

Vaginal microbiota and the pathobiosis path: its role in the evolution of HPV cervical intraepithelial neoplasia and cancer

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Summary

Human microbiome study has become pivotal during the last years; eubiosis, pathobiosis, and balanced ecosystem are now leading terms in comprehension of Health and Disease in humans. Vaginal ecosystem is a balance system in which microbiome is in constant dynamic profile in relation to the symbiotic equilibrium from different species and cells (human cells, lactobacilli and other bacteria, virus or fungi). Previous experiences demonstrated how it is possible to restore human vaginal ecosystem by using a long-time course of a selected population of vaginal symbiotic containing lactobacillus rhamnosus BMX 54 plus lactose. Taking into account the leading role of lactobacilli in human vaginal microbiota and the pathobiosis pathway from eubiosis to pathology until cancer (eubiosis derangement versus a more aggressive and dangerous pathobiosis), the authors hypothesized that exogenously manipulation of vaginal microbiome by a selected symbiotic could stop the pathobiosis pathway leading to a new health system. Surprisingly a recent published clinical trial showed a consistent percentage of clearance of PAP-smear abnormalities and HPV-DNA clearance obtained in women affected by concomitant vaginitis/bacterial vaginosis and HPV infections treated for long-term course with vaginal tabs of lactobacillus rhamnosus BMX 54 plus lactose. Since exogenous vaginal manipulation of microbiome could be a new and promising tool for eubiosis restoration in vaginal ecosystem, and since "pathobiosis pathway" could be interrupted by long-term use of vaginal probiotics, it seems possible to confirm the hypothesis that eubiosis replacement in vaginal microbiome could lead to counter infections such as HPV and its detrimental-related pathologies in women. Vaginal probiotics application could represent a safe, less expensive, and effective treatment to control HPV infections.

Key words: Vaginal microbiota; Pathobiosis path; HPV; Cancer; Cervical intraepithelial neoplasia.

Increasing interest in human microbiome has recently been focused on "eubiosis": symbiotic balance between human cells, lactobacilli and other species of bacteria, fungi or virus [1-4]. Human cells are lesser than other microbiota population (1 to 10 ratio) and considering microbioma genic expression (microbioma increases and modulates more than 100 times the human DNA active gene), this relationship becomes smaller [1-4].

Microbioma "continuously" changes information between species and this allows any "living organism" to follow its natural destiny: a programming and ordinate "cells duplication" [1-4]. The "dynamism" of the microbiota system gives to the cells a continuum in progression and regression with a clinical result of healthy and pathologic status [1-4].

Considering the vaginal ecosystem "lactobacillocentric", the authors have hypothesized a "pathobiosis path" well marked from a progressive but reversible deviation from the ideal balance of the system (predominance of lacto-

bacillus species) to its modification to predominance of other bacterial, viral or fungi species with the disappearance of lactobacillus species [1-4]. The "message's coding"-DNA-mediated lost the coordination between symbiotic species of vaginal microbiome leading to different clinical pathologies during the steps of the "pathobiosis path" up to an uncontrolled cellular reproduction or an atypical cellular apoptosis [1-4].

The vaginal ecosystem possesses its own characteristics, but it is in correlation with other border ecosystems such as the urinary, intestinal, and cutaneous systems [1-4]. Molecular biology studies are in agreement that lactobacilli, lactobacillus crispatus mostly, play the role of "director" in the vaginal eubiosis system [1-4]. The mechanisms by which Lactobacilli are able to stabilize eubiosis seems to be through direct competition with other species, together with direct production of antimicrobial factors such as lactic acid, bacteriocins, and hydrogen peroxide [5-7].

The present authors hypothesized that the "pathobiosis

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path” begins from a specific reactivity of lactobacillus species which, following pathogen damage, leads to a clinical phenomenon known as “doderlein cytolysis”: this cytolysis represents the first reaction of the system able to reset the vaginal natural eubiosis. If otherwise the “dysbiosis” goes on, we assist to a progressive substitution of *L. crispatus* with other lactobacilli, such as *L. Jenseni* and/or *L. gasseri*.

Clinically, we observe an increase of vaginal pH and candida species transform themselves from sporigen forms to indirect fluorescence antibody (IFA). This could represent a new defensive strategy of the vaginal ecosystem able to counteract the aggression of other pathogens from other ecosystems: at this stage women are affected by “acute symptomatology”. If these “defence mechanisms” are not able to restore vaginal eubiosis, lactobacilli progressively disappear leaving only the last lactobacilli barrier population, *Lactobacillus iners*, which is able to adapt itself to unfavourable pH conditions: we clinically assist a pathology known as “bacterial vaginosis”. *Lactobacillus iners* is capable of surviving metronidazole treatment and to counteract the aggression of other bacteria species. When *Lactobacillus iners* (the last lactobacilli defence system) disappears we observe a vaginal microbiome with an exponential increase of other bacteria and virus with the typical clinical manifestations of vaginal pathology. Concluding, a modified microbioma could be responsible for anomalies in the gene code allowing for different and serious illnesses, including cancer [8-10].

Microbiome manipulation and restoration can be performed by using bacterial supplements, probiotics, prebiotics and/or symbiotics, diet or antibiotic treatment [8-10]. Gene decoding and metagenomic in eubiosis and in dysbiosis reveals the association between a serious dysbiosis in the microbiome and appearance of different cancers [8-10]. In these cases we assist in specific alterations in the mucous barrier (the so-called “leaky-gut” for the intestinal system and “leaky-vag” for the vaginal system) in which different bacterial species could survive [11-12].

HPV infection has been recognized as the main cause of cervical cancer in women [13-23]. In recent years the hypothesis of “pathobiosis path” has become more and more evident in HPV-related pathologies and also in pre-cancerous lesions related to an increase in vaginal pH [11-14].

In the present clinical Department of Gynaecological, Obstetric and Urologic Sciences of “La Sapienza”, Rome University, the authors have analyzed how a vaginal microbiome modulation by a specific vaginal symbiotic administration (*Lactobacillus rhamnosus* BMX 54 plus lactose vaginal tabs) could be able to stop the progression of vaginal dysbiosis and restore healthy vaginal eubiosis [1].

The results of this study have been analyzed in order to clinically confirm the “pathobiosis path” through the manipulation of the vaginal microbiome by long-term treatment with a vaginal symbiotic (normogin: *Lactobacillus*

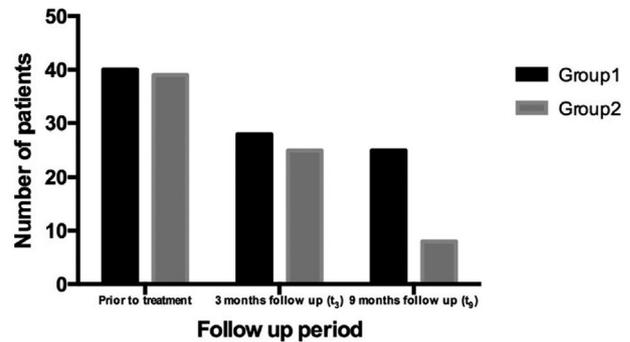


Figure 1. — Trend of HPV abnormalities resolution in the two groups (group 1 - short probiotics implementation group, n = 60; group 2 - long probiotics implementation group, n = 57; $p = 0.041$ [1]).

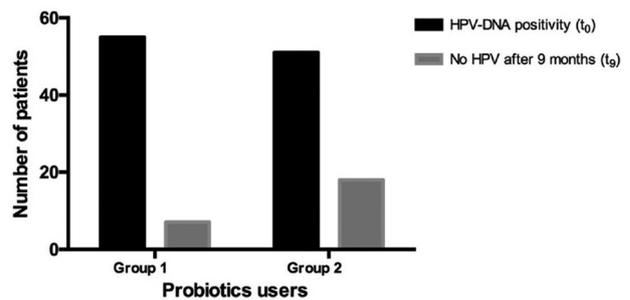


Figure 2. — HPV clearance at the end of follow up period (t₉) in the two groups (group 1 - short probiotics implementation group, n = 60; group 2 - long probiotics implementation group, n = 57). HPV clearance was significantly higher in long term probiotics users than in the other group ($p = 0.044$. t₀: before treatment; t₉: after 9 months follow up [1]).

rhamnosus BMX 54 plus lactose) in women with dysbiosis and HPV infection. The initial hypothesis was to verify if vaginal microbiome manipulation was, per se, able to modulate and control viral infection through vaginal eubiosis re-establishment [1].

The present authors analyzed and reviewed the collected data belonging to their previous published article performed on a total of 117 women affected by vaginitis or bacterial vaginosis with concomitant HPV infection referring to their Department between February 2015 and March 2016. The women were divided in two different arms: arm 1 standard of care (metronidazole 500 mg twice a day for 7 days or fluconazole 150 mg orally once a day for 2 days) plus short-term (three months) symbiotic (*Lactobacillus rhamnosus* BMX 54 plus lactose) vaginal application (60 women) versus arm 2 standard of care plus long-term (9 months) of the same symbiotic treatment (57 women) [1]. A median follow-up of 14 (ranged 9–30) months indicated that the women in arm 2 (long-term *Lactobacilli* treatment) demonstrated a double probability (79.4% vs. 37.5%, $p < 0.041$) to solve HPV-related cytological anomalies when compared

to arm 1 (short-term Lactobacilli treatment). (Figure 1). At the same time, arm 2 women demonstrated a statistically significant ($p < 0.044$) probability to present a negative HPV-DNA test when compared to arm 1 (31.2% vs. 11.6%) (Figure 2) [1]. Results from the vaginal bacterioscopy, Amsel Criteria, vaginal PAP-test, and HPV-DNA test obtained in the group of women undergoing long-term vaginal treatment with lactobacillus rhamnosus BMX 54 plus lactose seem to uphold the hypothesis of vaginal pathobiosis path and the option to restore vaginal ecosystem eubiosis by topical long-term application of this symbiotic which seems to confirm this hypothesis [1].

Another recently published article indicated that the role of vaginal microbiota restoration in preventing sexually transmitted diseases confirms the activity of the same vaginal symbiotic treatment [24]. Vaginal microbiome “eubiosis” seems to be a determinant in the health of the female genital tract: although HPV was well and diffusely distributed, only a few women presented recurrent infections leading to pre-cancer lesions [1]. If we hypothesize that “pathobiosis path” is related to lactobacillus disappearance and this bacterial loss allows the virus to replicate “unconditionally and uncontrolled”, it seems obvious to think of cervical intraepithelial neoplasia (CIN) lesions and cancer lesions as a consequence of long-term course of “uncontrolled” viral infection [19-21].

Cervical cancer represents the most frequent neoplasia amongst women in the developing countries and the second cause of cancer in women worldwide [20]. Cervical cancer progression can be diagnosed through different pre-cancer lesions as CIN 1, 2 or 3 [13]. The time limit between pre-cancer lesions and cancer is usually between ten to 20 years, but only a few women develop a neoplasia [1, 21, 22]; 90% of the HPV related abnormalities spontaneously disappeared [18, 25].

It is reasonable to think that the association between vaginal dysbiosis and HPV could be related to a more aggressive HPV infection without “eubiosis control” [25-28]. Physiologically, a well-balanced vaginal ecosystem. “lactobacillocentric” is able to modulate the defence mechanisms of the microbioma, constraining virus, but also bacteria and fungi, to “symbiotically live together” without infections and commencement of the “pathobiosis path” [17, 29-33]. Also the “recurrent pathologies” concept could be related to the “pathobiosis path” according to recent advances in molecular biology [34, 35].

The role of probiotics, prebiotics, and symbiotics could be “speculated” as “potential anti-cancer agents” in accordance with their ability to balance the vaginal ecosystem by counteracting the “pathobiosis path”. The results obtained by using a symbiotic vaginal approach in HPV women with vaginitis/vaginosis seems to confirm this hypothesis; “pathobiosis path” interruption, by using a long-term vaginal course of a selected vaginal probiotic (lactobacillus rhamnosus BMX 54) able to restore “vagi-

nal eubiosis”, could be a new and strategic “safe approach” to control HPV infection progression and consequently HPV-related CIN and cancer.

The present authors selected lactobacillus rhamnosus vaginal administration because it represents the unique lactobacillus strain that has been recognized to be able to colonize human vaginal microbiota once exogenously applied [36]; thus, lactobacillus rhamnosus BMX 54, a specifically selected lactobacillus strain deposited to the Pasteur Institute under the Budapest Treaty, has previously shown clinical evidence of effectiveness when vaginally applied by restoring the vaginal balanced ecosystem [1, 37].

To the present authors’ knowledge this is the first clinical trial able to demonstrate a control of HPV infection obtained by using vaginal probiotics: if these preliminary results are confirmed in controlled, randomized, and double-blinded clinical trials, the “pathobiosis path hypothesis” could become “more than an hypothesis” and this safe, less expensive, and effective vaginal approach to restore “eubiosis” could become a new and strategic approach to control also HPV-related CIN and cancer [1].

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