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Effects and Mechanisms of *Lactobacillus* Probiotics in Maintaining Women's Vaginal Health: A Review

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Abstract

Probiotics are beneficial living microorganisms. They are useful to vaginal health when consumed in suitable doses. The dominant species included in probiotics are *Lactobacillus*. We can get these microbes naturally or commercially utilize them as functional food. *Lactobacillus* species form 70% of inhabitant bacteria in females compared to other warm-blooded animals. The prevalent *Lactobacillus* species are *L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*. Because of the development of antibiotic resistance mechanisms, the beneficial relationship between humans and probiotics has been discussed for several years. *Lactobacillus* prevents the attachment of pathogens by using several mechanisms, for example, by increasing estrogen levels, production of lactic acid, lowering pH 4.0-4.5 to provide an acidic environment, release of hydrogen peroxide, bacteriocins and bacteriocins-like substances, adhesion and colonization properties. *Lactobacillus* prevent the entry and attachment of pathogenic bacteria, thus not only helping to control and manage different gynecological conditions like bacterial vaginosis, endometriosis, vulvovaginal candidiasis and genitourinary syndrome of menopause but also infections that can spread through sexual contacts such as gonorrhea, chlamydia, genital herpes, Human papillomavirus infections (HPV). Overall, the use of probiotics in improving vaginal health is beneficial, easy and cost-effective but the use of probiotics in HPV clearance is not quite beneficial. So, more work should be done on HPV preventive vaccines.

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Introduction

Probiotics can be defined as live microorganisms providing many positive outcomes on health status, and that's why due to their beneficial effects they have got attention for their possible importance in women's health [1]. Probiotics can only improve human health when they are ingested in suitable doses [2]. In the vagina, *Lactobacilli* are the most dominant bacteria connected with the lining of the vagina and the purpose of this adhesion is to prevent the attachment of other pathogenic bacteria [3]. We can naturally obtain these microbes from yogurt, kimchi, kefir, sauerkraut, and kombucha as well as through dietary supplements [1]. Probiotics may help to control and manage female reproductive diseases such as polycystic ovary syndrome, bacterial vaginosis, endometrial diseases, gestational diabetes mellitus [4], vulvovaginal Candidiasis, cervical intraepithelial neoplasm (CIN), infections that can spread through sexual contacts such as gonorrhea, chlamydia, genital herpes, human papillomavirus infections (HPV) in the women reproductive health [1]. Probiotics also provide benefits during pregnancy, for example, they can reduce the rate of occurrence of gestational diabetes [5], group B streptococcal colonization [6] and lactational mastitis [7].

Probiotics are also involved in preventing and treating gynecologic cancer [8]. The imbalance of normal flora of the female reproductive tract is due to two reasons. It may be due to infection-causing pathogens and may be due to adopting a lifestyle that is unhealthy [9]. Their vaginal health is particularly associated with abundance of vaginal L*actobacilli* [10]. To understand the relationship between normal flora and human health, there is an increase in the number of scientific research that occurs vastly, that's why the importance of probiotics has grown remarkably, providing many curative benefits [11]. Because of the progression of resistance mechanisms to antibiotics, curative benefits of medications would have limited competency. The beneficial relations between probiotics and the health of humans have been a topic of discussion in present years [12]. Probiotics are suggested to be used as medicinal products as they have no bad effects on the human body. They make the human immune system strong and improve its ability to adapt to many diseases [13]. They have selective features such as anti-diabetes, anti-obesity, anti-inflammatory and anti-angiogenic capabilities, that's why, are being used medically. However, before using probiotics, it is important to know about the safety of probiotics [12]. The vaginal tract is a unique fluctuating habitat that is constantly affected by alternation, which ultimately determines how the microbial species interacts with other organisms and similarly how they interact with the host [14]. Residential *Lactobacillus* species can produce lactic acid that prevents infections caused by other pathogenic bacteria. The vaginal microbiota profile varies among women of different races [15]. Many factors affect the normal microflora for example things like what you eat, your genes, how you live, taking antibiotics, and getting older can all affect it. There are many chronic conditions like obesity, type 2 diabetes, oxidative stress, non-alcoholic fatty liver diseases, cardiovascular issues, and immune-related disorders that are associated with an imbalance in the microbiome which is known as Dysbiosis [12]. Probiotic bacteria are classified into two groups for example first one is *Lactobacillus* and the second one is *Bifidobacteria*. *Lactobacillus* is a Gram-positive bacterium. It is a facultative anaerobe. It is found in the vagina and gastrointestinal system [16].

The objective of this review article is to analyze the probiotic effects of *Lactobacillus* in the women's vaginal health. This review focuses on the *Lactobacillus* role in treating many gynecological conditions rather than using conventional medicines, which may have many side effects. This review also focuses on human vaginal microbiome composition, factors that maintain the human vaginal microbiome, and the mechanism through which the *Lactobacillus* plays a role as normal microbiota of the vagina.

Vaginal Microbiome

The microenvironment of the vagina is colonized with normal flora. It also has endocrine control and a protective barrier which is a mucosal epithelial. In healthy women's vaginas, about 95% of colonization takes place by normal microbial flora and most commonly *Lactobacillus* is present. In the total vaginal microbiome, *Lactobacilli* often exceed 70% [17]. The microbiome of the upper female genital tract differs from the lower female genital tract. The bacterial count is 10^2-10^4 folds in higher concentration in the lower female genital tract as compared to the upper female genital tract [18]. Vaginal *Lactobacilli* prevent the attachment of pathogens in the vagina by keeping their number in balance in the vagina, but several predisposing variables can disrupt the number of this normal microbial flora. For example, these variables include menstruation, pregnancy, intercourse and antibiotics use without their specific need. *Lactobacillus* species form five different community state types (CSTs) that

can impact the normal microbial community in the vagina. That's why the structure of vaginal normal flora plays a very critical role in finding vaginal health [19]. In *Lactobacilli*, the phenomenon behind producing lactic acid is the breakdown of glycogen. This production of lactic acid will decrease the pH to less than 4.5, which creates an acidic environment. The presence of *Lactobacilli* and a low pH in the human vaginal microbiome may reduce illness [20].

Human Vaginal Microbiome Composition

The vagina, cervix, uterus, fallopian tubes and ovaries are the different components that are parts of the genital tract. The function of the cervix is that it joins the upper reproductive tract and vagina [21]. Normal flora, which is present in the female reproductive system can affect genital or vaginal health (Table 1). These bacterial communities also help in protecting against urogenital infections along with maintaining vaginal health. *Lactobacillus* species are divided into 5 distinct community state types (CSTs). They include *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*, but CST IV differs from all the other types. The three species of *Lactobacillus*, for example, *L. crispatus*, *L. gasseri*, and *L. jensenii* have the capacity of producing lactic acid and H_2O_2. They also acidify the vaginal environment by lowering the pH below 4.5. They stop the proliferation of other viruses and bacteria [3, 22]. *Lactobacillus* species have been classified through genotyping of 16SrRNA gene-based amplicon sequencing. To gain an understanding of vaginal normal flora more deeply, a

Table 1 Summary of clinical studies of *Lactobacillus* in vaginal infections.

Vaginal diseases	Common *Lactobacillus* species	Study design	Conclusions	Reliability of results	References
Bacterial vaginosis	*L. rhamnosus* GR-1 *L. fermentum* RC-14	Randomized placebo-controlled trials included 59 premenopausal women	Probiotics vs placebo 96% of patients exhibit healthy vaginal ecology	Strong	[37]
Vulvovaginal Candidiasis	*L.gasseri* *L.scripts*	23 Randomized controlled trials on patients	Probiotics vs placebo 64% reduced chances of recurrent vaginal candidiasis	Strong	[1]
Endometriosis	*L. acidophilus* *L. plantarum* *L. fermentum* *L. gasseri*	Randomized pilot placebo-controlled trials on 37 women	Probiotics vs placebo Change in overall score in LactoFem^R was 7.33 and in control was 4.11	Strong	[66]
Genitourinary syndrome of menopause	*L. acidophilus*	3 randomized clinical trials	Probiotics plus 0.03 mg vaginal estriol are more efficient in the treatment.	Strong	[1]
Sexually transmitted infections					
Chlamydia	*L. brevis* *L. salivarius*	Controlled experimental designs	*L. brevis* shows higher activity as compared to *L. salivarius*	Strong	[75]
Human papillomavirus (HPV)	*L. rhamnosus* GR-1 *L. reuteri* RC-14	Randomized double-blinded Placebo-controlled trial on 121 women	Probiotic vs placebo No role in HPV clearance	Weak	[80]
Gonorrhea	*L. crispatus*	Clinical trials on 104 women	*In vitro* assessment of probiotics A vaginal lubricant containing *L. crispatus* shows preventive action against gonorrhea.	Strong	[1,90]
	L. acidophilus	*In vitro* experiments on 100 married healthy women	Decrease in the plaque synthesis level by HSV-1 on HeLa cells		[88]
Genital Herpes	*L. brevis*	Randomized double-blind controlled trials on 81 women	Multi-strain probiotics or acyclovir Side effects of acyclovir, so probiotic treatment was more efficient	Strong	[89]

comprehensive meta-analysis using 1312 publically available databases of metagenomic sequencing of healthy vaginal microbiomes was conducted. This meta-analysis found *Lactobacillus*, the most dominant taxa, but alongside, many other genera were also dominantly involved in the composition of vaginal microbiota. They include *Gardnerella*, *Vibrio* and *Atopobium*. It helps us in understanding the structure and activities of the vaginal normal flora. This analysis also sheds light on its role in vaginal health and disease prevention [23].

Maintenance of the Human Vaginal Microbiome

Estrogen influences on vaginal microbiota

Among the host factors that play a very crucial role in regulating the vaginal microbiota, one is estrogen. The estrogen affects the number of bacteria in the vaginal normal flora and as a result, it promotes the generation of glycogen in the vaginal epithelium [24]. The growth of vaginal epithelial cells is dependent on the higher concentration of estrogen and halfway through the cycle in the vaginal lining, the concentration of intrinsic glycogen also increases. This results in an increase in the number of bacteria that produce lactic acid [25]. The aging process of the vagina depends upon different factors. It may be due to a shortage in supplies of estrogen, alternations in the microbiome and changes in genes that are relevant to the modification of DNA [26]. The conversion of extracellular estrogen in lactic acid is done by *Lactobacillus* species. The overall lactic acid generation by the vaginal lining is a major factor that determines the vaginal pH [27]. However, pathogenic bacteria can cause problems by stimulating inflammation, biofilm formation and releasing toxins and enzymes but *Lactobacillus* species maintain vaginal health by producing an acidic environment [24]. Before puberty, the microbial composition of the vagina primarily consists of anaerobes. Examples of these anaerobic bacteria are *Escherichia coli,* Diphtheroid, and coagulase-negative *Staphylococci*, with less glycogen [28]. Persistent parental estrogen has effects on the female reproductive tract that includes the vagina and vulva during the several weeks of life. A higher concentration of estrogen is present in the vaginal lining, so during the initial 24 hours of birth, *Lactobacillus* which is lactic acid-producing bacteria dominates the vaginal mucosa [29]. *Lactobacilli* expand in an acidic environment ranging from pH 3.5 to 4.5 and compete with other anaerobic bacteria. *Lactobacilli* dominance diminishes after menopause but can be increased with vaginal estrogen replacement treatment. *Lactobacilli* prevail and strengthen the normal flora of the vagina during pregnancy [30]. Menstruation changes vaginal microbial multiplicity, *L. crispatus* is reduced 100-fold and there is an increase in the number of *L. iners*, *G. vaginalis*, *P. Livia* and *A. vaginae*. The usual acidic pH in fertile women is influenced by several factors for example estrogen, glycogen, and *Lactobacillus*. By increasing estrogen, glycogen after the menopause is also increases. *Candida albicans* infection increases, which uses glycogen as a source of food [31].

Vaginal Defense Mechanisms Determined by *Lactobacillus*

Probiotics utilize their beneficial effects by using different mechanisms. The first one is sustaining the epithelial barrier, improving adhesion, inhibiting the binding of pathogens, exempting harmful microorganisms, yielding antimicrobial compounds and regulating the immune system [32]. There are following antimicrobial agents are produced, for example, hydrogen peroxide, bacteriocins and biosurfactants by *Lactobacilli* when they attach to epithelial cells of the vagina, which obstruct the multiplicity of the pathogens. *Lactobacilli* sustain the epithelial barrier of the vagina by the generation of mucus and building a durable microbial film, serving as a protective shield against disease-causing agents, thereby avoiding their establishment and penetration represented in Fig. 1 [1].

Lactic acid

Synthesis of lactic acid is caused by *Lactobacillus*. This lactic acid then can pass through the membrane barrier of lipopolysaccharide-containing bacteria which is its outer layer and as a result, it becomes sensitive to be influenced by many other stimuli [23]. Lactic acid does not allow the attachment of harmful bacteria. Examples include *Trichomonas vaginalis*, *Candida* and bacterial vaginosis by maintaining a vaginal pH of 4.0 to 4.5. Lactic acid enters the pathogen cell and lowers the pH inside the cell, inhibiting growth and cooperation [33]. The increase in the number of *Lactobacillus* produces various acidic substances and bioactive antifungal agents. They include lactic acid, benzoic acid, propionic acids, formic acid, butyric acid, hexanoic, caproic acids, phenylacetic acid, H_2O_2, monohydroxy

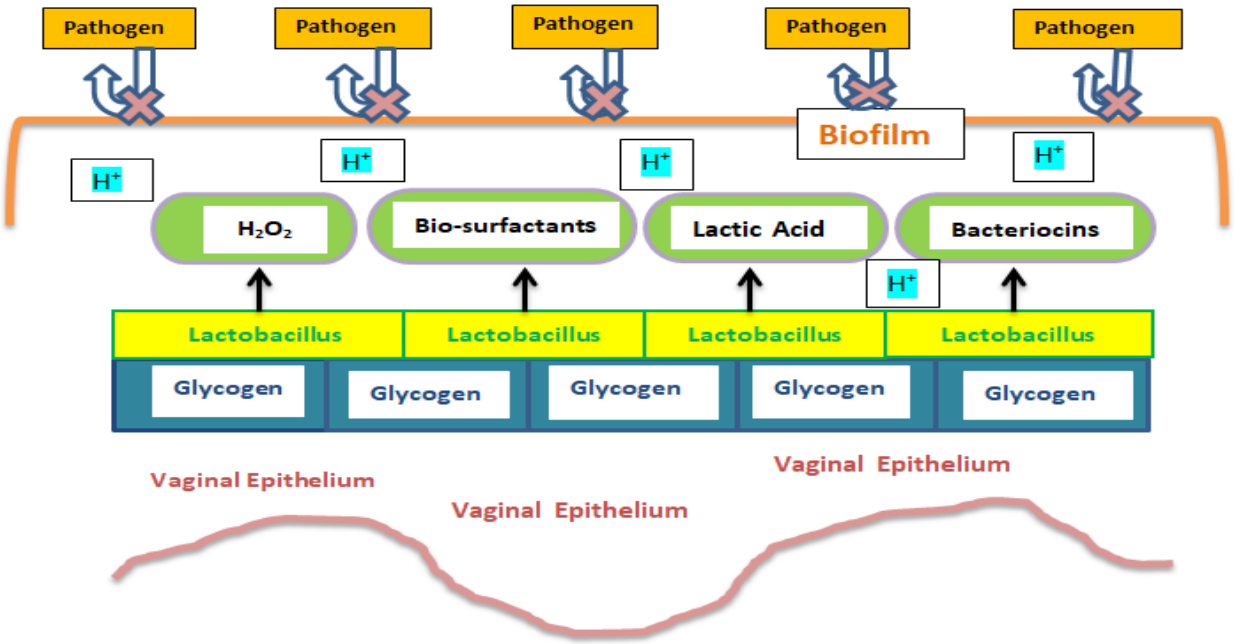

Fig. 1 Vaginal defense mechanism determined by *Lactobacillus*. The first one is the maintenance of epithelial barrier, improved adhesion, inhibits the binding of pathogens, exclude harmful microorganisms, yielding antimicrobial compounds such as hydrogen peroxide, bacteriocins, and bio-surfactants. Production of lactic acid by the metabolism of glycogen reduces the pH ranging from 3.5 to 4.5. Microbial biofilm plays an important role in the maintenance of the epithelial barrier and serves as a protective shield against pathogenic bacteria. It was adopted from a previously published article [1].

octadecenoic acid, CO_2, cyclic dipeptides, phenolics, bacteriocins, fungicides, uterine, ethanol, diacetyl and hydroxyl fatty acids [34]. Those bacteria that can cause fermentation of sugars are thick peptidoglycan layer bacteria. The metabolic process of every organism becomes different from each other when as primary carbon source glucose is used [16]. Lactic acid produced by bacteria can perform direct pathogen-suppressing effects by breaking off the plasma membrane of harmful bacteria, for example, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* [35].

Hydrogen peroxide (H_2O_2)

Another important defense component produced by vaginal lactic acid-producing bacteria is H_2O_2 [36]. Hydrogen peroxide is an activating chemical that is hazardous for anaerobic micro-organisms that are particularly catalase-deficient bacteria [37]. In the process of terminal oxidation, the conversion of oxygen to water is an important step but lactic acid-producing bacteria are unable to perform this step because they lack haematin. This is the reason that flavo-proteins cause the conversion of oxygen directly to H_2O_2 [38]. The most common species of the vagina that produces 94% to 95% of the H_2O_2 are *L. crispatus* and *L. jensenii* [39]. Different studies related to epidemiology demonstrated that in the prevention of diseases that spread through sexual activities, most prominently H_2O_2-producing *Lactobacillus* plays an important role [40]. This excess production of H_2O_2 can exert many harmful effects on the vaginal bacteria, particularly those with a reduced expression of H_2O_2 degrading enzymes such as catalase-peroxidase, for example, *Prevotella*, *Peptostreptococus*, and *Gardnerella* [38]. Furthermore, some *Lactobacillus* species create H_2O_2, giving them a competitive edge over other possible vaginal colonists organisms in the presence of oxygen, while the environment of the female reproductive tract is predominantly anaerobic which may limit H_2O_2 generation in vivo [23].

Bacteriocins and bacteriocin-like substances

Bacterial ribosomes are the sites of bacteriocins production, which are defined as antimicrobial peptides. The most important function of these antimicrobial peptides is that they prevent the growth of bacterial strains that show similar characteristics as they possess. Bacteriocins have numerous structures, working mechanisms, biosynthesis, process of self-immunity and regulation of genes [41]. These

compounds have biotechnological significance because they can exhibit anti-microbial efficacy against pathogenic and degrading micro-organisms [42]. Bacteriocins are classified into primary classes based on their physical and structural properties. However, those produced by thick peptidoglycan layer bacteria especially lactic acid bacteria (LAB) are of particular significance, due to the utilization of multiple strains of gram-positive bacteria, in a variety of industrial uses, that are conventionally known as safe classification [43]. Bacteriocins are classified into three types and this classification is based on their production and molecular composition. Bacteriocin-like substances (BLS) have properties comparable to bacteriocins but do not completely meet the standards. Research highlights that those *Lactobacillus* species that produce bacteriocins and those that produce BLS from cell culture suspensions have antifungal properties [44]. From vaginal cultures, the isolated *Lactobacillus* was found to contain specific bacteriocin genes. For example, Gassericin E from *L. gasseri* inhibits other species such as *G. vaginalis in vitro* [23]. They are distributed into four different groups on the base of their size. Bacteriocins inhibit DNA and protein synthesis by eradicating cell wall integrity. They perform their activity against vaginal infectious agents such as *Gardnerella vaginalis*, *Escherichia coli*, and *Candida albican*. Bacteriocins and antibiotics have similar inhibitory actions but the difference is noticed in their production, working mechanism, lethality and resistance mechanisms. Furthermore, bacteriocins are working well at a vaginal pH of 4.0 to 7.0, and drop significantly outside of this range [33].

Adhesion and colonization

Lactobacillus species also develop another defense substance called adhesins, which aid in their inhabitation to the epithelial linings and at the same time inhibits the inhabitation of epithelial lining with the micro-organisms that disrupt the normal vaginal flora, for example, *G. vaginalis* and *Escherichia coli*. Furthermore, *L. crispatus* helps to prevent candidiasis by supporting mucosal defense, and this support is done by the regulation of Toll-like receptors, interleukin 8, and b-defensins [23]. Adherence is a key pathogenic factor that causes the vaginal infection. The purpose of this adherence is the removal of the pathogenic micro-organisms such as *C. albicans, G. vaginalis*, *E. coli*, *Streptococcus agalactia*, and *S. aureus*. *C. albicans* and *G. vaginalis* are attached to vaginal mucosal cells, while the other pathogenic organism like *E. coli* and *Streptococcus agalactia* do not bind to the vaginal epithelial cells. The first two species that can adhere are considered as vaginal pathogens while the other two species are considered as opportunistic pathogens [33]. By depletion in cells epithelium membrane of cholesterol, there is reduction occurs in the bacterial adhesion forces. Thus, the cells of epithelium can modulate the membrane that interferes with bacterial adherence [45]. *Lactobacilli* adhesive characteristics vary by their type of strain instead of species, according to molecular analysis [46].

Exploring the Vaginal Probiotic Potential of Lactic Acid Bacteria

The terminology of "Probiotic" comes from the Latin words "pro" and "bios" meaning "life". "Russian Nobel laureate Elie Metchnikoff first gave the concept of probiotics in 1907. This concept of probiotics provides the information that they have many curative effects. There were two scientists in 1965 named Lilly and Stillwell who presented the word "probiotic" [37]. Probiotics are beneficial live microorganisms, this definition of probiotics was first introduced by the World Health Organization and the curative effects are visible only when ingested in sufficient quantity, providing well-being advantages to the carrier [47]. New treatments against multidrug resistance infections are possible by lactic acid bacteria that show the property to secrete specie specific secondary metabolites [48]. The most common probiotics on which studies are done are *Lactobacillus* and *Bifidobacterium*. However, other species, such as *Enterococcus faecium* and *Enterococcus faecalis*, have demonstrated probiotic activity [49]. There has been meaningful eagerness among women about the application of lactic acid bacteria for preserving urogenital well-being. See Fig. 2 representing the requirements for the choice of probiotics [37].

Role of *Lactobacillus* in Gynecological Conditions

Bacterial vaginosis

Bacterial vaginosis (BV) ranks among prevailing and frequently occurring disorder that affects females for the duration of their reproductive cycles. The disruption of the natural microbiome is also known as dysbiosis and it is considered a key factor for the occurrence of bacterial vaginosis (BV). Dysbiosis is recognized by a bacterial overpopulation that grows

in little or no presence of oxygen [50]. An increase in the number of anaerobic bacteria is linked with bacterial vaginosis, which may negatively impact the epithelial cells of the vagina and raise the chance of Human Papillomavirus infection [51]. In a normal healthy vagina, the *Lactobacilli* dominate and they work hard to maintain the balance and keep everything safe by producing lactic acid. At the same time, some pathogenic bacteria like *Gardneralla vaginalis* and *Mycoplasma hominis* will present in fewer numbers because the *Lactobacillus* dominates. However, there is a decrease in *Lactobacillus* number when a person is infected with bacterial vaginosis. Alongside, there is a rise in pathogenic bacteria like *Gardneralla vaginalis* and *Mycoplasma hominis* [52]**.** The bacteria in the bacterial vaginosis microbiota build a biofilm on the epithelial layer and secrete a toxic substance that can eradicate epithelial cells. *Gardneralla vaginalis* produces enzymes known as proteolytics that break down proteins as well as decarboxylase into amino acids [53]. There are following signs of bacterial vaginosis (BV), for example, fish-scented discharge can cause serious anxiety [54], a burning feeling when urinating, and itching inside and outside the vagina [51]. Research was carried out to study the curative effects of *L. rhamnosus* vaginal application. First women patients were treated with the antibiotic metronidazole that was given orally to prevent this disease from occurring again and again. This study included forty-nine women. These women were positive with BV. Some of them underwent a seven-day treatment of metronidazole and were also given *L. rhamnosus* 40mg dose for six months. The results concluded that 96 percent of patients exhibited beneficial vaginal colonization and decreased the risk of BV infection [37]. A study was conducted on vaginal probiotics to check its effects on post-BV treatment. 95 women were provided vaginal tablets containing *L. gasseri* LN40, *L. fermentum* LN99, *L. casei* subsp. *L. rhamnosus* LN113, and *P. acidilalactici* LN23 at doses ranging from 10^8 to 10^9 viable cells per capsule, and the duration of giving this capsule was kept 5 days. At the initial steps, probiotic strains persisted 89% in women, and 53% of this probiotic strain resided after one menstruation. Nine percent remained resided after six months. Probiotics reduced the foul-smelling screens and showed potential for higher cure rates [55]. Lactic acid has been proposed as a potentially beneficial effect in the cure of bacterial vaginosis (BV) by stabilizing the genital microbiota and disrupting harmful bacterial biofilms [56].

Vulvovaginal candidiasis

Candida albicans causes vulvovaginal candidiasis. They exist as symbionts. They are considered as they can be changed from non-pathogenic to pathogenic and this depends upon the variety of changes that take place in the host. About 10-15% of women carry Candida without any symptoms, while about 70-75% experience at least one-time vulvovaginal infection during their lives and 50% of the women experience re-occurrence [57]. We can recognize it by biofilm formation when the Candida cell's attachment to the host epithelium takes place [58]. In the United States, bacterial vaginosis is the second most common disease. Millions of women globally still suffer from vulvovaginal candidiasis [59]. Azole is considered beneficial in the treatment of vulvovaginal candidiasis. It is a short-duration local therapy. Nystatin is also used as local therapy but it is less curative when we make its comparison to Azole [60]. Antifungal therapy is used as a recent treatment method. The studies considered two strains of probiotics which are effective against *Candida albicans*. An example is *L. gasseri*. A recent meta-analysis observed 2212 women participants. Then they performed 23 randomized controlled trials on these patients. The conclusion was that recurrent vaginal candidiasis by the use of probiotics is prevented at six months and there is a 64% less chance of happening vaginal candidiasis in women who use probiotics as compared to women who do not use probiotics [1].

Endometriosis

Those tissues that have a composition similar to the endometrium outside the uterus, are affected by an inflammatory condition, called endometriosis which leads to the formation of lesions in these tissues, predominantly impacting women in their reproductive years [61]**.** By studying the immune-pathophysiology of endometriosis, it is not still sure what is the main causative agent of it but one concept is that the immune system is not working properly. When the immune system is working properly it can remove any misplaced tissue in the body for example during mensuration blood flows back into the body but when the immune system not working properly the tissue is not removed and develops outside the uterus [62]. This syndrome seems to be dependent on the estrogen level for its development which predominantly affects women of reproductive age [63]. The hallmark symptom associated with endometriosis is pain [64]. The pain that is linked or

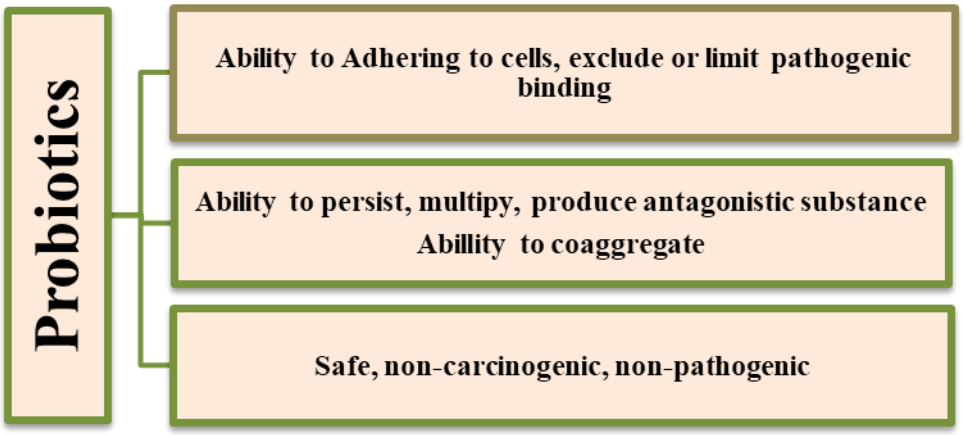

Fig. 2 Requirements in the choice of probiotics. They should be safe, non-invasive, non-carcinogenic, non-pathogenic, ability to adhere cells, co-aggregate, persist, multiply and produce antagonistic substances. This figure was adapted from a manuscript published earlier [37].

associated with endometriosis encompasses with following conditions such as painful menstruation, pain during sexual intercourse, painful defecation, and difficulty in urination alongside prolonged pelvic pain [65]. Those individuals who are suffering from endometriosis may endure intense pain similar to the suffering experienced by cancer patients. Participants received LactoFem® capsule and this capsule comprised 10^9 colonies of four distinct types of strains of *Lactobacillus* bacteria which include *L. acidophilus*, *L. plantarum*, *L. fermentum* and *L. gasseri*. Research indicated that *Lactobacilli*, especially *L. gasseri*, have the potential to enhance IL-12 production, modulate host immunity, and influence the microbiome which could potentially lead to pain relief [66]. The underlying mechanisms of endometriosis are still not debatable, that affect the reproductive health of women. The study found that to reduce menstrual pain and dysmenorrhea in endometriosis patients *L. gasseri* OLL2809 tablet was effective. IL-12 production at a high level by probiotics contributes to pain relief without adverse effects [67].

Genitourinary syndrome of menopause (GSM)

By declining estrogen levels, the tissues of the urogenital tract undergo thinning, dryness and inflammation, particularly in women experiencing menopause, and this phenomenon is exhibited by the genitourinary syndrome of menopause [1]. This disorder ailment involves genital manifestations like irritation, dryness, and burning, urinary indications such as painful urination, frequent urinary infections, and sexual discomfort encompassing discomfort and dedication [68]. Vulvovaginal atrophy (VVA) is more common in postmenopausal women [69]. GSM occurs by the transformation occurring in the labia, introitus, clitoris, vagina, urethra and bladder. The epidemiology was that the ratio by which they affect women is 84% while in a US study, 57% of sexually active middle-aged women express VAA signs. GSM has been diagnosed in 65% of women in one-year post-menopause and 85% post-menopause. Key indications include vaginal desiccation (100%), pain in sexual intercourse (78%), burning/itching (57%), and difficulty in urination (32%) [70]. The objective of GSM treatment is to provide relief to the patient from symptoms. The first line of treatment in women who do not perform any sexual activities includes extended-release lubricants of the vagina and a short-term use of genital estrogen. Treatment options currently available include non-hormonal vaginal lubricants for sexual activity, genital lubricants for long duration, systemic hormonal therapies, and supplementation genital estrogen therapies in low amounts, such as creams and intra-vaginal tablets [71]. Multiple studies indicate *L. acidophilus* when combined with ultra-low dose genital estriol (E3) at 0.03mg demonstrates efficacy in alleviating GSM symptoms [1].

Role of *Lactobacillus* in Sexually Transmitted Infections

Chlamydia

On a global scale, sexually transmitted infections can be transmitted through sexual contact (STIs) and Chlamydia is the most commonly transmitted sexual

disease. The causative agent is *Chlamydia trachomatis* stands as the most prominent bacterial agent worldwide [72]. There are 131 million new cases of genital infection caused by *C. trachomatis* reported each year. Adolescents and young adults aged 15 to 24 hours exhibit the highest rates of reported cases of chlamydia infection. Young women tend to report chlamydia infection more frequently than young men. *C. trachomatis* is living inside the cell with a distinctive biphasic life cycle [73]. Swift antibiotic treatment appears to be the most effective approach for treating chlamydia and their purpose is to prevent serious complications such as female infertility, ectopic pregnancy, neonatal infections like ophthalmitis, and chronic pelvic pain. Identifying and providing timely for Chlamydia infection can be challenging due to various factors that may impede early and swift detection [74]. A Study was conducted that aimed to investigate that is there any association present between vaginal *Lactobacilli* and *Chlamydia trachomatis*. *L. brevis* and *L. salivarius* two strains were used in this study. The conclusion was that *Lactobacillus* particularly *L. brevis* significantly inhibits *C .trachomatis* multiplication at the various stages of the infection cycle such as inhibitory Chlamydial bodies, adsorption to epithelial cells, and intracellular replication [75]. A discovery was made by Foschi et al. that the *C. trachomatis* ability to cause infection is hindered by *L. crispatus* and can produce biosurfactants that target the *Chlamydia trachomatis* elementary bodies (EBs). Here the role of biosurfactants was that they disrupt the elementary bodies (EBs) membrane making them non-communicable [1].

Gonorrhea

Neisseria gonorrhoeae causes Gonorrhea, which is the second most prevalent disease and can spread through sexual contact epidemiology [76]. The occurrence of Gonorrhea is more common in women than men. There were 301174 cases documented in the United States in 2009, with a prevalence rate of 99.1 cases per 100,000 individuals but in the previous years is 10.5% decline was reported [77]. The emerging issue is that Gonorrhoea develops resistance to multiple drugs which makes it challenging to treat Gonorrhea [78]. The primary treatment option that was used for treating gonorrhea is Penicillin but TEM beta-lactamases that were encoded by plasmids were discovered in the 1970s and 1980s then there was a continuous increase in rates of penicillinase-producing *N. gonorrhoeae* [79]. A discovery was made by Foschi et al. that a probiotic strain such as *L. crispatus* uses different mechanisms to hinder the proliferation and survival of *N. gonorrhoeae*. These different mechanisms include pH below 4.0, which represents an acidic environment, hydrogen peroxide production and the production of biosurfactants. In another preclinical experimentation, *L. crispatus* containing vaginal gel is created, which is used to prevent gonorrhea. When exposed to gel there is a notable reduction in the growth of gonorrhea bacteria [1].

Human papilloma virus (HPV)

HPV infection significantly increases the risk of vaginal intraepithelial neoplasia. The most common HPV-associated vaginal lesions were HPV16 (24.2%), HPV52 (24.2%), HPV53 (16.1%), HPV58 (14.5%), and HPV66 (14.5%) [80]. In the declining HPV infection rate with age in youth, the major factor that plays its role is HPV-specific immunity. The poorly defined immunological factors are used primarily to determine the viral outcomes. Risks increase with smoking, multiple childbirth and prolonged use of oral contraceptives. *Chlamydia trachomatis*, chronic inflammation and nutritional factors may also influence. HPV vaccination is the prime prevention method undergoing large-scale trials [81]. The fourth most common cancer among women as per statistics is cervical cancer, which is ranked by the World Health Organization (WHO). HPV mostly affects sexually active women and the ratio by which it affects is 99% of cervical cancer. The infection will be cured in 6 to 24 months but the duration of clearance of infections may be 6-24 months. If the infection is prolonged, it will increase the cancer risk. HPV clearance occurs by two strains of probiotics, which are *Bifidobacteria* and *Lactobacillus* used for HPV clearance [82]. HPV becomes persistent by dysbiosis in the vaginal microbiota [83]. Another research performed in 2019 aims to assess the impact of probiotic strains *L. rhamnosus* GR-1 and *L. reuteri* RC-14 on genital high-risk HPV clearance and cervical smear quality. However, no significant difference was found in HR-HPV clearance between the probiotic and controls [84]. There are limitations of HPV preventive vaccines, which include that they may prevent high-risk HPV infections but they do not treat cervical cancer. The researchers are working on new ones that might be used to treat cervical cancer. These therapeutic vaccines target the HPV E6 and E7 oncoproteins. Bacterial vector vaccines especially *Lactobacillus* based are promising. For example, *L.*

casei (IGMKK16E7) therapeutic HPV vaccine with E7 endogenous expression is developed and BLS-M07 expressing HPV16 E7. Vaccines are not implemented and are still in the stage of trials. *Lactobacillus* are used as engineering bacteria in the development of the curative HPV vaccine [21]. Currently, there is limited efficacy of probiotics in clearing HPV and no conclusive results can be drawn from it but improvements can be made by performing additional high-quality randomized controlled trials to validate these results and identify the potential predictors of viral persistence [1].

Genital herpes

Genital Herpes is caused by Herpes Simplex virus (HSV). HSV is covered by an envelope. Its genome is double-stranded DNA. HSV-1 and HSV-2 are two main types and their route of transmission from one person to another is sexual activities [85]. In the United States, during the period of 2005 and 2010, in the individual age group 14 to 49 years, the prevalence of HSV-1 and HSV-2 was 53.9% and 15.7%, respectively. It is also more common in immune-compromised patients. The major risk factor for human immunodeficiency virus (HIV) is genital HSV infection [86]. For the patients expressing the symptoms of active lesions, the confirmation of HSV infection is done with the help of polymerase chain reaction (PCR)[87]. To treat HSV infections, acyclovir 200 mg five times each day and 1 g valacyclovir and 125 mg famciclovir twice daily are administered. To prevent HSV shedding in recurrent infections, valacyclovir is taken at 500 mg twice daily. The complete suppression of HSV is still not achieved even if we use higher doses of antiviral medications than standard [86, 87]. Acyclovir, famciclovir (Famvir), and valacyclovir (Valtrex) are used as three nucleoside analogs. Their mechanism of action is that they hinder the viral DNA replication. This treatment procedure for HSV-1 and HSV-2 remains constant [87]. Another research was carried out that aimed to observe the inhibitory results of *L. acidophilus* culture supernatant on HSV-1 replication. The conclusions are made that there is a significant reduction in the HSV-1 replication by the activity of *L. acidophilus* culture supernatant which shows its antiviral potential[86]. The research was conducted to make a comparison of *Lactobacillus brevis* and acyclovir in treating HSV-2 herpes infections in women. Iranian women who met the specific criteria were selected to either take *Lactobacillus brevis* or oral acyclovir 400 mg for 6 months. The patients were observed for two consecutive recurrences post-treatment. In terms of primary and secondary efficacy endpoints, there was no significant difference was found between acyclovir and probiotics but acyclovir causes some adverse effects in patients. As compared to acyclovir probiotic therapy appears to be more beneficial because of its low cost, easy assessment, and affordability [89].

Conclusions

In conclusion, probiotics are beneficial live micro-organisms that play very important in improving vaginal health by expressing a variety of mechanisms, including increasing estrogen levels, production of lactic acid, lowering pH, hydrogen peroxide, bacteriocin production, adhesion and colonization properties. *Lactobacillus* strains dominate the vagina according to their efficacy in the treatment of specific vaginal abnormal conditions. A variety of studies ensures that probiotics can be given as an additional food substance or as a dietary source to prevent many gynecological conditions. In these studies, there is strong evidence to treat diseases like bacterial vaginosis (BV), vulvovaginal candidiasis, Endometriosis, GSM, chlamydia, gonorrhea and genital herpes, but weak evidence was found against the role of probiotics in the clearance of human papillomavirus (HPV). There should be more work done on HPV preventive vaccines to treat cervical cancer by using engineered *Lactobacillus* bacteria. Overall, the use of probiotics in improving the vaginal health is beneficial, easy, and cost-effective.

Conflict of interest

The Author has no conflict of interest relevant to this article.

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