



# Does probiotics work for bacterial vaginosis and vulvovaginal candidiasis

Yue Han and Qing-ling Ren

## Abstract

The different *Lactobacillus* strains of probiotics have been applied to the treatment and prevention of bacterial vaginosis and vulvovaginal candidiasis. The experimental data demonstrated that it works well via reducing the number of harmful bacteria, maintaining the acidic microenvironment, inhibiting the immune response, and so on, to restore the vaginal microecology. However, the clinical data indicated that it is not sufficient to support the use of probiotics in the intervention of vulvovaginal candidiasis rather than bacterial vaginosis. Hunting for novel probiotic strains and uncovering the precise mechanism of probiotics, especially with the new concept gut–vagina axis, to maintain the homeostasis of vaginal microbiota should be a great challenge in the future.

## Addresses

The Affiliated Hospital of Nanjing University of Chinese Medicine, 155 Hanzhong Road, Nanjing, Jiangsu, China

Corresponding author: Ren, Qing-ling ([yfy0047@njucm.edu.cn](mailto:yfy0047@njucm.edu.cn))

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## Introduction

Probiotic is known as the ‘good’ bacteria, originally from a combination of ‘pro: for, bios: life’ in Greek and Latin civilizations. It was the first time to be defined as microorganisms and/or substances secreted from living microorganisms to confer better health directly or indirectly or to enhance the growth of others in 1965 [1]. In 2002, the Food and Agriculture Organization proposed the latest description of probiotics to be ‘live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host’ [2]. In 2014, probiotics came to the consensus definition,

which is ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’ [3]. Currently, the common probiotics include *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, *Escherichia*, and *Bacillus*. Increasing evidence demonstrated that probiotics have been integrated into life processes and recognized as beneficial microorganisms via restoring physiological homeostasis and host function, including reducing hostile bacteria, regulating immunity system, preventing infection, and promoting overall health of the gastrointestinal tract, and it has frequently been considered one of the most common consumed food supplements worldwide with a constantly growing multi-billion dollar industry and widely recommended by physicians, especially gastroenterologists [4].

Probiotics have also been applied to ward off female reproductive illness by maintaining the vaginal microbiome in balance. As we have known that the harmonious balance of vaginal microbiota is crucial for a robust host-microbial interaction that promotes a healthy vaginal ecosystem for the management of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), sexually transmitted infections (STIs), trichomoniasis, human papillomavirus infection, *Chlamydia trachomatis* infection, HIV susceptibility, and genital herpes infection. BV is the most common cause of abnormal vaginal discharge in women at ages 15–44, and there is a strong link between BV and preterm delivery, sexually transmitted infections, *Chlamydia trachomatis* infection, HIV, and other genital infections, characterized by the loss or sharp decline in the total number of *Lactobacillus* and a corresponding 100–1000 fold increase in the concentration of facultative or obligate anaerobic microbes, such as *Gardnerella*, *Prevotella*, *Atopobium*, *Mobiluncus*, *Bifidobacterium*, *Sneathia*, *Leptotrichia*, and BV-associated bacteria 1–3 [5]. The global prevalence of BV was reported to be from 23% to 29% across different regions (Europe and Central Asia, 23%; East Asia and Pacific, 24%; Latin America and the Caribbean, 24%; the Middle East and North Africa, 25%; sub-Saharan Africa, 25%; North America, 27%; South Asia, 29%) with the annual estimated cost of burden \$4.8 billion [6]. VVC is the second most common cause of vaginitis (after BV) caused by overgrowth of *Candida* species in the female lower

genital tract and most commonly caused by *Candida albicans*. An estimated 70–75% of women worldwide will take at least one episode of VVC in their lifetime, and 40–50% of the VVC cases will experience multiple episodes [7]. Apart from VVC can be associated with substantial morbidity, including significant genital discomfort (pain, itching, burning), reduced sexual pleasure and activity, psychological distress (stress, depression, anxiety), embarrassment, reduced physical activity, and loss of productivity, it also might be associated with increased risk of premature rupture of membranes, preterm labor, chorioamnionitis, and congenital cutaneous candidiasis during pregnancy and contribute substantially to enhanced susceptibility to HIV infection [7]. A variety of treatments are currently available, and a relatively high effectiveness in the relief of symptoms typically associated with acute infections, however, are generally unable to offer a long-term protective barrier against possible recurrences. Owing to BV and VVC being the first and second most common cause of vaginitis and the onset factor of other female sicknesses, consideration of administering probiotic organisms to restore vaginal microecological imbalance has been implied to be as an alternative strategy for the prevention and treatment of them in restoring reproductive health, we would like to only focus on the state of arts in probiotics and BV, VVC in this review.

### Potential mechanism of action of probiotics in bacterial vaginosis and vulvovaginal candidiasis

BV is characterized by loss of or significant decline in lactic acid-producing *Lactobacilli* (good bacteria) and higher overgrowth of other pathogenic bacteria such as *Gardnerella* spp., *Atopobium* spp., *Prevotella* spp., and *Mobiluncus* spp. (harmful bacteria) [8]. Under this condition, the imbalanced microbiota in the vagina is very typical, resulting in a higher level of pH and lower lactic acid level. When the *Lactobacillus* spp. (*Lactobacillus crispatus*, *Lactobacillus gasseri*, *Lactobacillus iners*, *Lactobacillus jensenii*, and so on) is lost or reduced in the vagina and the vaginal pH fails to be maintained at the normal range 3.8–4.5, the normal vaginal barrier is destroyed via hydrolytic enzymes (e.g. sialidase and prolidase), and, subsequently, enhanced immune responses will happen, accompanying with a set of proinflammatory chemokines and cytokines (interleukin (IL)-6, IL-8, IL-1 $\alpha$ , IL-1 $\beta$ , TNF- $\alpha$ , and so on) were released, which successively contribute to persistent infection potentially caused by a mixture of difficult-to-treat pathogens, especially when some of them possess the ability to form biofilms [9,10]. Hence, administering probiotics containing high abundance of *L. spp.* could be a possible choice in restoring normal vaginal microflora. For instance, use of probiotic *Clostridium butyricum* WZ001 with vaginal infusion can not only promote the growth of *Lactobacillus* and inhibit the

growth of pathogenic bacteria but also reduce the inflammatory response induced by *Escherichia coli*, decrease the increased phosphorylation of nuclear factor-kappa B p65 in vaginal tissue and inflammatory cytokines, IL-1 $\beta$ , TNF- $\alpha$ , and IL-6 in the serum levels, which is helpful for the restoring of the vaginal microecological environment [11]. Another study with the BV mouse model induced by *Gardnerella vaginalis* (GV), oral, or intravaginal administration of different probiotics [*Lactobacillus rhamnosus* HN001 (L1); *Lactobacillus acidophilus* La-14 (L2); and probiotic mixture (PM; L1, L2, and lactoferrin RCXTM L2)] indicated that L1, L2, or probiotic mixture (PM) significantly inhibited GV-induced epithelial cell disruption and myeloperoxidase activity. The levels of L1 and L2 were higher in intravaginally administered mice than in orally administered mice. When L1 or L2, one of the PM ingredients, was orally or intravaginally applied individually, more L2 was detected than L1. L1 and L2 also significantly inhibited the adherence of GV to HeLa cells (a human cervical cancer cell line) and GV growth *in vitro*. Oral administration was more effective against GV-induced BV than intravaginal administration although the data indicated that both oral and intravaginal administration of L1, L2, or PM significantly inhibited GV-induced epithelial cell disruption, myeloperoxidase activity, nuclear factor-kappa B activation, IL-1 and TNF- $\alpha$ , IL-17, and ROR $\gamma$  expression, and increased IL-10 and FOXP3 expression. It suggested that anti-BV effect of L1, L2, and PM attenuated GV-induced vaginosis by regulating both vaginal and systemic innate and adaptive immune responses rather than direct competition or killing of GV in the vagina [12].

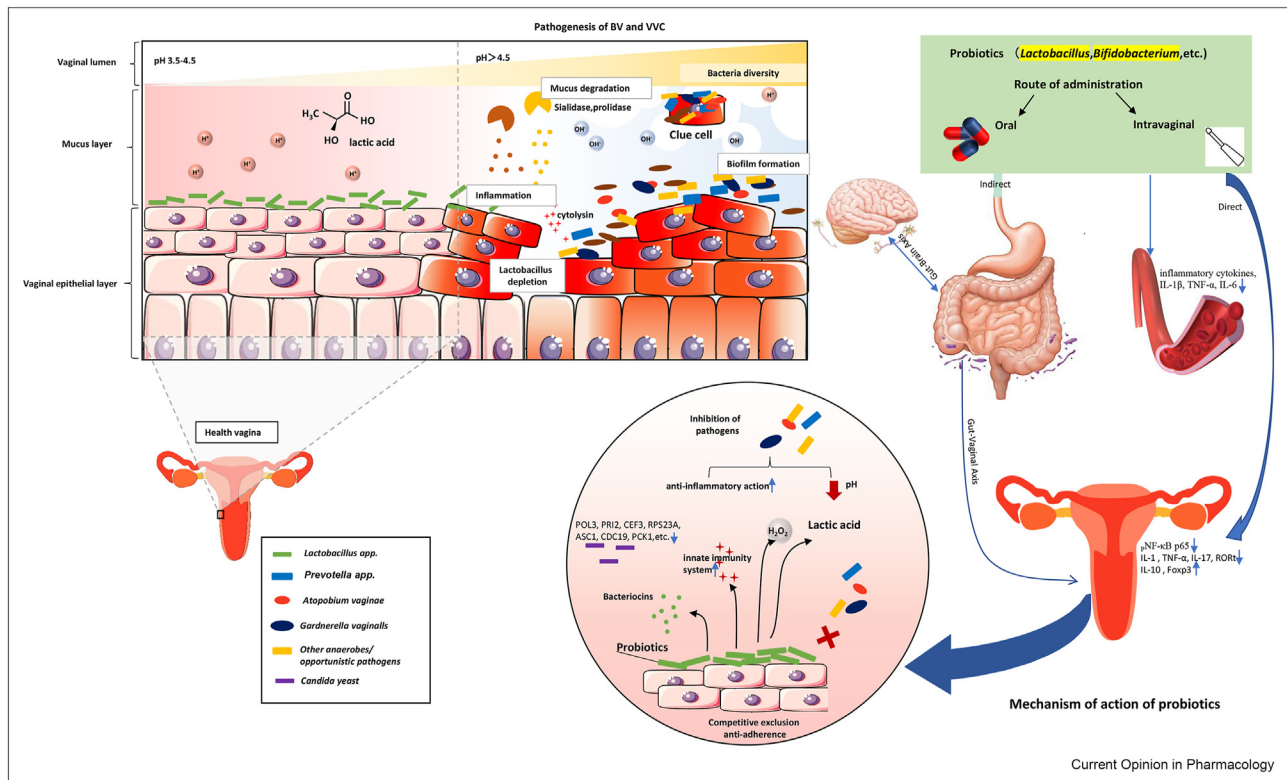
VVC is an infection mainly caused by *Candida* yeast, one of the most common vaginal inhabitants, which frequently cocolonizes the vagina with *Lactobacillus*. The development of VVC is usually attributed to the disturbance of the balance between *Candida* vaginal colonization and the host environment by physiological or nonphysiological changes. Several host-related and behavioral risk factors have been proposed as predisposing factors for VVC. The former includes pregnancy, hormone replacement, uncontrolled diabetes, immunosuppression, antibiotics, glucocorticoid use, and genetic predispositions. The latter consists of use of oral contraceptives, intrauterine devices, spermicides, and condoms, and some habits of hygiene, clothing, and sexual practices [13]. The mechanism by which *Candida* spp. produces inflammation remains unknown. However, most *in vitro* and animal studies have revealed that *L. spp.* exerts an inhibitory effect on the growth, morphological transition, virulence, and biofilm formation of *C. albicans* [14–16]. The metabolites of *L. spp.*, including organic acids, hydrogen peroxide, bacteriocins, and biosurfactants, all contribute to these antifungal effects [17,18], especially the secretion of lactic and

other organic acids is recognized as the main mechanism by which *Lactobacilli* impedes candidal virulence. *Lactobacilli* were also reported to compete with the attachment of *C. albicans* to host cells, resulting in reduced adhesion to epithelial surfaces [19]. The inhibitory effects of *L. spp.* on the growth of *Candida* yeast in VVC implied that it should be an underlying mechanism to explain the application of probiotics in VVC. The *in vitro* studies demonstrated that both *Lactobacillus* strains (*Lact. rhamnosus* GR-1 and *Lact. reuteri* RC-14) generate inhibitory effects against *C. albicans*, [20,21]. The first study on the antagonistic effects of probiotic *lactobacillus* strains against the non-*C. albicans* *Candida* species *Candida glabrata* was published in 2015 [22]. The probiotic strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 were investigated to clarify whether they were against VVC-causing *C. glabrata*. It indicated that both strains exhibited potent antagonistic activities against all of the tested *C. glabrata* strains. These *lactobacilli* exhibited antifungal effects, including those attributed to their aggregation abilities, and their presence caused the cessation of growth and eventual cell death of *C. glabrata*. It seems that the two probiotic strains, *Lact. rhamnosus* GR-1 and *Lact. reuteri* RC-14, would be helpful against *C. albicans* and non-*C. albicans* *Candida* species *C. glabrata* in VVC. *In vitro* studies indicated that use of probiotics reduced the virulence of

*Candida* by inhibiting biofilm formation and may provide additional benefit to antifungals. *L. rhamnosus*, *L. casei*, and *L. acidophilus* significantly reduced levels of *C. albicans* biofilms at the initial colonization phase and the later maturation phase of biofilm development. The transcriptome analyses revealed the molecular mechanisms of probiotic interference by *L. rhamnosus* GR-1 and *L. reuteri* RC-14 toward the opportunistic fungal pathogen *C. albicans*. The list of downregulated genes presented the growth inhibitory effects of the *lactobacilli* including DNA replication (POL3, PRI2), translation (CEF3, RPS23A, ASC1), glycolysis (CDC19), and gluconeogenesis (PCK1) [21]. Probiotics have also been shown to downregulate genes involved in synthesis of ergosterol and genes associated with a drug efflux pump involved with fluconazole resistance [23].

To date, *Lactobacillus* spp. has attracted more attention in the research of probiotic bacteria. It has been considered potential promising probiotics to protect the vaginal environment of BV and VVC with the following common mechanism (Figure 1): production of lactic acid that provides pathogenic exclusion ability by lowering pH to 3.5–4.5 and contribution to indirect pathogenic inhibition by efficient acid cervicovaginal human mucus; production of bacteriocins which are AMPs and proteins, produced as a response of imbalance

Figure 1



The pathogenesis of BV and VVC and the potential mechanism of probiotics for the prevention and treatment of BV and VVC. BV, bacterial vaginosis; VVC, vulvovaginal candidiasis.

of the vaginal microbiome, to protect the host against microbial invasion blocking the colonization of vaginal pathogens as a result of displacement and exclusion competition by *Lactobacillus* bacteria, and promotion of immunomodulation mechanisms by provoking the innate immunity system, producing hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and stimulating anti-inflammatory action. In addition, oral or intravaginal administration of different species of *Lactobacillus* has shown to increase the number of vaginal *Lactobacilli* which presumably provides a mechanical barrier against *Gardnerella vaginalis* and prevents the adhesion of the pathogens to the vaginal epithelium [24–31]. The *Lactobacillus* species produces lactic and acetic acid and H<sub>2</sub>O<sub>2</sub>, maintains the vaginal pH around 4.5 or less, hampers the growth of pathogenic bacteria and *C. albicans* and is thus considered protective against VVC and BV [32]. Owing to the production of lactic acid by probiotics, the electrochemical potential of cell membranes and intracellular protein denaturation of harmful microflora are neutralized. Probiotics destroy pathogens as a result of their competing for nutrients and for receptors in the mucosa and epithelium. They also produce bacteriocins that remove pathogens from the digestive tract. They are heterogeneous chemicals that inhibit the activity of pathogenic bacteria — *Staphylococcus aureus*, *Salmonella enteritidis*, *Bacillus cereus*, or *Pseudomonas aeruginosa* — that are mainly produced by *L. acidophilus*. Bastani confirmed the potential efficacy of *lactobacilli* as a nonchemotherapeutic means to restore and maintain normal urogenital flora and showed that probiotic bacteria especially *L. acidophilus*, *L. rhamnosus* GR-1, and *Lactobacillus fermentum* RC-14 when administered over 10<sup>8</sup> colony forming unit (CFU) for 2 months can most appropriately normalize vaginal flora, help cure the existing infection, and prevent recurrence of BV. Longer periods of probiotic administration may be useful for long-term control of BV relapses after conventional therapy with metronidazole [33]. Current data implied that application of probiotics works for restoring the vaginal microecology in BV and VVC. However, the detailed and precision mechanisms are still remaining unclear.

### Beneficial or not in clinic

Probiotics have been reported to be useful when used either vaginally or orally. One of the first reports of probiotic effectiveness by using well-characterized probiotic strains in treating BV was provided by Anukam et al. [34]. They studied the effect of capsules containing *Lactobacillus rhamnosus* and *L. reuteri* on 40 Nigerian women with bacterial vaginosis. The results demonstrated a 90% recovery rate in women with BV after probiotic administration. This finding may lead to the use of probiotic capsules as self-use therapy over the shelf. Another study evaluated the efficiency of *Lactobacillus* containing vaginal tablets in treating bacterial BV and in restoring vaginal microbiota. A double-blind,

placebo-controlled clinical study was carried out by Mastromarino et al. [35] in 2008. Thirty-nine women with BV were enrolled in the trial and were divided randomly into two groups. The test group received *Lactobacillus* vaginal tablets (*L. brevis*, *L. salivarius*, and *Lactobacillus plantarum*) for 7 days daily and was compared with the placebo group. The results proved a remarkable improvement of the vaginal microbiome as 83% of the women in the test group were absolutely free of BV after the therapy period and 17% showed an intermediate level of vaginal flora. The previous experimental data were confirmed by a study conducted by Ya et al. in 2010 that showed the efficacy of exogenous administration of *Lactobacillus* probiotics in treating BV and restoring a healthy vaginal microbiome. These data were followed by many other studies that all verified the eminence of exogenous probiotics in curing bacterial vaginosis and restoration of vaginal normal flora. Ling et al. [36] proved that probiotic administration has better effect, as long-term therapy, than the classical antibiotic (metronidazole) in treating BV. They found that probiotics enhance vaginal homeostasis in a gradual manner by suppressing the overgrowth of harmful bacterial strains, thus providing steady restoration of the vaginal microbiome. A recent study aimed to investigate the efficiency of long-term *Lactobacillus* administration in restoring vaginal microbiota. The study used 250 nonpregnant women with BV over 9 months. The patients were divided into two groups: group A received standard antibiotic treatment (metronidazole, 500 mg orally twice a day for 7 days), whereas group B received antibiotics followed by vaginal tablets containing *Lactobacillus rhamnosus* BMX 54. Patients were evaluated after 2, 6, and 9 months (T0, T2, T6, and T9) in terms of recurrences rates of BV, vaginal symptoms, re-establishment of healthy vaginal flora, vaginal pH, and treatment tolerability. These data suggested the pivotal role of *Lactobacillus* as a prophylactic therapy in suppressing BV recurrence, after antibiotic treatment [37]. In addition, current evidence indicated that although oral administration of probiotics reduced recurrent BV, however, direct vaginal application may provide more rapid treatment [38,39].

Currently, increasing studies proved that regulated use of probiotics, administered both orally and vaginally, is effective in the prevention and treatment of vaginal infections such as BV and VVC [39–43] and found that administration of probiotics is helpful to restore the loss and recovery of endogenous vaginal *Lactobacilli* by competitively, biochemically, and immunologically replacing pathogens and re-establishing vaginal homeostasis [44]. The long-term lower recurrence rate observed in women treated with probiotics could be attributed to their capacity to steadily re-establish vaginal homeostasis [45]. However, most of the studies were focused on treating BV as the most

common dysbiosis; a few studies focused on VVC [46]. And the clinical benefits have also been debated and concluded by systematic analysis and reviewed that the probiotics hold promise for BV cure and prevention but much less so for VVC cure and prevention because a couple of studies of probiotics for the treatment of VVC have conflicting results, possibly caused by differences in probiotic strain, delivery mechanism, and treatment schedules [47–49]. And the benefit of probiotics for VVC, to some extent, depends on whether patients have acute infection, recurrent infection, or are at increased risk for infection [50]. In 2019, Buggio et al. [51] also made a conclusion at their latest publication that despite increasing marketing of probiotics for the treatment of VVC and prevention of preterm birth robust evidence demonstrating a beneficial effect is scarce. Moreover, there was considerable heterogeneity among the different studies in terms of route of administration, strain/s of probiotic adopted, and length of probiotic use. Before recommending the systematic use of probiotics to treat bacterial vaginosis and VVC and prevent preterm birth, high-quality research is needed. Professional medical associations should issue recommendations defining if, when, and how probiotics should be used for gynecological disorders.

## Perspective

Use of probiotics in the prevention and treatment for BV and VVC has been well-reviewed and debated [51]. Here, we just concluded and proposed with following issues. Beyond the mentioned *Lactobacillus* species in this review, the food market is full of a great number and different pharmaceutical forms of probiotics, such as capsules (EcoVag, Gynophilus, LACTIN-V, and Probiacac Vaginal), tablets (Gynoflor, ActiCand 30, Normogin, and Florisia™), pessaries or powders (LACTIN-V powder, with a vaginal applicator) for vaginal administration; capsules (LaciBios Femina, Lactogyn, prOVag, and Provinorm) and tablets for oral application. What is the different efficacy and mechanism between them is worthy of keeping concern in the future investigation. A recent study [52] indicated that several new vaginal strains exhibited better probiotic profiles than current commercial strains after comparing 57 vaginal *Lactobacillus* strains from young African women to strains from commercial probiotic products for vaginal health. Another latest study demonstrated the *Lactocaseibacillus rhamnosus* TOM 22.8 strain, isolated from the vaginal ecosystem of a healthy woman, exhibited a broad spectrum of antagonistic activity against vaginal pathogens, adhesion capacity to both the vaginal VK2/E6E7 (The VK2/E6E7 cell line was established in 1996 from the normal vaginal mucosal tissue taken from a premenopausal woman undergoing anterior-posterior vaginal repair surgery) and the intestinal Caco-2 cells, anti-inflammatory and antioxidant activities, suggesting its promising probiotic features. The oral or vaginal strain

administration presented a significant pathogen reduction after 10 days of administration and a maintenance of eubiosis up to 30 days after the end of the treatment [53]. It implied that in the future it would be possible to find out more probiotics to improve treatment outcomes for BV and VVC. The current idea on the probiotic application was derived from the opinion that the predominant bacteria in the vagina is *Lactobacillus*. Shall we try to test other common probiotics *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, *Escherichia*, and *Bacillus* for the prevention or treatment of BV and VVC?

Since the Human Microbiome Project launched in 2007 [54], the microbial communities across several different sites on the human body nasal passages, oral cavity, skin, gastrointestinal tract, and urogenital tract have been investigated with 2 phases [55–57] and provided us a variety of new concept and findings to update our knowledge in the relationship between microorganisms and human beings. Among the 3 key results of this project published in Nature and Nature Medicine [31,58,59], the study on the vaginal microbiome and preterm birth demonstrated that women who delivered preterm exhibited significantly lower vaginal levels of *L. crispatus* and higher levels of BV-associated bacteria 1, *Sneathia amnii*, TM7-H1, a group of *Prevotella* species and nine additional taxa. It suggested that the vaginal microbiome would be a crucial factor to maintain or promote female health and how to maintain the homeostasis of the vaginal microbiome by means of probiotics would be the next focus to reveal the mechanism. One issue we cannot neglect in future research is the fluctuation of vaginal microbiota owing to that the vaginal ecosystem is influenced drastically by many factors during a woman's life cycle, such as hormonal changes, menstrual cycle, pregnancy, menopause, age, sexual behavior, antibiotic treatment, hygienic habits, and ethnicity [60–63]. For instance, the level of *L. crispatus* is dropped 100 times during the menstrual cycle, whereas the level of *L. iners* and many other *Lactobacillus* strains increased in women with healthy vaginal microbiota. Finally, the crucial role of the vaginal microbiome in BV and VVC has come to agreement [5,64]. However, what is the impact of cross talk between the female gut and genital tract microbiota which was freshly proposed on the mechanism of probiotics in the treatment for BV and VVC? Owing to the delivery way of probiotics for the treatment of BV and VVC includes oral and vaginal way, whether they will generate similar or different effects on the gut, and/or vaginal microbiota, and their cross talk (gut–vagina axis) [4,65–68]. Whether it would be possible that vaginal administration is locally or directly to reduce the number or replace the harmful bacteria, restore the acidic vaginal microenvironment by the good ones, whereas the oral administration of probiotics is indirectly or entire body responses via the regulation of gut



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