ABSTRACT: Infections of the lower urinary tract occur frequently in young women, during pregnancy, and in pre- and post-menopausal women. Multiple drug resistance arising in different microbial isolates has complicated the therapeutic management of urinary tract infections. Because of the chronic nature of these urinary tract infections and the potential for antibiotic resistance, a natural remedial approach for prevention and treatment is desirable. In this milieu, several recent clinical researches have nominated probiotics as a promising natural option for long-term prevention of these ailments.

KEY WORDS: Lactobacillus, menopause, probriotics, urinary tract infections, UTI, vaginosis.

INTRODUCTION

Annually, the prevalence of urinary tract infection, bacterial vaginosis, and yeast vaginitis, estimated to influence one billion women each year (the rate for urinary tract infection alone is 0.5 cases per person per year), means that the probability of infection is high. Indeed, the presence of pathogens dominating the vagina increases several folds the likelihood that a woman will develop a symptomatic infection. In short, an abnormal microbiota may indeed lead to a symptomatic vaginal or bladder infection. The concept of restoring the Lactobacillus content of the vaginal microbiota as a barrier to prevent infection was first conceived by Canadian urologist Andrew Bruce in the early 1970s. Extensive research since then has shown that certain Lactobacillus strains are able to colonize the vagina following vaginal suppository use (Cadieux et al., 2002) and reduce the risk of urinary tract infection, yeast vaginitis (Reid et al., 1995; Gil et al., 2010; Verdenelli et al., 2014), and bacterial vaginosis (Reid et al., 2001a,b). Although most patients react to antimicrobial treatment, the reappearance rate is quite high in many cases and several cases are also accompanied with side effects. Some parts of the female reproductive system, particularly the vulva and vagina, are characterized by a normal microbiota that includes intense populations of different species of fungi and bacteria. A few generalizations have also been made about what constitutes the commensal vaginal microflora of a hale and hearty, post/pre-menopausal, sexually vital, non-pregnant female. The vaginal microflora is usually dominated by the lactic acid bacteria (LAB), which is preferably appropriate to the vaginal atmosphere. As facultative or obligate anaerobes, they flourish in a low O₂ environment such as the vagina that usually has only 2% of the O₂ content of outside air. Lactobacilli are also acidophilic, and therefore can easily tolerate the acidic pH of the vagina. Though, the optimum pH for their growth is pH 6.0, the range of tolerance varies from pH 3.5 to 6.8. During most part of the menstrual cycle, the vaginal pH is maintained around 4.0, but usually increases to near-neutrality for two days at the commencement of menstruation. Since LAB produce lactic acid, they also contribute to the acidity of the vagina. These bacteria colonize well to the vaginal cells and seldom cause infections in women. Also, their growth is encouraged on secretions from the vaginal mucosa, as well as shedded vaginal epithelial cells. Besides LAB, the usual vaginal microbiota also comprises bacteria Gardnerella vaginalis, and fungi Candida albicans. Although, these microorganisms usually occur in healthy individuals, either of them can cause infections of the vagina and vulva (such as vaginosis, vulvovaginitis, vaginal candidiasis, vaginitis) if their populations flourish too much (Gil et al., 2010; Verdenelli et al., 2014). It has been recommended that these infections often
occur in situations where LAB does not dictate the microflora (e.g., when antibiotics kill the bacteria), and therefore, when the vaginal pH is elevated. Indeed, *G. vaginalis* growth is inhibited below pH 4.5, but it grows well between pH 6.0 and 6.5. Similarly, *C. albicans* can also grow well among pH 5.1 to 6.9. It is significant to note that *C. albicans* can stay alive in a low, acidic pH environment (pH 2), but its growth is inhibited under such acidic environment. Apart from antibiotics, certain behavioral factors such as frequent douching may reduce the vaginal *Lactobacillus* counts and augment the numbers of *G. vaginalis*, since douching fluids hold antimicrobial compounds that may influence these bacteria. Douching has also been linked with an increased risk of vaginosis, cervical cancer, pelvic inflammatory disease, HIV attainment, and pre-term delivery of babies by pregnant women. Overall, it appears that the safeguarding of “natural” vaginal health may involve conditions that support vaginal resident populations of LAB while discouraging vaginal populations of *C. albicans* and *G. vaginalis*.

### TABLE 1. Effective probiotic lactobacilli against UTIs.

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>Lactobacillus rhamnosus</em> GR-1</td>
<td>Anukam et al., 2006</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> GR-1</td>
<td>Reid et al., 2001b</td>
</tr>
<tr>
<td><em>L. reuteri</em> RC-14</td>
<td>Anukam et al., 2006</td>
</tr>
<tr>
<td><em>L. acidophilus</em></td>
<td>Hallén et al., 1992</td>
</tr>
<tr>
<td><em>L. acidophilus</em> NCDO 1748</td>
<td>Fredricsson et al., 1989</td>
</tr>
<tr>
<td><em>L. fermentum</em></td>
<td>Eriksson et al., 2005</td>
</tr>
<tr>
<td><em>L. fermentum</em> B-54</td>
<td>Eriksson et al., 2005</td>
</tr>
<tr>
<td><em>L. fermentum</em> BR11</td>
<td>Heineman et al., 2000</td>
</tr>
<tr>
<td><em>L. gasseri</em></td>
<td>Eriksson et al., 2005</td>
</tr>
<tr>
<td><em>L. gasseri</em> 335</td>
<td>Mastromarino et al., 2002</td>
</tr>
<tr>
<td><em>L. salivarius</em> FV2</td>
<td>Mastromarino et al., 2002</td>
</tr>
<tr>
<td><em>L. crispatus</em></td>
<td>Antonio et al., 1999, Zhou and Bent, 2004</td>
</tr>
<tr>
<td><em>L. jensenii</em></td>
<td>Antonio et al., 1999</td>
</tr>
<tr>
<td><em>L. brevis</em> CD2</td>
<td>Mastromarino et al., 2002</td>
</tr>
<tr>
<td><em>L. iners</em></td>
<td>Zhou and Bent, 2004 ; Burton et al., 2003</td>
</tr>
<tr>
<td><em>L. reuteri</em> NCIB 11951</td>
<td>Heinemann et al., 2000</td>
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</table>

### TABLE 2. Different case studies of probiotics in UTIs.

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>120 children with persistent primary vesicoureteral reflux</td>
<td><em>L. acidophilus</em></td>
<td>Effective as trimethoprim/sulfamethoxazole in reducing the rate of UTI.</td>
<td>Lee et al., 2007</td>
</tr>
<tr>
<td>Recurrence of UTIs</td>
<td><em>L. rhamnosus</em> GR-1 and <em>L. fermentum</em> B-54</td>
<td>Reduced UTI recurrences from an average of 6 to 1.6 per year.</td>
<td>Reid et al., 1995</td>
</tr>
<tr>
<td>Randomized, placebo-controlled trial of 42 healthy women</td>
<td><em>L. rhamnosus</em> GR-1 and <em>L. fermentum</em> RC-14</td>
<td>Women in the <em>Lactobacillus</em> treatment group had a greater number of vaginal Lactobacilli.</td>
<td>Reid et al., 2001b</td>
</tr>
<tr>
<td>46 premenopausal women with episodes of BV and/or vaginal candidiasis</td>
<td>Probiotic yoghurt versus pasteurized one</td>
<td>60% reduction in BV episodes among patients consuming probiotic yoghurt after one month; 25% reduction in subjects receiving pasteurized yoghurt</td>
<td>Shalev et al., 1996</td>
</tr>
<tr>
<td>84 women in the first trimester of pregnancy to observe the effect on BV</td>
<td>Probiotic yoghurt containing <em>L. acidophilus</em></td>
<td>Release of an amine fishy odor; vaginal pH greater than 4.5; clue cells in the vaginal fluid; milky homogenous vaginal discharge absent in 88%</td>
<td>Neri et al., 1993</td>
</tr>
<tr>
<td>BV in 57 young women</td>
<td><em>L. acidophilus</em></td>
<td>BV symptoms absent in 57%</td>
<td>Hallén et al., 1992</td>
</tr>
<tr>
<td>40 women (ages 18 to 50) with BV; single-blind study</td>
<td><em>L. rhamnosus</em> GR-1 and <em>L. reuteri</em> RC-14</td>
<td>BV cure rate of 88% in the probiotic group</td>
<td>Anukam et al., 2006</td>
</tr>
<tr>
<td>Randomized, double-blind, multicentric, placebo-controlled trial, including a total of 544 subjects (with vaginal infection)</td>
<td><em>L. rhamnosus</em> GR-1 and <em>L. reuteri</em> RC-14</td>
<td>Restitution to balanced vaginal microbiota was reported in 40 subjects (26.9%) in the placebo group, compared to 243 subjects (61.5%) in the probiotic group</td>
<td>Vujic et al., 2013</td>
</tr>
<tr>
<td>268 subjects, randomized, double-blind, placebo-controlled 6 month trial</td>
<td>Oral metronidazole 400 mg b.i.d. for 7 days followed by vaginal pessary containing <em>L. acidophilus</em> KS400 ≥10⁷ CFU</td>
<td>BV cure rate 72% compared to 73%</td>
<td>Bradshaw et al., 2006</td>
</tr>
</tbody>
</table>
Supporting this observation, probiotic therapy, in which LAB (particularly *Lactobacilli*) are applied directly to the vagina or consumed orally, could aid in increasing the number of LAB in or around the vagina, and serve the patients with vaginitis or vaginosis (Table 1 and 2).

Traditional and modern medicine have also suggested to use fermented foods an alternative for the prevention and treatment of malnutrition, chronic diarrhea, gastrointestinal diseases and other conditions, since consumption of these foods provides an entry route for the common biological agents that comprise the beneficial intestinal flora, also referred as probiotics (Fitzgerald et al., 2006; Parvez et al., 2006; Nagpal et al., 2012a; Shibly and Mishra, 2013). Probiotics are live microorganisms present in foods which when ingested in sufficient quantities can have specific physiological benefits such as anti-pathogenic, anti-diarrheal, anti-carcinogenic, anti-diabetic, anti-cholesterolemic etc. to individual health (Fuller, 1991; Goldin, 1998; Isolauri, 2001; Ouwehand, et al., 2002; Sanders, 2003; O’Sullivan et al., 2005; Salminen et al., 2005; Kumar et al., 2009; 2010; 2012a, b; Nagpal et al., 2007; 2012b; Sanders et al., 2013). An important group of probiotics that are used traditionally are LAB, i.e., *Lactococcus, Streptococcus, Lactobacillus* and *Bifidobacterium*.

At times, during UTI, gut bacteria such as *E. coli* may take up residence in the vagina and displace LAB. Women take antimicrobial medications to fight these infections, and these medications wash out most of the bacteria, including both probiotic and pathogenic bacteria. However, in some cases, the pathogenic bacteria grow back to cause a recurrent UTI. In this context, probiotics could help to re-flourish the natural bacterial environment to make it hard for the pathogenic bacteria to cause another infection (Martin et al., 2012; Verdenelli et al., 2014). Almost 20% of women who experience a UTI have a recurrence of the infection, and about 30% of these women also experience a second recurrence. Generally, the UTIs are caused when bacteria pass through the urinary tract by wiping from back to front after a bowel movement (thereby pushing the bacteria closer to the urethra), by making sex (where germs already in the vagina can be pushed into the urethra), and due to waiting too long to urinate (the urine allows a breeding ground for bacteria). The microbial species that usually dwell in the vaginal tract play a vital function in the maintenance of good health and prevention of infection. Almost fifty different microbial species have been recovered from the vaginal route (Redondo-Lopez et al., 1990; Anukam et al., 2005; Oakley et al., 2008). Studies in *vitro* and in women have observed that a multispecies microflora, frequently related with bacterial vaginosis (BV), is present in opaque biofilms (Reid et al., 1990a; Heinemann and Reid, 2005; Swidsinski et al., 2005), while a LAB dominant microbiota may be evenly distributed on the epithelium (Reid et al., 1990b; Heinemann and Reid, 2005).

**WOMEN OF CHILD BEARING AGE (PREMENOPAUSAL WOMEN)**

The microbiota of healthy woman is normally dominated by LAB, the most frequently occurring ones are *L. iners, L. crispatus, L. gaseri, L. jeneseni*, followed by *L. fermentum, L. acidophilus, L. plantarum, L. brevis, L. vaginalis, L. casei, L. delbrueckii, L. salivarius, L. rhamnosus* and *L. reuteri* (Anukam et al., 2005; Heinemann and Reid, 2005; Fredricks et al., 2005; Hill et al., 2005; Antonio et al., 1999; V’asquez et al., 2002). However, the potential of LAB to stick to epithelial cells and inhabit the vagina is influenced by different factors such as vaginal pH, hormonal changes (estrogen) and glycogen content. The colonization of LAB to vaginal epithelial cells may be increased with elevated concentrations of estrogen and changes in vaginal microflora during menstrual cycle (Chen et al., 1988). With reduced estrogen levels linked with menopause, there is also a decline in LAB present in the vaginal route of menopausal women (Heinemann and Reid, 2005; Burton et al., 2003; Raz et al., 2003). Several behavioral risk factors are at play in young women who suffer from recurrent UTIs, for instance, increased frequency of intercourse, new sexual partners and use of spermicides (Anukam et al., 2006). Intercourse and spermicide experience could increase the rate of vaginal and periurethral colonization with *E. coli*. When a first UTI is caused by *E. coli*, the risk of a second infection within 6 months is greater than when a first infection is cause by another uropathogen (Foxman et al., 2000). Dysfunctional voiding patterns in which there is increased tone of the external sphincter during micturition can also be associated with recurrent UTI in otherwise urologically normal Women (Amsel et al., 1983). In young women, recurrent UTI may also be attributed to some non-behavioral risk factors such as a history of UTI before age 15 and a maternal history of UTI, also hinting at the involvement of anatomic and genetic factors (Anukam et al., 2006). Most women with recurrent UTIs do not have any functional or anatomic abnormalities of the urinary tract, and extensive radiologic and cystoscopic examination is not indicated (Hooton, 2001; Colli et al., 1997).

**MENOPAUSAL WOMEN**

In premenopausal women, almost 90% of the vaginal microflora is LAB, which protects against colonization with uropathogens such as *E. coli*. However, since estrogen loss at menopause results in thinning of the vaginal epithelium and decreased amounts of glycogen, the resulting environment is antagonistic to LAB, and hence the LAB numbers decrease. This results in an elevated vaginal pH, and hence, an increased tendency for colonization with uropathogens (Gupta and Stamm, 1999). Women who are non-secretors of histocompatibility blood-group antigens are at augmented risk of recurrent UTI, possibly as a result of the attachment of P-fimbriated *E. coli* to glycolipids on vaginal and uroepithelial cells (Gupta and Stamm, 1999). Non-secretor status is a more significant risk factor in postmenopausal than in premenopausal women. Postmenopausal women who suffer from incontinence and have major pelvic floor prolapse and prominent post-void residual volumes are at augmented risk for recurrent UTI (Gupta and Stamm, 1999). Other significant
factors for recurrent UTI in postmenopausal woman are diabetes mellitus and a previous history of UTI (Kane and Pierce, 2001).

Postmenopausal women are also more vulnerable to UTI, however it is hypothesized that LAB colonization on vagina could serve protection from these pathologies (Raz et al., 2003). The vertical mechanism(s) by which these probiotic bacteria do this are still unclear, it appears to involve an ability to colonize and populate the vaginal epithelium and mucin layer, thereby inhibiting the pathogens from taking over (Reid et al., 2001b; Osset et al., 2001; Heinemann et al., 2000; H’utt et al., 2006), to diminish pathogen virulence (Laughton et al., 2006; Medellin et al., 2007), and to transform host defenses (Kim et al., 2006). Hormone replacement therapy (HRT) could also change the bacterial microflora of the vaginal tract of postmenopausal women, help in restoring a LAB dominated state, and reduce the incidences of UTIs (Raz et al., 2003). Taking combination of conjugated equine estrogen and progesterone as HRT, only 1 to 3 species of bacteria, generally LAB, could be established in the vaginal mucosa of most of the women (Heinemann and Reid, 2005). In postmenopausal women not getting HRT, vaginal mucosa could be populated with several organisms, many of which could have pathogenic potential, for example Bacteroides, Gardnerella, Prevotella (linked with BV), and Enterococcus and E. coli (linked with UTIs) (Heinemann and Reid, 2005). While a vaginal route dominated by LAB appears to defend the host against different vaginal infections, it does not fully prevent colonization by other microbial species. Pathogenic microorganisms may still coexist with these commensal organisms such as LAB, since G. vaginalis, a pathogen associated with BV, has been observed in vaginal samples which also contain species of LAB (Burton and Reid, 2002). Fascinatingly, G. vaginalis could be displaced beyond assessable limits within 21 days, following a single intravaginal instillation of probiotic LAB (Burton et al., 2003). From different experiments exposing the diversity of vaginal microbiota, it is evident that the balance between a healthy and diseased state involves some sort of balance or see-saw effect which can swing in either direction depending on a number of factors, such as douching, hormone levels, sexual practices, as well bacterial interactions and host defenses (Bruce et al., 1973; Reid et al., 2000).

**BACTERIAL VAGINOSIS**

BV is a disease with unfamiliar etiology, characterized by reduced LAB count and elevated numbers of anaerobes and gram-negative rods (Fredricks et al., 2007). It is one of the most common vaginal infections, and the most general symptom is malodors discharge. In comparison, the gut is inhabited with more than eight hundred species of microorganism, most of which are shed in feces, and many of these are well ready to be pathogenic (or opportunistic pathogenic). Regardless of the close nearness of the vagina to the anus, the diversity of microflora present in the vagina is much lower than in the gut, possibly due to the poor receptivity of the vagina, different nutrient availability compared to the gut, and antagonism with indigenous microorganisms. Some species (such as E. coli and Streptococci) that are usually observed in the gut, can also be found in the vagina, indicating that proper nutrients, receptors and O2 tension are present for these microorganisms to flourish.

Many pathogenic microorganisms are able to communicate a disease to the vagina such as yeast vaginitis, BV and UTIs, thereby collectively causing predictable one billion or more cases per year (Reid and Bruce, 2006; Foxman et al., 2000; Allsworth et al., 2007; Sobel, 2007). However, it is also speculated that the causative microorganisms can be transmitted by sexual associates. Yeast vaginitis is characterized by irritation, white discharge and local itching. The majority of infections are caused by C. albicans, C. krusei, C. glabrata and C. tropicalis (Sobel, 2007). It is generally diagnosed by microscopic examination of dense numbers of yeast cells on a vaginal smear, by physical examination and by the presence of a white, mucous-like yeast discharge. However, the beginning of infection does not appear to require the yeast displacing or killing off the LAB, since LAB are frequently found in patients with yeast vaginitis.

UTIs occur when pathogenic bacteria rise from the vagina and replicate, from time to time, within the bladder urothelium (Reid and Bruce, 2006; Hooton et al., 1996; Imizraliohi et al., 2008). These infections are common among women, with an estimated fifty percent suffering at some time in their life. Signs and symptoms involve dysuria, suprapubic pain, pyuria, frequent and sporadic hematuria, and painful micturition. Asymptomatic bacteriuria is also a frequent incidence, mostly among the elderly. The most regular pathogen is E. coli, followed by Staphylococcus saprophyticus, and Enterococcus faecalis (Imizraliohi et al., 2008). Diagnosis can be done by presence of signs and symptoms, and by urine samples containing over 10^5 organisms/ ml of the pathogens. In some cases, E. coli may infect the bladder epithelium and form thick biofilms that are recalcitrant to antibiotics (Rosen et al., 2007). In women with no history of UTIs, the vagina and perineum is most frequently colonized by LAB (Bruce et al., 1973); while in women with recurrent UTIs, there is an inverse relationship between LAB and E. coli (Gupta et al., 1998). suggesting that LAB could play a role in combating these infection. BV is the most common urogenital disorder among women of reproductive age. The vaginal microflora of BV patients typically contains a broader range of species than that observed in healthy subjects, with Bacteroides spp., Atopohium vaginae, Mobiluncus spp., G. vaginalis, Mycoplasma hominis, Megaspheira, Prevotella and Peptostreptococcus being the mostly prevalent ones (Oakley et al., 2008; Reid et al., 2004; Hill, 1993; Burton et al., 2004; Martinez et al., 2008; Anukam et al., 2007). BV is related with multiple species of bacteria that arise in majority of cases, and basically consists of a high vaginal pH (>4.5) and depletion of LAB. It affects women of different age groups, and is commonly asymptomatic (Klebanoff et al., 2004). When signs and symptoms do occur, they include discharge, fishy odor and
vaginal pH above 4.5. Indeed, this forms the basis of the Amsel criteria for BV diagnosis, i.e. the presence of at least 3 of the following 4 criteria: (1) Fishy smell or release of amine upon addition of 10% potassium hydroxide, (2) a vaginal pH higher than 4.5, (3) at least 20% of clue cells detected (which are vaginal cells occupied by gram-negative rods), and (4) vaginal discharge is milky and homogeneous (Amsel et al., 1983). In addition, Nugent score, a gram-staining method, has also been used, which is based on the morphology of bacteria present in vagina swab samples (Nugent et al., 1991). A healthy woman score is given to samples showing predominantly gram-positive rods indicative of LAB, while the presence of predominantly small and curved shaped gram-negative rods and gram-positive cocci, along with the absence of LAB, is indicative of BV. The BV Blue test is another kit used to diagnose BV that relies upon detection of sialidase released by pathogens associated with the condition (Myziuk et al., 2003; Milani et al., 2003). During pregnancy, BV can also raise the risk of pre-term labor and low birth weight (Gravett et al., 1986; Jacobsson et al., 2002). Other problems connected with BV include UTI, pelvic inflammatory disease and augmented susceptibility to sexually transmitted diseases, including HIV (Sevankanbo et al., 1997; Cherpes et al., 2003; Sharami et al., 2007; Gallo et al., 2008). The organisms associated with BV form thick biofilms on the vaginal epithelium that are linked with increased resistance to lactic acid and H₂O₂, which are usually antagonistic to planktonic organisms (Patterson et al., 2007). The expression of certain inflammatory factors such as IL-8 and IL-1 are also stimulated by these biofilms in the host (Simhan et al., 2005). Although, any clinically protective role of H₂O₂ produced by LAB against BV has not been established or proven; the augmented prevalence of H₂O₂-producing vaginal LAB in healthy women has speculated it as a self-protective factor (Hillier et al., 1992). It has also been observed that women with more H₂O₂-producing vaginal LAB such as L. crispatus or L. jensenii have a significantly lower incidence of BV than women with a different vaginal microflora (Antonio et al., 1999).

**Identification of microorganism**

In women with symptoms of BV, microarrays have shown the expression of a number of inflammatory genes such as TNF-2 allele that augment the risk of preterm delivery. Although, culture-based techniques allow strains to be identified and used for further experimentation, the huge challenge is the ability to grow numerous bacterial species. Consequently, next-generation techniques are being exploited to recognize the diverse range of the vaginal microflora. This has been accomplished by non-culture based methods and analyzing the microbial ribosomal DNA sequences (Oakley et al., 2008; Fredricks et al., 2005), using a combination of PCR and denaturing gel gradient electrophoresis (Anukam et al., 2005; Heinemann and Reid, 2005; Burton et al., 2002; Burton et al., 2003; Devillard et al., 2004), and by using degenerate, universal PCR primers (Hill et al., 2005). It is also generally observed that the microflora present in the vaginal mucosa differ between premenopausal women and those who have gone through menopause.

**Altered immunity in bacterial vaginosis**

Innate immunity plays an important role in the shift from a healthy state to BV (Witkin et al., 2007). The suggested mechanisms are microbial induced inhibition of Toll-like receptor (TLR) expression, pro-inflammatory immunity, lack of 70-kDa heat-shock protein production, and a shortage in vaginal mannose-binding lectin (MBL) concentrations decreasing the capacity for pathogen inhibition. In postmenopausal women, BV is associated with apparent reduced expression of host antimicrobial factors (Dahn et al., 2008). It has also been observed that probiotic L. rhamnosus GR-1 introduction to the vagina of premenopausal women could result in gene expression changes and enhanced expression levels of some antimicrobial defenses (Kirjavainen et al., 2008).

Bacteria present in the vaginas of women without BV sustain vaginal epithelial cell TLR activation at a steady level, resulting in sufficient cytokine production to slow down the proliferation of unusual BV-linked bacteria. Examination of vaginal secretions of women with LAB-dominated microflora is accompanied with lower concentrations of pro-inflammatory cytokines viz. TNF-α, IL-6 and IL-1β (Donders et al., 2003). However, this capability to prevent replication of abnormal microflora could be diminished, and the possibility of developing BV could be augmented under conditions in which vaginal TLR expression is inhibited. Vaginal bacteria could inactivate TLRs by the stimulation of immunosuppressive anti-inflammatory cytokines, such as IL-10, and also by direct inhibition of pathogen-associated molecular pattern–TLR interaction (Netea et al., 2004). Secretory leukocyte protease inhibitor, a protein secreted by vaginal epithelia, inhibits neutrophil elastase activity, down-regulates the pro-inflammatory immune responses of monocytes and macrophages, and also predominantly inhibits TLR activation (Greene et al., 2004). However, microbial products such as proteases and other degradative products produced by BV-related bacteria may directly react with and enzymatically inactivate epithelial cell TLRs. In addition, an accumulation of unsaturated fatty acids in the vagina by the action of BV-linked bacteria and the alteration of host fatty acids by these organisms could also result in the blockage of local TLR4 and TLR2 activation and further obstruct the development of an effective antimicrobial immune response (Lee et al., 2003). Subsequently, in the absence of an effective immune response, atypical microorganisms present in the vagina in low numbers could be free to multiply. It has been shown that a polymorphism in the gene coding for TLR4, which results in noticeably reduced TLR activity, is linked with an altered vaginal immune response and growth of BV in pregnant women (Genc et al., 2005). Hsp70 has been recognized as
a constituent of extracellular vaginal fluids in women with recurrent vulvovaginal candidiasis and in a subset of women with BV (Giraldo et al., 1999; Genc et al., 2005). Additional studies have also exposed that hsp70 is linked with high vaginal levels of nitric oxide (Genc et al., 2006). Thus, by activating TLRs and stimulating nitric acid production, hsp70-producing women may be more efficient at countering the presence of abnormal and potentially pathogenic vaginal flora; and hence, may have a decreased risk for BV. However, the variables associated with hsp70 release into the vaginal secretions of some women but not in others still remain to be determined. It is proposed that MBL, in addition to functioning as an antimicrobial agent in the vagina, is a main factor preventing the migration of bacteria to the uterus and fallopian tubes in women with BV. MBL binding to microbial surfaces renders the organisms willing to attach to collectin receptors present on epithelial cells and on antigen-presenting cells (Neth et al., 2002). This sequesteration of bacteria prevents their transport from the vagina to the upper genital tract. MBL could also bind to clue cells, indicating the interaction of MBL with BV-related bacteria (Pellis et al., 2005). Therefore, situations favoring a decrease in MBL bioactivity could be anticipated to increase the possibility of bacterial proliferation and migration within the genital tract. MBL degradation could be brought about by the elaboration of bacterial proteases. In addition, the gene coding for MBL (mbl2) is polymorphic at several loci, and carriage of the variant alleles at these polymorphic loci is associated with the production of an unstable MBL protein that is rapidly degraded (Lipscoume et al., 1995). Greatly reduced concentrations of MBL have been measured in both serum and vaginal secretions from women positive for MBL variant alleles (Babula et al., 2004).

Mucosal immune system helps in protection against pathogens, prevention of the penetration by foreign antigens, maintenance of mucosal homeostasis and induction of oral tolerance. Moreover, the mechanisms of innate immunity and the activation of B cells for mucosal immunity could be more important than the adaptive immune response involving the T-cell population (Revaz and Nardelli, 2005). However, the knowledge of different requirements to induce an effective immune response at the mucosal sites would certainly help to define immunotherapeutic approaches and to combat diseases. Nevertheless, probiotic bacteria could be a good choice to improve the mucosal immune system, especially if the mechanisms through which they work are fully elucidated. L. casei has been found to interact in-vitro with the epithelial cells through TLR-2 and induce IL-6 release (Vinderola et al., 2005). Also, a significant increase in the number of positive cells for mannose-receptor has been observed for all periods of L. casei administration, either in lamina propria or in isolated cells of the Payer’s patches (Maldonado and Perdigón, 2006).

**PATHOPHYSIOLOGY OF RECURRENT UTIs IN WOMEN**

The healthy female urogenital flora consists of numerous microbial species, among which LAB, especially *L. crispatus* (Antonio et al., 1999; Zhou and Bent, 2004), *L. jensenii* (Antonio et al., 1999) and *L. iners* (Zhou and Bent, 2004; Burton et al., 2003) predominate in healthy pre-menopausal women. It has been observed that there is an elevated incidence of vaginal LAB in women without any history of UTIs (Bruce et al., 1973). Besides LAB, other microorganisms also prevail among the vaginal microflora of some healthy women, such as *Megasphaera spp.*, *Atopobium spp.* and *Leptotrichia spp.* (Zhou and Bent, 2004). However, estrogens could also encourage the colonization of vagina with LAB and decrease the vaginal pH, thus controlling the development of pathogens (Stamm and Raz, 1999). This could be one potential reason why postmenopausal women are more prone to urogenital infections than premenopausal women. Moreover, vaginal microflora often changes considerably during the menstrual cycle, even in women without any episodes of UTI (Keane et al., 1997). In patients with UTI, the microflora of the urethra and the vagina are colonized frequently by uropathogens, particularly *E. coli* and other members of Enterobacteriaceae. Uropathogens produce many virulence factors, including adhesins, siderophores and haemolysins, and their potential to cause infection is associated with their adhesion to urogenital cells, to each other (autoaggregation) and probably to other organisms (coaggregation) (Reid and Sobel, 1987). Even though the adhesion of the uropathogens on the urogenital epithelium has been established in many studies, it is, however, still not yet well excavated how they manage to survive the passage through natural microflora.

**PROBIOTICS TO PREVENT AND TREAT UROGENITAL INFECTIONS**

A number of probiotics have been studied for effectiveness in prevention of recurrent UTIs. Because *E. coli*, the principal pathogen involved in UTIs, travels from the intestines and/or vagina to inhabit the normally sterile urinary tract, improving the gut or vaginal flora can impact the urinary tract (Huang et al., 2013; Homayouni et al., 2014; Borges et al., 2013; Mastromarino et al., 2013). Many women experience chronic UTIs, and a new approach using probiotics could provide long-term relief, at least for some if not all. The reduction of vaginal *Lactobacillus crispatus*, the main organism associated with the healthy vagina, has also been linked with these infections, since its absence could allow harmful bacteria to flourish, suggesting that replenishing the microflora may be helpful. Moreover, the recent emergence and spread of antibiotic resistance is also adding fuel to the fire, thereby further pressing on the need to develop non-antibiotic remedies to prevent and/or treat UTIs. It has been suggested that restoring the LAB in women with recurrent UTIs could normalize the vagina and prevent UTIs (Table 1 and 2). It is not surprising that alternative treatments are gaining significant interest from the patients, since antimicrobial treatment of urogenital infections is not always successful, and problems may persist due to bacterial and yeast resistance, recurrent infections as well as side effects (Schmitt
et al., 1992). The reason for UTI recurrences could be that antimicrobials fail to abolish the pathogens, perhaps because of biofilm resistance, or that the virulent microorganisms come back from their source (gut, sex partner etc.) and attack the host whose defenses are suboptimal. Although, many BV, UTIs and yeast vaginitis patients experience a recurrence, young girls who suffer from UTIs are more expected to have repeated episodes in adulthood (Reid, 2001; Stamm et al., 1999). Recurrent infection could also be attributed to the eradication of commensal organisms in the vagina by the antimicrobials, thereby increasing the vulnerability to recolonization by pathogens (Reid et al., 1990a,b; Hooton et al., 1991). This is one of the major reasons for considering the use of probiotics, i.e., to reload the good microflora as an independent or combined way to lower the risk of re-infection.

**Mechanisms of action of probiotics**

Lactobacilli are involved in maintaining the normal vaginal microflora by preventing the overgrowth of pathogenic and opportunistic organisms (Ronnqvist et al., 2006; Martin et al., 2012; Verdenelli et al., 2014). The basis for use of probiotics in BV treatment emerged in 1973, when healthy women with no history of UTI were reported to have lactobacilli in their vagina (Reid et al., 2003). Lactobacillus organisms that predominate in the vagina of healthy women spread from their rectum and perineum and form a barrier to the entry of uropathogens from vagina into the bladder (Reid et al., 2001a,b). The principal mechanisms by which lactobacilli exert their protective functions are a) stimulation of the immune system; b) competition with other microorganisms for nutrients and for adherence to the vaginal epithelium; c) reduction of the vaginal pH by the production of organic acids, especially lactic acid; and d) production of antimicrobial substances, such as bacteriocins, and hydrogen peroxide (Aroutcheva et al., 2001). The hydrogen peroxide microbial metabolite represents one of the most effective protective agents against pathogens. It has been observed that 70% to 95% of lactobacilli present in the vaginal flora of healthy women produce hydrogen peroxide. This percentage drops to 5% in women affected by vaginal infections (Eschenbach, 1989). Although, the in-vitro studies may or may not be clinically valid, such studies may at least be useful for clarifying the potential of probiotics to obstruct the growth of uropathogens (Verdenelli et al., 2014). Also, the in-vitro experiments of interactions between microorganisms could be simplified compared with the complexity of interactions within the urogenital microflora. Nevertheless, despite such boundaries, there has been ample evidence generated from in-vitro studies to elucidate the mechanism(s) of
action of probiotics in preventing UTIs (Fig. 1 and 2). Several in-vitro studies have shown that particular LAB strains have the potential to hamper the adherence, growth and colonization of the female urogenital epithelium by uropathogenic bacteria. This communication could be significant in the maintenance of a natural urogenital microflora and prevention of infections in women. Using glass and sulfonated polystyrene polymers both of which are hydrophilic, LAB have been used to coat biomaterial surfaces, thus declining the adhesion of uropathogens (Hawthorn and Reid, 1990). Precoating the polymers with LAB significantly reduced the adhesion of E. coli and Staphylococci. Surlactin, a biosurfactant from E. coli L. polymers with LAB significantly reduced the adhesion of uropathogens (Hawthorn and Reid, 1990). Precoating the coat biomaterial surfaces, thus declining the adhesion of both of which are hydrophilic, LAB have been used to in women. Using glass and sulfonated polystyrene polymers of a natural urogenital microflora and prevention of infections.

**TABLE 3. Randomized controlled trials of probiotics in pregnancy.**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil droplets of <em>L. reuteri</em> ATCC 55730 or placebo</td>
<td>Treated infants had less IgE-associated eczema at 2 years of age than those from the placebo group</td>
<td>Abrahamsson et al., 2007</td>
</tr>
<tr>
<td>LGG or placebo</td>
<td>Infants whose mothers received LGG showed significantly higher occurrence of <em>B. breve</em> and lower occurrence of <em>B. adolescentis</em> than those from the placebo group</td>
<td>Gueimonde et al., 2006</td>
</tr>
<tr>
<td>LGG or placebo</td>
<td>At 2 years of age, the frequency of atopic eczema in the LGG group was half that of the placebo group. At 4 years of age, the frequency of atopic disease remained lower in the LGG group versus placebo</td>
<td>Kalliomäki et al., 2001</td>
</tr>
<tr>
<td>LGG or placebo</td>
<td>Dietary counseling with probiotics resulted in higher concentrations of linoleic and dihomo-γ-linolenic acids compared with dietary counseling and placebo or with placebo alone</td>
<td>Kaplas et al., 2007</td>
</tr>
<tr>
<td>Probiotics and galactooligosaccharides, or placebo</td>
<td>Probiotics may improve response to Hib immunization</td>
<td>Kukkonen et al., 2006</td>
</tr>
<tr>
<td>Probiotics and galactooligosaccharides, or placebo</td>
<td>Probiotic treatment showed no effect on the incidence of all allergic diseases by age 2 years, but significantly prevented atopic eczema</td>
<td>Kukkonen et al., 2007</td>
</tr>
<tr>
<td>Fermented milk containing <em>L. johnsonii</em></td>
<td>Oral administrations of probiotics can restore vaginal flora in pregnant women</td>
<td>Nishijima et al., 2005</td>
</tr>
<tr>
<td>LGG or placebo</td>
<td>LGG increases the immunoprotective potential of breast milk when administered during pregnancy and lactation</td>
<td>Rautava et al., 2002</td>
</tr>
</tbody>
</table>
then directly administered *L. reuteri* or placebo for two years. At the end of the trial, the authors reported that infants treated with *L. reuteri* had significantly less IgE-associated eczema at two years of age and therefore may possibly have a reduced risk of developing later respiratory allergic disease (Abrahamsson et al., 2007). Besides, myriad evidences for several probiotic strains are now available that the intake of probiotics during pregnancy and after delivery could be beneficial for the gut and metabolic health of mothers as well as the infant.

**CONCLUSION**

Infections of the lower urinary tract are common occurrences, predominantly in sexually active young women, during pregnancy, and in pre- and postmenopausal women. The predictable approach, after urine dipstick or culture, is to treat with antibiotics at the first sign of an infection. In addition, women with chronically recurring infections are often prescribed long-term antibiotic treatment, contributing to gut and vaginal dysbiosis and antibiotic resistance. In this milieu, probiotics may prove to be an independent or adjunct effective prophylactic approach for combating such recurrent infections.

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