MICROBIOTA AND UROGENITAL INFECTIONS
Lower urogenital tract infections are extremely common amongst women and appear to be closely related to a dysbiosis of the urinary or vaginal microbiota. Contrary to a still widely held belief, urine is never sterile; it is instead home to a unique microbiota. And a loss of diversity in this microbiota is a risk factor for urinary tract infections. On the other hand, the vaginal microbiota, when balanced, has low diversity and is dominated by a small number of lactobacilli. A loss of this dominant flora is associated with infections of the lower urogenital system. Hence the efforts of science to regulate vaginal and urinary microbiomes using probiotics (bacteria, yeasts) or even vaginal microbiota transplants.
Urinary tract infections

Uncomplicated lower urinary tract infections are much more common amongst women than amongst men. Rarely linked to a structural abnormality, they occur when a pathogen from the digestive system—often Escherichia coli—travels up the urinary tract and colonizes it. The most common bacteria are Escherichia coli, Proteus, and Proteus mirabilis, but other possible pathogens include Staphylococcus, Enterococcus, and Pseudomonas. The bacteria have a number of appendages that allow them to adhere to the urinary tract wall. They produce toxins and enzymes that are harmful to the host’s immune system. Biofilms form inside the urinary system, which results in persistent infection. Biofilms are even more common in the case of women, who have a greater number of bacterias adhere (cells of the vaginal epithelium to which a large number of bacteria adhere) on microscopy, copy of vaginal secretions. The Nugent score, a microscopic examination of a Gram stain of vaginal secretions, is also used in many countries and classifies the bacterial flora into three groups: healthy, if the score is between 0 and 3, intermediate if the score is between 4 and 6, and indicative of bacterial vaginosis if the score is greater than 6. Three such scores are available: (a) presence of clue cells (cells of the vaginal epithelium to which a large number of bacteria adhere) on microscopy, copy of vaginal secretions, (b) absence of clue cells (cells of the vaginal epithelium to which a large number of bacteria adhere) on microscopy, copy of vaginal secretions, (c) presence of clue cells (cells of the vaginal epithelium to which a large number of bacteria adhere) on microscopy, copy of vaginal secretions. The Nugent score, a microscopic examination of a Gram stain of vaginal secretions, is also used in many countries and classifies the bacterial flora into three groups: healthy, if the score is between 0 and 3, intermediate if the score is between 4 and 6, and indicative of bacterial vaginosis if the score is greater than 6. Some authors believe that BV may actually be a set of common clinical signs and symptoms caused by a wide range of pro-inflammatory bacteria, coupled with a host-dependent immune response. As a result, some experts prefer to refer to it as polymicrobial vaginosis.

Vaginal infections

Bacterial vaginosis and vulvovaginal candidiasis are two very common gynecological infections. The first is a bacterial infection which may be caused by a wide range of pro-inflammatory bacteria, while the second is due to the spread of a fungus. BACTERIAL VAGINOSIS

Bacterial vaginosis (BV) is the most frequently reported microbiological syndrome amongst women of reproductive age. The Amsel criteria, although controversial, remains the standard method for diagnosing bacterial vaginosis and is based on the presence of at least three of the following clinical criteria: (1) thin, homogeneous vaginal discharge; (2) vaginal pH > 4.5; (3) amine (fishy) odor on adding potassium hydroxide to a vaginal smear; (4) presence of clue cells (cells of the vaginal epithelium to which a large number of bacteria adhere) on microscopy; copy of vaginal secretions. The Nugent score, a microscopic examination of a Gram stain of vaginal secretions, is also used in many countries and classifies the bacterial flora into three groups: healthy if the score is between 0 and 3, intermediate if the score is between 4 and 6, and indicative of bacterial vaginosis if the score is greater than 6. Some authors believe that BV may actually be a set of common clinical signs and symptoms caused by a wide range of pro-inflammatory bacteria, coupled with a host-dependent immune response. As a result, some experts prefer to refer to it as polymicrobial vaginosis.

VULVOVAGINAL CANDIDIASIS

Vulvovaginal candidiasis (VVC), so called because it is linked to the spread of fungi (more specifically, yeasts) of the Candida genus, is considered the second most common vaginal infection after BV. 70%-75% of women are thought to have been affected at least once in their lifetime, 50% twice, and...
The microbiota seems to play an important role in infections of the lower urogenital system, whether it is the urinary microbiota (urine is not sterile) in the case of urinary tract infections, or the vaginal microbiota, in the case of bacterial vaginosis and vulvovaginal candidiasis. However, there is a difference: while diversity is favorable in the first case, it is unfavorable in the latter.

**URINE IS NOT STERILE**

Historically, urine was considered sterile but recent scientific discoveries have shown that this is not the case: 562 bacterial species have been identified in the human urinary microbiota. Of these, 352 species (62.6%) have been linked to at least one report of infection among humans, including 225 (40.0%) described as the causal agent of urinary tract infections. The eight bacteria most commonly implicated in UTIs are *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterococcus faecalis*. Conversely, bacteria that secrete lactic acid, namely *Lactobacillus* and *Streptococcus*, are believed to play a protective role against pathogens: lactic acid lowers the pH of urine (≈ 4.5), resulting in a microenvironment that is unfavorable to most pathogenic bacteria, while lactobacilli produce antibacterial metabolites (hydrogen peroxide and bacteriocins).

**Dysbiosis-related urinary tract infections**

Although long considered sterile, the urinary tract is actually home to a microbiota containing over 500 bacterial species. A loss of diversity in this microbiota seems to be a risk factor for urinary tract infections.

**CLINICAL CRITERIA FOR VAGINAL INFECTIOUS DISEASES**

| **Bacterial vaginosis (BV)**  
| *Gardnerella vaginalis*  |
| Thin, homogeneous, fishy odor vaginal discharge  
| No inflammation |
| **Vulvovaginal candidiasis (VVC)**  
| *Candida albicans*  |
| Whitish, pasty leukoema, adherent to vaginal wall  
| Vaginal pruritus and burning sensation  
| Vulvar erythema and edema with scratching lesions  
| Ucerations, fissures  
| Dyspareunia and dysuria |

Dysbiosis-related infections of the lower genital tract

Unlike the urinary microbiota and many other microbiotas, the vaginal microbiota, when healthy, has low diversity and is mostly dominated by a few lactobacilli. A dysbiosis where lactobacilli lose their predominance has been linked to infections of the lower genital tract (bacterial vaginosis, vulvovaginal candidiasis).

A HEALTHY VAGINAL MICROBIOTA: LOW DIVERSITY AND DOMINATED BY LACTOBACILLI

The vaginal microbiota consists mainly of lactobacilli with a protective role. Despite considerable variability among women, in general, five types of communities have been categorized, depending on whether they are dominated by L. crispatus, L. gasseri, L. iners or L. jensenii, or have few or no lactobacilli and a significant quantity of strict anaerobic bacteria (Megasphaera, Prevotella, Gardnerella, Atopobium, and Sneathia) known to be characteristic of bacterial vaginosis.

Therefore, while a high number of microbial communities is usually an indicator of health for several microbiotas (digestive microbiota, etc.), the vaginal microbiota is balanced when it has low diversity and is dominated by one or a few species of lactobacilli. In women of childbearing age, hormones promote the proliferation of lactobacilli. Estrogen levels induce the deposition of large amounts of glycogen, the main source of energy for lactobacilli; on the vaginal walls. From adolescence to the menopause, high estrogen levels promote colonization of the vagina by lactobacilli which metabolize glycogen, produce lactic acid and maintain intravaginal health by lowering the pH level.

BACTERIAL VAGINOSIS: WHEN G. VAGINALIS DRIVES OUT LACTOBACILLI

Despite more than sixty years of research, the etiology of BV remains unknown. Nevertheless, research seems to point more and more to the dysbiosis theory according to which dominant lactobacilli are replaced by polymicrobial flora derived from numerous bacterial genera (Gardnerella, Atopobium, Prevotella, etc.) G. vaginalis is in effect present in 90% of symptomatic subjects and 45% of normal subjects, whereas Lactobacillus sp. is found in 70% of apparently healthy subjects and 40% of symptomatic subjects. Consequently, G. vaginalis has been suspected of being the main pathogen in BV. However, there is long-standing disagreement in this regard, since this virulent bacterium has also been found...
in virgin girls and in sexually active women with normal vaginal microbiota, in other words, colonization by *G. vaginalis* does not always lead to BV. An explanation recently put forward may settle the debate: there is not one, but at least thirteen, different species of the genus *Gardnerella*, some of which may not be pathogenic. A mechanism for the development of the dysbiosis has even been suggested: *G. vaginalis*, transmitted sexually, spreads itself among healthy vaginal lactobacilli, such as *L. crispatus*, initiating the formation of a biofilm, a structure that further protects the pathogen from the hydrogen peroxide and lactic acid secreted by the lactobacilli. By reducing the redox potential of the vaginal microbiota, *G. vaginalis* gradually reduces the lactobacilli population in favor of strict anaerobic bacteria such as *P. bivia* and *A. vaginae*. *G. vaginalis* and *P. bivia* seem to facilitate each other’s development, the former providing amino acids to the latter and the latter ammonia to the former. Lastly, both pathogens produce an enzyme that destroys the mucus in the vaginal epithelium, facilitating the adhesion of different bacteria associated with BV, such as *A. vaginae*, and potentially causing a polymicrobial infection.

**VULVOVAGINAL CANDIDIASIS: A PROLIFERATION OF CANDIDA**

Vulvovaginal candidiasis could be linked to an imbalance of the vaginal microbiota together with a proliferation of the fungus *Candida*, including *C. albicans* in 80%-92% of cases, and to a lesser extent *C. glabrata*, *C. tropicais*, *C. parapsilosis* and *C. krusei*.

Exposure to antibiotics, whether local or systemic, is thought to be one of the main factors leading to vulvovaginal candidiasis. The reduction of certain bacterial species, lactobacilli or not, that control the replication and virulence of Candida yeasts apparently allows the fungi already present in the vagina to multiply and induce infection. Future studies involving new sequencing technologies are needed to characterize in further details the interaction between vaginal microbiota, these yeasts and the occurrence and recurrence of vulvovaginal candidiasis.

**HEALTHY VAGINAL MICROBIOTA: A SAFEGUARD AGAINST STIs**

The vaginal microbiota also plays an important role in maintaining vaginal health and protecting the host against the acquisition and transmission of sexually transmitted infections. Vaginal microbiota with a small number of bacterial communities and dominated by lactobacilli (in particular *Lactobacillus crispatus*) are those most associated with vaginal health, whereas increased diversity seems to be associated with lower resilience to imbalances and higher susceptibility to STIs such as herpes (BV increases the risk of herpes and vice versa), papillomavirus (increased prevalence and likelihood of contracting HPV, delayed elimination, increased severity of cervical intraepithelial dysplasia), HIV (increased risk of acquisition and transmission), and other infections (gonorrhea, chlamydia and trichomoniasis).

**Urinary area: efficacy to be confirmed in clinical trials**

In order to endow the urinary microbiota with the diversity it requires to be in equilibrium and reduce the development of bacterial resistance to antibiotics, the use of probiotics and cranberry has also been considered. Different strains of lactobacilli have demonstrated their potential, although further clinical trials are expected.

Since lower urogenital infections seem closely related to a dysbiosis of the urinary or vaginal microbiota, treatments attempt to regulate the microbiota in order to prevent or even cure these conditions.

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normal, balanced flora\(^2\). According to the literature, probiotics have proven effective in the treatment and prevention of urogenital infections\(^3\). Certain lactobacilli (L. rhamnosus, L. fermentum and L. reuteri) have been shown to have a beneficial effect in treating urinary tract infections\(^4\). An inhibitory effect on E. coli has been demonstrated in vitro, with certain strains of lactobacilli (L. rhamnosus and L. plantarum) possessing antimicrobial properties against this bacterium\(^5\). Therefore, the data increasingly suggests that probiotics may be used as a first step in the regulation of urinary microbiota in order to reduce the risk of, or treat, certain urinary infections, particularly since they are safe, better tolerated than antibiotics and frequently requested by patients\(^6\). However, further clinical trials involving large numbers of patients will be required to obtain clear evidence on the preventive and curative role of probiotics in urinary tract infections\(^7\).

### Vaginal area: efficacy confirmed

In order to maintain a lactobacilli-dominated protective flora, the use of topical or oral probiotics to prevent or treat vaginal infections, as well as a first clinical trial focused on vaginal microbiota transplants, have been initiated. Results are positive.

**PROBIOTICS: IMPORTANCE OF BACTERIA AND YEASTS**

Since a vaginal microbiota dominated by lactobacilli is considered optimal, vaginal probiotics unsurprisingly contain strains of this genus, which vary depending on the probiotic in question (L. acidophilus, L. crispatus, L. reuteri, L. rhamnosus). A review of 22 commercially available topical vaginal probiotics carried out in early 2019\(^8\) highlighted their potential for the prevention and treatment of BV, but much less so for the prevention and treatment of vulvovaginal candidiasis. None of the studies reported any major safety concerns. Probiotic strains have never been detected in the vagina beyond the period of administration, suggesting they do not colonize the environment in a sustained manner. In addition to probiotics for local use, oral probiotics have gained considerable importance. Four strains (L. crispatus, L. gasseri, L. jensenii and L. rhamnosus) out of 127 vaginal lactobacilli studied are noteworthy for their ability, in vitro, to acidify the environment, inhibit the growth of G. vaginalis and C. albicans and survive gastrointestinal transit\(^9\). But what about in vivo? Consumed in the form of a yoghurt drink by patients suffering from BV (twice a day for four weeks), these same four strains led to a 100% recovery (Amsel criteria), compared to a 65% recovery in the placebo group\(^10\).

In addition to bacteria, certain yeasts (C. albicans, C. glabrata, S. cerevisiae, S. bayanus, C. krusei, C. tropicalis, C. parapsilosis, S. sake) may also be of interest, particularly C. albicans, which modifies the structure of the connective tissue and can naturally secrete capric acid, which modifies the structure of C. albicans, reducing its adhesion capacity, inhibiting its filamentation and hindering its ability to form biofilms\(^11\).

**VAGINAL MICROBIOTA TRANSPLANTS: PROMISING FIRST CLINICAL TRIALS**

In October 2019, the results of a first exploratory trial testing the transplant of vaginal microbiota from donors as a therapeutic alternative in five patients with symptomatic, untreatable and recurrent BV were published in *Nature Medicine*\(^12\). Four patients showed a full long-term remission (sometimes requiring several transplants or even a change of donor) up to the end of the follow-up period (5 to 21 months after the transplant), with a marked improvement in symptoms, Amsel criteria and the microscopic appearance of vaginal fluids, as well as the reconstruction of lactobacilli-dominated vaginal microbiota. The remaining patient is still in incomplete remission, while no adverse side-effects were observed. Accordingly, the researchers recommended further trials evaluating the therapeutic efficacy of vaginal microbiota transplants.
Dr Jean-Marc Bohbot, an infectologist specializing in urogenital infections, is head of the Sexually Transmitted Infections Department at the Alfred Fournier Institute (Paris). He is also the author of books aimed at the general public on sexually transmitted infections and the vaginal microbiota.

**DR JEAN-MARC BOHBOT**

Do we not overestimate the importance of microbiota in the urogenital area?

In recent years we have come to understand the urogenital microbiota more clearly. We now know that it can be a factor in infections, in urinary disorders related to the menopause and even in tumors. The urogenital microbiota and its disruptions must be taken into account in patient management and probiotics must be part of the therapeutic arsenal. Although probiotics are obviously not our only weapon, they are indispensable, since anti-infectious treatments do not treat the cause of recurrence, i.e. the dysbiosis.

What role do you think probiotics can play today against urinary tract infections?

Urinary tract infections are closely linked to imbalances in three microbiomes: the urinary microbiota, since urine is not sterile; the vaginal microbiota, with which the urinary microbiota shares many similarities; and the gut microbiota, from which the pathogens involved in urinary tract infections originate (e.g. E. coli, which passes from the anus to the vulvar vestibule and then to the bladder).

Conventional antibiotic treatment is justified for single UTI episodes. On the other hand, for recurrent UTIs (more than four episodes per year), it is essential, after having ruled out functional causes (e.g. a tumor of the bladder), to question the patient about possible disorders of the gut microbiota (constipation, etc.) and/or vaginal microbiota, the latter acting as a protective barrier between the digestive and urinary systems. The prevention of recurrence involves treatment for three to six months with intestinal probiotics administered orally, if a dysbiosis of the intestinal microbiota is present, and/or vaginal probiotics, ideally administered vaginally. These treatments may be combined with the use of cranberry, which reduces the level of E. coli in the bladder.

What about vaginal infections?

There are two types of vaginal infections: endogenous infections resulting from changes in endogenous microorganisms (bacteria or fungi) and exogenous infections contracted during sexual intercourse.

For endogenous infections, in the case of a single episode, an antifungal vaginal suppository or antibiotic treatment may suffice. However, where there is a risk of recurrence, the dysbiosis must be treated for several months with gynecological probiotics.

Probiotics also have a role to play in exogenous infections, since the less balanced the vaginal microbiota, the greater the risk of acquiring a sexually transmitted infection (STI), and the higher the risk of an unfavorable outcome. For example, the papillomavirus is four to five times more likely not to be completely eliminated, and progresses more rapidly to potentially cancerous forms, when a dysbiosis exists. It is therefore important to test for an imbalance of the vaginal microbiota in infected women through a simple measurement of acidity (the pH should be between 3.5 and 4.5) and then by vaginal sampling where the pH is above 4.5. Where there is an imbalance, laboratory-tested and clinically approved probiotics should be prescribed.

A vaginal dysbiosis also increases the risk of contracting HIV. Although the acidity of lactobacilli helps destroy the virus, an inflammatory state increases the presence of lymphocytes, the cells targeted by HIV.

Lastly, what can we expect from vaginal microbiota transplants?

The results of just over twenty cases of vaginal microbiota transplants have been published. Although these results are interesting, they are not yet conclusive. The idea of treating recurrent bacterial vaginosis through a microbiota transplant still raises concerns as regards the criteria for selecting donors—particularly since the absence of symptoms does not mean that the donor’s flora is balanced—and the indications for the recipient.

It will most likely be known within a year or two whether vaginal microbiota transplants can be used as a last resort.
Microbiota and urogenital infections

Urinary tract infections, bacterial vaginosis and vulvovaginal candidiasis, which are widespread in women, are the most common types of lower urogenital tract infections. In the urinary area, infections are due to the colonization by a pathogen originating in the gastrointestinal tract, most frequently Escherichia coli, that travels up the urinary tract. In the gynecological area, infections are related to bacterial (bacterial vaginosis) or fungal (vulvovaginal candidiasis) proliferation. These female urogenital disorders are largely caused by urinary or vaginal dysbioses. The urinary tract has long been considered a sterile environment, but it actually hosts a specific microbiota. Its imbalance is a risk factor for UTIs, allowing opportunistic bacteria to colonize the bladder. On the contrary, a healthy vaginal microbiota displays low diversity and is dominated by one or several species of lactobacilli. While vulvovaginal candidiasis seems to be associated to an imbalance in the vaginal microbiota, the exact etiology of bacterial vaginosis is still unknown. However, the hypothesis that dysbiosis promotes colonization by a polymicrobial flora (Gardnerella, Atopobium, Prevotella…) seems to be the most plausible.

How can a protective microbiota be restored and kept healthy? What challenges tomorrow’s therapies must overcome? Is it already conceivable to prevent, or even treat, some lower genital infections by modulating microbial ecosystems? The objectives of this paper are to explain the current state of knowledge, and to question and clarify the value of probiotics (bacteria, yeasts) in the urogenital field.