] and if this occurs late in the third trimester, it may result in premature labor.

Yeast vaginitis

Abbott reported a high incidence, increased propensity for recurrence, and the increasing prevalence of non-albicans vaginitis which have underscored the need to better understand the epidemiology and pathogenesis, and to develop more accurate, rapid diagnostics and effective treatments.[33]

Even though treatment of yeast vaginitis, mainly with topical antimycotic drugs, is reasonably effective[34] recurrences are extremely frequent. Resistance to these drugs is increasing because many women self-diagnose, self-treat, and resort to over-the-counter antifungal medications.[35]

Synergy between an abnormal vaginal microbiota and the spread of human immunodeficiency virus in women

The data from 2001 reveals that the proportion of women between 15 and 24 years of age, living with HIV / AIDS is 62% worldwide (and 67% in the sub-Saharan Africa).[36] The main reasons are multifactorial, namely, lack of awareness and access to health information, rape, and dependence on men for housing and income, diminished educational opportunities, low male use of condoms, and young age at first intercourse.

Multiple studies have also shown that the absence or depletion of *Lactobacilli* in the vagina, associated with overgrowth of anerobic pathogens causing BV, results in significantly increased risk for HIV, gonorrhea, chlamydia, and herpes simplex virus infections.[37–41]

PROBIOTICS FOR VAGINAL AND BLADDER HEALTH

Lacidophilus NCFM for the gut[42] and *L rhamnosus* GR-1 and *L fermentum* RC-14 for the urogenital tract[43] have been extensively studied since the commercial introduction of *L. casei*, in the 1930s.

One must understand that simply showing an absence of *Lactobacilli* associated with disease does not mean that application of *Lactobacilli* to the vagina will prevent or treat that ailment. Colonization of that particular strain over a sufficient period of time (maybe days or weeks) may be necessary to confer health benefits to the host[42–45] However, longer-term colonization for months or years may not be necessary if the person's own *Lactobacilli* recolonize or the exogenous therapy is re-administered.

Reid *et al*., and Gardiner *et al*., suggest that insertion of *Lactobacilli* into the vagina via a pessary or capsule is an effective means of boosting the content of the flora and overcoming some pathogens or reducing their ability to dominate. This seems to be true for UTI pathogens.[46,47]

There is only anecdotal evidence to suggest that *Lactobacilli* can treat yeast vaginitis.[48,49] Use of skim milk-based preparations can also be effective,[50] but compliance may be a problem for some women which would negate the potential benefits. Reid *et al*. suggested an oral dosage which seems to require around 10^9 viable bacteria once or twice weekly, although a once-per-day vaginal protocol for three days might initially be required to deal with the urogenital tract.[51]

Most urogenital microflora originate from the gut. Studies have shown that the daily oral intake of *L rhamnosus* and *L fermentum* can modify the vaginal flora.[51,52] Administration of the probiotic organisms even normalized the flora, opening opportunities of a possible long-term therapy for pregnant women and those susceptible to UTI.[53]

The interactions among microbes at the vaginal mucosal surface has not been elucidated to date. A recent study conducted by Rachmilewitz D *et al*., on a dextran sulfate-induced mouse colitis model suggested that DNA extracted from probiotic organisms and *E. coli* could mediate anti-inflammatory activity and ameliorate disease through toll-like receptor 9 signaling.[54,55]

Although the actual mechanisms of action of probiotics in the vagina have not been proven they are probably multifactorial. *Lactobacilli* have been shown to produce biosurfactants and collagen-binding proteins that inhibit pathogen adhesion.[56,57] This may explain why vaginal mucosa is dominated by *Lactobacilli*, they can still be less receptive to pathogens. Mack *et al*, Pathmakanthan *et al* and Pessi *et al* mentioned cell-to-cell communication as another probable mechanism of action which may involve the signaling of mucus production, which in turn acts as a barrier to pathogens or as the signaling of anti-inflammatory cytokine production.[58–60] However, the question still remains as to how the normal flora becomes susceptible to infection. Rapid epithelial turnover could be the answer to it. Due to this, new surfaces are exposed to these pathogens. Also perianal or anal pathogens can gain easy access to the urinary tract via the bladder courtesy a small length of the urethra. This contributes to the change in milieu and makes one susceptible to infection.

Uehling *et al*., introduced the concept of the vaginal mucosal vaccine, which contained nonviable bacteria.[61] Although the results were encouraging it was still hard to believe that nonviable bacteria could ‘tickle’ the immune system, when live pathogens present in the vagina, in large numbers, in patients with recurrent UTI, did not.

PRACTICAL APPLICATION

At present, the practical application of probiotics to improve urogenital health is difficult. Although, what one can do is be vigilant regarding the same. A simple vaginal swab sent for bacterial culture or Gram staining will definitely help to ascertain whether the number of *Lactobacilli* are depleting with rising age or not. It will also help to catch the notorious gram-negative bacilli and treat them accordingly. Avoiding the injudicious use of antibiotics and discouraging over-the-counter prescription of drugs should be the 'mantra' adopted by all. Gregor Reid *et al*., mentions about an independent, third-party survey of more than 100 urologists attending the American Urological Association Annual Conference about 10 years ago, where almost 80% of the urologists stated that they would offer probiotics to patients with recurrent UTIs if available (unpublished data).[62]

ROUTE OF ADMINISTRATION

The known modes of administration are orally, vaginally, and so on. Insertion of *Lactobacilli* into the vagina via a pessary or capsule is an effective means of boosting the content of the flora and overcoming some pathogens or reducing their ability to dominate. This seems to be true for treatment of BV and possibly UTI pathogens. Reid and colleagues found that combination of *lactobacilli* strains reduce both yeast and bacterial pathogens in the vagina even when taken orally. It also provides a better cure rate when used with metronidazole instead of an antibiotic alone. A daily oral dose of 108 viable probiotic lactobacilli can restore and maintain the urogenital health of women.[63,64]

CONCLUSION

As the days go by science and medicines will touch new milestones, which will include probiotics. It is also necessary to make available valid and conclusive data on the various probiotic strains available commercially and their role in treating urogenital infections. The value of a probiotic cannot be taken on face value. Probiotics must not be considered a panacea in treating urogenital infections. However, the available data promises that it will be a strong option for improving and maintaining urogenital health.

Footnotes

Source of Support: Nil

Conflict of Interest: None declared.

REFERENCES

1. Carter PB, Cauci S, De Buysscher EV. Vol. 12. Herborn Dill, Germany: Old Herborn University Seminar Monograph; 1999. Top scientists put out call to action on women's health; pp. 5–6.

2. Anukam KC, Osazuwa EO, Ahonkhai I, Reid G. 16S rRNA gene sequence and phylogenetic tree of Lactobacillus species from the vagina of healthy Nigerian women. Afr J Biotechnol. 2005;4:1222–27.

3. Cribby S, Taylor M, Reid G. Vaginal microbiota and the use of probiotics. Interdiscip Perspect Infect Dis. 2008;2008:256–490. [PMCID: PMC2662373] [PubMed: 19343185]

4. Burton JP, Reid G. Evaluation of the bacterial vaginal flora of twenty postmenopausal women by direct (Nugent Score) and molecular (polymerase chain reaction and denaturing gradient gel electrophoresis) techniques. J Infect Dis. 2002;186:1777–80. [PubMed: 12447763]

5. Antonio MA, Hawes SE, Hillier SL. The identification of vaginal Lactobacillus species and the demographic and microbiologic characteristics of women colonized by these species. J Infect Dis. 1999;180:1950–6. [PubMed: 10558952]

6. Yoshimura T, Okamura H. Short term oral estriol treatment restores normal premenopausal vaginal flora

to elderly women. Maturitas. 2001;39:253–7. [PubMed: 11574185]

7. Devillard E, Burton JP, Hammond JA, Lam D, Reid G. Novel insight into the vaginal microflora in postmenopausal women under hormone replacement therapy as analyzed by PCR-denaturing gradient gel electrophoresis. Eur J Obstet Gynecol Reproductive Biol. 2004;117:76–81. [PubMed: 15474249]

8. Galask RP. Vaginal colonization by bacteria and yeast. Am J Obstet Gynecol. 1988;158:993–5. [PubMed: 3284368]

9. Burton JP, Cadieux P, Reid G. Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. Appl Environ Microbiol. 2003;69:97–101. [PMCID: PMC152440] [PubMed: 12513982]

10. Vásquez A, Jakobsson T, Ahrné S, Forsum U, Molin G. Vaginal *Lactobacillus flora* of healthy Swedish women. J Clin Microbiol. 2002;40:2746–9. [PMCID: PMC120688] [PubMed: 12149323]

11. Chan R C Y, Bruce A W, Reid G. "Adherence of cervical, vaginal and distal urethral normal microbial flora to human uroepithelial cells and the inhibition of adherence of gram-negative uropathogens by competitive exclusion," The Journal of Urology. 1984;131(3):596–601. [PubMed: 6422061]

12. Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. N Engl J Med. 1993;329:753–6. [PubMed: 8350884]

13. Bruce AW, Chadwick P, Hassan A, van Cott GF. Recurrent urethritis in women. Can Med Assoc J. 1973;108:973–976. [PMCID: PMC1941341] [PubMed: 4633489]

14. Bruce AW, Chadwick P, Hassan A, van Cott GF. Recurrent urethritis in women. Can Med Assoc J. 1973;108:973–976. [PMCID: PMC1941341] [PubMed: 4633489]

15. Seddon JM, Bruce AW, Chadwick P, Carter D. Introital bacterial flora - effect of increased frequency of micturition. Br J Urol. 1976;48:211–8. [PubMed: 938873]

16. Reid G. The scientific basis for probiotic strains of Lactobacillus. Appl Environ Microbiol. 1999;65:3763–6. [PMCID: PMC99697] [PubMed: 10473372]

17. Reid G, Bruce AW. Selection of Lactobacillus strains for urogenital probiotic applications. J Infect Dis. 2001;183(Suppl 1):S77–80. [PubMed: 11171021]

18. Klebanoff SJ, Watts DH, Mehlin C, Headley CM. Lactobacilli and vaginal host defense: Activation of the human immunodeficiency virus type 1 long terminal repeat, cytokine production, and NF-kappaB. J Infect Dis. 1999;179:653–60. [PubMed: 9952372]

19. Donders GG, Bosmans E, Dekeersmaecker A, Vereecken A, Van Bulck B, Spitz B, et al. Pathogenesis of abnormal vaginal bacterial flora. Am J Obstet Gynecol. 2000;182:872–8. [PubMed: 10764465]

20. Bengmark S. Gut microbial ecology in critical illness: is there a role for prebiotics, probiotics, and synbiotics? Curr Opin Crit Care. 2002;8:145–151. Available at: http://www.fao.org/es/ESN/Probio/probio.htm. FAO/WHO. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report. 2001 . [PubMed: 12386516]

21. Hooton TM, Scholes D, Hughes JP, Winter C, Roberts PL, Stapleton AE, et al. A prospective study of risk factors for symptomatic urinary tract infection in young women. N Engl J Med. 1996;335:468–74. [PubMed: 8672152]

22. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. Urinary tract infection: self-reported incidence and associated costs. Ann Epidemiol. 2000;10:509–15. [PubMed: 11118930]

23. Scholes D, Hooton TM, Roberts PL, Stapleton AE, Gupta K, Stamm WE. Risk factors for recurrent urinary tract infection in young women. J Infect Dis. 2000;182:1177–82. [PubMed: 10979915]

24. Gupta K, Hillier SL, Hooton TM, Roberts PL, Stamm WE. Effects of contraceptive method on the vaginal microbial flora: a prospective evaluation. J Infect Dis. 2000;181:595–601. [PubMed: 10669343]

25. McGroarty JA, Soboh F, Bruce AW, Reid G. The spermicidal compound nonoxynol-9 increases adhesion of Candida species to human epithelial cells in vitro. Infect Immun. 1990;58:2005–7. [PMCID: PMC258759] [PubMed: 2160437]

26. McGroarty JA, Chong S, Reid G, Bruce AW. Influence of the spermicidal compound nonoxynol-9 on the growth and adhesion of urogenital bacteria in vitro. Curr Microbiol. 1990;21:219–23.

27. Raz R, Gennesin Y, Wasser J, Stoler Z, Rosenfeld S, Rottensterich E, et al. Recurrent urinary tract infections in postmenopausal women. Clin Infect Dis. 2000;30:152–6. [PubMed: 10619744]

28. Raz R. Hormone replacement therapy or prophylaxis in postmenopausal women with recurrent urinary tract infection. J Infect Dis. 2001;183(Suppl 1):S74–6. [PubMed: 11171020]

29. Stamm WE, Hooton TM. Management of urinary tract infections in adults. N Engl J Med. 1993;329:1328–34. [PubMed: 8413414]

30. Reid G, Seidenfeld A. Drug resistance amongst uropathogens isolated from women in a suburban population: laboratory findings over 7 years. Can J Urol. 1997;4:432–7. [PubMed: 12735807]

31. Krieger JN. Complications and treatment of urinary tract infections during pregnancy. Urol Clin North Am. 1986;13:685–93. [PubMed: 3535210]

32. Nicolle LE. Prophylaxis: recurrent urinary tract infection in women. Infection. 1992;20(Suppl 3):S203–5. [PubMed: 1490746]

33. Abbott J. Clinical and microscopic diagnosis of vaginal yeast infection: a prospective analysis. Ann Emerg Med. 1995;25:587–91. [PubMed: 7741332]

34. Sobel JD. Vaginitis. N Engl J Med. 1997;337:1896–1903. [PubMed: 9407158]

35. Dun E. Antifungal resistance in yeast vaginitis. Yale J Biol Med. 2000;72:281–5. [PMCID: PMC2578967] [PubMed: 10907778]

36. New York: UNICEF; [Accessed February 26, 2004]. UNICEF, UNAIDS, and WHO 2002. Cited in: Lopez, V. M. 2002. “HIV and Young People. A Review of the State of the Epidemic and Its Impact on World Youth.” Report prepared as input for: UNICEF. 2003. World Youth Report 2003. Available at: http://www.unfpa.org/swp/2003/english/ch3/

37. Mbizvo EM, Msuya SE, Stray-Pedersen B, Sundby J, Chirenje MZ, Hussain A. HIV seroprevalence and its associations with the other reproductive tract infections in asymptomatic women in Harare, Zimbabwe. Int J STD AIDS. 2001;12:524–31. [PubMed: 11487393]

38. Sewankambo N, Gray RH, Wawer MJ, Paxton L, McNaim D, Wabwire-Mangen F, et al. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. Lancet. 1997;350:546–50. [PubMed: 9284776]

39. Taha TE, Hoover DR, Dallabetta GA, Kumwenda NI, Mtimavalye LA, Yang LP, et al. Bacterial vaginosis and disturbances of vaginal flora: Association with increased acquisition of HIV. AIDS. 1999;12:1699–706. [PubMed: 9764791]

40. Wiesenfeld HC, Hillier SL, Krohn MA, Landers DV, Sweet RL. Bacterial vaginosis is a strong

predictor of Neisseria gonorrhoeae and Chlamydia trachomatis infection. Clin Infect Dis. 2003;36:663–8. [PubMed: 12594649]

41. Cherpes TL, Meyn LA, Krohn MA, Lurie JG, Hillier SL. Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis. Clin Infect Dis. 2003;37:319–25. [PubMed: 12884154]

42. Sanders ME, Klaenhammer TR. Invited review: the scientific basis of Lactobacillus acidophilus NCFM functionality as a probiotic. J Dairy Sci. 2001;84:319–31. [PubMed: 11233016]

43. Reid G, Cook RL, Bruce AW. Examination of strains of lactobacilli for properties which may influence bacterial interference in the urinary tract. J Urol. 1987;138:330–335. [PubMed: 3599250]

44. Reid G, Millsap K, Bruce AW. Implantation of Lactobacillus casei var rhamnosus into the vagina. Lancet. 1994;344:1229. [PubMed: 7934561]

45. Cadieux P, Burton J, Gardiner G, Braunstein I, Bruce AW, Kang CY, et al. Lactobacillus strains and vaginal ecology. JAMA. 2002;287:1940–1. [PubMed: 11960535]

46. Reid G, Bruce AW, Taylor M. Instillation of Lactobacillus and stimulation of indigenous organisms to prevent recurrence of urinary tract infections. Microecol Ther. 1995;23:32–45.

47. Gardiner GE, Heinemann C, Bruce AW, Beuerman D, Reid G. Persistence of *Lactobacillus fermentum* RC-14 and L rhamnosus GR-1, but not L rhamnosus GG in the human vagina as demonstrated by randomly amplified polymorphic DNA (RAPD) Clin Diag Lab Immunol. 2002;9:92–6. [PMCID: PMC119863] [PubMed: 11777835]

48. Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. Ann Intern Med. 1992;116:353–7. [PubMed: 1736766]

49. Shalev E, Battino S, Weiner E, et al. Ingestion of yogurt containing Lactobacillus acidophilus compared with pasteurized yogurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis. Arch Fam Med. 1996;5:593–6. [PubMed: 8930233]

50. Bruce AW, Reid G. Intravaginal instillation of lactobacilli for prevention of recurrent urinary tract infections. Can J Microbiol. 1988;34:339–43. [PubMed: 3138016]

51. Reid G, Buerman D, Heinemann C, Bruce AW. Probiotic Lactobacillus dose required to restore and maintain a normal vaginal flora. FEMS Immunol Med Microbiol. 2001;32:37–41. [PubMed: 11750220]

52. Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. FEMS Immunol Med Microbiol. 2001;30:49–52. [PubMed: 11172991]

53. Reid G, Tieszer C. Preferential adhesion of bacteria from a mixed population to a urinary catheter. Cells Materials. 1993;3:171–6.

54. Rachmilewitz D, Katakura K, Karmeli F, Hayashi T, Reinus C, Rudensky B, et al. Toll-like receptor 9 signaling mediates the anti-inflammatory effects of probiotics in murine experimental colitis. Gastroenterology. 2004;126:520–8. [PubMed: 14762789]

55. Hopkin M. Probiotic bacteria health boon. Nature. 2004;432:427.

56. Heinemann C, van Hylckama Vlieg JE, Janssen DB, Busscher HJ, van der Mei HC, Reid G. Purification and characterization of a surface-binding protein from *Lactobacillus fermentum* RC-14 inhibiting Enterococcus faecalis 1131 adhesion. FEMS Microbiol Lett. 2000;190:177–80. [PubMed: 10981710]

57. Gan BS, Kim JG, Reid P, Cadieux, Howard JC. Lactobacillus fermentum RC-14 inhibits *Staphylococcus aureus* infection of surgical implants in rats. J Infect Dis. 2002;185:1369–72. [PubMed: 12001060]

58. Mack DR, Michail S, Wei S, McDougall L, Hollingsworth MA. Probiotics inhibit enteropathogenic *E. coli* adherence *in vitro* by inducing intestinal mucin gene expression. Am J Physiol. 1999;276(4 Pt 1):G941–50. [PubMed: 10198338]

59. Pathmakanthan S, Li CK, Cowie J, Hawkey CJ. *Lactobacillus plantarum* 299: Beneficial *in vitro* immunomodulation in cells extracted from inflamed human colon. J Gastroenterol Hepatol. 2004;19:166–73. [PubMed: 14731126]

60. Pessi T, Sutas Y, Hurme M, Isolauri E. Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. Clin Exp Allergy. 2000;30:1804–8. [PubMed: 11122221]

61. Uehling DT, Hopkins WJ, Elkahwaji JE, Schmidt DM, Leverson GE, et al. Phase 2 clinical trial of a vaginal mucosal vaccine for urinary tract infections. J Urol. 2003;170:867–9. [PubMed: 12913718]

62. Reid G, Bruce AW. Could probiotics be an option for treating and preventing urogenital infections? Medscape Womens Health. 2001;6:9. [PubMed: 11698931]

63. Reid G, Beuerman D, Heinemann C, Bruce AW. Probiotic lactobacillus dose required to restore and maintain a normal vaginal flora. FEMS Immunol Med Microbiol. 2001;32:37–41. [PubMed: 11750220]

64. Reid G, Charbonneau D, Erb J, Kochanowski B, Beuerman D, Poehner R, et al. Oral use of *Lactobacillus rhamnosus* GR-1 and *L.fermentum* RC-14 significantly alters vaginal flora: Randomized, placebo-controlled trial in 64 healthy women. FEMS Immunol Med Microbiol. 2003;35:131–4. [PubMed: 12628548]