Women

AND THEIR MICROBIOTA

BIOCODEX
Microbiota Institute

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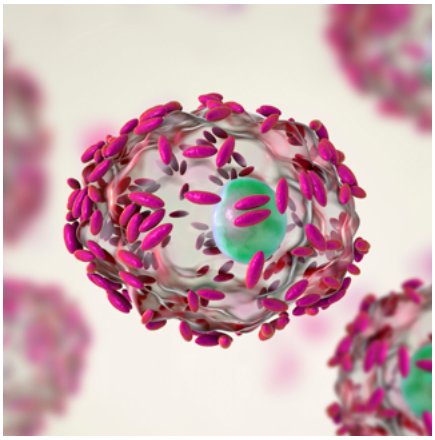
1. MICROBIOTA, VULVOVAGINAL INFECTIONS AND CYSTITIS

Vaginal microbiota plays an essential role in women's health. It is mainly composed of lactobacilli, and protects women throughout their life not only from different pathogens (bacteria, fungi, viruses originating in the intestinal microbiota or from the outside), but also from imbalances in the vaginal microbiota (dysbiosis) responsible for several urogenital disorders and infections. The most common diseases are cystitis, vaginosis and sexually transmitted infections (STI). The good news is that it is possible to prevent their onset or recurrence thanks to some hygiene rules or a targeted intake of probiotics intended to restore the microbial balance.

Is bacterial vaginosis a disease?

Although it affects nearly 20% of women in France[1] and millions of women every year in the world, bacterial vaginosis remains under-diagnosed and poorly treated because of its unclear definition.

Bacterial vaginosis has been described as an infection, an inflammatory disease, a dysbiosis (microbiota imbalance), a syndrome, or even a normal situation. A proper definition has not yet been found and it keeps causing controversy among the scientific community. Canadian microbiologist, Gregor Reid[2] reminded that while the disease had been discovered in 1954 and defined as an infection caused by *Gardnerella vaginalis*, the term "bacterial vaginosis" only appeared in 1983. But the fact that the responsible bacterium can also be found in healthy women without causing vaginosis undermines this theory. Six years later, vaginosis was described as "*a complex change in vaginal microorganisms associated with malodorous discharge and no visible inflammation*". Some time later, researchers observed an increase in inflammation markers and classified it as an inflammatory disease. However, this definition was invalidated in 2010 due to lack of evidence. More recently, the term "dysbiosis" was added to the list. In conclusion, after nearly 65 years of research, no consensus has been reached yet.



Gardnerella vaginalis.

1 Collège national des gynécologues et obstétriciens français (CNGOF)

2 Reid G. Is bacterial vaginosis a disease? Applied Microbiology and Biotechnology (2018) 102:553–558 https://doi.org/10.1007/s00253-017-8659-9

Poorly defined, poorly treated

According to the literature, vaginosis is not a disease as generally understood, that is to say a deterioration of health characterized by specific signs. It presents as a range of symptoms (inflammation, foul vaginal odor, increase in bacterial diversity, etc.) which vary greatly from one woman to another. In some cases, it may cause no symptoms at all.

Nevertheless, its diagnosis, prevention and management depend on its definition. To this day, only a pharmacologic approach receives financial support from health authorities, thus excluding alternative avenues aiming at restoring and maintaining the flora, such as probiotics and prebiotics. Gregor Reid believes this to be an aberration and calls for the term "vaginosis" to be abandoned and replaced by a term which better describes the different disorders it encompasses. According to him, "vaginal dysbiosis" or "vaginal inflammation" would help provide a more appropriate treatment.

INTIMATE HYGIENE DON'TS [3]

- Vaginal douches
- Too frequent washing
- Use of chemical antiseptics
- Washing with water only
- Use of intimate hygiene soap and deodorant
- Use of panty liners or tampons outside periods

Escherichia coli or the influence of intestinal microbiota on urinary tract infections

Due to anatomical reasons, women are much more prone to urinary tract infections (UTI) than men. The most frequent form in women is cystitis, a bladder infection caused by the bacterium *Escherichia coli*.

Escherichia coli is naturally present in our intestinal microbiota but can become a pathogen by using some of its infectious properties such as the ability to adhere to the bladder. It is then referred to as uropathogenic *Escherichia coli* (UPEC). Urinary tract infections occur when the urogenital area is contaminated by the fecal flora. Bacteria can colonize the urethra exclusively (causing urethritis), spread to the bladder and cause acute cystitis, or reach the kidneys (causing pyelonephritis). This bacterial migration from the anal area to the urogenital system raises two questions: are the responsible strains different from a genetic standpoint or do they need to adapt when they migrate from the intestines to the bladder? For preventive purposes, could it be possible to predict the risk of contracting a UTI using fecal *Escherichia coli* samples?

No adaptation is required

Several studies[4, 5] from a Danish team provided us with some answers. The researchers observed that the fecal strains of *Escherichia coli* in patients with UTI were the same as those found in their own urine samples and also the same as in healthy women. The only differences were a few minor genetic variations. In other words, *Escherichia coli* is able to migrate from the intestines to the bladder without needing to adapt at all. The evidence thus showed that fecal microbiota composition cannot predict the risk of developing a urinary tract infection. Then, what are the causes of UTIs? UPEC-mediated urinary tract infection probably results from a combination of factors related to the bacteria (ability to adhere to intestine cells, virulence...) and to the host's immune status, creating an infection-prone environment.

3 Microbiote vaginal, la révolution rose, Jean-Marc Bohbot & Rica Etienne

4 Nielsen KL, Stegger M, Kiil K, Godfrey PA, Feldgarden M, Lilje B, Andersen PS, Frimodt-Møller N. Whole-genome comparison of urinary pathogenic Escherichia coli and faecal isolates of UTI patients and healthy controls. Int J Med Microbiol. 2017 Dec;307(8):497-507. doi: 10.1016/j.ijmm.2017.09.007. Epub 2017 Sep 14. PMID: 29031453

5 Nielsen KL, Stegger M, Godfrey PA, Feldgarden M, Andersen PS, Frimodt-Møller N. Adaptation of Escherichia coli traversing from the faecal environment to the urinary tract.. Int J Med Microbiol. 2016 Dec;306(8):595-603. doi: 10.1016/j.ijmm.2016.10.005. Epub 2016 Nov 4. PMID: 27825516

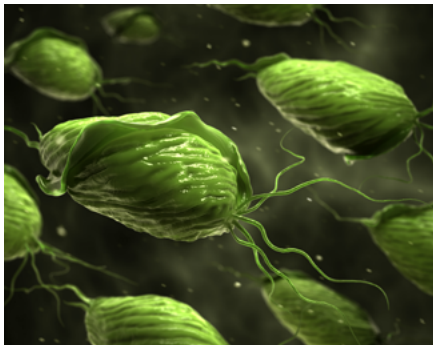
Are certain women predisposed to STIs?

Several epidemiological studies have found a correlation between bacterial vaginosis, vulvovaginal candidiasis, colonization of the vaginal microbiota by pathogenic bacteria, and sexually transmitted infections (STIs). Based on these results, a Dutch researcher[6] investigated whether their vaginal microbiota predisposes certain women to acquire STIs.

A healthy vaginal microbiota is composed of various microorganisms, where lactobacilli generally predominate. However, advances in molecular biology have shown that not all lactobacilli provide the same degree of protection: *Lactobacillus crispatus*, for example, is associated to an anti-inflammatory profile and seems to afford protection against pathogenic germs. On the contrary, *Lactobacillus iners* seems to promote an imbalance of the vaginal microbiota (dysbiosis) favorable to bacterial vaginosis, similarly to pathogenic bacteria.

Microbiota, vaginosis and STIs: dangerous liaisons

Vaginosis, vaginal candidiasis, colonization of the vaginal microbiota by pathogens, and STIs share many biological and behavioral factors which could explain their interrelationships. Although vaginosis and vaginal candidiasis are not STIs per se (since they can occur without intercourse), a study by Janneke Van de Wijgert showed that sexual transmission of the responsible organisms most certainly plays a role in their development. Moreover, dysbiosis and vaginosis weaken the vaginal mucosal barrier and leads to cervicovaginal inflammation, which increases the risk of HIV infection.



Trichomonas vaginalis.

The risk of contracting sexually transmitted infections thus depends, at least in part, on the health of the vaginal microbiota. By preserving their microbial flora, women could limit their risk of developing STI. Future research needs to focus on determining how the vaginal microbiota can expose women to a higher risk of STI, in order to better screen and treat them, especially with local probiotics.

6 Van de Wijgert JHHM. The vaginal microbiome and sexually transmitted infections are interlinked: Consequences for treatment and prevention. PLoS Med. 2017 Dec 27;14(12):e1002478. doi: 10.1371/journal.pmed.1002478. eCollection 2017 Dec. PMID: 29281632

2. REPRODUCTIVE HEALTH, PREGNANCY AND POSTPARTUM

Role of the vaginal microbiota in the health of the reproductive system and that of future generations[7]

For several years, investigators have focused on the intestinal microbiota, but they are now widening their research to the vaginal bacterial community which could play a major role in the health of women and their babies. Some scientists[8] believe microbes to be the most precious legacy a mother passes on to her child.

The composition of the vaginal microbiota changes throughout women's lives: while it has a particularly low content of *Gardnerella vaginalis, Prevotella* and lactobacilli before puberty, it becomes almost exclusively colonized by lactobacilli after puberty. Lactobacilli ensure women's health by fighting against pathogens. Their decrease is associated to various gynecological disorders, such as premature delivery, infertility, sexually transmitted infections or even pelvic inflammatory diseases. Shortly before menopause, hormonal variations cause significant changes in the composition of the vaginal microbiota which finds a different balance after menopause.

Microbes and reproduction

Vaginal microbiota also seems to play a role in conception, whether it is natural or by means of *in vitro* fertilization (IVF). The presence of *Gardnerella vaginalis* and *Atopobium vaginae* in the vaginal microbiota was associated to lower success rates, while treatment of bacterial vaginosis, which is frequent in infertile women, improves pregnancy rate. Success also depends on the proportion of lactobacilli in seminal fluids as well as the presence of some species in the Fallopian tubes and the uterine mucosa (endometrium), whose microbiota could either favor or limit chances of embryo implantation.

The baby's health starts in the uterus

The baby's immune and metabolic systems could be determined during intrauterine life through its exposure to maternal microbes which are present in the placenta and amniotic liquid and can also be found in the newborn's first stools (meconium). At present, it is unknown if the placenta hosts its own microbiota. It is known however, that it is similar to the maternal oral flora, which could explain why pregnant women with periodontal diseases have an increased risk of facing pregnancy-related complications. Moreover, changes in its composition are associated to premature births.

7 Younes JA, Lievens E, Hummelen R, van der Westen R, Reid G, Petrova MI.Women and Their Microbes: The Unexpected Friendship. Trends Microbiol. 2018 Jan;26(1):16-32. doi: 10.1016/j.tim.2017.07.008. Epub 2017 Aug 23. PMID: 28844447

8 "Women and their Microbes", conferences organized in Amsterdam in 2015 and 2016.

Risks and benefits

Although the mother is a source of microbes for her baby, other factors come into play to modulate the baby's microbiota. Use of antibiotics by the mother (especially in the second or third trimesters of pregnancy) as well as cesarean delivery (because the newborn does not come into contact with the mother's vaginal microbiota) are associated with an increased risk of childhood obesity. On the contrary, probiotics seem to benefit the mother and her unborn child, according to the researcher Jessica Younes. In pregnant women, they could reduce the risk of premature delivery, gestational diabetes, postpartum depression or urinary and vaginal infections; whereas in the newborn child they could limit colic, predisposition to developing allergies (atopy), resistance to antibiotics and could also reduce—or even eliminate—the risk of necrotizing enterocolitis which is a fatal disease. Finally, breastfeeding or formula feeding could play a significant role in the child's microbiota composition, although its impact on childhood health remains unknown.

Cervical microbiome in postpartum HIV-positive women

So far, the study of the postpartum cervicovaginal microbiota community has been neglected in HIV-positive women while it is known that they have an increased risk of contracting human papillomavirus (HPV) infection which is the major risk factor for cancerous lesions.

For the first time[9], scientific data were obtained regarding the cervicovaginal microbiota in postpartum HIV-positive women. They showed that postpartum HIV-positive women have a highly diverse microbiota, just like postpartum HIV-negative women. They also showed that immunodeficiency caused by HIV and cervicovaginal dysbiosis are suspected to play a role in the onset of precancerous lesions.

HIV and increased risk of lesions

Vaginal microbiota dominated by *Lactobacillus crispatus* is associated with a decreased risk of HIV infection and, in HIV-positive women, to a decreased risk of HPV infection. On the contrary, vaginal dysbiosis with higher bacterial diversity and decreased levels of lactobacilli increases the risk of HIV and HPV acquisition, cervical precancerous lesions and cervical cancer. It is also known that a change in vaginal microbiota composition occurs during the postpartum period: higher bacterial diversity and decreased levels of *Lactobacillus crispatus*. As a result, postpartum HIV-positive women have a vaginal microbiota that could expose them to a higher risk of HVP infection and thus to a higher risk of cervical precancerous lesions and cervical cancer.

What is the role of the microbiota?

To test this hypothesis, Brazilian researchers analyzed the vaginal microbiota of 80 young HIV-positive women on antiretroviral therapy at 6 and 12 months postpartum: four types of microbiota were identified, including three with a high microbial diversity, but none dominated by *Lactobacillus crispatus*. The researchers observed an overabundance of specific bacteria in cases of cervical precancerous lesions, especially *Moryella* and *Schlegellela*. They also detected an increased content of *Gardnerella vaginalis* in women whose lesions had regressed during the monitoring period, but not in women who had developed lesions.

Lactobacillus crispatus.

It is not clear from these findings whether the identified bacteria are the cause or the result of cervical precancerous lesions, but this study still highlighted HIV-positive women' susceptibility and described the type of microorganisms involved. Their exact role in the development of lesions has yet to be determined, bearing in mind that cervical cancer is the 4th most common type of female cancer, causing more than 200,000 deaths every year in the world.

9 Curty G, Costa RL, Siqueira JD, Meyrelles AI, Machado ES, Soares EA, Soares MA. Analysis of the cervical microbiome and potential biomarkers from postpartum HIV-positive women displaying cervical intraepithelial lesions. Sci Rep. 2017 Dec 12;7(1):17364. doi: 10.1038/s41598-017-17351-9. PMID: 29234019

3. AFTER MENOPAUSE

Menopause is characterized by estrogen deficiency, which reduces bone mineral density (BMD) and changes the bone structure, thus exposing women to a higher risk of osteoporosis and fracture. This period of hormonal changes is also associated with various autoimmune and inflammatory diseases. Understanding the involvement of the microbiota and its interactions with estrogen levels and the immune system could lead to preventive and/or curative treatments for menopausal women as an alternative to the standard hormone replacement therapy (HRT).

Natural remedies against osteoporosis?

To prevent the risk of osteoporosis and fracture, menopausal women can decide between HRT or a treatment combining calcium and vitamin D. But in the US, where the types of hormones and their doses are different from those used in France, THM remains controversial since it is suspected to increase the risk of some female hormone-dependent cancers, including breast cancer. What are the alternatives?

According to a Chinese study[10], probiotics associated with isoflavones (naturally occurring plant compound) could be a low-risk and effective alternative treatment for osteoporosis.

Efficacy of probiotics has been demonstrated in mice

Non-clinical tests have shown that the intestinal microbiota plays a role in bone metabolism regulation. First possible mode of action: through an interaction with the immune and/or endocrine (i.e. hormones) systems, both involved in bone metabolism. Second possible mode of action: by aiding calcium absorption, a mineral essential to bone formation and strength. The microbiota could thus promote bone formation as well as limit bone loss, although to a lesser extent.

All these results have led researchers to assess the benefits of probiotics to prevent osteoporosis and evaluate their efficacy in an animal model. Probiotics play a role at two levels: on the one hand, they increase microbiota diversity by restoring the intestinal barrier and modulating the immune response; and on the other hand, they stimulate calcium absorption and production of estrogen-like compounds. These promising results still have to be confirmed in women.

10 Xu X, Jia X, Mo L, Liu C, Zheng L, Yuan Q, Zhou X. Intestinal microbiota: a potential target for the treatment of postmenopausal osteoporosis. Bone Res. 2017 Oct 4;5:17046. doi: 10.1038/boneres.2017.46. eCollection 2017.

KEY FIGURES

OSTEOPOROSIS

50% of French women with osteoporosis sustain a fracture after the age of 75.
(Source : Société française de Rhumatologie)

Fractures of the femoral neck are **3 times more** frequent in women than in men aged 65 and over, every year in France.
(Source : Santé Publique France)

Combination with isoflavones

Isoflavones are compounds naturally found in some plants, especially soy and red clover. They have anti-osteoporotic effects which are boosted by the concomitant administration of probiotics. They mimic certain modes of action of estrogens while counteracting others, i.e. they limit disorders associated with menopause while still protecting against breast cancer. A Danish study[11] showed that the combination of isoflavones and probiotics associated with calcium and vitamin D supplementation was more effective than calcium and vitamin D supplementation alone to reduce osteopenia (loss of bone density).

As a monotherapy or as a combination therapy with an anti-osteoporosis drug, probiotics could offer an alternative treatment to women who would rather use natural remedies to limit the progression of osteoporosis.

11 Lambert MNT, Thybo CB, Lykkeboe S, Rasmussen LM, Frette X, Christensen LP, Jeppesen PB. Combined bioavailable isoflavones and probiotics improve bone status and estrogen metabolism in postmenopausal osteopenic women: a randomized controlled trial. Am J Clin Nutr 2017;106:909–20. doi: https://doi.org/10.3945/ajcn.117.153353.

Oral and intestinal microbiota: towards a different management of menopause

Estrogen deficiency caused by menopause also increases the risk of several autoimmune and inflammatory diseases. More and more research studies have highlighted the role of the oral and intestinal microbiota in their development. Could prebiotics and probiotics be used as a supplement to hormone replacement therapy (HRT)?

Many women complain about dry mouth during menopause. According to an international team of researchers[12], this lack of saliva, associated to the drop in estrogen levels, could have harmful effects such as changes in composition of the oral microbiota, dysbiosis (imbalance of the microbial flora) and onset of inflammatory disorders (e.g. gingivitis or periodontitis) which create a loss of tooth support that potentially leads to tooth loss.

Drop in estrogen levels and diseases

Since female sexual hormones affect the composition of the different body floras, especially the intestine microbiota, a drop in estrogen levels impairs the microbial balance and promotes the onset of autoimmune diseases. This could explain why some autoimmune diseases affect women more than men (lupus, Sjögren syndrome, rheumatoid arthritis) or why they occur at specific moments of their hormonal lives, i.e. after their menstrual period or during their reproductive period (asthma). Moreover, microbiota alteration due to estrogen deficiency leads to metabolic changes. The one most feared by women is abdominal weight gain, which is a proven risk factor for type 2 diabetes. Finally, intestinal microbiota could affect breast cancer risk through its effects on estrogens produced by fatty tissue in menopausal women.

Composition of oral and/or intestinal microbiota and estrogen deficiency have been successfully linked to all these disorders that are likely to occur during menopause. That is why researchers are encouraged to continue investigating and study the effect of prebiotics and probiotics and their potential use as a monotherapy or in combination with HRT.

12 Vieira AT, Castelo PM, Ribeiro DA, Ferreira CM. Influence of Oral and Gut Microbiota in the Health of Menopausal Women. Front Microbiol. 2017; 8: 1884. Published online 2017 Sep 28. doi: 10.3389/fmicb.2017.01884. PMCID: PMC5625026

4. MICROBIOTA AND CANCER

Microbiota and breast cancer

Breast cancer is the most common female cancer in the world. In addition to genetic factors and already identified risk factors such as tobacco and alcohol consumption, other less known elements are also most certainly involved. Recently, several studies have highlighted the direct and indirect role of microbiota in the development of breast cancer. Here is the explanation[13].

Before menopause, estrogens are synthesized by ovaries and later, other tissues take over (fatty tissue, brain, hypothalamus). A portion of estrogens produced are subject to chemical reactions of detoxification by the liver (which makes molecules harmless to the body) and then excreted in the bile. They are then transferred to the intestines where they are deconjugated by the microbiota before being reabsorbed by the tissues or released into the blood flow. Depending on the composition of the microbiota, this reabsorption means that hormonal metabolites with differentiated estrogenic activity are released back into the blood flow. It seems that breast cancer risk is contingent, as least partly, on the nature and ratio between metabolites and estrogens.

This “deconjugation” is led by bacterial genes, mainly an enzyme which is involved in the degradation of complex sugars and whose activity can be modulated by diet and by the intestinal microbiota. Blocking the actions of this enzyme could thus decrease the level of active estrogens released back into the blood flow and reduce breast cancer risk. This is precisely the hypothesis that a team of American scientists is currently testing in mice.

Breast microbiota

Some researchers also discovered a microbiota in the breast tissue. Its composition, and more specifically the abundance or lack of specific bacterial families, seems to be different whether the host is suffering from breast cancer or not. Other researchers made a similar finding in the intestinal microbiota, whose composition seems to vary depending on the stage of cancer. The alteration of the intestinal microbiota (dysbiosis) as a starting point for breast cancer is an avenue seriously considered by researchers.

Is there a link between the different microbiotas?

At this time, all these assumptions are leads worthwhile to be investigated. Further research should focus on determining if there are links between the different microbiotas, and whether these links lead them to act together to generate an environment favoring the development of breast cancer.

13 Mani S. Microbiota and Breast Cancer. Prog Mol Biol Transl Sci. 2017;151:217-229. doi: 10.1016/bs.pmbts.2017.07.004. Epub 2017 Aug 31. PubMedPMID: 29096895

KEY FIGURES

BREAST CANCER

54,000
new cases every year in France
(Source: INCa, Santé Publique France)

1 out of every 4 cancers in women in the world
(Source : IARC | OMS)

571,000
deaths every year in the world
(Source : IARC | OMS)

Professor Laurence Zitvogel is Head of the Laboratory of Tumor Immunology and Cancer Immunotherapy, a joint research center (Inserm, Gustave Roussy, University Paris-South). Along with her team, she discovered that the efficacy of cancer therapies is influenced by the microbiota.

Through which mechanisms does the microbiota influence the efficacy of chemotherapy or immunotherapy?

We have shown that chemotherapy leads to intestinal permeability, which facilitates the spread of bacteria into the immune system. Although this downside is the source of many adverse events (nausea, diarrhea, vomiting), it is also paradoxically very useful since it stimulates the immune system and boosts the effect of the antitumor drug.

As for immunotherapy, its aim is to mobilize the immune system against tumors, and its success also seems to depend on the intestinal microbiota which acts on three main mechanisms:

- Its composition impacts the distribution of lymphocytes (white blood cells) in the digestive tube, and thus impacts the endogenous defense system, as was recently shown in several articles.
- Some of its metabolites could activate the immune system, although it has yet to be demonstrated.
- It activates mechanisms of intestinal barrier repair which contribute to treatment efficacy.

How are these findings going to change the management of cancers treated by chemotherapy or immunotherapy?

Probiotics used as complement to the existing therapeutic arsenal (surgery, radiotherapy, chemotherapy, hormone therapy, immunotherapy) could become a sixth therapeutic modality against cancer. When there is no infection, we will avoid prescribing prophylactic antibiotics (preventive treatment), while in case of infection, we will postpone immunotherapy. Our objective is to identify patients with an intestinal dysbiosis and to restore their microbiota before starting immunotherapy or chemotherapy.

> **“PROBIOTICS USED AS COMPLEMENT COULD BECOME A SIXTH THERAPEUTIC MODALITY AGAINST CANCER.”**

What are the research avenues currently considered to modulate the composition of the intestinal microbiota?

Probiotics administration and fecal transplant (transplant of the microbiota from a healthy individual into the patient’s digestive tract) are the main avenues under investigation to restore a microbiota which is likely to hinder treatment. These bacteria, called “oncomicrobiotics” are not intended to increase the efficacy of chemotherapy or immunotherapy on their own. Their aim is to prepare the organism of a patient with dysbiosis to respond positively to these immunomodulating treatments. More and more biotechnology companies dedicate part of their research efforts to the development of “anticancer” probiotics. However, only multidisciplinary research followed by large-scale clinical trials will be able to identify which are the “friendly” bacteria and to assess their efficacy within the context of chemotherapy or immunotherapy. We can expect progress in the foreseeable future.

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