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The Vaginal Microbiota in Women Health and Disease: Current Understanding and Future Perspectives - A Review

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Abstract

Vaginal Microbiota varies within individuals and between human populations. Colonization by Lactobacillus in the female genital tract is thought to be critical for maintaining genital health. Nevertheless, little is known about how genital microbiota influence host immune function and modulate disease susceptibility. This article discusses the advancement of new strategies for disease diagnosis and personalized treatments to promote health and improve the quality of women's lives. Previous research on the use of vaginal products for sexual intercourse available over the counter used by women has primarily focused on gels, creams and ointments composed by natural origin drugs/excipients and applied by means of an applicator. General concerns and misconceptions related to use of vaginal products were rare. More research is needed from the medical community to guide women clarifying therapeutic outcomes of vaginal products.

Key Words: Microbiome, Health, Lactobacilli, Genital Tract, Quality of Life, Immune regulation, Reproductive age

Introduction

The human body hosts complex microbial communities whose combined membership outnumbers our own cells by at least a factor of ten. Microorganisms that inhabit surfaces and cavities exposed or connected to the external environment at each body site includes ecological communities of microbial species that exist in a mutualistic relationship with the host(1) The kinds of organisms present are highly dependent on the prevailing environmental conditions and host factors and hence vary from site to site. Moreover, they vary between individuals and over time (2). Together, our ~100 trillion microbial symbionts (the human microbiota) endow us with crucial traits; for example, we rely on them to aid in nutrition, resist pathogens, and educate our immune system (3).

The microbiota normally associated with the human body have an important influence on human development, physiology, immunity, and nutrition (4). These microbial communities are believed to constitute the first line of defence against infection by competitively excluding invasive nonindigenous organisms that cause diseases. Despite their importance, surprisingly little is known about how these communities differ between individuals in composition and function, but more importantly, how their constituent members interact with each other and the host to form a dynamic ecosystem that responds to environmental disturbances. Major efforts are now underway to better understand the true role of these communities in health and diseases (5).

Vaginal microbiota form a mutually beneficial relationship with their host and have major impact on health and disease (4). The bacterial communities that reside in human vagina are an example of this finely balanced mutualistic association. In this relationship, the host provides

benefit to the microbial communities in the form of the nutrients needed to support bacterial growth. This is of obvious importance since bacteria are continually shed from the body in vaginal secretions, and bacterial growth must occur to replenish their numbers. Some of the required nutrients are derived from sloughed cells, while others are from glandular secretions. The indigenous bacterial communities, on the other hand, play a protective role in preventing colonization of the host by potentially pathogenic organisms, including those responsible for symptomatic bacterial vaginosis, yeast infections, sexually transmitted infections (STI), and urinary tract infections (6)(7)(8). Lactobacilli have long been thought to be the keystone species of vaginal communities in reproductive-age women. These microorganisms benefit the host by producing lactic acid as a fermentation product that lowers the vaginal pH to ~3.5–4.5 (9). While a wide range of other species are known to be members of vaginal bacterial communities, their ecological functions and influence on the overall community dynamics and function are largely undetermined. The vaginal ecosystem is thought to have been shaped by co-evolutionary processes between the human host and specific microbial partners, although the selective forces (traits) behind this mutualistic association are still not clear.

This review seeks to inform the scientific community about the background of new developments and update the progress of new strategies for disease diagnosis and personalized treatments to promote health and improve the quality of women's lives. This cannot be accomplished without addressing a fundamental issue as to what constitutes a 'normal' and 'healthy' vaginal microbiota and understanding its function in health and diseases.

Dynamics of vaginal Microbiota

The female genital tract is anatomically made up of a succession of cavities (Fallopian tubes, uterine cavity, endocervix, and vagina) that correspond with the exterior through the vulvar cleft. This structure accommodates the menstrual flow and acts as the passage of foetus delivery; in addition, it allows sexual intercourse and also the entrance of pathogenic micro-organisms that are potentially harmful to the process of reproduction. Vaginal microflora undeniably presents one of the most important defence mechanisms for the reproductive tract by maintaining a healthy environment and preventing the proliferation of micro-organisms foreign to the vagina (10). The vagina and its distinctive microflora form a finely balanced ecosystem, with the vaginal environment controlling the microbial types present while the microflora in turn control the vaginal environment (11) (12).In obstetrics and gynaecology the bacterial flora of the female reproductive tract is thefocus of the study of infectious disease, as it is noted that many pelvic infections involve bacteria residing on the cervical-vaginal epithelium. The vaginal flora contains a large variety of bacterial species, including both aerobic and anaerobic organisms (Table 1 & Table 2) (13).

The development of the biota of the vagina is linked with maturation and deposition of glycogen in the vaginal epithelium from acidogenic and acidouric micro-organisms. An important distinction must be made between the flora of the vulva, vestibulum and that of the vagina proper. Immediately following parturition and during the first few days of puerperium, the vaginal flora is reasonably distinct from that of the vulval flora (14).

The means by which vaginal microbiomes help prevent urogenital diseases in women and maintain health are poorly understood. In a study directed by Ravel et al. (15) to understand vaginal microbiome of reproductive age woman, the vaginal bacterial communities of 396 asymptomatic North American women who represented four ethnic groups (white, black, Hispanic, and Asian) were sampled and the species composition characterized by pyrosequencing of barcoded 16S rRNA genes. The communities clustered into five groups: four were dominated by Lactobacillusiners, L. crispatus, L. gasseri, or L. jensenii, whereas the fifth had lower proportions of lactic acid bacteria and higher proportions of

Vol. 11 (2) 190-205 April 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)

strictly anaerobic organisms, indicating that a potential key ecological function, the production of lactic acid, seems to be conserved in all communities(Fig. 1).

The normal microbial flora of the vagina plays a dynamic role in the prevention of genital and urinary tract infections in women. Thus, an accurate understanding of the composition and ecology of the ecosystem is crucial to understanding the aetiology of these diseases. Prior studies on the microbial flora of the human vagina indicate that micro-organisms normally present in the vagina play a pivotal role in preventing successful colonization by 'undesirable' organisms. This is inclusive of those organisms responsible for bacterial vaginosis, yeast infections, sexually transmitted diseases and urinary tract infections. Furthermore, epidemiologic studies have undoubtedly established that abnormal vaginal microbial communities and lower genital tract infections are considerably associated with an increased risk of HIV infection (11).

A healthy host-vaginal microbiome refers to an ecosystem in which a functional equilibrium is created through reciprocal and mutually beneficial interactions amongst the host and her resident micro-organisms. This healthy equilibrium functions to provide a barrier to both new colonization by pathogenic organisms and the overgrowth of organisms that are otherwise commensal (16). The innate immune system of the female reproductive tract is highly important in the prevention of ascending genital infections that can threaten healthy pregnancy and foetal development. The mucosa of the lower genital tract has to selectively support a habitat for resident commensal microbes (while simultaneously inhibiting the growth of potential pathogens) whereas the upper genital tract must remain aseptic (12).

Immune regulation of Female Genital tract

The vaginal innate immune system represents the first line of defence against foreign organisms and pathogenic microbes. Through its chief components, a natural balance is maintained and disease is averted. Many recent advances

Fig 1. Representation of vaginal bacterial community groups within each ethnic group of women. The number of women from each ethnic group is in parentheses (adapted from Ravel et al. PNAS 2011; 108: 4680-4687)

have furthered our knowledge of this intricate equilibrium and the contribution of each element to the final homeostasis. Several adverse obstetric and gynaecologic conditions have been traced to abnormalities in the vaginal environment (17). Besides, female reproductive tract is immunologically unique in its requirement for tolerance to allogeneic sperm and, in the upper tract, to the conceptus. However, it must also be appropriately protected from, and respond to, a diverse array of sexually transmitted pathogens. Some of these infections can be lethal (e.g. Human Immunodeficiency Virus (HIV), Human Papilloma Virus (HPV)), and others (e.g. Chlamydia trachomatis and Neisseria gonorrhoeae) can have potentially devastating reproductive sequelae. Interactions between a host and a pathogen are complex, diverse and regulated, and are a function of the individual pathogen, and host immunity. Although there is undoubtedly commonality in the mucosal immune response, there is also evidence of a degree of site-specificity in immune mechanisms, dependent upon the function and anatomical location of an organ (18).

Events leading to a state of local immune-suppression (such as sexual intercourse or local induction of an allergic response) create suitable conditions for the proliferation of micro-organisms and also enable their transformation. These situations result in the rise of symptomatic vaginitis. Besides the protective effects of the endogenous vaginal flora, the protection against potentially pathogenic micro-organisms is also completed by the local components of both innate immunity and acquired immunity. The innate immune system is the most primitive branch of the immune system as it has been conserved throughout evolution (19) (10). One of its major characteristics is its ability to recognize molecular patterns associated with the pathogens (PAMPs) in the invading microbes, in place of recognizing specific antigens. Recognition of these molecular structures permits the immune system to distinguish between infectious non-self and non-infectious self (20).

Innate immunity acting of female genital tract (FRT) is represented by, firstly, soluble factors (such as mannose-binding lecithin [MBL], components of the complement, defensines, secretory leukocytes protease inhibitor [SLPI], nitric oxide), components in association with the membrane ('Toll-likereceptors') and, lastly, phagocytic cells (20). The recognition of a PAMP by a component of the innate immunity activates a sequence of events resulting in the release of pro-inflammatory cytokines and the activation of the acquired immune system, that is, the activation of lymphocytes T and B. A summary of the cellular origin, target cell lineages and function of each family of molecules is shown in Fig 2. It is important to note that, while the activation of the innate immune system occurs directly after the recognition of the pathogen, multiple days are required for the acquired immunity to become functional. The layer of the vagina constituted of epithelial cellsestablishes the initial contact point between micro-organisms and the host's genital tract. These epithelial cells possess 'Toll-like receptors' (TLR) on their surface and, thus, are imperative components of innate vaginal immunity. Eleven TLRs have already been identified, with each having a different specificity. The TLR1 and TLR2 complexes recognize lipoproteins and peptidoglycan present on the surface of gram-positive bacteria. Moreover, TLR3 is specific for the double DNA chain, which is an intermediate in the replication cycle of multiple viruses, while TLR4 recognizes the liposaccharide component of the gram-negative bacteria wall. Additionally, TLR5 reacts with flagellins, an important component of the bacterial flagella, and TLR9 has the capacity of differentiating DNA sequences containing the CpG dinucleotide in the non-methylated state (noting that in humans the DNA sequence is highly methylated, but the TLR9 reacts only with the CpG of bacteria which is non-methylated, thus, being specific for them). Vaginal cells also emit molecules with potent non-specific antimicrobial activity. A class of these molecules include positively-charged peptides, known as defensines, which rapidly bind negatively-charged bacterial surfaces. This binding action results in

Vol. 11 (2) 190-205 April 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)

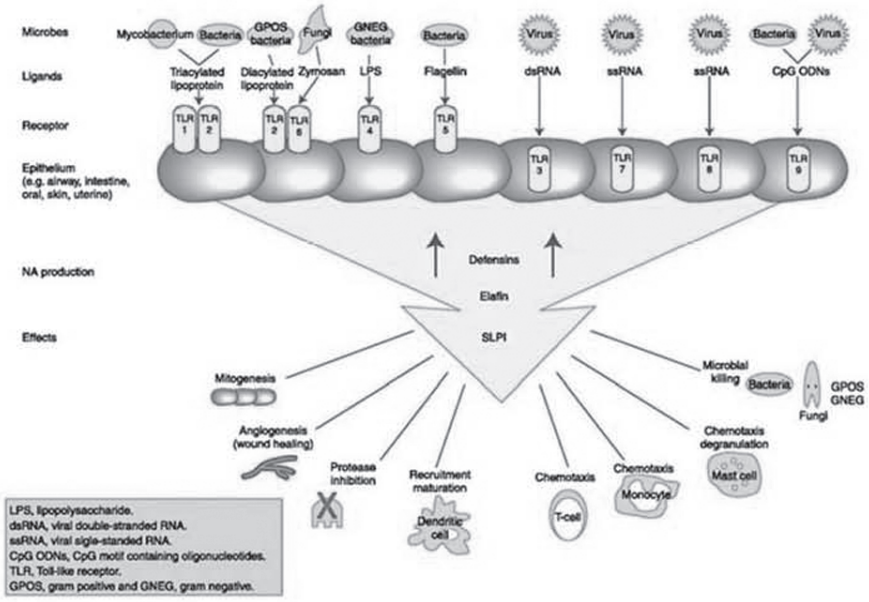

Figure 2 Summary illustrating the cellular origin, target cell lineages and function of the natural antimicrobial peptides, defensins and toll-like receptors(adopted from Andrew W Horne et al. Reproduction 2008;135:739-749)

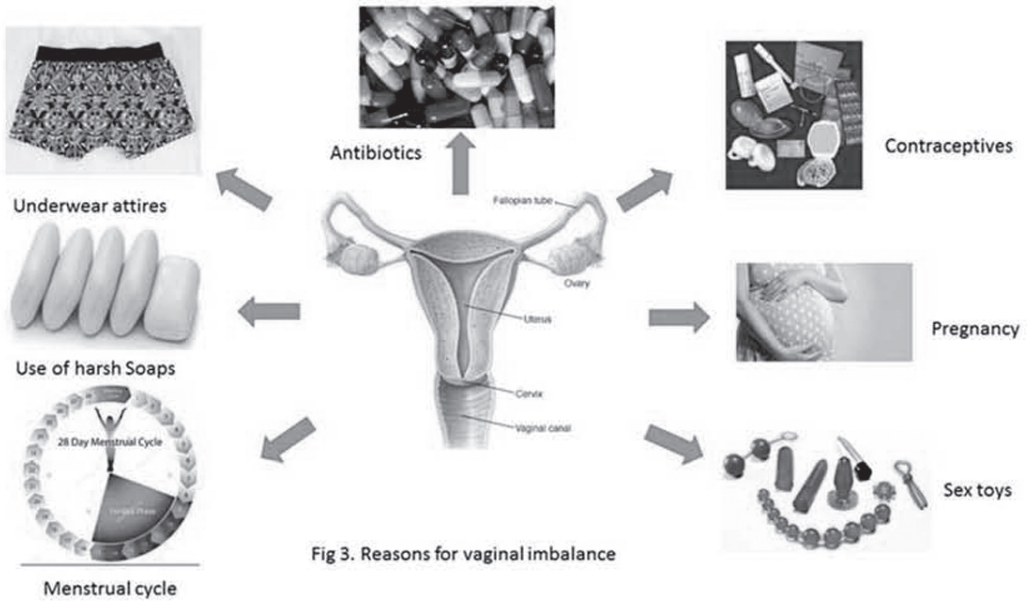

Fig 3. Reasons for vaginal imbalance

Vaginal Microbiota

the disturbance of the micro-organism membrane and in cellular lysis. Another class of molecules is represented by the SLPI, which has the capability of inhibiting enzymes that break proteins (proteases), destroying both gram-positive and gram-negative bacteria and hindering the action of the human immunodeficiency virus. The production of SLPI additionally occurs in the uterine tube. Levels of this protein (SLPI)in the vagina are decreased in women with bacterial vaginosis,signifying that the SLPI, as a constituent of the innate immune system, plays a pivotal role in the vaginal homeostasis maintenance. Besides SLPI, other constituents of the innate immune system protecting against infections by retrovirus (particularly HIV) have been characterized in the female genital tract. Uterine or tubarian cells produce CCL20/ MIP3 alpha chemokine and in vitro studies with cell cultures show an increase in the production of this protein after stimulation with double chain of synthetic DNA homologue to the viral RNA chain. Antibodies with the ability of identifying and binding specific microbial antigens are located in the vagina through transudation of the systemic circulation. Following binding comes the microbial death by either complement-dependent mechanism oropsonisation. Furthermore, a constituent of the mucosa's immune system is found in the reproductive tract. Antibodies producing B-lymphocytes are present in both the endocervix and the vagina, locally producing both IgC and IgA classes of antibodies. The local antibodies amplification represents a rapid mechanism for fighting pathogenic micro-organisms, without having to wait for the commencement of systemic immune response. Antibodies formed locally and present in the vagina possibly differ from the systemic ones. It is also possible to identify antibodies in the cervical-vaginal secretion that are undetectable in peripheral blood (21).

Factors that impact on vaginal microbiota in women of reproductive age Lifestyle factors

***Alcohol abuse*:** Sexual dysfunction is a condition that is caused by various different factors (Fig 3). Substance abuse, particularly alcoholism is known to be a factor for both men and women who are suffering from sexual dysfunction. Alcoholism is known to cause severe damage to the liver. A damaged liver can impact on the body's ability to regulate hormone distribution. In males, this can cause testicular atrophy, impotence and sterility. Women alcoholics can suffer from ovarian functionality issues, menstrual abnormalities and changes to sexuality (22).

Recent study by Loganantharaj et al.(23) to evaluate the immunological and virological changes in the genital microenvironment of females exposed to chronic alcohol suggest that chronic alcohol consumption negatively impacts the female genital micro-environment through the following mechanisms: increasing vaginal inflammation, decreasing beneficial bacterial species and increasing adverse bacterial morphotypes.

***Underwear attires*:** Genital hygiene is the major component of women's health and is very important for the protection of reproductive health (24). Both the type and cleanliness of the underwear garment, as well as the frequency with which it is changed, are important factors affecting the risk of a woman obtaining a urinary infection. Nylon and synthetic underwear is unable to absorb perspiration as effectively as cotton underwear. This causes the perineum to remain humid and leads to an increased risk of genital tract infections. Frequent changing of underwear is recommended in preventing genital and urinary infections. Tight-fitting jeans and nylon underwear should be avoided as they tend to trap moisture and aid bacterial grow.

***Harsh soap products*:** Washing the genital region more than once daily or using different commercial soap products may increase risk for vaginal infection by disturbing the genital flora and is therefore not recommended by the International Society for the Study of Vulvo-Vaginal Disease (25). Infection is most likely caused due to the reduced acidity of the vagina, either endogenously through hormones or exogenously through vaginal unhygienic practices (such as the usage of harsh

soaps or intra-vaginal cleansing "using fingers or douche"), poor menstrual hygiene and the usage of reusable cloth.

Antibiotics **:** The balance of the vaginal ecosystem depends on lactobacilli whose biofilm protects against microorganisms that are not normally present or are subdominant in vaginal flora. Lactobacillus species maintain the vaginal ecosystem in a healthy condition by production of antimicrobial substances. Depletion of lactobacilli in the vagina results in bacterial vaginosis (BV), where the normal flora is replaced by several bacterial pathogens, usually Gardnerella vaginalis and obligate anaerobes (26).Since the early 1980s, metronidazole has been used widely in the treatment of BV with good clinical results (27). Various preparations allowing a vaginal or oral administration and different regimens have been studied. In a clinical study conducted by Koumans et al.(28) the cure rates of women given antibiotics were higher (58%–100%) than the cure rates of women given placebo (5%–29%) when evaluated 4 weeks after treatment. Clindamycin is a second antimicrobial agent for the treatment of BV. This lincosamide antibiotic, a subclass of the larger family of macrolide antibiotics, has various treatment preparations including vaginal (ovule and cream) and oral. In a recent meta-analysis, intravaginal treatment (0.1%, 1%, 2% clindamycin cream twice daily for 5 days; 2% clindamycin cream at bedtime for 7 days) showed benefits to BV treatment with lower treatment failure compared with placebo (relative risk: 0.25; 95% confidence interval: 0.16–0.37)(29). Until recently, the mainstay therapy consisted of either metronidazole or clindamycin. A recent alternative has been the use of tinidazole. However, many clinical studies suggest that Probiotics should be seriously considered as part of the approach to disease prevention, and as an adjunct to antimicrobial treatment. Current recommendations do not advocate treatment of asymptomatic BV.

Hormonal fluctuations

Menstrual cycle : The vaginal microbial community plays a dynamic role in maintaining women's health. Understanding the precise bacterial composition is challenging because of the diverse and difficult-to-culture nature of many bacterial constituents, necessitating culture-independent methodology (30). During a natural menstrual cycle, physiological changes could have an impact on bacterial growth, colonization, and community structure. The relative compositional stability of the vaginal microbiome is quite remarkable, given the variability in the host ecosystem associated with the menstrual cycle, sexual contact, and introduction of bacteria from the skin and external environment. In particular, the menstrual cycle creates an ever-changing vaginal environment, with ovulation, menses, and corresponding fluctuations of oestrogen and progesterone levels affecting bacterial attachment to the vaginal epithelium cervical mucus production, pH and redox potential and glycogen levels (31). Moreover, an increase in mucosal glycogen, superficial keratinization of the mucosa and a shift of vaginal pH from 7 to 4.5-6, due to lactic acid production from the glycogen by Doderlein's bacilli (Lactobacilli), occurs at puberty or after oestrin. This results in an antigonococcal environment. During the menstrual cycle, hormonal deviationsinterfere in the substrate of different micro-organisms. These variations, with the addition of menstrual blood, lead to changes in vaginal pH. However, the levels of Lactobacillus remain constant throughout the menstrual cycle. The non-Lactobacillus bacteria levels increase during the proliferative phase of the menstrual cycle and the concentrations of Candidaalbicans become higher in the pre-menstrual period (32).Menstruation and sexual activity have been shown to have negative effects on the stability of the vaginal microbiota (33). The secretory phase of the menstrual cycle, which is characterized by high concentrations of oestrogen and progesterone, appears to be more stable in terms of microbial community composition.

Pregnancy : The vaginal microbiome in pregnancy plays an important role in both maternal and neonatal health outcomes (34). Pregnancy is accompanied by a shift in the bacterial community

structure of the vagina to a composition that is typically dominated by one or two species of Lactobacillus. These bacteria are believed to inhibit pathogen growth through secretion of antibacterial bacteriocins as well as the production of metabolites such as lactic acid that help to maintain a low, hostile pH (35).The maternal vaginal microbiome may also be an important source of pioneer bacteria for the neonatal gut microbiome, which have a profound effect on host system metabolism and immunity (36).Fluctuations in hormone levels in females can also cause the vaginal environment to adjust to one that is highly susceptible to infection and this can happen during pregnancy or while breastfeeding.

In pregnant woman, levels of oestrogen, mainly in the form of estriol, rise steadily over the course of pregnancy, with urine levels rising from a low in the first trimester of around 4 µg/24 hours to a high in the third trimester of up to 50,000 µg/24 hours (37). Increased oestrogen levels during pregnancy strengthen a Lactobacillus-dominant microbiota however, simultaneously, the occurrence of vulvovaginal candidiasis increases in comparison to that of non-pregnant women. The reason for this increase has been suggested to be the somewhat suppressed cell-mediated immunity in pregnant women which leads to an increased susceptibility to pathogens such as C. albicans (32). Oestrogen likewise stimulates the deposition of glycogen in vaginal epithelial tissue resulting in metabolized glucose within the vaginal epithelium and this is then converted to lactic acid via cellular metabolism (38). Recent data on changes in the vaginal microbiota during pregnancy are sparse and are meaningless unless the time of sampling is specified.

Use of Contraceptives

Currently, emerging scientific data (39) suggests that some commonly used contraceptives may increase risk of sexual HIV acquisition and transmission. There are several biologically plausible mechanisms by which hormonal contraceptives (HC) could increase HIV risk including disrupting epithelial barriers (thinning of the epithelium or altering epithelial integrity), causing changes in inflammatory responses that could in turn enhance HIV replication locally (40) or altering the vaginal microbiota which itself effects local immunity and genital inflammation.

Vaginal cells release molecules with potent non-specific antimicrobial activity. A class of these molecules, known as defensines, include positively-charged peptides that rapidly bind negatively-charged bacterial surfaces. This binding results in the disruption of the microorganism membrane and in cellular lysis (40). In women presenting with infections, HBD-1 and HBD-2 human defensines are produced by the vaginal epithelial cells. The production of HBD-2, but not that of HBD-1, is stimulated by oestrogens and inhibited by progesterone. This proposes that the usage of oral contraceptives may decrease the release of HBD-2, thereby increasing the susceptibility to infections. This suggests that the use of oral contraceptives may decrease the release of HBD-2, increasing, thus, the susceptibility to infections. Another class of molecules is represented by the SLPI, which possesses the capacity of inhibiting enzymes that break proteins (proteases), destroying gram-positive and gram-negative bacteria and blocking the action of the human immunodeficiency virus.

External influences such as spermicidal agents deplete the lactobacilli flora, especially strains producing hydrogen peroxide. The vaginal insertion of tampons, diaphragms and intrauterine devices (IUDs) can also disturb the microflora (41). A classic example was the usage of highly absorbent tampons to which Staphylococcus aureus strains producing toxic shock toxins, attached and resulted in morbidity and mortality. It is acknowledged that pathogen adhesion to IUDs can be associated with infections, however, when lactobacilli are the dominant organisms on IUDs, infections may not arise. Spermicides contain an ingredient called nonoxynol-9 which may cause a chemical irritation to both the vaginal and urethral mucosa as well as changes in the normal vaginal flora (42). Patients with recurrent Urinary tract infections (UTIs) should avoid diaphragms and

Vol. 11 (2) 190-205 April 2017, ISSN 0973-8916 (Print), 2230-7303 (Online)

spermicide coated condoms, as well as other barrier agents containing nonoxynol-9 such as foam, suppositories, and sponges (43).Periodic condom usage has been shown to increases the risk of vaginal inflammatory states, but with normal microbiota [44].

Sexual devices

Vaginal lubricants : Vaginal lubricants in particular are widely available and frequently used by women in order to allow the minimizing of dyspareunia or to enhance sexual pleasure (45). A large number of women use these feminine hygiene products daily. For some it is part of their daily cleansing or bathing. Feminine hygiene products or methods may disturb the normal pH level of 4.5 in the vagina, which is vital for maintaining the healthy vaginal immune barrier environment. Through the change of pH or the direct bactericidal properties, these products and practices may affect the composition of the normal vaginal microbiome, which is important for the healthy mucosal environment and protection against yeast infection or other sexually transmitted pathogens.

Lactobacilli and, especially, L. crispatus are among the bacteria that are most common in healthy women and characteristic for the healthy vaginal environment (46). Despite their intrinsic antimicrobial potential however, vaginal lactobacilli fail to retain dominance in a considerable number of women, resulting in overgrowth of the vaginal epithelium by other bacteria, as observed, most typically, with anaerobic polymicrobial overgrowth in bacterial vaginosis, or less commonly, with overgrowth by streptococci, including group A and group B streptococci, by bifidobacteria, or by coliforms such as E. coli (47). Vaginal products remain harmful to the Lactobacillus bacteria and should therefore be utilised with caution. Some feminine hygiene products may change the vaginal immune barrier by negatively effecting epithelial cell integrity, survival of beneficial Lactobacillus species in the vaginal microenvironment, and changing the ability of the vaginal epithelial cells to produce any protective or inflammatory immune mediators, such as, IL-8(48).

Use of foreign devices (Sex toys) : The HIV/AIDS pandemic has elevated international responsiveness on ways to reduce the risk of STIs including HIV, through the use of condoms and potentially microbicides. It is documented that the use and effectiveness of such methods is not only a question of availability and knowledge, but is also closely linked to socio-cultural beliefs about health, hygiene and sexuality, often expressed through women's vaginal practices that are widespread in much of the world. Concern has also been raised about whether vaginal practices could have harmful effects such as increasing the susceptibility to sexually transmitted or reproductive tract infections (49).

Sexual practices involving the transmission of vaginal fluid such as sex toys (after use) allow the sexual transmission of some agents associated with bacterial vaginosis (50). Furthermore, sharing of sex toys is alsoassociated with decreased quantities of H2O2-producing lactobacilli and greater risk of colonization with G. vaginalis. In a cross sectional study by Mitchell et al. (51) on women reporting sex with women who provided information on sexual behaviours it was noticed that the use of sex toys is consistently associated with differences in vaginal ecosystem and increases the risk of bacterial vaginosis, either by decreasing colonization with protective H_2O_2-producing lactobacilli or by increasing the presence of bacterial associated species like Gardnerella vaginalis.

The menace of sharing sex toys (e.g. dildos, butt plugs and other objects meant to be inserted into the rectum and/or vagina) varies according to whether they are covered with condoms, and whether they are properly cleaned (i.e., disinfected) between use with different partners. Sex toys come into direct contact with rectal and/or vaginal mucosal membranes, which can both transmit HIV and are susceptible to infection (52). Shared an uncleaned dildo or other toy can transfer infectious fluids from an HIV-positive person to an uninfected person, and poses a high transmission risk. If sex toys are used with new, clean condoms

for each partner, or are properly cleaned (i.e., disinfected) before use by the uninfected partner, they pose a negligible risk of transmission. Note that using the same condom with both partners is effectively the same as not using one at all.

There is zero HIV transmission risk attached to using sex toys if they are not shared. However, sex toys are often used before or after other sexual activities. As with fingering, fisting and other forms of play, any tissue damage or inflammation that results from sex toy use can affect the risk of infection during other activities.

Vaginal infections : Many factors can disturb the natural balance in the vagina, and if they become too intense, the vaginal flora can no longer adapt, which may result in a vaginal infection. The number of lactobacilli in the vagina is usually then reduced and the pH is also often raised to values above 4.4. Vaginal complaints such as itching, burning and discharge, can be signs of a bacterial or yeast infection and thus of an imbalance in the vaginal flora (53). The scope of the article is very limited to discuss in detail the common vaginal infections and therefore bacterial vaginosis is discussed in brief.

Bacterial vaginosis : Bacterial vaginosis (BV) is the most common cause of vaginal infection in women of childbearing age. While the aetiology and transmissibility of BV remain unclear, there is strong evidence to suggest an association between BV and sexual activity (54). Bacterial vaginosis is characterised by imbalance of the vaginal microbiota with a notable reduction of lactobacilli species, an overgrowth of a mixture of mostly endogenous obligate anaerobic bacteria spp. and elevated pH level in the vagina. The total number of bacteria associated with BV is increased 100-1000 fold when compared to normal levels. Bacterial vaginosis is a polymicrobial syndrome resulting from a decreased concentration of protective lactobacilli and an increase in pathogenic bacteria (55). As a result, there is both a qualitative and quantitative change of the microbiota associated with BV. The presence of anaerobic bacteria gives both rise to amines and an elevated pH, which further encourages the growth of anaerobic bacteria. Among the bacterial spp. commonly identified in BV are Gardnerella vaginalis, Atopobiumvaginae, Prevotella spp., Mobiluncusspp., Mycoplasma hominis and Urea plasmaspp.The list of BV-associated bacteria is growing since new species are being revealed through the use of cultivation independent methods of detection (56) (Table 1). Bacterial vaginosis causes no complications in most cases, however it does present some serious health risks. Multiple studies have shown an association between BV and an increased susceptibility to STDs such as HIV-1, Herpes simplex virus, HPV, N. gonorrhoeae, and C. trachomatis. Bacterial vaginosis has also been associated with a higher risk of endometritis, pelvic inflammatory disease and post-operative (hysterectomy, legal abortion) infections (57). Potential mechanisms by which bacterial vaginosis might increase HIV transmission comprise effects on local immune mediators. Additionally, hydrogen peroxide produced by lactobacilli can inhibit HIV in vitro, and is absent in most women with bacterial vaginosis. If bacterial vaginosis is established as an important risk factor for HIV spread, its control will become an important public health issue in many countries. Approximately 50% of women are asymptomatic. Bacterial vaginosis is characterised by a thin, homogeneous white discharge, a vaginal pH of above 4.5, a positive amine test and the presence of clue cells detected microscopically. Bacterial vaginosis increases a woman's risk of acquiring HIV, increases complications in pregnancy and may also be involved in thepathogenesis of pelvic inflammatory disease. Bacterial vaginosis has also been associated with an increased incidence of non-gonococcal urethritis in male partners in one small study(58).

Prevention Recommendations for healthy Vaginal Microbiota : Probiotic therapy is an exciting avenue that has been pursued for the treatment of vaginal dysbiosis for many years (59). However, the use of probiotics for the treatment of vulvovaginal atropy, vaginal dryness, and

Table 1. Prevalence of aerobic (facultative) isolates reported in vaginal flora studies published in the literature (adapted from Bryan Larsen, and Gilles R. G. MonifClin Infect Dis. (2001); 32:e69-e77)

	Prevalence in Vaginal Flora		
Aerobic Isolate	Low	Mean	High
Gram Positive Rods			
Diptheroids	3	40	80
Lactobacilli	18	60	90
Gram Positive cocci			
Staphylococcus aureus	0	2	25
Staphylococcus epidermidis	5	50	95
Staphylococcus species			
α-Hemolytic	8	20	38
β-Hemolytic	3	15	22
Nonhemolytic	0	20	32
Group D	2	28	45
Gram Negative Rods			
Escherichia coli	3	18	33
Klebsiella and Enerobacter species	0	10	20
Proteus species	0	5	10
Pseudomonas species	0	0.1	3

restoration of a healthy vaginal microbiota in women is a relatively new concept. Probiotics (including Lactobacillus spp.) potentially work through a variety of mechanisms to reinstate homeostasis by enhancing epithelial barrier function, commensal colonization, blocking adhesion of pathogenic bacteria, reducing pH, influencing antimicrobial peptide production/ secretion and overall mucosal immunity and vaginal health (60).

Both oral and vaginal routes of delivery have been pursued in clinical studies for reinstating vaginal homeostasis by delivery of Lactobacillus-based probiotic formulations and exhaustive efforts have likewise been made toward the administration of drugs, via alternative routes, that are poorly absorbed after the oral administration. The vagina as a route of drug delivery has been known since ancient times (61). In recent years, the vaginal route has been rediscovered as a potential route for systemic delivery of peptides and other therapeutically important macromolecules. However, successful delivery of drugs through the vagina remains a challenge, primarily due to the poor absorption across the vaginal epithelium. The rate and extent of drug absorption after intravaginal administration may vary depending on formulation factors, vaginal physiology, age of the patient and menstrual cycle. Suppositories, creams, gels, tablets and vaginal rings are commonly used vaginal drug delivery systems (62).

Vaginal drug delivery often ideal because it allows the use of lower doses, maintains steady drug administration levels, and requires less frequent administration than the oral route. With vaginal drug administration, absorption is unaffected by gastrointestinal disturbances, there is no first-pass effect, and use is discreet.

Conclusion

Research about the vaginal microbiome has advanced over the last decade. Light microscopy and advanced culture techniques revealed healthy vaginal microbiomes are dominated by Lactobacillus species. Next-generation sequencing technology has allowed scientists to

further categorize and understand the complexity and diversity of microbes that inhabit the vaginal cavity. Overall, Lactobacillus dominance is strongly correlated to vaginal health and homeostasis in both pre- and postmenopausal women. Disruptions in vaginal flora, such as BV, are related to a number of poor obstetrical and gynaecologic outcomes.

The U.S. Food and Drug Administration (FDA) and the European Medicine Agency (EMA), as well as most of the other regulatory bodies around the world, cautions women regarding the use of lubricants and other medical devices easily available over the counter (OTC) as these products lack extensive pre-clinical and clinical testing as otherwise required for drug products. There is lack of data on the safety of vaginal OTC lubricants, moreover different in vitro and in vivo animal studies indicate that water-based lubricants may induce changes to the vaginal environment and mucosa that can lead to toxic effects and, eventually, enhancement of the transmission of sexually transmitted pathogens, such as HIV.

With greater consciousness of the chemicals used in popular feminine hygiene products, the unique characteristics of the vaginal region, and the potential for health disparities among various groups of women through culturally determined use of these products, it is apparent that more studies are needed to understand their effects on vaginal microbiota.

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Conflict of interest

The authors declare no conflict of interest.

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