Int. j. adv. multidisc. res. stud. 2023; 3(4):512-518

Received: 07-06-2023
Accepted: 17-07-2023

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

Vaginal Microbiota and Probiotics for the Prevention and Treatment of Recurrent Vulvovaginal Infections

[1] María Ángeles Moreno-Pérez, [2] María Jesús Ramírez-Expósito, [3] José Manuel Martínez-Martos

[1, 2, 3] Department of Health Sciences, Experimental and Clinical Physiopathology Research Group CTS-1039, School of Health Sciences, University of Jaén, Jaén, Spain

Corresponding Author: **José Manuel Martínez-Martos**

Abstract

Women's obstetric and reproductive health has been under increasing investigation in recent years. A problem of high prevalence and importance is vaginal infections and the recurrence to which they tend to lead. The most frequent are bacterial vaginosis, which is involved in 40-50% of vaginitis cases, and vulvovaginal candidiasis, which accounts for about 20%. Recent studies examine the role of the vaginal microbiota in disease and the relationship between dysbiosis (altered state of the microbiota) and vaginal infections and how the incidence could be prevented or reduced by restoring the natural vaginal microbiome. Multiple reproductive health consequences arise from microbial invasions and alterations in the vaginal tract, making it an issue to be addressed. The uncontrolled use of antibiotics and antifungals to treat such problems not only leads to drug resistance but also to the potentiation of dysbiosis. This review aims to analyse the efficacy of complementary therapies to traditional therapies to treat vaginal infections, such as the use of probiotics to restore the vaginal ecosystem.

Keywords: Bacterial Vaginosis, Candidiasis, Dysbiosis, Lactobacillus, Microbiome, Probiotics, Vaginal Infections, Vaginal Microbiota

1. Introduction

Recent studies by the Human Microbiome Project (HMP), which is dedicated to the profiling of microbiota communities in humans, have highlighted the relevance of the microbiome in the context of health and its influence on disease [1]. Vaginal infections are a field about which information is still unknown, and that is why, being at the peak of scientific research, the way is being opened to explore new treatments and prevention strategies, investigating their relationship with the imbalance of the vaginal microbiota.

1.1 Vaginal Microbiome

The female genital tract is colonised by a wide variety of micro-organisms that are in dynamic equilibrium and protect the vaginal mucosa from invasion by pathogens. This host assemblage is referred to as the microbiota, microbiome or vaginal flora. Both bacterial communities, e.g. Lactobacillus and other anaerobic bacteria, and fungal communities, mainly represented by Candida albicans, cohabit [2].

The variation in the balance of colonisation of these species in the vaginal flora is due to physiological conditions, such as the ovarian cycle or the phases of a woman's sexual and reproductive life [3]. However, certain habits or actions can contribute to the pathological imbalance of the communities that inhabit the mucosa. In addition, there are genetic and environmental factors that would determine which communities one is more prone to have depending on ethnicity, geographical location, age, etc [4].

1.2 Natural Defence Mechanisms of the Female Genital Tract

The anatomical and physiological characteristics of the genital organs - short distance between anus and urethra and moist environment - increase the risk of infection. Nature has developed a series of defence mechanisms against potentially pathogenic micro-organisms that vary throughout the life cycle. In the newborn girl, it is the acidic pH that hinders the excessive proliferation of such invaders, under the influence of the mother's oestrogen from the intrauterine period and following colonisation by acid-forming bacteria (Lactobacillus acidophilus). During the following months, the level of oestrogens and the community of acidifying bacteria gradually decrease, so that the pH alkalinises to a neutral pH. Although your body has not yet developed the necessary molecules that trigger the pH-acidifying processes, there are other barriers that

protect against infection. The narrowness of the vagina, the tightness of the vaginal walls, the folds and the presence of the hymen are physical mechanisms that compensate for the lack of biochemical defences during this phase. During puberty, oestrogen levels increase and new layers of tissue form in the vaginal epithelium, between which accumulate cells capable of synthesising glycogen that allows the growth of acid-forming bacteria. In maturity, future defences will develop, including intensified exfoliation of genital epithelial cells, formation of secretions, specific pH [5].

2. Healthy Microbiota vs. Dysbiosis

The microbiome itself determines the immunity and protection that the entrance to the vagina will present. Scientific evidence has shown an association between vaginal dysbiosis, or imbalance in microbial composition, and an increase in vaginal infections, from bacterial and fungal infections such as bacterial vaginosis (BV) and vulvovaginal candidiasis (VVC), to sexually transmitted infections such as trichomoniasis, human papillomavirus (HPV), Chlamydia, human immunodeficiency virus (HIV) and even genital herpes infections [6-11] (Table 1).

Table 1: Summary of factors increasing or decreasing the risk of dysbiosis

		Reference
Lactobacillus community	Reduces risk	[12]
High concentration of oestrogens	Increases risk	[13]
Basic pH	Increases risk	[5]
Diet high in simple HC	Increases risk	[16]
Diet high in saturated fat	Increases risk	[5]
Exogenous chemicals	Increases risk	[15]
Douching	Increases risk	[12]
Antioxidant-rich diet	Reduces risk	[5]

Lactobacillus species, including Lactobacillus Crispatus, Lactobacillus Gasseri, Lactobacillus Iners and Lactobacillus Jensenii, are abundant in a healthy vaginal microbiota [6]. The characteristics of these particles confer antioxidant, anti-biofilm, and immunomodulatory properties [12]. In addition, they have pathogen inhibitory power by acidifying the medium through the conversion of glycogen into lactic acid after a metabolic process and antimicrobial activity thanks to their ability to produce hydrogen peroxide (H2O2) [2, 13].

It is important to maintain an adequate level of these bacteria because, as mentioned above, the vaginal flora also contains a wide variety of potentially pathogenic micro-organisms, and an alteration of the microbial community can overstep the boundary between symbiosis and invasion, increasing the risk of excessive proliferation and adherence to the epithelium, with the possibility of infection. Former hosts become infectious agents. This state of mismatch and imbalance of the microbiota is called dysbiosis, and can be the consequence of multiple factors.

2.1 Factors that may Alter the Balance of the Vaginal Microbiota

The human vaginal microbiome can be altered by a variety of causes. These causes may be purely physiological or associated with habits and/or behaviour.

In relation to physiological causes, it has been shown that a high oestrogen condition influences the microbiota in such a way that its immunity is lower compared to a lower oestrogen condition [13]. This could be explained by understanding the reproductive role of ovarian cycles. In the luteal phase, the organism prepares to receive a foreign body which will be the spermatozoon that must fertilise a previously matured egg. If the microbiota's defence in this phase were too reactive, it would attack this foreign body and fertilisation would not take place. Therefore, among the mechanisms that the body triggers to enhance fertility and to receive the future fertilising sperm is an increase in oestrogen. It is for this reason that women tend to get infections mostly in this phase of the cycle, as well as during pregnancy and in other situations where oestrogen levels are high, such as oestrogen replacement therapy or taking oral contraceptives with high oestrogen concentrations [2, 14].

Behaviourally, numerous studies confirm the association between dysbiosis and antibiotic use, douching, unprotected sex and exposure to endocrine disruptors or exogenous chemicals, the latter especially when the contact is direct through "hygiene" products [12, 15].

Diet has also been shown to play a role in determining the composition of the microbiota. For example, it has been shown that constant exposure to hyperglycaemic states after meals can increase the risk of infection by pathogens. The oxidative stress to which cells are exposed following hyperglycaemia and the consequent production of free radicals trigger inflammatory processes that may affect the immune response against microbial overpopulation [16]. A diet high in fat, especially saturated fat, can increase vaginal pH, which again favours the growth of these pathogens. On the other hand, a diet with adequate levels of folic acid, vitamins D, E, A and C, beta-carotene, calcium and betaine prevents inflammation and improves the immune response [5]. Finally, there are different compositions of microbiota that depend on the individuality of the person, which in turn depend on genetic and environmental factors. A vaginal microbiota dominated by the genus *Lactobacillus Iners* is more prone to dysbiosis than one dominated by *Lactobacillus Crispatus* [17].

Although vaginal dysbiosis is a complex and multifactorial process, identifying some of the causes that increase the risk is crucial for the prevention and early treatment of the conditions it can trigger.

3. Vaginitis

Vaginitis refers to the condition where the vaginal mucosa is inflamed and, normally, this inflammation is accompanied by a vaginal discharge with signs of abnormality. When, in addition, the vulva is involved, we speak of vulvovaginitis. Symptoms of vulvovaginitis may include vulvovaginal itching or irritation, dysuria, dyspareunia and light bleeding. It is usually caused by vaginal infections, but can also be caused by allergic reactions, after contact with irritating substances or vaginal atrophy [18].

3.1 Most Prevalent Vaginal Infections

Bacterial vaginosis (BV) is the most prevalent type of infection, comprising 40-50% of diagnosed vaginitis and affecting approximately 29% of women of reproductive age at least once in their lifetime [5, 18]. Recurrence rates of this infection are currently high at approximately 80% after effective treatment [19], which implies the need and insistence on finding alternative means of treatment to eradicate further occurrences or, alternatively, to find out the

latent cause(s) by which this type of infection is derived in order to prevent its occurrence.

The bacterium that usually causes this infection is *Gardnerella vaginalis* and is part of the vaginal tract [20]. An overgrowth of this bacterium and an imbalance with the bacteria that keep the vaginal environment acidified and protected from external agents causes the infection. Fifty per cent of women who are infected are symptomatic, experiencing vaginal itching, abnormal, greyish, strong-smelling discharge with increased vaginal pH [19]. The clinical diagnosis of this condition is made through the conjunction of symptomatology, physical anamnesis and laboratory tests, which meet the Amsel criteria [18] (Table 2). Gram staining is the most accurate diagnostic test, as *Gardnerella vaginalis* has a high sensitivity and specificity by this test [18].

Table 2: Amsel criteria for the diagnosis of vulvovaginitis

At least 3 of the 4 conditions must be met for the diagnosis of BV.	pH >4.5
	Amine test +
	Presence of key cells
	Increased, homogeneous, greyish, fishy-smelling vaginal discharge.

Scientific evidence has confirmed that BV (Table 3) is associated with an increased risk of contracting sexually transmitted infections such as gonorrhoea, trichomoniasis, chlamydia, human immunodeficiency virus, herpes simplex and human papillomavirus [20]. On the other hand, Salah *et al* [21] have stated that bacterial vaginosis has a strong association with infertility problems: it is estimated that 1 in 5 patients suffering from infertility have had bacterial vaginosis.

Table 3: Summary of characteristic signs and symptoms of bulbovaginitis, vulvovaginal candidiasis and trichomoniasis

	Bacterial vaginosis	**Vulvovaginal candidiasis**	**Trichomoniasis**
Colour of secretion	Greyish	Off-white	Greenish/yellowish,
Odour of secretion	Strong, fishy	Invaluable	Foul
Consistency or texture of secretion	Abundant	Lumpy	Sparkling
pH	>4.5	<4.2	>4.5
Other	-	Itchy discharge	Itchy discharge
According to [19, 22, 23].			

Secondly, vulvovaginal candidiasis (VVC) (Table 3) accounts for 20-25% of diagnosed vaginitis [5]. It affects up to 75% of women of childbearing age at least once in their lifetime, and about 9% suffer recurrences (at least 3 episodes per year) [2, 14]. It is most often caused by the fungus *Candida albicans*, which inhabits the respiratory, gastrointestinal and, of course, genitourinary mucosa. Like BV, it is produced by a microbe that regularly but asymptomatically inhabits the vagina and, likewise, its presence causes symptoms following uncontrolled invasion, which inflames the mucosa and produces virulence factors, triggering yeast infection with possible symptoms of vulvovaginitis and an itchy, whitish, lumpy-textured vaginal discharge [22]. Diagnosis of VVC is made by the expression of characteristic signs and symptoms, the presence of potassium hydroxide in vaginal discharge samples or DNA hybridisation probe testing [18].

The third most common cause of vaginitis is trichomoniasis (Table 3), comprising 10-15% of all diagnosed vaginitis [5], ahead of chlamydia and gonorrhoea. It is an infection caused by the parasite *Trichomonas vaginalis*. The symptoms caused by this infection are similar to those mentioned above, but it is characterised by a greenish or yellowish vaginal discharge with a foul odour, foamy consistency and itching. There is evidence that this condition is associated with preterm birth, low birth weight infants, premature rupture of the placental membrane and neonatal death [23].

4. Healthy Vaginal Microbiota and Role in Fertility Reproductive and Obstetric Health

Evidence suggests that a healthy vaginal microbiota, i.e., one with a balanced composition of host microorganisms and a high concentration of lactobacilli, supports women's reproductive health. In addition to the health problems associated with vaginal infections discussed above, an imbalance of the communities colonising the vulvovaginal, cervicovaginal and endometrial mucosa is also associated with hormonal, metabolic and reproductive disorders [24]. Salah *et al* [21] confirmed the association between infertility and atypical microbiota in the course of infectious bacterial invasion, concluding that detection and treatment of bacterial vaginosis improved pregnancy rates. Similarly, one study observed that low concentrations of lactobacilli could be implicated in implantation failure and miscarriage of *in vitro* fertilised embryos [25]. Dysbiotic microbiota have also been associated with preterm birth [12]. On the other hand, there is scientific evidence of symptomatic improvement of polycystic ovary syndrome when the vagina is largely colonised by lactobacilli, which can be given by external administration [24].

All of these statements justify the need to address the situation through in-depth, specialised research that addresses all of these scenarios and finds safe and effective treatments, or educates people to identify that this problem exists and that it may be responsible for many conditions for which no answers have been found.

5. Treatment of Vaginitis and Related Dysbiosis

Currently the application of treatment for the restoration of vaginal eubiosis is not very common, mainly because dysbiosis of the microbiota is not considered pathology, despite increasing studies showing its close relationship to the disease. It is then a question of acute pathology, for example: in the case of vaginal infections, the infectious agent would be fought against. If it were a bacterium, it would be treated with antibiotics, and if it were a fungus, with antifungals. In the case of non-infectious vaginitis, treatment does address the underlying cause. For example, in atrophic vaginitis, hormonal treatments are applied [18]; in the case of an allergic reaction, the allergen is withdrawn, etc. This begs the question: if the underlying cause of many infectious vaginitis is due to previous dysbiosis, why not apply therapies that restore microbial normality?

5.1 Conventional Treatment

The most current clinical practice guidelines on the treatment of vaginitis postulate the use of antimicrobial drugs to fight the infection. For BV, oral metronidazole, intravaginal metronidazole and intravaginal clindacimin are the drugs of choice. Although they have been shown to be effective, they are not valid options for long-term treatment, as they do not prevent future recurrences and are quite common [19]. On the other hand, antifungal formulations

such as azoles are chosen for VVC, the drugs of choice being miconazole, clotrimazole and fluconazole. These formulations are usually in the form of topical cream or vaginal ovules, which are administered directly into the vagina and attack the invading host more effectively. They have also been found to be ineffective in the long term [26].

Since relapses are so common in women who have already been infected at least once, it is vitally important to find a treatment alternative that does not generate resistance. The continued and frequent use of antibiotics and antifungals creates resistance to these drugs [12], making them less effective each time and endangering the person using them for three reasons: the need for an antibiotic for another more severe infection, the alteration of the microbiota due to their use, as mentioned above, and the elimination of beneficial bacteria.

This is why the use of probiotics is being widely studied as a safer treatment alternative to antimicrobials and prevention of future relapses, in order to prevent obstetric and gynaecological complications arising from dysbiosis in states of infection.

5.2 Use of Probiotics as a Therapy to Restore the Vaginal Ecosystem

Here we propose to review the use of probiotics based on the properties of *Lactobacilli* that confer the ability to create a protective barrier in the vaginal flora. *Lactobacilli* are gram-positive, microaerophilic, acid-inhabiting molecules that do not produce spores and are capable of producing lactic acid, bile salt hydrolases, organic acids and antimicrobial compounds, which gives them pathogen inhibitory activity. They also have antibiofilm and antioxidant activity, i.e. they protect from potentially pathogenic bacteria by shedding their protective matrix, and from oxidative damage produced by free radicals, which is very interesting in terms of health [12]; they have also been shown to modulate the immune system [27]. These molecules adhere to the epithelium and colonise it in competition with other micro-organisms. They are usually present in considerable quantities as part of the healthy vaginal microbiota and, thanks to their characteristics, the vagina's moist environment, which is susceptible to invasion by pathogens, is protected. They can also be provided through the diet, by eating fermented foods such as yoghurt, kefir, sauerkraut, certain pickles, etc. In this way, the benefits of these micro-organisms, which will colonise the microbiota once ingested, are conferred.

Recent research proposes external administration of different strains of probiotics, including the genus *Lactobacillus*, as a preventive method or as a treatment for vaginitis, via oral and intravaginal supplementation. It is hypothesised that external probiotic supplementation could restore the balance of the colonising species of microbiota, bringing them back to a state of healthy eubiosis. A randomised, placebo-controlled pilot study conducted in 2017 demonstrated that oral administration of different probiotic strains would be sufficient to colonise the vaginal tract and maintain the same antimicrobial properties as detected *in vitro* [28]. This information provides the basis for studying the effect of colonisation of these species on different types of vaginitis. In 2011, a trial was proposed that looked at the recurrence rate of urinary tract infections in women who had had an infection and had been treated with antimicrobials, comparing a group given *Lactobacillus crispatus* intravaginally with a group given placebo. The results confirmed that those receiving placebo had a higher recurrence rate [29]. However, the limitations of this study, as it did not specify the type of infection suffered and, therefore, the causative microorganism was unknown, do not determine the effectiveness of the probiotic on one microbe or another in a totally reliable manner.

Table 4: Effects of probiotics administered during or after episodes of bacterial vaginosis

Methods	Observed effects	Reference
In this study the sample was chosen from women diagnosed with BV. They were divided between those on metronidazole and those on metronidazole combined with probiotics (Lactobacillus rhamnosus BMX 54) intravaginally.	Patients who supplemented with probiotics showed a replacement of the vaginal microbiota after two months and a decrease in pH at 9 months. The recurrence rate was significantly low compared to the group not taking probiotics.	[32]
A liquid co-culture assay simulating vaginal tract secretions was performed where Lactobacillus strains (*L. acidophilus* GLA-14 and *L. rhamnosus* HN001) were incubated in combination with bacterial strains (*G. vaginalis, A. vaginae, E. coli and S. aureus*) separately.	The microbicidal activity of the lactobacilli against the proposed bacterial strains was demonstrated, causing complete inhibition of their growth at 24h and 48h depending on the strain.	[30]
A prospective, double-blind, randomised, prospective trial was conducted in women with at least two episodes of BV in the past year effectively treated with metronidazole. Lactobacillus crispatus was administered intravaginally for 4 menstrual cycles to prevent recurrence of BV.	41% of people taking placebo had at least one recurrence of BV compared to 20% of those taking probiotics. Lactobacillus administration had no side effects.	[31]
Patients with BV treated with metronidazole for the previous 7 days were randomised into a control group receiving only behavioural EPS and case groups given intermittent metronidazole and vaginal capsules containing various strains of *Lactobacillus.*	The recurrence rate of patients treated with intermittent metronidazole in conjugation with vaginal probiotics was significantly decreased compared to the control group.	[33]

The most recent research examines the effect of lactobacilli against specific micro-organisms. Here we will summarise the conclusions of studies that have looked at the impact on bacterial vaginosis on the one hand and vulvovaginal candidiasis on the other. The interest in testing the efficacy of such treatments stems from the need to find alternatives to commonly used drugs because resistance develops as infections recidide. Thus, the results of several recent trials in which probiotics are administered during or after episodes of bacterial vaginosis are summarized in Table 4. It can be confirmed that external administration of certain doses of probiotic strains could transform the vaginal microbiota to a healthier state. The studies analysed used the intravaginal route as the means of administration, so the variable of

absorption that could modify the effect, as in oral administration, should not be considered. It therefore shows that the propensity for recurrence in episodes of bacterial vaginosis decreases considerably when antimicrobial treatment is combined with probiotic treatment. This means that long-term recovery is improved with probiotic species supplementation [29-33].

Vulvovaginal candidiasis has also been studied to test the efficacy of probiotics for prevention and treatment. Table 5 shows the results of some of the most recent trials evaluating this intervention. They involved women who had or have had at least one episode of vulvovaginal candidiasis and studied the effect of oral probiotics on symptomatology, fungal load and recurrence of the disease. It has been shown that lactic acid inhibits the growth of *Candida albicans* and that colonisation of the vaginal microbiota by lactobacilli prevents recurrences of VVC. Although studies confirm that a single probiotic treatment is sufficient to combat mild infections, i.e., with moderate fungal concentrations and no previous history of resistance to treatment, more research with more representative samples is needed [34-38].

Table 5: Efficacy of probiotics for prevention and treatment of vulvovaginal candidiasis

Methods	Observed effects	Reference
Randomised, placebo-controlled study. A sample of 93 women with a history of recurrent VVC was selected. A treatment regimen of oral *L. plantarum* capsules was administered to one group and placebo control to another.	At the end of treatment, there was a higher colonisation of lactobacilli in the intervention group compared to the control group. It was also associated with improvement of VVC-associated symptoms. The efficacy of the treatment in preventing recurrences is suggested.	[40]
A study was conducted in 48 women with confirmed VVC and a history of recurrences. One group received placebo and the other probiotics and lactoferrin as adjuvant treatment to clotrimazole.	Women treated with probiotics showed a marked improvement in symptoms (itching and discharge) and recurrence rates were 33.3% after 3 months compared to 91.7% for those treated with clotrimazole alone.	[38]
Lactobacillus strains isolated from MRS and *C. albicans* were cultured in broth with yeast extract.	A specific protein with anti-hyphal activity was found in *L. rhamnosus, L. casei and L. paracasei* that had the ability to break down chitin, a molecule present in the hyphae formed by C. albicans and responsible for its virulent expression.	[34]
Twenty women diagnosed with acute VVC were selected. For 10 days they followed a gel treatment containing three strains of lactobacilli.	Of the 20 women, nine did not need to complete their fluconazole treatment; the rest did. Those who needed rescue medication were found to have a more severe infection (higher fungal concentrations at baseline, more hyphae and more previous infections). *C. albicans* concentrations in both groups (treated and untreated with fluconazole) were similar at the end of treatment, with those taking fluconazole showing a reduction of lactobacilli in their vaginal microbiota. No probiotic-related side effects were observed.	[37]
The study sample consisted of a total of 78 pregnant women with a clinical diagnosis of VVC. Fifty percent were treated with probiotics and 50% with placebo, but all of them were additionally treated with clotrimazole.	Patients who received probiotics in addition to treatment showed a significant reduction in *C. albicans* and *C. glabrata* concentration after 8 weeks of treatment compared to placebo.	[35]

5.3 Risk and Safety of Probiotics

As an interesting therapeutic option, it is necessary to determine the safety of its application and possible side effects in different groups and strains. Given that clinical trials have so far reported no cases of side effects related to probiotic intake, *in vitro* and animal studies have not shown a major risk, and the length of time they have been in use, especially as they are found in foods consumed on a daily basis, it can be assumed that their use is generally safe. However, it is risky to confirm that there is no risk. In fact, the FDA (Food and Drug Administration) has identified some populations that would be at potential risk of side effects from probiotic ingestion [39]: a) people undergoing treatment with immunosuppressive drugs, autoimmune diseases or organ or stem cell transplantation; b) people with active bowel disease or acute abdomen and c) persons receiving chemotherapy or radiotherapy for neutropenia.

6. Role of Nursing

As health professionals, nurses have a leading role in addressing this situation. Firstly, there is a very important area in our profession called primary prevention, which is not addressed often enough as it should be. In the Western society in which we live, we have prioritised the cure of diseases over the prevention of disease, moving away from healthy habits and towards pills and interventions once the disease occurs. The information we have about microbiota and what happens when we alter them is very valuable in this regard. We have got closer to the underlying cause of many of the conditions that have hitherto been considered opportunistic and we know how to prevent them. We must promote health by teaching how to care for our microbiota and the impact it has on our lifestyle.

Secondly, our duty to address health on a holistic level means that we must ask ourselves in what other areas, apart from the physiological, the health problem may be affecting the person suffering from it. In this sense, a vaginal infection with symptoms of vaginitis, including itching, burning and pain, among others, is an obstacle to socialising, having sex, going to work, etc. If this problem affects the same person too often, it is clear that they cannot fully enjoy a healthy sexuality, with all the psychological effects that this may entail. We have also seen that it is a very frequent cause of alterations in a woman's fertility, so it is important to identify it and intervene as early as possible, before the problem becomes more serious, as we have seen in situations of premature rupture of membranes, miscarriage, etc.

7. Conclusions

To date, research suggests the effectiveness and safety of probiotics in combination with antimicrobial drugs for the prevention of recurrences and restoration of altered microbiomes. Everything points to the emergence of a new avenue of research worth investigating. An alternative to conventional medicine, where the cure is not achieved by external applications, but by boosting the immune system's own defence mechanisms. There is a tendency in the new generations to generate healthy habits aligned with what our organism, as animals, needs. Knowledge of our body at the most microscopic level and its functioning, together with research into the properties of the elements of nature, is a very valuable tool in favour of our health. Without demonising Western medicine, it is suggested a balance where Western and ancestral medicine complement each other, and prioritise the preservation of health over the cure of disease.

8. References

1. Lloyd-Price J, Mahurkar A, Rahnavard G, Crabtree J, Orvis J, Hall AB, *et al*. Strains, functions and dynamics in the expanded Human Microbiome Project. Nature. 2017; 550(7674):61-66.
2. Rosati D, Bruno M, Jaeger M, Ten Oever J, Netea MG. Recurrent Vulvovaginal Candidiasis: An Immunological Perspective. Microorganisms. 2020; 8(2).
3. Al-Nasiry S, Ambrosino E, Schlaepfer M, Morre SA, Wieten L, Voncken JW, *et al*. The Interplay between Reproductive Tract Microbiota and Immunological System in Human Reproduction. Front Immunol. 2020; 11.
4. Gupta VK, Paul S, Dutta C. Geography, Ethnicity or Subsistence-Specific Variations in Human Microbiome Composition and Diversity. Front Microbiol 2017; 8:p1162.
5. Mizgier M, Jarzabek-Bielecka G, Mruczyk K, Kedzia W. The role of diet and probiotics in prevention and treatment of bacterial vaginosis and vulvovaginal candidiasis in adolescent girls and non-pregnant women. Ginekol Pol. 2020; 91(7):412-416.
6. Eastment MC, McClelland RS. Vaginal microbiota and susceptibility to HIV. AIDS. 2018; 32(6):687-698.
7. McKee KS, Carter KA, Bassis C, Young VB, Reed B, Harper DM, *et al*. The vaginal microbiota, high-risk human papillomavirus infection, and cervical cytology: Results from a population-based study. Gynecol Pelvic Med. 2020; 3.
8. Mitra A, MacIntyre DA, Marchesi JR, Lee YS, Bennett PR, Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? Microbiome. 2016; 4(1):p58.
9. Shannon B, Gajer P, Yi TJ, Ma B, Humphrys MS, Thomas-Pavanel J, *et al*. Distinct Effects of the Cervicovaginal Microbiota and Herpes Simplex Type 2 Infection on Female Genital Tract Immunology. J Infect Dis. 2017; 215(9):1366-1375.
10. Van de Wijgert J. The vaginal microbiome and sexually transmitted infections are interlinked: Consequences for treatment and prevention. PLoS Med. 2017; 14(12):e1002478.
11. Ziklo N, Vidgen ME, Taing K, Huston WM, Timms P. Dysbiosis of the Vaginal Microbiota and Higher Vaginal Kynurenine/Tryptophan Ratio Reveals an Association with Chlamydia trachomatis Genital Infections. Front Cell Infect Microbiol. 2018; 8:p1.
12. Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. Microb Cell Fact. 2020; 19(1):p203.
13. Moulton VR. Sex Hormones in Acquired Immunity and Autoimmune Disease. Front Immunol. 2018; 9:p2279.
14. Al-Nasiry S, Ambrosino E, Schlaepfer M, Morre SA, Wieten L, Voncken JW, *et al*. The Interplay between Reproductive Tract Microbiota and Immunological System in Human Reproduction. Front Immunol. 2020; 11:p378.
15. Aguilera M, Galvez-Ontiveros Y, Rivas A. Endobolome, a New Concept for Determining the Influence of Microbiota Disrupting Chemicals (MDC) in Relation to Specific Endocrine Pathogenesis. Front Microbiol. 2020; 11:p578007.
16. Van Ende M, Wijnants S, Van Dijck P. Sugar Sensing and Signaling in Candida albicans and Candida glabrata. Front Microbiol. 2019; 10:p99.
17. Verstraelen H, Verhelst R, Claeys G, De Backer E, Temmerman M, Vaneechoutte M. Longitudinal analysis of the vaginal microflora in pregnancy suggests that L. crispatus promotes the stability of the normal vaginal microflora and that L. gasseri and/or L. iners are more conducive to the occurrence of abnormal vaginal microflora. BMC Microbiol. 2009; 9:p116.
18. Paladine HL, Desai UA. Vaginitis: Diagnosis and Treatment. Am Fam Physician. 2018; 97(5):321-329.
19. Coudray MS, Madhivanan P. Bacterial vaginosis-A brief synopsis of the literature. Eur J Obstet Gynecol Reprod Biol. 2020; 245:143-148.
20. Ravel J, Moreno I, Simon C. Bacterial vaginosis and its association with infertility, endometritis, and pelvic inflammatory disease. Am J Obstet Gynecol 2021; 224(3):251-257.
21. Salah RM, Allam AM, Magdy AM, Mohamed A. Bacterial vaginosis and infertility: Cause or association? Eur J Obstet Gynecol Reprod Biol. 2013; 167(1):59-63.
22. Willems HME, Ahmed SS, Liu J, Xu Z, Peters BM. Vulvovaginal Candidiasis: A Current Understanding and Burning Questions. J Fungi (Basel). 2020; 6(1).
23. Carlier Y, Truyens C, Deloron P, Peyron F. Congenital parasitic infections: A review. Acta Trop. 2012; 121(2):55-70.
24. Lopez-Moreno A, Aguilera M. Vaginal Probiotics for Reproductive Health and Related Dysbiosis: Systematic Review and Meta-Analysis. J Clin Med. 2021; 10(7).
25. Garcia-Grau I, Perez-Villaroya D, Bau D, Gonzalez-Monfort M, Vilella F, Moreno I, *et al*. Taxonomical and Functional Assessment of the Endometrial Microbiota in a Context of Recurrent Reproductive Failure: A Case Report. Pathogens. 2019; 8(4).
26. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, *et al*. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016; 62(4):e1-e50.
27. Maldonado Galdeano C, Cazorla SI, Lemme Dumit JM, Velez E, Perdigon G. Beneficial Effects of Probiotic Consumption on the Immune System. Ann Nutr Metab.

2019; 74(2):115-124.
28. Mezzasalma V, Manfrini E, Ferri E, Boccarusso M, Di Gennaro P, Schiano I, *et al*. Orally administered multispecies probiotic formulations to prevent uro-genital infections: A randomized placebo-controlled pilot study. Arch Gynecol Obstet. 2017; 295(1):163-172.
29. Stapleton AE, Au-Yeung M, Hooton TM, Fredricks DN, Roberts PL, Czaja CA, *et al*. Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis. 2011; 52(10):1212-1217.
30. Bertuccini L, Russo R, Iosi F, Superti F. Effects of Lactobacillus rhamnosus and Lactobacillus acidophilus on bacterial vaginal pathogens. Int J Immunopathol Pharmacol. 2017; 30(2):163-167.
31. Bohbot JM, Darai E, Bretelle F, Brami G, Daniel C, Cardot JM. Efficacy and safety of vaginally administered lyophilized Lactobacillus crispatus IP 174178 in the prevention of bacterial vaginosis recurrence. J Gynecol Obstet Hum Reprod. 2018; 4 7(2):81-86.
32. Recine N, Palma E, Domenici L, Giorgini M, Imperiale L, Sassu C, *et al*. Restoring vaginal microbiota: Biological control of bacterial vaginosis. A prospective case-control study using Lactobacillus rhamnosus BMX 54 as adjuvant treatment against bacterial vaginosis. Arch Gynecol Obstet. 2016; 293(1):101-107.
33. Van de Wijgert J, Verwijs MC, Agaba SK, Bronowski C, Mwambarangwe L, Uwineza M, *et al*. Intermittent Lactobacilli-containing Vaginal Probiotic or Metronidazole Use to Prevent Bacterial Vaginosis Recurrence: A Pilot Study Incorporating Microscopy and Sequencing. Sci Rep. 2020; 10(1):p3884.
34. Allonsius CN, Vandenheuvel D, Oerlemans EFM, Petrova MI, Donders GGG, Cos P, *et al*. Inhibition of Candida albicans morphogenesis by chitinase from Lactobacillus rhamnosus GG. Sci Rep. 2019; 9(1):p2900.
35. Ang XY, Mageswaran UM, Chung YLF, Lee BK, Azhar SNA, Roslan NS, *et al*. Probiotics Reduce Vaginal Candidiasis in Pregnant Women via Modulating Abundance of Candida and Lactobacillus in Vaginal and Cervicovaginal Regions. Microorganisms. 2022; 10(2).
36. Martens MG, Maximos B, Degenhardt T, Person K, Curelop S, Ghannoum M, *et al*. 107. A Phase 3, Randomized, Double-Blind Study to Evaluate the Efficacy and Safety of Oteseconazole (VT-1161) Oral Capsules versus Fluconazole and Placebo in the Treatment of Acute Vulvovaginal Candidiasis Episodes in Subjects with Recurrent Vulvovaginal Candidiasis (ultraViolet). Open Forum Infectious Diseases. 2021; 8(Supplement 1):S66-S67.
37. Oerlemans EFM, Bellen G, Claes I, Henkens T, Allonsius CN, Wittouck S, *et al*. Impact of a lactobacilli-containing gel on vulvovaginal candidosis and the vaginal microbiome. Sci Rep. 2020; 10(1):p7976.
38. Russo R, Superti F, Karadja E, De Seta F. Randomised clinical trial in women with Recurrent Vulvovaginal Candidiasis: Efficacy of probiotics and lactoferrin as maintenance treatment. Mycoses. 2019; 62(4):328-335.
39. Doron S, Snydman DR. Risk and safety of probiotics. Clin Infect Dis. 2015; 60 Suppl 2:S129-S134.
40. Vladareanu R, Mihu D, Mitran M, Mehedintu C, Boiangiu A, Manolache M, *et al*. New evidence on oral L. Plantarum P17630 product in women with history of recurrent vulvovaginal candidiasis (RVVC): A randomized double-blind placebo-controlled study. Eur Rev Med Pharmacol Sci. 2018; 22(1):262-267.