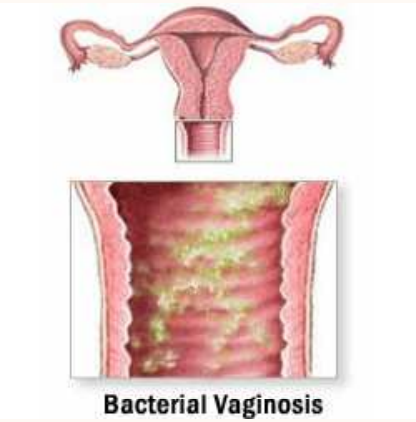
Effectiveness of the two microorganisms *L. fermentum* LF15 and *L. plantarum* LP01 in women diagnosed with Bacterial Vaginosis (BV): a pilot study.

Franco Vicariotto, M.D.

Naples, September 23rd, 2014

Two innovative applications of lactobacilli in the genito-urinary tract

- Use of selected lactobacilli in women diagnosed with Bacterial Vaginosis



- The possible usefullness of lactobacilli in women with acute uncomplicated cystitis



The Vaginal MICROBIOTA

The composition of the urogenital microflora is crucial for the health and well-being of women.

In the vaginal environment many different groups of microorganisms, either commensals, opportunistic pathogens or probiotics, coexist in equilibrium with each other and with the guest.

Lactobacilli are believed to promote a healthy ecosystem by producing lactic acid, hydrogen peroxide, and bacteriocins

Several factors can cause an imbalance in the vaginal microflora eliciting vaginitis, the most 'frequent' of which is Bacterial Vaginosis

Bacterial Vaginosis (BV)

(BV) is a polymicrobial syndrome (not an infection)

BV is highly prevalent, affecting on average from 10 to 30% of women

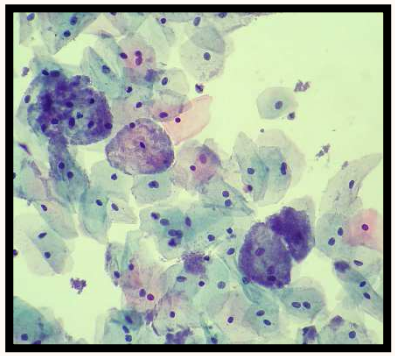
One of its most important causative agent is *Gardnerella vaginalis*

Women with BV may have a malodorous vaginal discharge or local irritation and pain at sexual intercourse

BV can be very dangerous during pregnancy ,it may cause premature birth

About half of the women with BV are asymptomatic

and should not be treated



Current treatment strategies of BV

Administration of antibiotics, either orally or topically.

The use of the two specific antibiotics metronidazole or clindamycin almost always gets good care in the acute episodes (antibiotic medication is effective in up to 90% of cases)

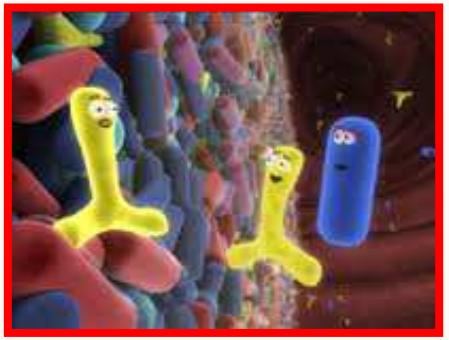
But the real problem are the relapses that can also cause psychological harm to the woman and take her to a wrong self-treatment

About 25% of women will develop BV again within four weeks.
Long-term recurrence rates have been shown to be greater than 70%!

Here is the rationale of use of natural probiotics to rebalance the vaginal environment and to obtain the patient compliance

Beneficial lactobacilli: an effective approach

In BV relapses, but also as prophylaxis and prevention, the use of lactobacilli, is consolidating with the aim of restoring the physiological balance of vaginal ecosystem.



There is a strong need for innovative treatments that should be specific, natural and mostly free of side effects

BALANCE



HEALTH

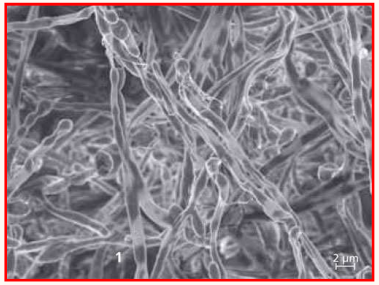
Bacterial biofilms: a huge challenge

Biofilms are associated with BV

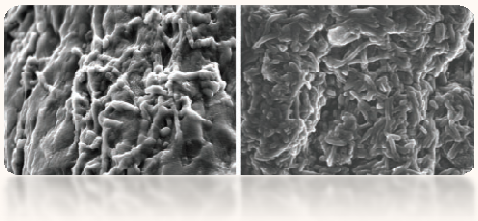
Bacterial biofilms are detectable in 90% of subjects with BV

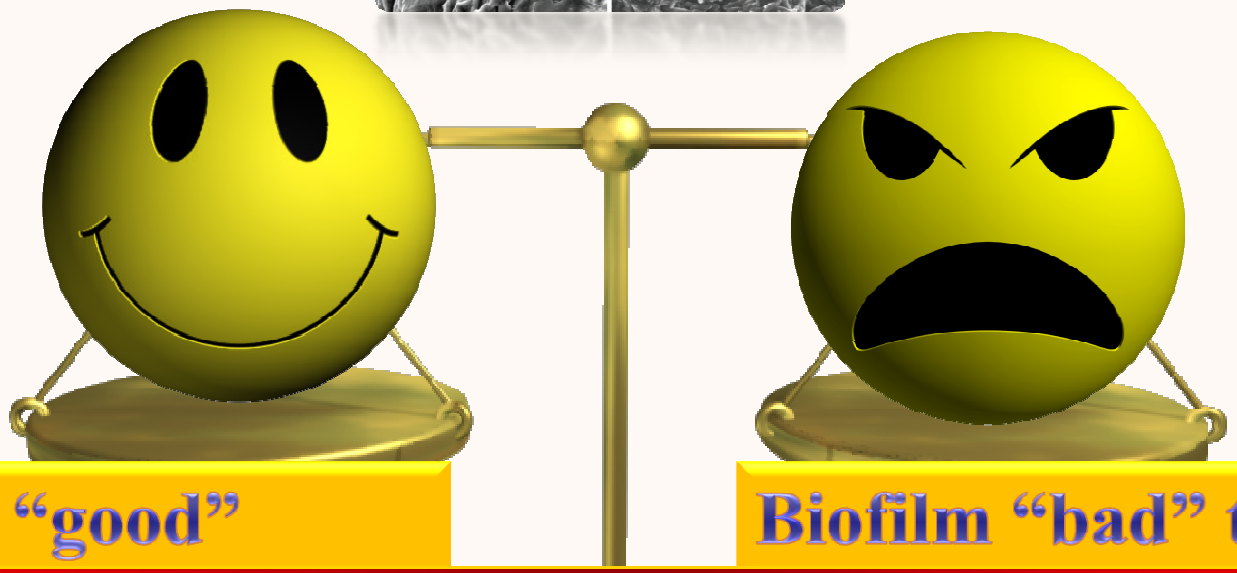
Adherent biofilms are attached to the vaginal epithelial surface

An adherent Gardnerella vaginalis biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole



Complex communities of microorganisms colonize human mucosal surfaces forming <u>biofilms</u>





Biofilms "good"

defendi

Biofilm "bad" that

a

The Lactobacilli are able to produce a protective biofilm that covers the vaginal mucosa

Harriott MM, Lilly EA,
biofilms on the vaginal mucosa. Microbiology. 2010

The use of live microorganisms with a probiotic value was indicated since some years by many gynaecologists as an alternative therapy or complementary to the use of traditional drugs with antibacterial or antimycotic activity.

These include the surging levels of multidrug resistance among pathogenic bacteria,

The increasing demands of consumers for natural substitutes for drugs,

The emergence of scientific and clinical evidence showing the efficacy and effectiveness of some probiotic strains.

A pioneering solution

Medical Device in tablets for vaginal use containing, the two lactobacilli *L. fermentum* LF15 and *L. plantarum* LP01 tara gum (a natural gelling ingredient) **fructo-oligosaccharides (FOS) and arabinogalactan.**

MECHANICAL PRIMARY ACTIVITY: the tara gum rapidly spreads over the surface of vaginal mucosa, creating a mechanical barrier that hinders the adherence of *Gardnerella vaginalis* and of other Gram-negative rods to the mucosa

ANCILLARY SPECIFIC ACTIVITY: the simultaneous production of bacteriolysins and organic acids by the two lactobacilli exerts a specific inhibitory activity against Gardnerella that reinforces the mechanical primary effect

Patent Application MI2013A000794

A completely natural approach

***L. plantarum* LP01 and *L. fermentum* LF15**
belong to species that are native of the vaginal environment

Normo-physiological conditions: prevalence of lactobacilli (~10^9 viable cells/g) belonging to the **"Döderlein's complex"**: *L. crispatus, L. gasseri, L. acidophilus, L. jensenii, L. rhamnosus, L. reuteri,* ***L. fermentum,*** *L. casei, L. paracasei,* ***L. plantarum****, and L. vaginalis*

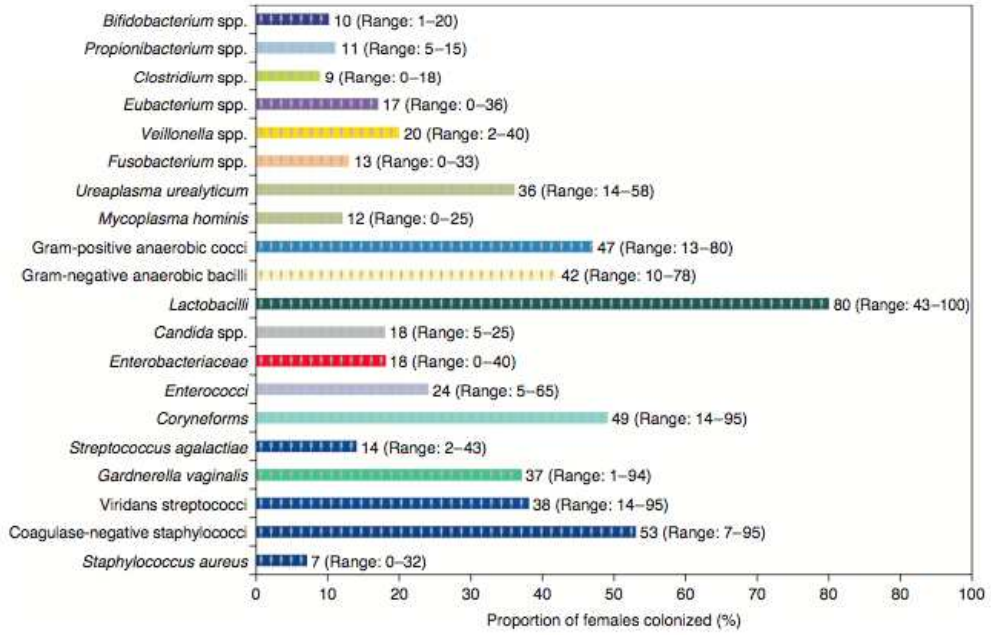
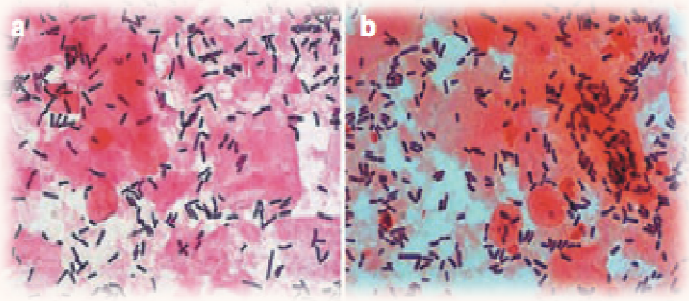


Fig. 6.19 Frequency of detection of various microbes in the vagina of post-menarcheal/pre-menopausal, healthy, nonpregnant females. The data shown are mean values (and ranges) derived from the results of 32 studies involving 1756 individuals (2, 4–34).



L. fermentum LF15 isolated by brushing from a healthy woman

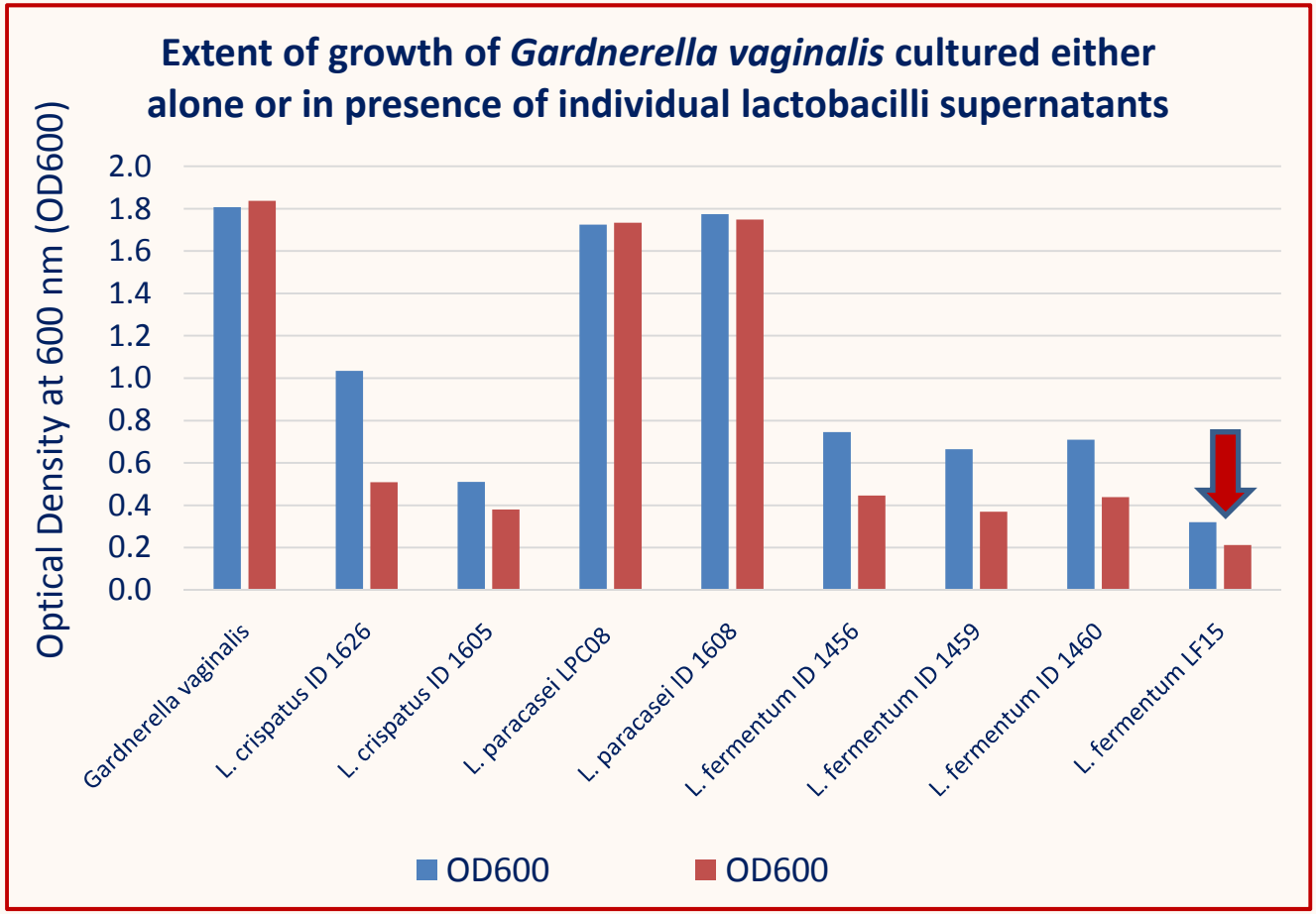
Overview of the main features of lactobacilli

Self-regulated

L. plantarum LP01 and *L. fermentum* LF15 are able to colonize the vaginal epithelium and produce antimicrobial molecules in full compliance with vaginal physiology

Properties of lactobacilli	Role in the vaginal colonization
Acidogenic (all)	Lowering of vaginal pH, inhibition of competitive microorganisms
Production of lactic and acetic acid	Bacteriostatic / bactericidal effect
Microaerophilic or anaerobic (all)	Survival at very low concentrations of oxygen
Production of hydrogen peroxide (*L. crispatus, L. jensenii, L. gasseri, L. delbrueckii, L. casei,* ***L. plantarum****, L. vaginalis, L. pentosus*)	Bactericidal effect towards catalase-negative bacteria
Biosurfactants production - natural surfactants - (*L. rhamnosus,* ***L. fermentum,*** *L. acidophilus*)	Prevents the adhesion of contaminating microorganisms to the epithelium
Presence of adhesins- glycoproteins (*L. acidophilus, L. gasseri,* ***L. fermentum***)	Adhesion to the vaginal epithelium
Competitive exclusion (all)	Competition for available nutrients and physical occupation of receptors on epithelial cells
Production of bacteriocins (*L. gasseri,* ***L. plantarum****,* ***L. fermentum****, L. casei, L. delbrueckii, L. reuteri, L. salivarius*)	Inhibition of the growth of contaminant microorganisms such as Gardnerella
Production of bacteriolysins (***L. fermentum***)	Specific antifungal activity free of side effects

In vitro antagonistic activity of selected lactobacilli towards *Gardnerella vaginalis*



***L. fermentum* LF15** showed the strongest *in vitro* activity after both 24 and 48 hours (82.2% and 88.5% inhibition, respectively) and **was selected for the subsequent human trial.**

The human trial design

35 female subjects diagnosed with BV according to the Nugent score

Pilot, randomized, placebo-controlled study

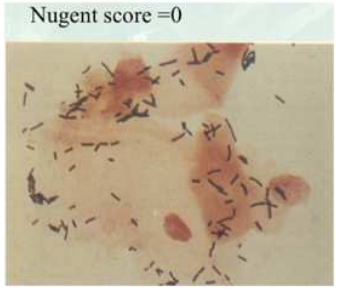
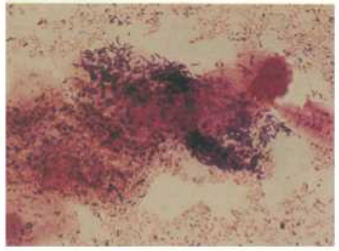
Group A (24 subjects) | **Group B (11 subjects)**

Active formulation | **Placebo**

- 1 tablet per day for 7 d
- 1 tablet every 3 days for the following 3 wk
- 1 tablet per week for the following 4 wk

Clinical examination and Nugent score quantified at enrolment (d_0), after 28 days (d_{28}) and at the end of the second month of relapses prevention (d_{56})

DIAGNOSIS OF VB (The Nugent score)

Table 4. Wet Mount - Nugent's Scoring	
Normal	**Abnormal**
Nugent score =0 	Nugent score = 10 
• Lactobacilli common • Few to no other organisms	• Polymicrobial preparations • Clue cells • Coccobacilli, Gram variable • Lactobacilli few or absent

The method of Nugent assesses the presence and relative amounts of three bacterial morphotypes, including Gram-positive rods (lactobacilli), Gram-negative and Gram-variable rods (*Gardnerella vaginalis* and *Bacteroides* species), and curved rods (*Mobiluncus* species).

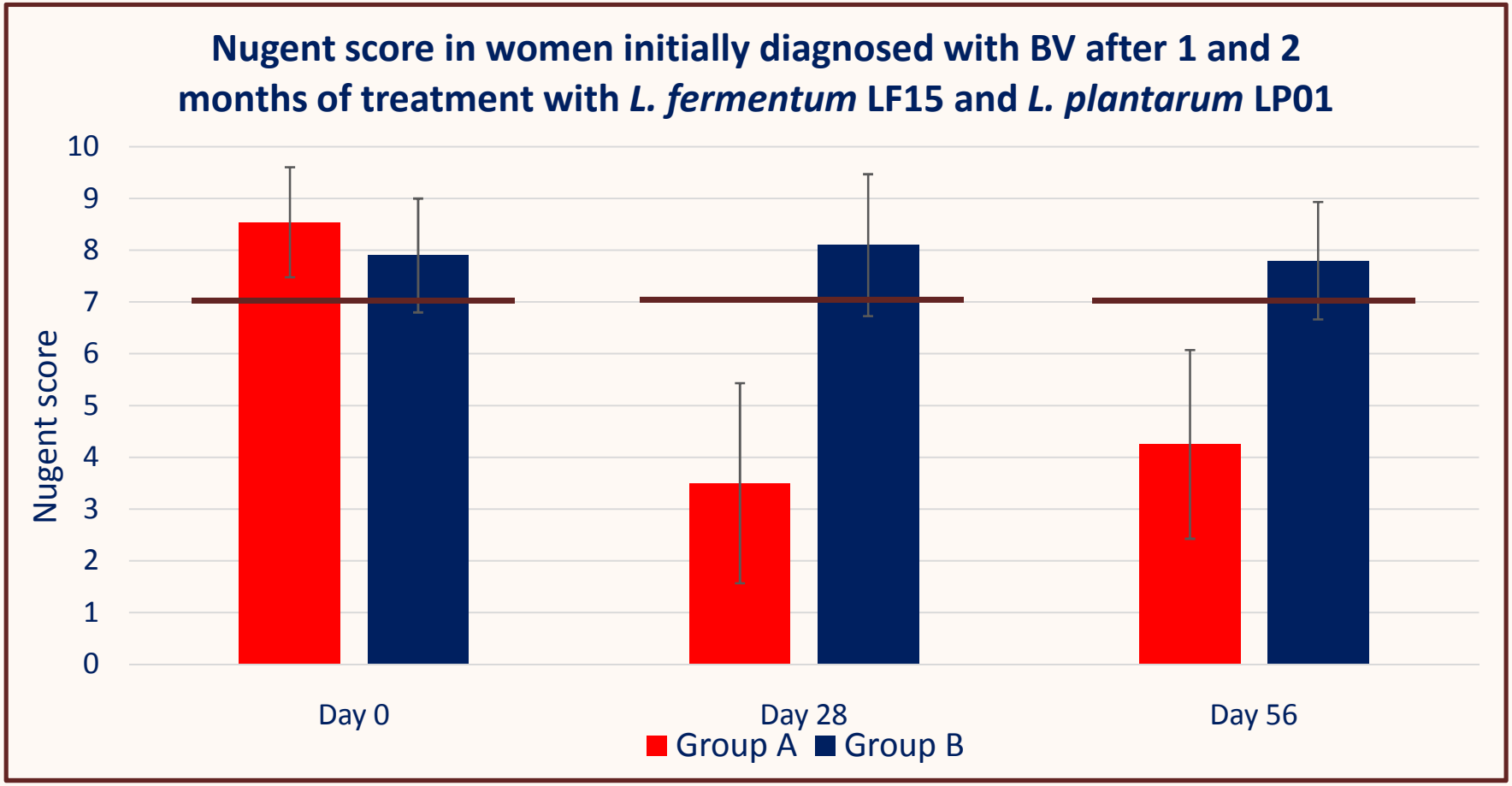
SCORE*	Lactobacillus morphotypes	*Gardnerella/Bacteroides* spp. Morphotypes	Curved Gram-variable rods
0	**4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

* Morphotypes were scored as the average number seen per oil immersion field (minimum of 10-20 fields were examined). Each morphotype was then given a score from the left hand column. The TOTAL SCORE was calculated by adding the individual morphotype scores = Lactobacillus + Gardnerella/Bacteroides + Curved Gram-negative rods.

** QUANTIFICATION SCALE: 0 = no morphotypes seen; 1+ = <1

The Nugent score is classified as Bacterial Vaginosis (≥7), intermediate situation (4-6), and healthy vaginal microbiota (≤3).

Results



At the baseline all the subjects recorded a score higher than 7.

After 28 and 56 days the mean Nugent score in the placebo (B) was still higher than 7, while in the active group(A) mean values of 3.50 and 4.25 were recorded, .

Conclusions (1)

- Bacterial Vaginosis (BV) is the most common cause of vaginal infection in women of childbearing age. Furthermore, BV is often asymptomatic since about 50% of women with this condition have no symptoms at all and the prevalence rate in apparently healthy women is around 10%.

- An association of the two strains *L. fermentum* LF15 and *L. plantarum* LP01 is able to effectively counteract Gardnerella acute infections and to significantly improve the related uncomfortable symptoms in a very high percentage of women.

- A slow release vaginal tablet seems a very effective way of delivering probiotics to the vagina.

Conclusions (2)

- The primary mechanism of the product is the establishment of a mechanical barrier mediated by the tara gum.

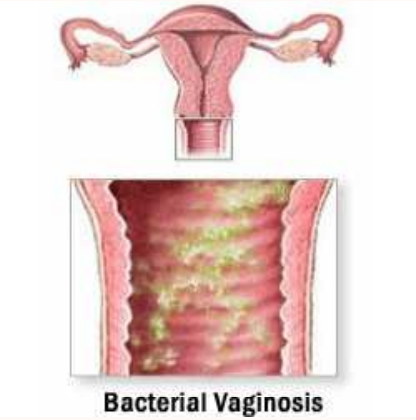


- A long-term physiological protection seems to be ensured thanks to the integration of the two lactobacilli in the vaginal microbiota and to their adhesion to the epithelial cells of the mucosa.

- In the light of the *in vitro* inhibitory activity against *E. coli*, their prospective use in aerobic vaginitis (AV) could also prove interesting.

Innovative applications of lactobacilli in the genito-urinary tract

- Use of selected lactobacilli in women diagnosed with Bacterial Vaginosis

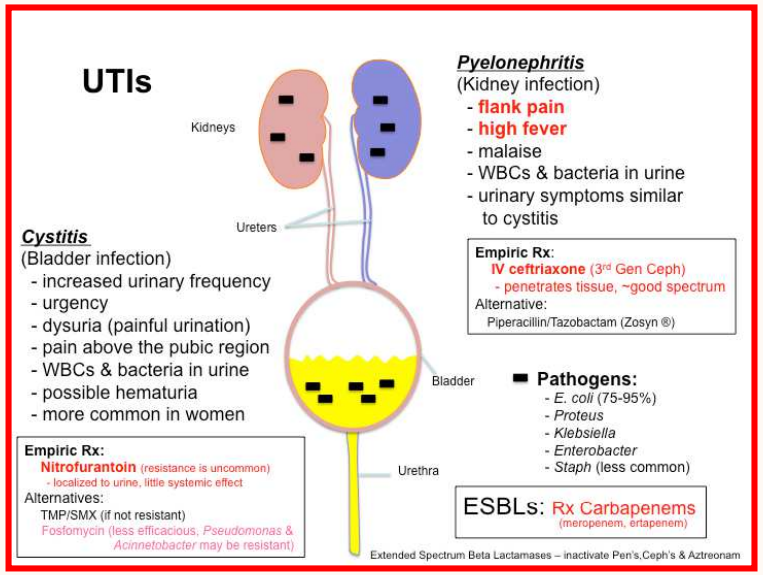


- The possible usefullness of lactobacilli in women with acute uncomplicated cystitis



Lactobacilli in acute uncomplicated cystitis

- Urinary Tract Infections (UTIs) are the most common bacterial infection in women. Most UTIs are (cystitis) caused by *Escherichia coli* (86%).



- About 50% of women experience at least one UTI in their lifetime.

- UTIs can be classified as UNCOMPLICATED (in the normal urinary tract of immunocompetent individuals, usually young healthy non-pregnant women) or

  COMPLICATED (individuals of all ages and sexes that are immunocompromised or have genitourinary tracts with abnormalities).

- The establishment of a biofilm plays a very important role in the onset and progression of *E. coli* infection.

The human trial design

35 premenopausal, non-pregnant women diagnosed with acute uncomplicated cystitis

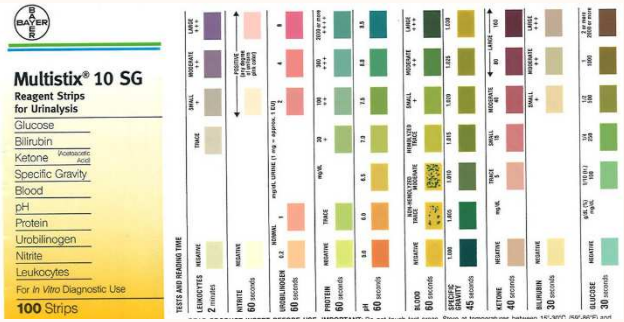
Pilot study

Active formulation: two selected lactobacilli, an innovative natural gelling complex, a cranberry extract and D-mannose

First month: two doses per day (acute treatment)
Second month: one dose per day (long-term treatment)

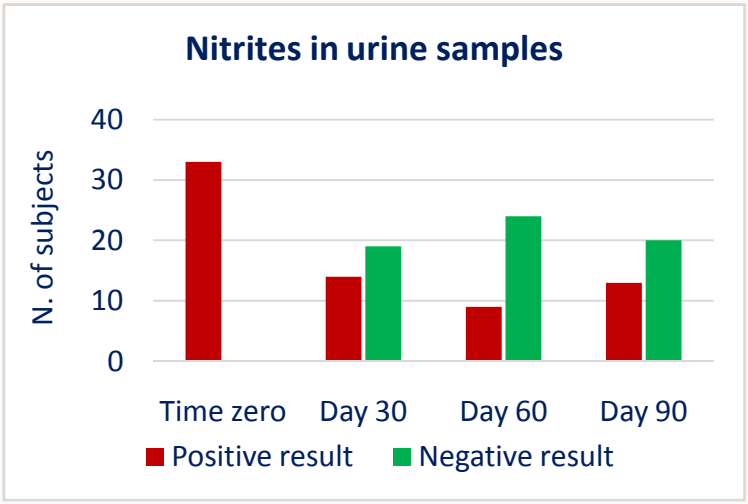
1. Urine dipstick testing at enrollment (d_0), after 30 days (d_{30}), at the end of the second month of treatment (d_{60}), and after one month of follow-up (d_{90})
2. UTI Symptoms Assessment questionnaire collected from each patient at each visit

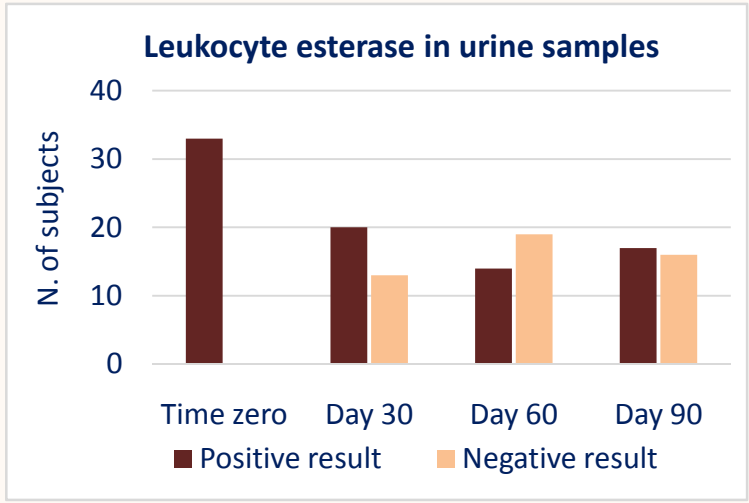
Urine dipstick test



Each test pad was referred to the corresponding row of color blocks.

The results were expressed as **positive** or **negative** (thresholds: ≥10 leukocytes/µL and 0.075 mg/dL nitrite ion).



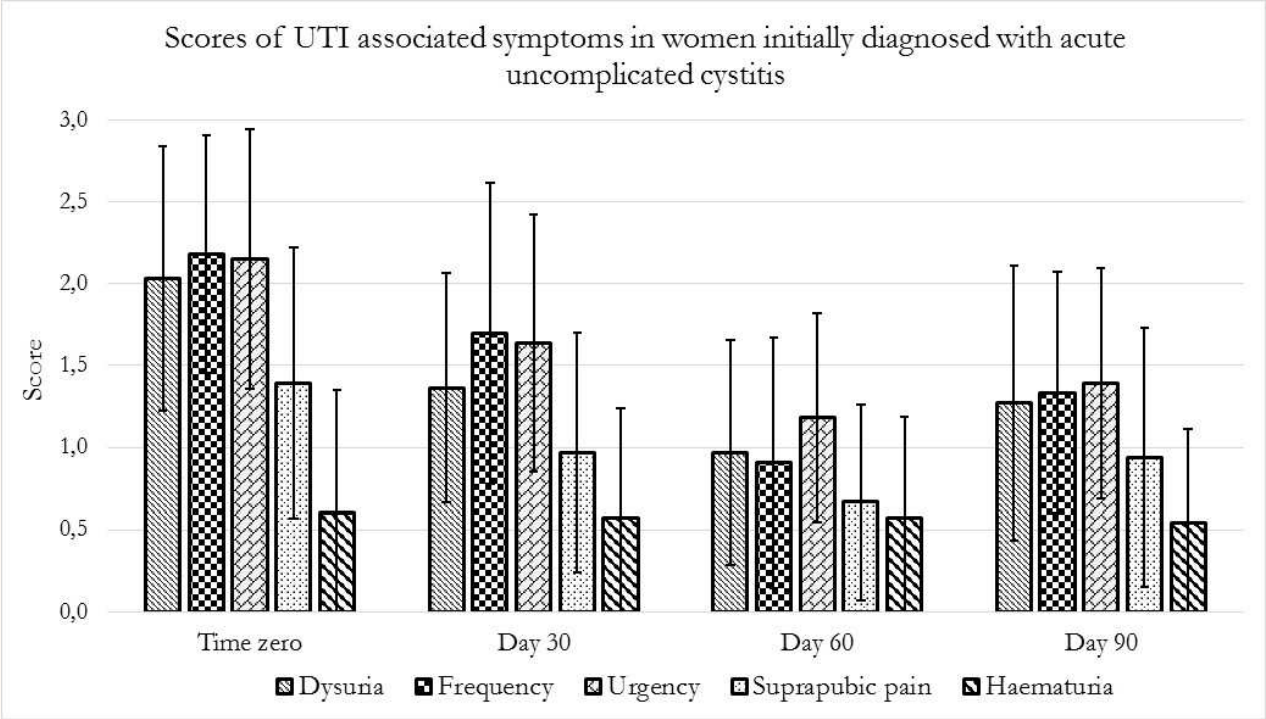


Nitrites and leukocyte esterase (diagnosis of cystitis)

were **positive** in 14 and 20 subjects after 30 days and in 9 and 14 women after 60 days,

At the end of the follow-up **positive** results for N and LE were recorded in only 4 and 3 out of 24 and 19 subjects with negative results after 60 days.

Severity of UTI symptoms



Our results highlighted a significant improvement of 4 out of 5 symptoms typically associated with UTI, namely dysuria, frequent voiding, urgency, and suprapubic pain. Haematuria remained almost unaltered throughout the study.

After 30 and 60 days significant improvements were recorded in dysuria, frequency of voiding, urgency, and suprapubic pain.

Conclusions

- In recent years natural approaches alternative to traditional pharmacological treatments have been investigated.

- Our results demonstrated the long-term ability of an association of cranberry dry extract, D-mannose, and two lactobacilli,to significantly improve the uncomfortable symptoms reported by women with acute cystitis.



- Future trials will be needed to investigate deeper the possible usefulness of this new association of natural ingredients in the alleviation of UTI associated symptoms and in the long-term prevention of possible recurrences.



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Thank you!



Take home message:
"The use of natural products is fundamental for intimate hygiene and for women's respect"