

ORAL MULTISPECIES PROBIOTIC SUPPLEMENT TO COLONIZE VAGINAL EPITHELIUM, IMPROVE BACTERIAL VAGINOSIS SYMPTOMS AND RESTORE VAGINAL MICROBIOTA.

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INTRODUCTION

Women Health - The burden of Vaginal dysbiosis

Dysbiosis is a condition characterized by a perturbed or imbalanced microbiota, where dominant species are overwhelmed by exogenous or minority ones. Several factors may influence microbiota composition, ranging from lifestyle habits to hormonal changes, to the use of therapeutic compounds or to the spread of infective microorganisms.

A vaginal microbiome not dominated by *Lactobacillus* species has been associated with severe diseases, such as increased sensitivity and transmissibility to HIV, HPV and other STD, increased risk of pelvic inflammatory disease, preterm birth and neonatal infections.

Vaginal dysbiosis affects every year **300 Million women worldwide**:

- 70% have experienced vaginal infections once
- 10% of them have frequent relapses

Antibiotic treatment is the standard therapy for vaginal infections, but sometimes failed to prevent recurrences. A major responsible for recurrences is the formation of a pathogenic biofilm over the vaginal epithelium. Biofilm acts as microbial reservoir and can provide a defensive barrier against anti-infective agents.

Bacterial vaginosis (anaerobic)

Prevalence: **20%**
> 50% of them are recurrent

Yeast vaginitis

3 in 4 women experience at least one episode

Aerobic vaginitis

Prevalence: from **5 to 10.5%**

IN-VITRO DOSSIER

Anti-microbial activity

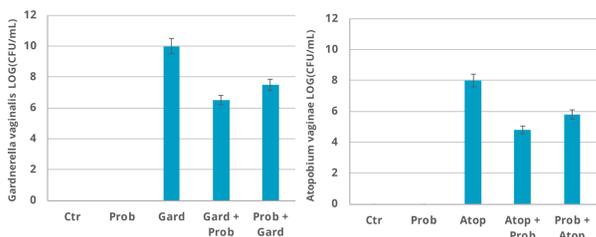
Probiotics transform nutrients into helpful metabolites and counteracts pathogens invasion through:

- Exclusion:** physical occupation of adhesion sites
- Displacement:** pathogen removal from adhesion sites
- Competition:** quicker settlement than pathogens in adhesion sites

Anti-biofilm activity

Selected species showed a strong inhibition of pathogen biofilms in different experimental models:

- Agar plate overlay assay against *E. coli* and *C. albicans*
- Ex-vivo reconstructed human vaginal epithelium (RHVE) against *G. vaginalis* and *A. vaginae*



Pathogen inhibition

- Enterococcus faecalis
- Staphylococcus aureus
- Candida albicans
- Escherichia coli
- Gardnerella vaginalis
- Atopobium vaginae

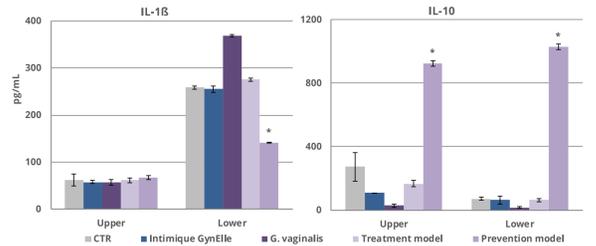
Strong inhibition

Adherent pathogens count over a reconstructed tissue model, (RHVE Episkin).
Ctr: control tissue. Prob: tissue treated with probiotic complex.
Gard/Atop: tissue infected with *G.vaginalis/A.vaginae*.
Gard/Atop+Prob: Tissue infected and then treated with probiotic complex.
Prob+Gard/Atop: Tissue preventively treated with probiotic and then infected.

Modulation of inflammation

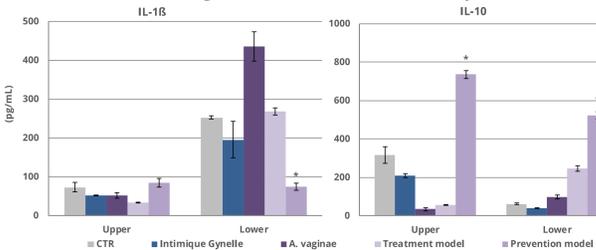
Probiotic strains show immunomodulating activity in a BALB 3T3 Fibroblasts model: ↓ TNF-α, ↑ IL-4 & antiox power

- Strong anti-inflammatory activity against pro-I cytokine IL-1β: -62% vs *G. vaginalis*-induced inflammation
- Excellent and significant modulation of anti-I cytokine IL-10: > 30 & 60 times vs *G. vaginalis*-induced inflammation



Protocol:
Cytokines quantification in a ex-vivo technique by a prevention model (previous application of GynElle probiotics followed by inoculum of pathogens) and by a treatment model (previous pathogen growth followed by exposure to GynElle probiotics or their acid metabolites)

- Strong anti-inflammatory activity against pro-I cytokine IL-1β: -83% vs *A. vaginae*-induced inflammation
- Excellent and significant modulation of anti-I cytokine IL-10: > 20 & 5 times vs *A. vaginae*-induced inflammation



Protocol:
Cytokines quantification in a ex-vivo technique by a prevention model (previous application of GynElle probiotics followed by inoculum of pathogens) and by a treatment model (previous pathogen growth followed by exposure to GynElle probiotics or their acid metabolites)

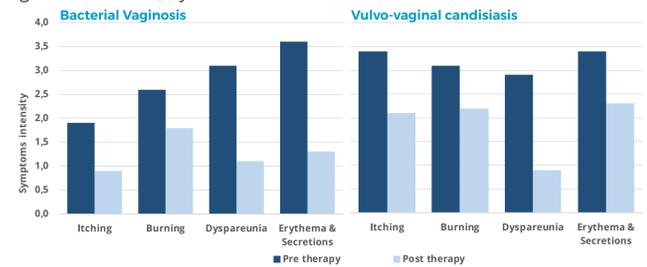
CLINICAL DOSSIER

1 - PILOT STUDY: VAGINAL CAPSULES

Symptoms improvement & microbiota restoration

Based on three *Lactobacillus* strains of SynBalance® line, this topically administered product, is useful in all cases of temporary alteration of vaginal microbiota, by:

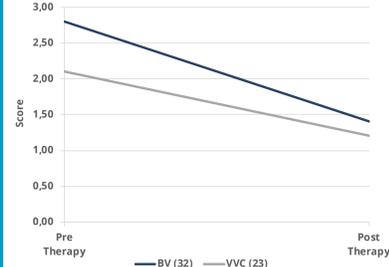
- Promoting the colonization of friendly species
- Facilitating the restoration of healthy physiologic conditions
- Inhibiting the growth of harmful microorganisms responsible for nonspecific vaginitis and vaginosis.



Restoration of vaginal healthy conditions

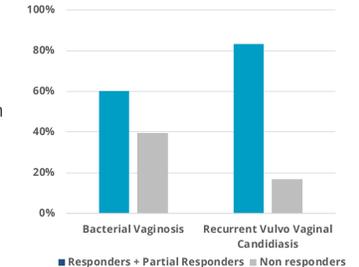
- Demonstrated efficacy in normalizing vaginal microbiota characterized by *Lactobacillus* species and absence of anaerobic microorganisms Gram-negative bacteria, fungal life or spores or *G. vaginalis*.
- Significant improvement up to 50%.

Microscopical Examination



Protocol:
Experimental open study (absence of Placebo as well as CTR) involving 30 women affected by BV & 23 affected by VVC. 10 days treatment (local use). Method: microscope analysis of vaginal swabs. Outcome: vaginal oedema/erythema, clinical symptoms (itching, burning, dyspareunia), pH, vaginal secretion, microscope evaluation. Analysis performed at T0, T10, T45 (telephone interview)

Percentage of subjects responding or not to MD treatment for Bacterial Vaginosis and Vulvo Vaginal Candidiasis. Recurrent: clinical history of more VVC episodes year.



Efficacy against recurrences

Recently, *Murina et al.* observed that the MD has a higher efficacy in treating recurrent episodes of vaginal candidiasis compared to sporadic ones.

The authors suggest that the anti-biofilm activity, of the ***Lactobacillus* complex**, has a major role in eradicating the pathogens when the antifungal agents fail to reach them.

REGULATORY CONTEXT

The Medical Device Regulation (EU) 2017/745 was officially published on May 5th 2017 and came into force on May 25th 2017. It repeals Council Directives 90/385/EEC and 93/42/EEC.



Manufacturers of currently approved medical devices will have a transition time of three years until May 26th 2020 to meet the requirements of the MDR. But this does not apply to probiotic based MD, since the new regulation clearly states that it does not apply to product containing viable organisms (Art.1, pt 6).

Probiotic-based Medical Devices will be interdicted in EU from May the 26th 2020.

CLINICAL DOSSIER

2 - VAGINAL COLONIZATION BY ORAL INTAKE

Aims

Assess the ability of two probiotic supplements to target vaginal microbiome for the management of vaginal dysbiosis by:

- Colonizing human vaginal epithelium
- Exerting in locus their antimicrobial activity

Methods

RDB three arms parallel pilot study involving 60 healthy women 18-45

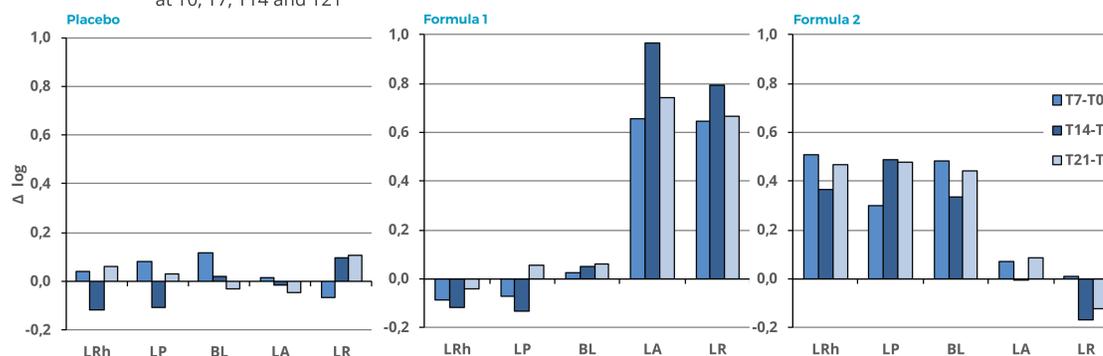
- Daily supplementation for 14 consecutive days with probiotics or placebo
- Real Time qPCR analysis on vaginal swabs collected at T0, T7, T14 and T21

Results

Significant increase of administered species DNA detected in vaginal swab.

These increments were significant also after one week from supplementation end (T21).

Probiotic species quantification by real time PCR with species specific primers over total DNA extracted by vaginal swab. Samples collected at four experimental time (T0, T7, T14 and T21). Histograms represent the delta of specific DNA detected compared to T0. The figure reports data of the five species supplemented during the trial. Probiotic formula 1: *L. acidophilus* (LA) and *L. reuteri* (LR). Probiotic formula 2: *B. lactis* (BL), *L. rhamnosus* (LRh) and *L. plantarum* (LP). Placebo: no probiotics.



CONCLUSIONS

Orally administered probiotic were able to modulate vaginal microbiota, increasing the number of administered species.

Microbiota modulation was observed also a week after end of supplementation, suggesting an active colonization from administered strains.

We assume that the efficacy demonstrated by topical administration will be confirmed through oral intake.

Further studies will follow.

References:

- Presti, I. et al. Evaluation of the probiotic properties of new *Lactobacillus* and *Bifidobacterium* strains and their in vitro effect. Appl Microbiol Biotechnol. 2015 Jul;99(13):5613-26.
- Mezzasalma, V. et al. Orally administered multispecies probiotic formulations to prevent uro-genital infections: a randomized placebo-controlled pilot study. Arch Gynecol Obstet (2017) 295: 163.
- Murina, F. and Vicariotto, F. Improvement of bacterial vaginosis and vulvovaginal candidiasis with a new *Lactobacillus* strains association: a pilot study. Int J Women's Health Care (in press).

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