The Surprising Power of Bacteria in the Vaginal Microbiome: How Lactobacillus is Crucial to Women's Health

Ashley Hurjak

Abstract

The purpose of this review article is to highlight the role of beneficial bacteria in promoting a healthy vaginal microbiome. The article describes the mechanisms for maintaining a healthy vaginal microbiome, the symptoms of a "dysbiotic" (disrupted) vaginal microbiome, and an overview of the vaginal diseases that can occur from a disrupted bacterial ecosystem inside this organ. Additionally, it outlines how to promote a healthy vaginal microbiome through lifestyle choices and a summary of current treatments available to patients who suffer from bacteria-related vaginal infections.

1.0 Introduction

For hundreds of years, women have struggled with vaginal infections (inflammation of the vaginal area that can result in pain and itching), which are caused by bacteria, viruses, or fungi (Saraf et al., 2021). Studies dating back to the 1800s (when the vaginal area was first routinely examined) have demonstrated that the beneficial bacteria found in the vaginal region act as the first line of defense for the female reproductive tract against biological pathogens such as harmful bacteria, viruses, and fungi (Saraf et al., 2021). Any disruptions to these "good" vaginal bacteria weaken the protective barrier against pathogens, which can lead to yeast infections, sexually transmitted infections (STI), and bacterial vaginosis (a condition when there is a surplus of harmful bacteria within the vaginal area) (Chee et al., 2020).

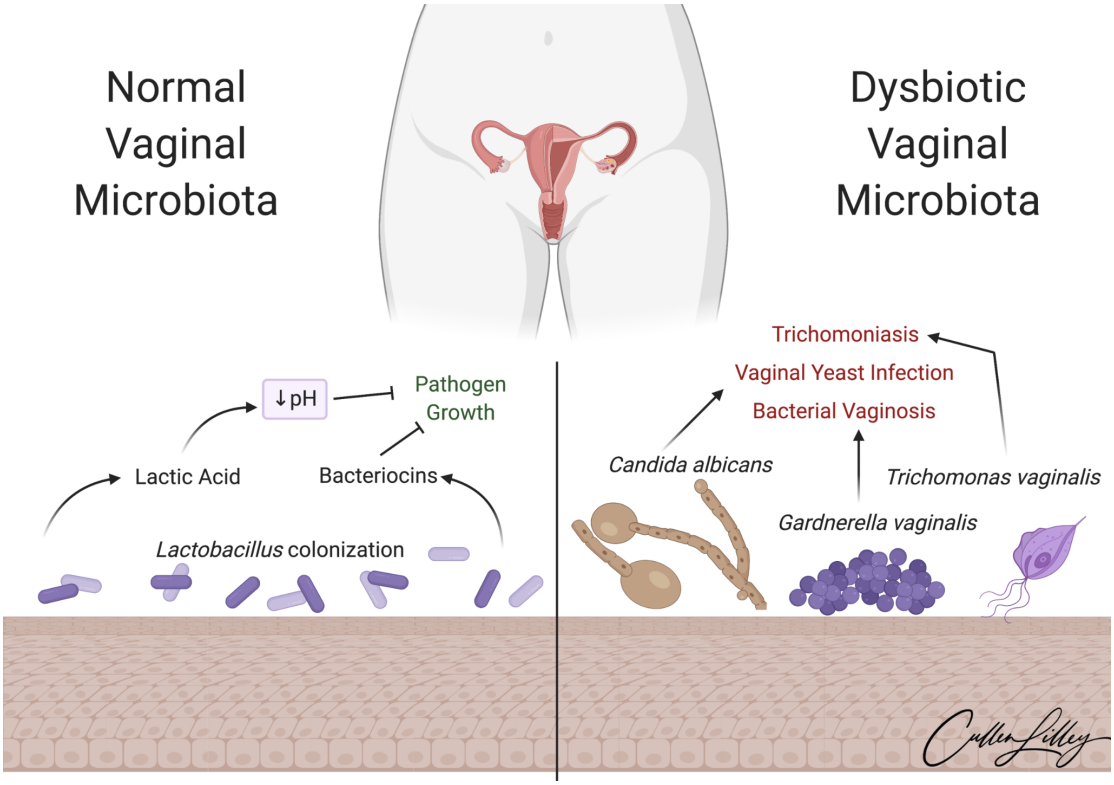
1.1 Healthcare Costs. Treating vaginal infections in the US healthcare system costs around $16 billion and affects 10 million women annually (Bitew et al, 2017). Bacterial vaginosis (BV) is estimated to cost around $4.8 billion and affects 21.2 million women annually (Peebles et al., 2019) Treating vaginal infections caused by viruses costs about $8 billion per year and there are about 110 million cases (CDC, 2013). Trichomoniasis, a sexually transmitted infection, is estimated to cost about $24 million per year (Sector et al., 2014). Fungal infections caused about 9 million outpatient visits nationally in the year 2017 and resulted in $2.4 billion in healthcare costs (Benedict et al, 2019). Specifically, invasive fungal infections (known as candidiasis) caused by a yeast strain named *Candida albicans* were responsible for 48% of fungi-related hospitalization and cost $1.4 billion of that overall $2.4 billion (Benedict et al., 2019). These healthcare costs are daunting for women, especially those living in poverty and lacking access to gynecological care.

1.2 Access to Healthcare. The vaginal health of women contributes significantly to their overall well-being, therefore simple and affordable access to healthcare services to maintain vaginal health is very important. Out of a survey of 3520 women across the globe in 2012, 44%

claimed they did not have access to a gynecologist (Nappi, Kokot-Kierepa, 2012). Sexually transmitted infections (STIs) and HIV testing, unwanted pregnancies, traumas, and chronic illnesses were among the vaginal illnesses due to lack of accessible care. (Ravi et al, 2017). Aside from hospitals and clinics, common care locations included jails, emergency rooms, and Planned Parenthood clinics (Ravi et al, 2017).

2.0 Background on the Vaginal Microbiome

A complete understanding of the vaginal microbiome is necessary to comprehend all of the components within a healthy vaginal microenvironment and their roles in the metabolic activities and the health of the host (Ravel & Brotman, 2016). A healthy vaginal microbiome depends on mutualism between the dominant bacterial species and the host. Under healthy conditions, beneficial bacterial communities coexist within human hosts in a mutually positive manner by preventing harmful bacteria from infiltrating the host's vaginal space. Comprehensive studies show that *Lactobacillus* bacterial species is the most prevalent species in the female vaginal microbiome (Gupta el al., 2019). Understanding the mutualistic relationship that exists between the host and the beneficial *Lactobacilli* colonies is significant because it provides insight on the mechanism of protection within the vaginal area (Chee et al., 2020). This group of bacteria provides protective barrier agents in the reproductive tract by releasing several compounds that prevent the overgrowth of harmful bacteria (Gupta et al., 2019). Once the *Lactobacilli* are within the specific vaginal environment, they release large amounts of lactic acid as a fermentation by-product and create a low pH environment to protect against pathogenic bacteria (unwanted bacteria), as shown in **Figure 1** (Ravel & Brotman, 2016).



https://www.pathelective.com/micromeded/vaginal-microbiota-and-dysbiosis

Figure 1: On the left is an example of a healthy vaginal microbiota with the right amount of lactic acid, bacteriocins (kill or inhibit bacterial strains), and a low pH to inhibit pathogen growth. This allows the *lactobacillus* colonies to stay healthy. However, on the right is an example of a dysbiotic vaginal microbiota with harmful pathogens, such as *Candida albicans*, *Gardnerella vaginilis*, and *Trichomonas vaginilis.* These harmful pathogens lead to vaginal diseases like vaginal yeast infections (candidiasis), BV, and Trichomoniasis.

2.1 *Lactobacillus* Strains as "good" bacteria. The healthy *Lactobacillus* strains thrive in the vaginal environment because of a specific anaerobic (without oxygen) nutritional environment catered to their needs. Some *Lactobacillus* species, including *L. crispatus*, *L. iners*, *L. gasseri*, and *L. jensenii*, appear to be unique to the human vagina (Ravel & Brotman, 2016). By finding ways to protect the *Lactobacilli*-bound area (the interior of the vaginal microbiota where the *Lactobacilli* is found), the vaginal microbiome can serve to protect the health of women from an increased risk of contracting bacterial infections (overgrowth of pathogenic bacteria) and yeast infections (overgrowth of fungi like *Candida albicans*). Furthermore, it may be possible to prevent 30% of new HIV cases if *Lactobacillus* bacteria predominate in the vaginal microbiome (Ravel & Brotman, 2016), as changing the microbiome to one in which *Lactobacillus* predominates is an alluring approach to lowering HIV incidence rates (Gustin et al, 2021).

Lactobacillus strains limit infections by producing a substance that reduces the formation

of “biofilms" (clusters of bacteria) by invading pathogens. Specific strains of *Lactobacillus* produce soap-like compounds called “biosurfactants" that prevent harmful pathogens (fungi, other bacteria) from sticking to the vaginal surface. The biosurfactants reduce the ability of the invading pathogens from forming the dense biofilms. Researchers have reported that these biosurfactants reduced the biofilm formation of vaginal pathogen *C. albicans* by 40 to 50% (Chee et al., 2020).

2.2 Promoting a Healthy Vaginal Microbiome. Since lifestyle, hygiene and diet affect the vaginal microbiome and allow healthy *Lactobacilli* to flourish, it is crucial for women to maintain good habits, cleanliness, and dietary choices (Ravel & Brotman, 2016). Consuming yogurt that contains *Lactobacillus* strains everyday is one way to maintain a healthy vaginal microbiome (Ravel & Brotman, 2016). Additionally, tight clothing reduces the flow of oxygen to the vaginal area, which can lead to the overgrowth of unwanted anaerobic species like *C. Albicans*, so women are advised to limit such clothing. Maintaining a healthy immune system also plays an important role in keeping the vaginal microbiome healthy. The epithelial and immune cells within the cervicovaginal mucus (vaginal fluid) maintain homeostasis (balance) with the vaginal microbiome and filter pathogens (Lehtoranta et. al, 2022).

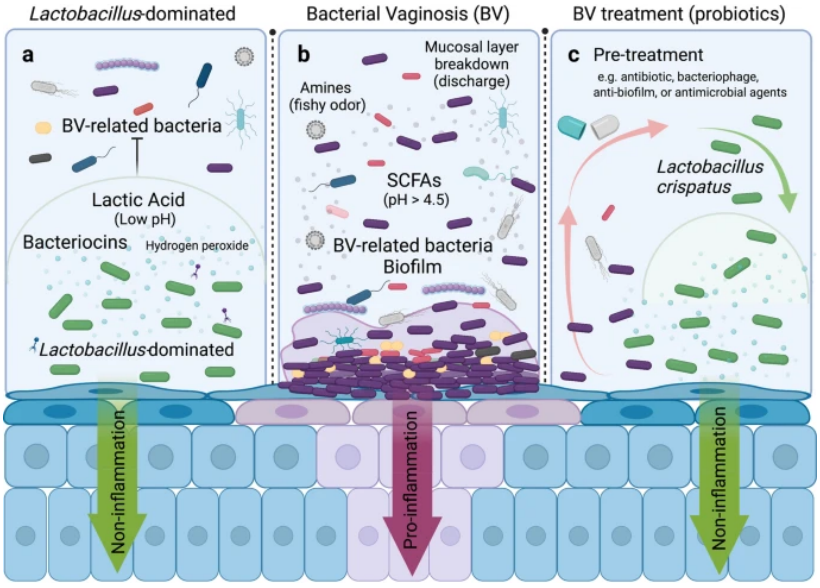
2.3 Vaginal Dysbiosis. If the vaginal microbiome lacks any of these beneficial *Lactobacilli* strains, a disorder can occur. A dysbiotic (unbalanced) environment can occur when the pH is too high in the vaginal space and no longer supports the healthy growth of *Lactobacilli* strains. There are other types of *Lactobacilli* that are harmful that can increase the risk of vaginal diseases, such as BV (Gupta et al., 2019). When the pH is not the optimal level, negative strains of bacteria can grow. The disruption or “dysbiosis” of these healthy species can lead to the invasion of pathogens that causes severe vaginal conditions such as BV, which is defined as the overgrowth of harmful bacteria in the vagina. This can lead to overgrowth of harmful bacteria like *Gardnerella vaginalis* and *Atopobium vaginae*, causing BV. Also, the presence of these harmful strains in the vaginal microbiota can put females at a higher risk of getting infected with STIs and with yeast strains like *Candida albicans*, which leads to a condition called vulvovaginal candidiasis (VVC). Recent studies have linked fungus biofilms to Candidiasis, particularly when therapy fails and recurrent Candidiasis occurs. As a result, several studies have supported the use of probiotics in treating various illnesses (Boahen et al., 2022). Vaginal Dysbiosis also leads to pelvic inflammatory disease and preterm birth (Gupta et al, 2019). Additionally, about 70% of women have Vaginal Dysbiosis within their lifetime without knowing (Gupta et al, 2019).

2.4 Treatment Challenges. When attempting to treat women for vaginal-related disorders, several difficulties may arise. The key to effective therapy is an accurate diagnosis, yet the misdiagnosis rate is close to 50%, increasing the chance of recurrence (Brown, Drexler, 2020). Oftentimes, BV has symptoms of vaginal discharge and color and can be confused with

symptoms from vaginal intercourse or menses, causing those women to misunderstand their need for treatment. (Brown, Drexler, 2020). It can also lead to a burning feeling during urination and itching around the vulva. Women's vaginal health is rarely routinely examined, even with symptoms such as pain, itching, and burning.

For asymptomatic, non-pregnant women, pelvic examination is no longer advised since it might lead to negative effects including false-positive test findings, overdiagnosis, anxiety, and unneeded expenses (Qin et al., 2020). A significant challenge for developing preventative approaches to improve women's health is that a large proportion of women who lack the *Lactobacillus* bacteria (10-42% of women) can remain asymptomatic for BV (Ravel & Brotman, 2016). Additionally, current guidelines from the US Centers for Disease Control do not support antibiotic treatment for these asymptomatic women (Ravel & Brotman, 2016).

About 75% of women experience VVC during their reproductive years (Azie et al., 2020). Additionally, candidiasis can lead to vaginal itching, pain during intercourse, discomfort while urinating, and abnormal vaginal discharge – the very same symptoms as BV. STIs can lead to unusual vaginal bleeding, blisters and sores around the genital area, and an unusual discharge from the vaginal area (Qin et al,. 2020). Furthermore, antibiotics and topical creams used to treat vaginal disorders can become less useful because of antibiotic resistance. This resistance occurs when bacteria become resistant to the medications meant to kill them. This means the germs survive even when treatment is applied.



https://www.nature.com/articles/s41522-022-00295-y/figures/1

Figure 2 This image showcases the healthy elements, such as a low pH and *Lactobacillus crispatus* that support a noninflammatory vaginal microbiome, creating a normal environment. Additionally, it showcases a dysbiotic or pro-inflammation environment caused by BV and a high

pH.

3.0 Medical Treatments for Vaginal Dysbiosis caused by Bacteria

Since Vaginal Dysbiosis can be caused by bacterial infections, viral infections, or fungal infections, different treatments have been developed that target the invading species. In the case of BV, medications that kill the pathogenic bacteria have been developed. One oral medication (metronidazole) diffuses into the vaginal microbiome and inhibits pathogenic bacteria from entering; the treatment requires 500 mg (1 pill) that is taken daily for 5-10 days (Chandrashekhar et al., 2021). Metronidazole was first released in 1959. Topical ointments (Clindamycin, Flagyl, Secnidazole) – have shown to be effective against BV although some treatments have a high rate of the infection returning (Chandrashekhar et al., 2021). These solutions also come with one large issue. Bacteria can grow resistance to the given treatments and they will not be effective anymore in treating BV. However, until the bacteria develop resistance to the antibiotic, these medications can be helpful in treating BV and they are very easy to access through a local pharmacy. What follows here are other approaches for treating BV.

3.1 Restoring *Lactobacillus*. BV is commonly treated with antibiotics which can cause recurrence and degrade the *Lactobacillus* populations within the vaginal microbiome (Lagenaur et al., 2021). One possible way to bring back *Lactobacilli* within the vaginal microbiome is to insert a probiotic strip containing this species within the vagina. LACTIN- V, a biological drug composed of *L. crispatus* (**Figure 2**) (a strain of healthy *L*actobacilli), could reduce pathogenic bacteria by repopulating the vaginal microbiome (Lagenaur et al., 2021). Additionally, it is a gelatin capsule which makes it very accessible to women around the world. Another strain of *Lactobacillus* taken orally, *Lactobacillus gasseri,* can also be used to restore *Lactobacilli* colonies that were destroyed from BV (Qi et al., 2022). A newer drug, *Lactobacillus rhamnosus BMX 54* + lactose) (NORMOGIN™), has demonstrated that it significantly reduces BV and restores a *Lactobacillus* dominated microbiome (Baldacci et al., 2020). These strategies have shown great results in the clinic by deteriorating BV.

3.2 Oral Betadine. Betadine, a treatment taken by mouth, is also used to treat vaginal dysbiotic disorders and has shown to be 75% effective (Ismatiloevna, 2022). It is better than other drugs, such as metronidazole, because it creates the conditions for *Lactobacillus* to thrive (Ismatiloevna, 2022). The repopulation of *Lactobacillus* happened to 96% of the patients that were tested with betadine (Ismatiloevna, 2022). However, betadine is a strong drug and should not be used unless it is certain that a vaginal disease is present.

3.3 Restoring Vaginal pH. When the vaginal microbiome encounters the issue of dysbiosis, the pH can rise creating an unbalanced environment. *Lactobacillus* colonies release lactic acid, which creates a specific pH. A lactic acid gel can also be utilized to restore the

optimal pH (4.5) (**Figure 2**) within the vaginal microbiome and eliminate pathogenic bacteria (Ross et al., 2023). This gel is meant to be used for 7 days. In studies comparing its effectiveness with metronidazole, the lactic acid gel did not work short-term, and metronidazole was more effective for short-term resolution (Ross et al., 2023). Unfortunately, both treatments had a common recurrence of BV, but the lactic acid gel had fewer side effects (Ross et al., 2023). It could be possible to use betadine with this gel as it is more efficient than metronidazole and the lactic acid gel could be a "booster" to yield quicker results.

3.4 Detecting BV. All of the treatments described above will work against bacteria that cause Vaginal Dysbiosis; however, diagnosing that these bacteria are the offending species can be very difficult. Before administering treatment to a patient, it is important to determine if pathogenic bacteria are present. Recent work to identify a feature in vaginal fluid that reveals BV has been discovered and studied for its detection possibilities. Sialidase is an enzyme present in vaginal fluid that can be used as such a biomarker for BV (Rodriguez et al., 2021). It can be used to detect a dysbiotic *Lactobacilli* environment and alert the patient of the presence of BV. Recent studies have demonstrated that this biomarker could be embedded in fibers that could be part of a panty-liner that changes color in the presence of the substrate for sialidase, thus enabling detection of BV without needing access to sophisticated lab tests (Rodriguez et al., 2021)

3.4 Palomacare. A new antibacterial vaginal gel called Palomacare, released in 2014, has shown efficacy against Vaginal Dysbiosis and relapse prevention (Losa et al., 2022). Palomacare has been recommended as a treatment for Vaginal Dysbiosis by 79% of gynecologists, 85% of patients observed an improvement in the healing process, and 84% of patients observed a reduction in Vaginal Dysbiosis recurrences (Losa et al., 2022).

4.0 Medical Treatments for Vaginal Dysbiosis caused by Fungal Infections (Candidiasis)

To target fungal infections that cause Vaginal Dysbiosis (Figure 1), researchers and clinicians have been studying ways to address yeast infections in the vagina caused by *Candida albicans* that are inexpensive, accessible without a doctor's prescription, and effective (Cateau et al., 2008). The medical literature refers to this condition as vulvovaginal candidiasis (VVC). Common antifungal topicals such as caspofungin and micafungin have been used since 2008 and reduced 70% of yeast biofilms (Cateau et al., 2008). However, these medications are only available by prescription, thereby limiting a patient's ability to get necessary treatment (Cateau et al., 2008). The chemicals ethanol, amphotericin, and echinocandins (antifungal) have demonstrated the reduction of *C. Albicans* with no bacteria resisting the given treatment. However, there are two issues with these chemical treatments: not enough testing has been done to affirm that it is completely safe for women and they would require a doctor's prescription.

Fluconazole is the first antifungal developed in 2014 for VVC that recurs after standard treatment; it controls symptoms of Candidiasis (itching) and is widely regarded as the first line of therapy for recurrent VVC (Sobel & Nyirjesy, 2021). Its drawbacks include the possibility of growing bacterial resistance of beneficial bacteria to this medication (Sobel & Nyirjesy, 2021). Oteseconazole, a topical fungal inhibitor, is a promising new therapy approach towards fungal infections(Sobel & Nyirjesy, 2021). Oteseconazole has a target goal of discarding fungal infections comparable to fluconazole (Sobel & Nyirjesy, 2021). A considerable improvement in the therapy of recurrent VVC may result from this combination of treatment (Sobel & Nyirjesy, 2021).

A new medication called ibrexafungerp is a drug that disrupts the formation of the fungal cell wall; if the cell wall forms improperly, then the fungus will die (Azie et al., 2020). This drug can be taken orally and has low risk of side effects as a treatment for VVC (Azie et al., 2020). It also protects against a broad range of *C. Albicans* species including those strains that are resistant to fluconazole (Azie et al., 2020).

5.0 Cutting-Edge Treatments for Vaginal Dysbiosis

Antibiotics and antifungals continue to be the primary line of treatment for Vaginal Dysbiosis despite the increasing resistance of microorganisms to them (Losa et al., 2022). However, small case studies involving the transplanting of healthy vaginal bacteria from the donor to patient are underway. This strategy allows a tissue graft to be inserted from donor to patient and results in a successful return of the vaginal flora (Losa et al., 2022). The first sample patient had a 91.3% of *Gardnerella* and a lack of *Lactobacillus* (Losa et al., 2022). Following the VMT treatment, the patient had their *Lactobacilli* colonies restored with no presence of *Gardnerella.*

In a case study with five patients, vaginal microbiota transplantation (VMT) with eubiotic (healthy) vaginal bacterial microbiota following the loss of pathogenic bacteria with antibiotics was effectively carried out; however, no VMT has been carried out without the use of antibiotics as it serves as a protective barrier regarding possible infection from the donor (Wrønding et al., 2023). The *Gardnerella vaginalis* bacteria dominated the vaginal microbiota to the extent of 90% (Wrønding et al., 2023). After one VMT, the microbiota completely changed, becoming 81.2% *L. crispatus* and 9% *L. jensenii*, and Vaginal Dysbiosis symptoms also disappeared at the same time (Wrønding et al., 2023). These findings suggest that VMT is a beneficial potential treatment for Vaginal Dysbiosis. However, it is administered by a catheter and is therefore not accessible to all women (Wrønding et al., 2023). The patient also needs to be matched with a donor which can create an accessibility issue (Wrønding et al., 2023).

The United States Food and Drug Administration has approved lactoferrin, a protein that defends the host's immune system, is a generally recognized as safe (GRAS) food additive

(Superti & De Seta, 2020). Lactoferrin has been categorized as a nutraceutical protein due to its capabilities for protecting the mucosa (soft tissue that lines reproductive organs) from infections and inflammations, as well as the present pharmacological and nutritional benefits (Superti & De Seta, 2020). Lactoferrin can act as a soluble that inhibits pathogenic bacteria binding to the cell's surface and promotes the return of *Lactobacillus* colonies (Superti & De Seta, 2020).

Conclusion: The vaginal microbiota is a very intricate environment that has many components regarding its health. Normal and dysbiotic conditions exist within the vagina due to many different factors such as changes in pH and exposure to pathogens. Vaginal Dysbiosis in women all over the world goes undiagnosed. Different treatments for Vaginal Dysbiosis include challenges such as recurring infections, antibiotic resistance, and delivery of the drugs themselves. It is imperative to keep researching treatments and improving the ways that Vaginal Dysbiosis can be diagnosed, along with making treatment more accessible and effective.

References

1. Azie, N., Angulo, D., Dehn, B., & Sobel, J. D. (2020). Oral Ibrexafungerp: an investigational agent for the treatment of vulvovaginal candidiasis. *Expert Opinion on Investigational Drugs*, *29*(9), 893-900. https://doi.org/10.1080/13543784.2020.1791820

2. Baldacci, F., Baldacci, M., & Bertini, M. (2020). *Lactobacillus* rhamnosus BMX 54+ Lactose, A Symbiotic Long-Lasting Vaginal Approach to Improve Women's Health. *International Journal of Women's Health*, 1099-1104. DOI: 10.2147/IJWH.S259311

3. Benedict, K., Jackson, B. R., Chiller, T., & Beer, K. D. (2019). Estimation of direct healthcare costs of fungal diseases in the United States. Clinical Infectious Diseases, 68(11), 1791-1797. https://doi.org/10.1093/cid/ciy776

4. Bitew, A., Abebaw, Y., Bekele, D., & Mihret, A. (2017). Prevalence of Bacterial Vaginosis and Associated Risk Factors among Women Complaining of Genital Tract Infection. International journal of microbiology, 2017, 4919404. https://doi.org/10.1155/2017/4919404

5. Boahen A, Than LTL, Loke Y-L and Chew SY (2022) The Antibiofilm Role of Biotics Family in Vaginal Fungal Infections. *Front. Microbiol*. 13:787119. doi: 10.3389/fmicb.2022.787119 https://doi.org/10.3389/fmicb.2022.787119

6. Brown, H., & Drexler, M. (2020). Improving the Diagnosis of Vulvovaginitis: Perspectives to Align Practice, Guidelines, and Awareness. Population health management, 23(S1), S3–S12. https://doi.org/10.1089/pop.2020.0265

7. Cateau, E., Rodier, M. H., & Imbert, C. (2008). In vitro efficacies of caspofungin or micafungin catheter lock solutions on *Candida albicans* biofilm growth. Journal of antimicrobial chemotherapy, 62(1), 153-155 .https://doi.org/10.1093/jac/dkn160

8. Chandrashekhar, P., Minooei, F., Arreguin, W., Masigol, M., & Steinbach-Rankins, J. M. (2021). Perspectives on existing and novel alternative intravaginal probiotic delivery

methods in the context of bacterial vaginosis infection. The AAPS journal, 23(3), 66. https://doi.org/10.1208/s12248-021-00602-z

9. Chee, W. J. Y., Chew, S. Y., & Than, L. T. L. (2020). Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health. Microbial cell factories, 19(1), 203. https://doi.org/10.1186/s12934-020-01464-4

10. Centers for Disease Control and Prevention. (2013, August 29). Incidence, prevalence, and cost of sexually transmitted infections in the United States. http://www.cdc.gov/std/stats/STI-Estimates-Fact-Sheet-Feb-2013.pdf

11. Gustin, A., Cromarty, R., Schifanella, L., & Klatt, N. R. (2021). Microbial mismanagement: how inadequate treatments for vaginal dysbiosis drive the HIV epidemic in women. Seminars in immunology, 51, 101482. https://doi.org/10.1016/j.smim.2021.101482

12. Gupta, S., Kakkar, V., & Bhushan, I. (2019). Crosstalk between vaginal microbiome and female health: a review. Microbial pathogenesis, 136, 103696. https://doi.org/10.1016/j.micpath.2019.103696

13. Ismatiloevna, Y. F. (2022). TREATMENT OF VAGINAL DYSBIOTIC DISORDERS IN PREGNANT WOMEN BEFORE CHILDBIRTH. *World Bulletin of Public Health*, *12*, 86-89. https://www.scholarexpress.net

14. Lehtoranta, L., Ala-Jaakkola, R., Laitila, A., & Maukonen, J. (2022). Healthy vaginal microbiota and influence of probiotics across the female life span. Frontiers in Microbiology, 13, 819958. https://doi.org/10.3389/fmicb.2022.819958

15. Losa, F., Palacios, S., Rodríguez, S. P. G., Baquedano, L., Khorsandi, D., & Muñoz, M. J. (2022). Vaginal dysbiosis management and the efficacy of a non-hormonal hyaluronic acid-based vaginal gel (Palomacare®) as an adjuvant treatment: the Palomascopia survey. *Obstet Gynecol Cases Rev*, *9*, 222. DOI: 10.23937/2377-9004/1410222

16. Nappi, R. E., & Kokot-Kierepa, M. (2012). Vaginal Health: Insights, Views & Attitudes (VIVA)–results from an international survey. Climacteric, 15(1), 36-44. https://doi.org/10.3109/13697137.2011.647840

17. Peebles, K., Velloza, J., Balkus, J. E., McClelland, R. S., & Barnabas, R. V. (2019). High Global Burden and Costs of Bacterial Vaginosis: A Systematic Review and Meta-Analysis. Sexually transmitted diseases, 46(5), 304–311. *DOI:* 10.1097/OLQ.0000000000000972

18. Qin, J., Saraiya, M., Martinez, G., & Sawaya, G. F. (2020). Prevalence of Potentially Unnecessary Bimanual Pelvic Examinations and Papanicolaou Tests Among Adolescent Girls and Young Women Aged 15-20 Years in the United States. doi:10.1001/jamainternmed.2019.5727

19. Ravi, A., Pfeiffer, M. R., Rosner, Z., & Shea, J. A. (2017). Identifying health experiences of domestically sex-trafficked women in the USA: A qualitative study in Rikers Island jail. https://doi.org/10.1007/s11524-016-0128-8

20. Qi, F., Fan, S., Fang, C., Ge, L., Lyu, J., Huang, Z., ... & Zhang, X. (2022). Orally administrated *Lactobacillus* gasseri TM13 and *Lactobacillus* crispatus LG55 Can Restore the Vaginal Health of Patients Recovering from Bacterial Vaginosis. *medRxiv*, 2022-12. https://doi.org/10.1101/2022.12.21.22283705

21. Ravel, J., & Brotman, R. M. (2016). Translating the vaginal microbiome: gaps and challenges. Genome medicine, 8, 1-3.] https://doi.org/10.1186/s13073-016-0291-2

22. Ross, J.D.C., Brittain, C., Anstey Watkins, J. et al. Intravaginal lactic acid gel versus oral metronidazole for treating women with recurrent bacterial vaginosis: the VITA randomized controlled trial. https://doi.org/10.1186/s12905-023-02303-5

23. Rodríguez-Nava, C., Cortés-Sarabia, K., Avila-Huerta, M. D., Ortiz-Riaño, E. J., Estrada-Moreno, A. K., Alarcón-Romero, L. D. C., ... & Morales-Narváez, E. (2021). Nanophotonic sialidase immunoassay for bacterial vaginosis diagnosis. ACS Pharmacology & Translational Science, 4(1), 365-371. DOI: 10.1021/acsptsci.0c00211

24. Saraf, V. S., Sheikh, S. A., Ahmad, A., Gillevet, P. M., Bokhari, H., & Javed, S. (2021). Vaginal microbiome: normalcy vs dysbiosis. Archives of Microbiology, 203, 3793-3802. https://doi.org/10.1007/s00203-021-02414-3

25. Secor, W. E., Meites, E., Starr, M. C., & Workowski, K. A. (2014). Neglected parasitic infections in the United States: trichomoniasis.
doi: 10.4269/ajtmh.13-0723

26. Sobel, J. D., & Nyirjesy, P. (2021). Oteseconazole: an advance in treatment of recurrent vulvovaginal candidiasis. *Future microbiology*, *16*(18), 1453-1461.
https://doi.org/10.2217/fmb-2021-0173

27. Superti, F., & De Seta, F. (2020). Warding off recurrent yeast and bacterial vaginal infections: Lactoferrin and lactobacilli. *Microorganisms*, *8*(1), 130.
https://doi.org/10.3390/microorganisms8010130

28. Wrønding, T., Vomstein, K., Bosma, E. F., Mortensen, B., Westh, H., Heintz, J. E., ... & Nielsen, H. S. (2023). Antibiotic-free vaginal microbiota transplant with donor engraftment, dysbiosis resolution, and live birth after recurrent pregnancy loss: a proof of concept case study. *eClinicalMedicine*.
https://doi.org/10.1016/j.eclinm.2023.10207