Corporate Collaborations 2015

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Probiotics in the treatment of (Vaginal Yeast Infection) VVC and (Bacterial Vaginosis) BV

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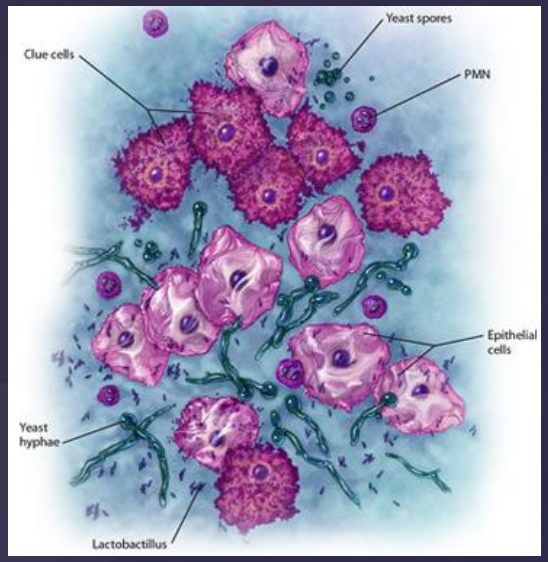
VAGINITIS

what is the problem?



Vaginal itching, discharge, and odor

are the symptoms more frequent and insistent

Women often call their practitioners after self-treating at home.

Self-diagnosis has been shown to be correct less than one-third of the time, leading to millions of dollars wasted on treating the wrong entity.

Diagnosis by phone has also been shown to be incorrect and damaging . The symptoms of an infectious vaginitis are often confused and/or complicated by irritation, allergy, or other systemic diseases

VulvoVaginal Candidiasis (VVC)

- 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

- Several factors can cause an imbalance in the vaginal microflora which, in turn, can lead to the onset of vaginal yeast infection

- The incidence is very high as 70-75% of women have at least a case of Candida vulvovaginitis (VVC) during the life.

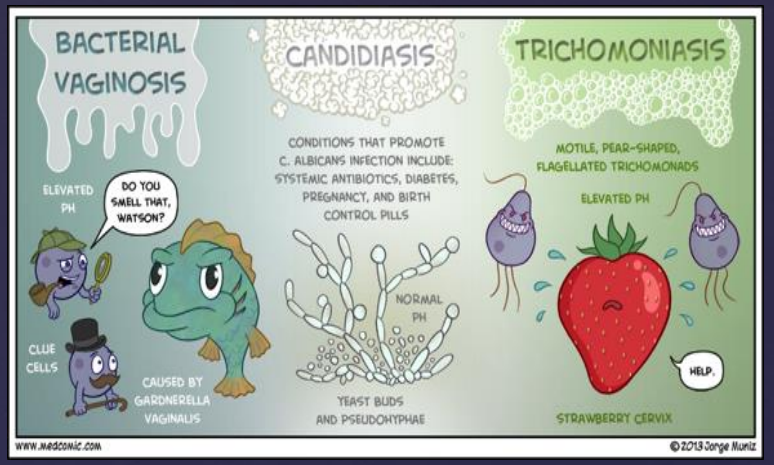
  Equally important is the fact that 40-50% of subjects, after the apparent resolution of the first infection episode, have one or more recurrences.

Bacterial Vaginosis (BV)

- BV is a polymicrobial syndrome
  an imbalance in the vaginal microflora
  (not an infection)

- BV is highly prevalent, affecting on average from 10 to 30% of women but the recurrences are the big problem
  One of its most important causative agent is Gardnerella vaginalis
- Women with BV may have a malodorous vaginal discharge , local irritation and pain at sexual intercourse

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



Current treatment strategies of BV and VVC

The use of the specific antibiotics: metronidazole or clindamycin for VB and azoli drugs are generally regarded as effective in the treatment of acute infections (effective in up to 90% of cases)

but they are frequently unable to offer a significant protection against possible recurrences

VVC and VB are very frequent
(the leading cause of gynecological examination in Western countries)
and they are often the cause of economic and psychological damage

Probiotic vs common antibiotic

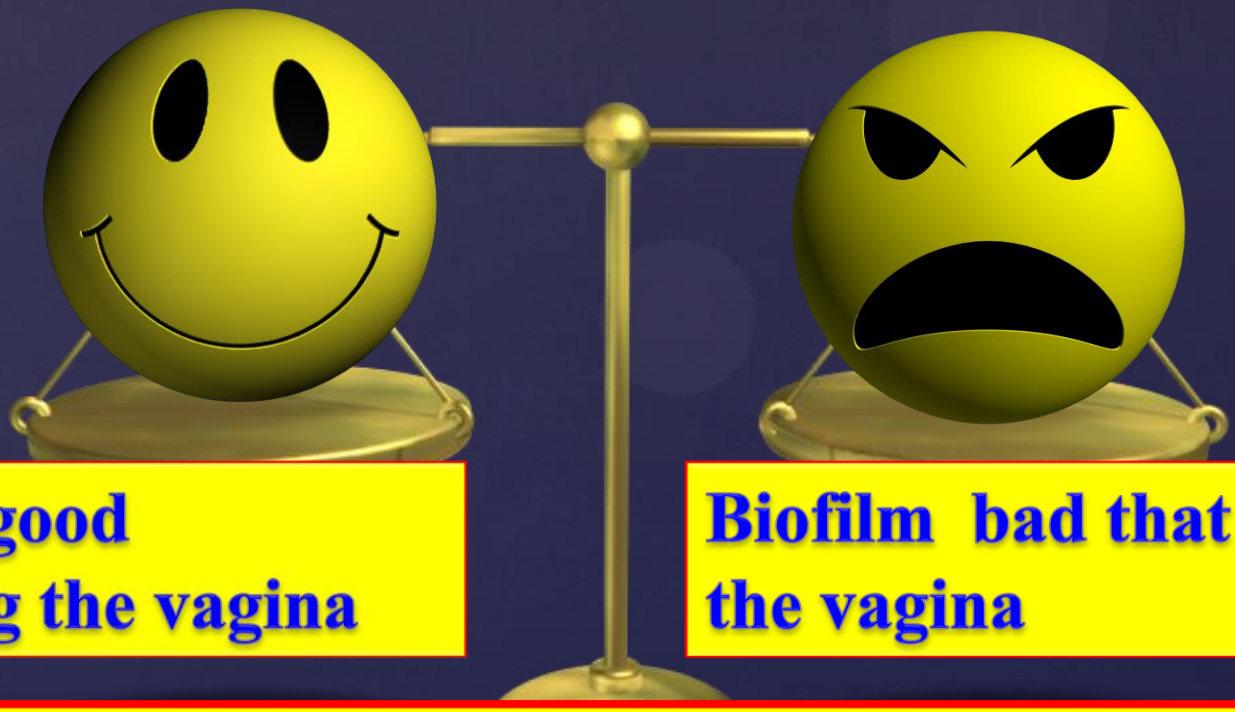
Function/ characteristic	Probiotic	Antibiotic/Antimicotic
Natural	YES	NO
Without side effects	YES	NO
Specific activity against pathogens	YES	YES (but it alter also commensal/positive bacteria)
Relapses prevention	YES	NO
Absence of possibile phatogen resistance development	YES	NO
Help to balance vaginal microflora	YES	NO

Beneficial lactobacilli: an effective approach

New topic probiotic strategy against VVC and VB

Very important to understand the failure of antibiotic therapy, especially in relapses are the BIOFILMS

Complex communities of microorganisms colonize human mucosal surfaces



Biofilm good defending the vagina

Biofilm bad that attacks the vagina

The lactobacilli are able to produce a protective biofilm that covers the vaginal mucosa and to penetrate into the biofilm of pathogens

Bacterial biofilms: a big challenge

Biofilms are associated with BV and VVC

Bacterial biofilms are detectable in 90% of subjects with BV and VVC

Adherent biofilms are tightly attached to the vaginal epithelial surface

An adherent *Candida albicans* o r*Gardnerella vaginalis* biofilm persists on the vaginal epithelium after standard therapy with oral antbiotics

Disruption of urogenital biofilms by lactobacilli

McMillan A, Dell M, Zellar MP, Human Microbiology and Probiotics, Lawson Health Research Institute, London, Ontario Canada. 2011

Abstract .

The classical therapy produced holes in the biofilm but did not eradicate the organisms.

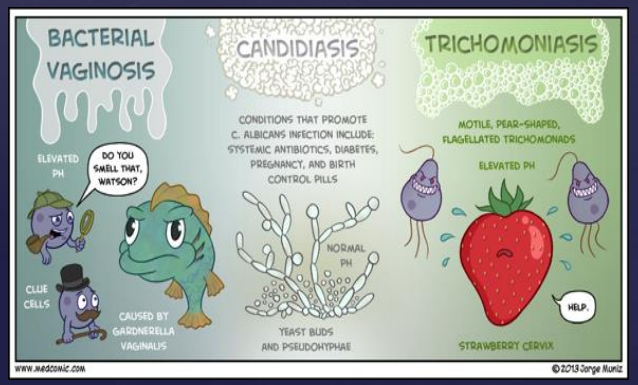
The findings provide some evidence of how lactobacilli probiotics might interfere with an aberrant vaginal microbiota, and strengthen the position that combining probiotics with antimicrobials could better eradicate pathogenic biofilms.

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

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

New probiotic strategy for VVC and VB treatment (topical medical device)

Three clinical studies published



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Vicariotto F, Del Piano M, Mogna L, Mogna G.

Effectiveness of the association of 2 probiotic strains formulated in a slow release vaginal product, in women affected by vulvovaginal candidiasis: a pilot study

J Clin Gastroenterol. 2012 Oct

An innovative solution for VVC

Medical Device in tablets for vaginal use able to produce CO_2 and containing ***L. fermentum*** **LF10** (400 million/cpr) and ***L. acidophilus*** **LA02** (400 million/cpr), fructo-oligosaccharides (FOS) and arabinogalactan (fibres).

A solution for the prevention and treatment of acute vaginal infections and recurrences caused by Candida based on two different mechanisms of action

PHYSICAL PRIMARY ACTIVITY:
the production of CO_2 creates an anaerobic environment able to significantly slow down the respirative metabolism of Candida

ANCILLARY SPECIFIC ACTIVITY:
the simultaneous production of bacteriolysins and lactic acid by the two lactobacilli exerts a specific inhibitory activity against Candida

A completely natural approach

Normo-physiological conditions: prevalence of lactobacilli 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*

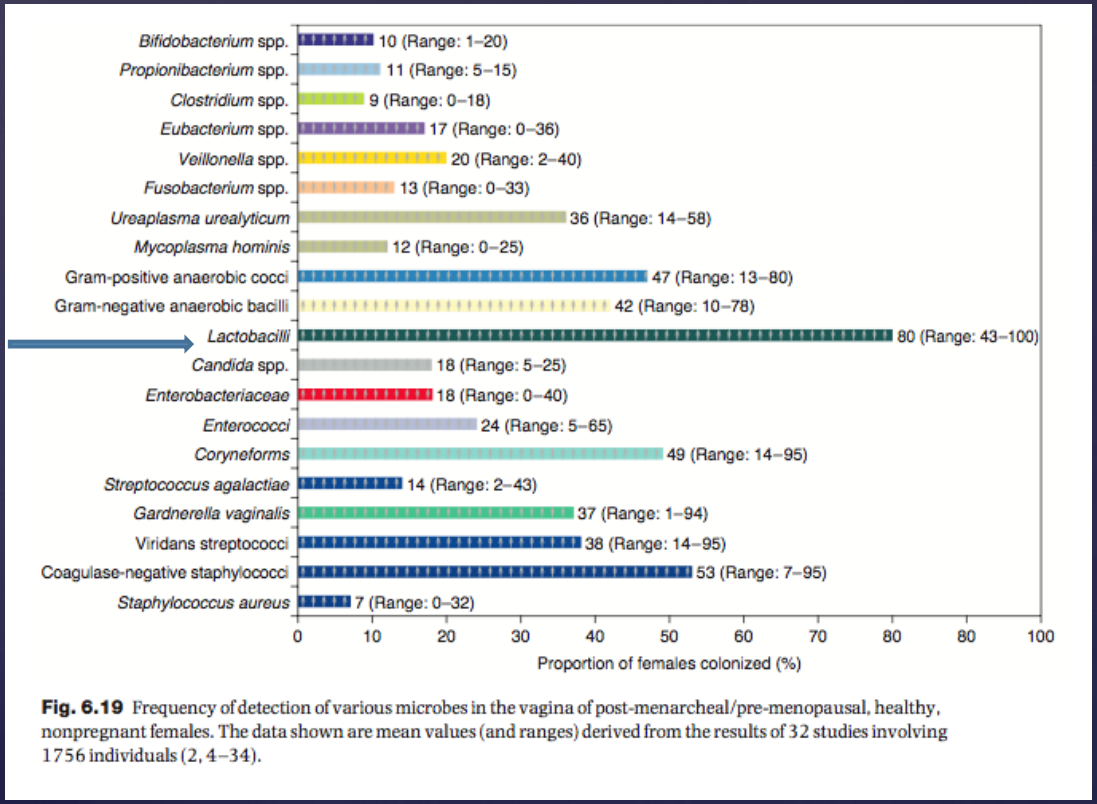
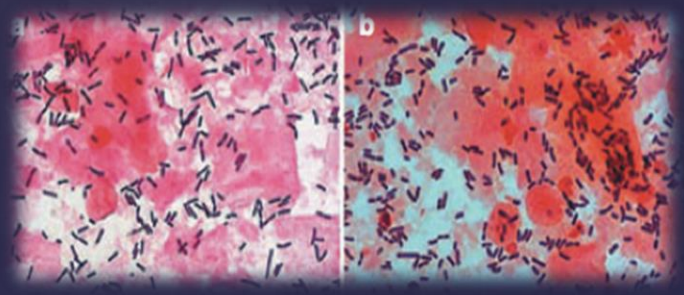


Fig. 6.19 Frequency of detection of various microbes in the vagina of post-menarcheal/pre-menopausal, healthy, nonpregnant females. The data shown are mean values (and ranges) derived from the results of 32 studies involving 1756 individuals (2, 4–34).



L. fermentum LF10 and L. acidophilus LA02 isolated by brushing from a healthy woman

L. acidophilus **and** ***L. fermentum*** **belong to species that are native of the vaginal environment**

Overview of the main features of lactobacilli

Self-regulated

***L. acidophilus* LA02 and *L. fermentum* LF10 are able to colonize the vaginal epithelium and produce antifungal 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
Production of hydrogen peroxide (*L. crispatus, L. jensenii, L. gasseri, L. delbrueckii, L. casei, L. plantarum, L. vaginalis, L. pentosus*)	Bactericidal effect
Biosurfactants production - natural surfactants - (*L. rhamnosus, **L. fermentum, L. acidophilus***)	Prevents the adhesion of contaminating microorganisms to the epithelium
Competitive exclusion (all)	Competition for available nutrients and physical occupation of receptors on epithelial cells
Production of bacteriocins and bacteriolysis (*L. gasseri, L. plantarum, **L. fermentum**, L. casei, L. delbrueckii, L. reuteri, L. salivarius*)	Inhibition of the growth of contaminant microorganisms

In vitro results against Candida (5 biotypes)

Time	Sample	C. albicans ATCC 10231	Candida parapsilosis ATCC 22019	Candida krusei ATCC 6258	C. albicans ATCC 90028	Candida glabrata ATCC 2001
Time 0	Yeast alone	2450	3100	1870	1300	1000
24 Hours	Yeast alone	3,860,000	600,000	660,000	4800,000	12,000,000
	Yeast + LF10 Inb. % growth	250 99.994 %	2700 99.550 %	2400 99.636 %	4000 99.917 %	12,600 99.895 %
	Yeast + LA02 Inb. % growth	260,000 93.264 %	260,000 56.667 %	280,000 57.576 %	470,000 90.208 %	2,280,000 81.000 %
48 Hours	Yeast alone	4,500,000	3,500,000	2,700,000	8,800,000	34,000,000
	Yeast + LF10 Inb. % growth	0 100 %	1100 99.969 %	3 100 %	9 100 %	12 100 %
	Yeast + LA02 Inb. % growth	61,000 98.644 %	120,000 96.571 %	1500 99.944 %	59,000 99.330 %	1,170,000 96.559 %

Lacotobacillus fermentum LF10 inhibits **different** Candida strains at 24 and 48 h *Lactobacillus acidophilus* LA02 inhibits Candida strains only after 48h due to lactic acid production and consequent acidifcation.

The results of the human pilot trial on VVC treatment (2012)

Vicariotto F . Effectiveness of the association of 2 probiotic strains formulated in a slow release vaginal product, in women affected by vulvovaginal candidiasis: a pilot s. J Clin Gastroenterol. 2012

Parameter	Time 0	Time 28	p (T28 vs. T0)	Time 56	p (T56 s. T0)	p (T56 vs. T28)
Total women with infection	30	4	<0.001	7	<0.001	
Total women without infection	0	26		23		
Percentage of healing	/	86.67%		76.67%		
Total women with recurrences	/	0		3		0.083
Percentage of recurrences	/	/		11.54%		

In the examination at the end of the first 4 weeks (t28) of product application, a complete healing and disappearance of Candida infection was found in 26 patients out of 30 (corresponding to 86.6%, p<0.001).
After 4 week at the end of treatment (t56) only 3 women had recurrences

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F. Murina , A. Graziottin , F. Vicariotto , F. De Seta
J. of Clinical Gastroenterology 2014

Can L. fermentum LF10 and L. acidophilus LA02 in a slow release vaginal product be useful for prevention of recurrent vulvovaginal candidiasis? A clinical study.

Can L. fermentum LF10 and L. acidophilus LA02 in a slow release vaginal product be useful for prevention of recurrent vulvovaginal candidiasis? A clinical study. 2014

58 patients with VVCR Score of Sobel >4
- positive test for Candida

Score of Sobel

symptom	Score
itch	0 = Absent 1= Slight 2= Medium 3= Intense
erythema	
edema	
ulcerations	

Am J Obstet Gynecol 2001;185:363-69.

F. Murina , A. Graziottin , F. Vicariotto , F. De Seta J. of Clinical Gastroenterology-2014,

Can L. fermentum LF10 and L. acidophilus LA02 in a slow release vaginal product be useful for prevention of recurrent vulvovaginal candidiasis? A clinical study. 2014

58-Score Sobel >4

Eradication

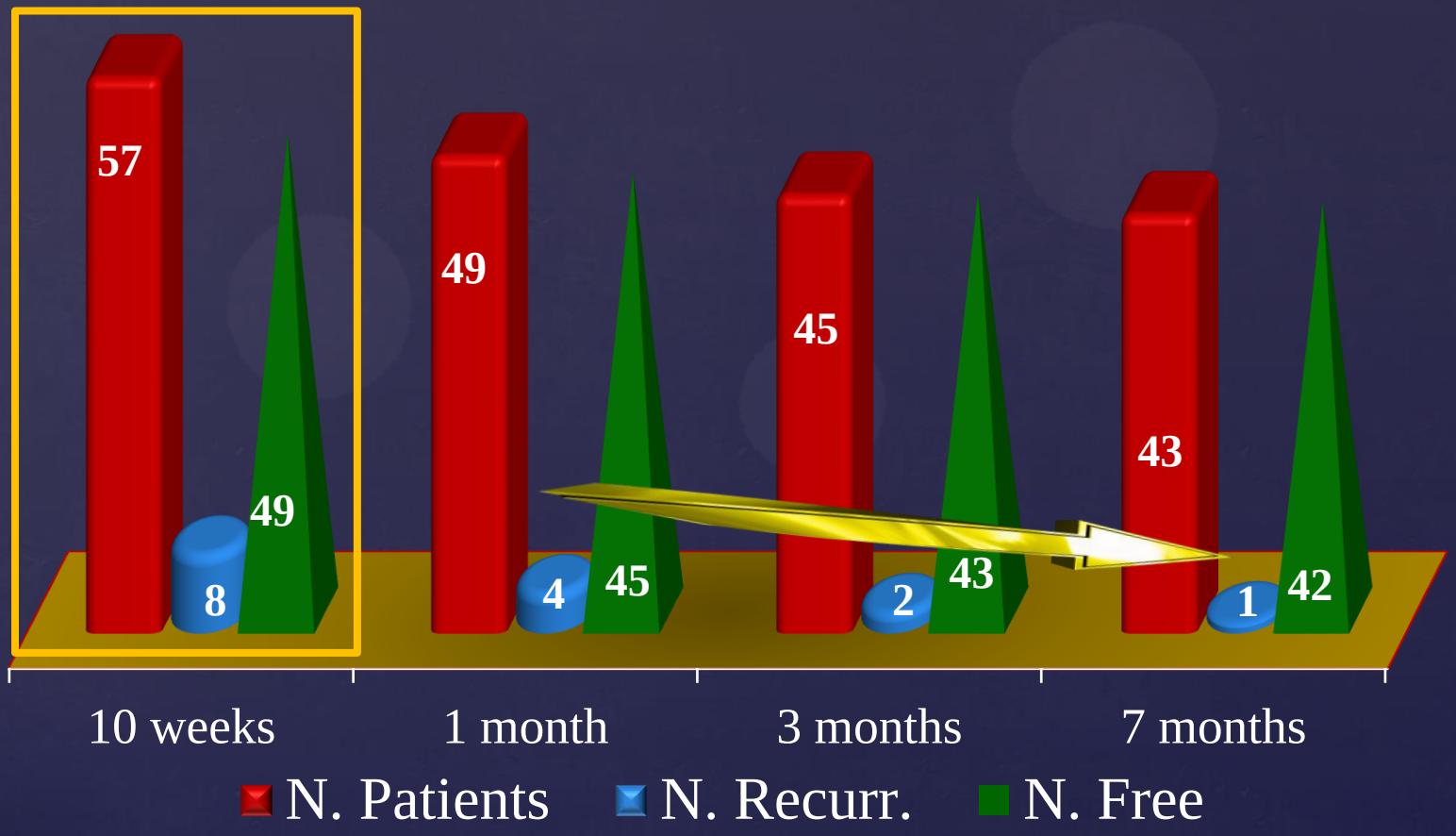
-oral fluconazole 200mg: 1/three times a week for 1 week

Prophylaxis

Lactobacillus fermentum and acidophilus
1 vaginal tablet every other day for 10 d.
and then one tablet every week for
10 weeks

F. Murina , A. Graziottin , F. Vicariotto , F. De Seta - Journal of Clinical Gastroenterology-2014, In Press

Can L. fermentum LF10 and L. acidophilus LA02 in a slow release vaginal product be useful for prevention of recurrent vulvovaginal candidiasis? A clinical study. 2014



F. Murina , A. Graziottin , F. Vicariotto , F. De Seta - Journal of Clinical Gastroenterology-2014

3

Vicariotto F, Mogna L, Del Piano M.

Effectiveness of the two microorganisms Lactobacillus fermentum LF15 and Lactobacillus plantarum LP01, formulated in slow-release vaginal tablets, in women affected by bacterial vaginosis:
a pilot study.
J Clin Gastroenterol. 2014

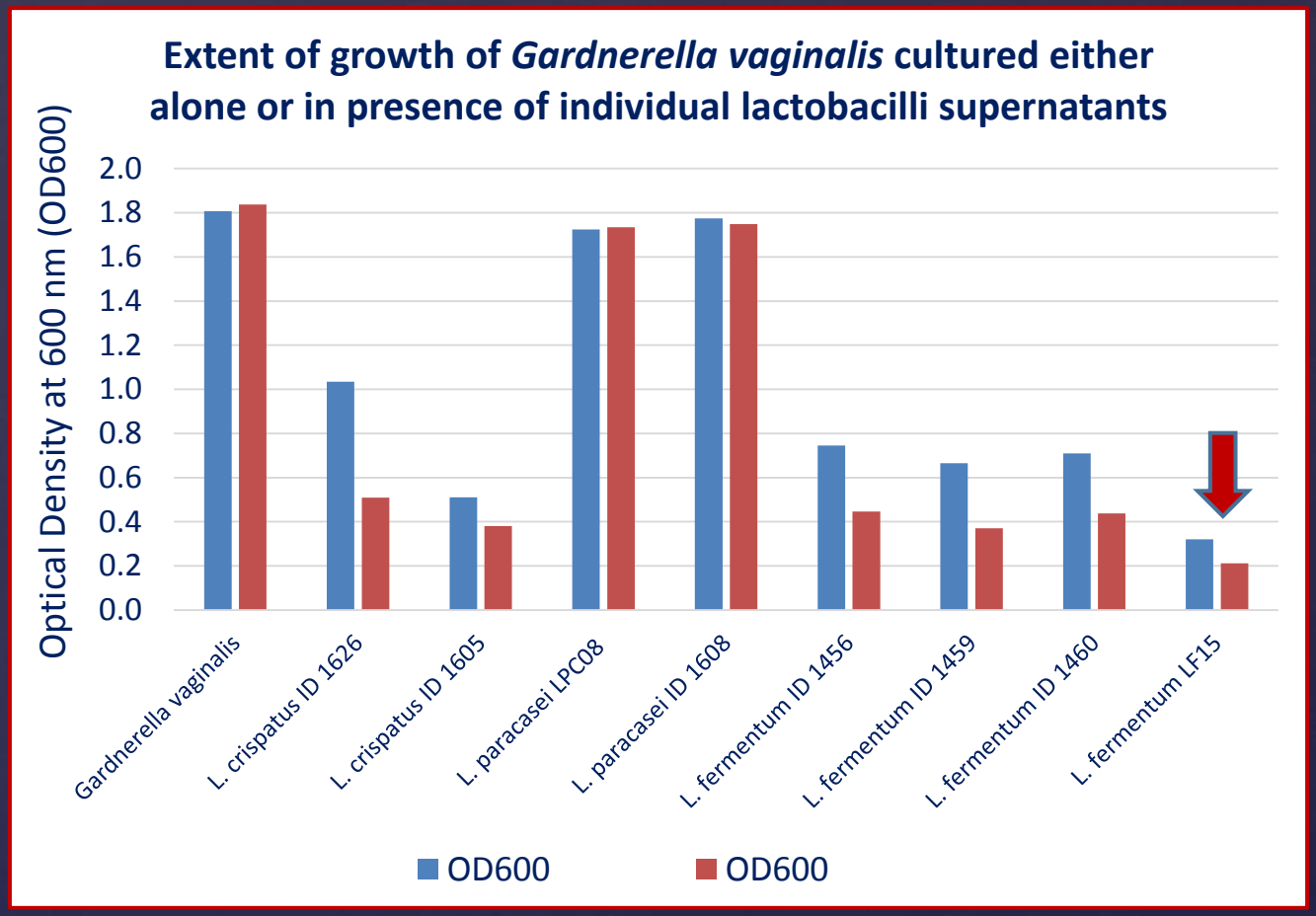
An innovative solution for BV

Medical Device in tablets for vaginal use containing tara gum (a natural gelling ingredient comprised of polysaccharides, mainly galactomannans), the two lactobacilli *L. fermentum LF15 (400 million/cpr) and L. plantarum LP01 (400 million/cpr)*, fructo-oligosaccharides (FOS) and arabinogalactan

MECHANICAL PRIMARY ACTIVITY: the tara gum rapidly spreads over the surface of vaginal mucosa, thus forming a hydrogel and 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 of the product exerts a specific inhibitory activity against Gardnerella that reinforces the mechanical primary effect

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 therefore selected for the human pilot trial in women diagnosed with Bacterial Vaginosis.

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)

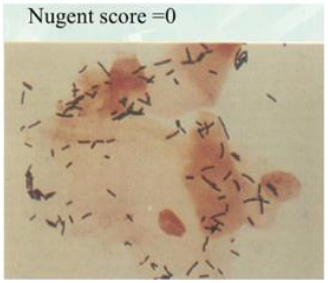
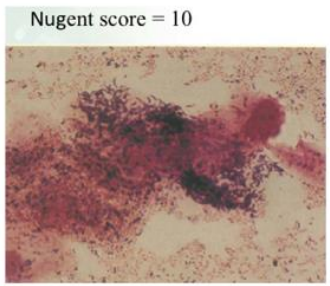
- 1 tablet per day for 7 d
- 1 tablet every 3 days for the following 3 wk
- 1 tablet per week for the following 4 wk

Active formulation

Placebo

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 For the human trial design

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 Nugent score)

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).

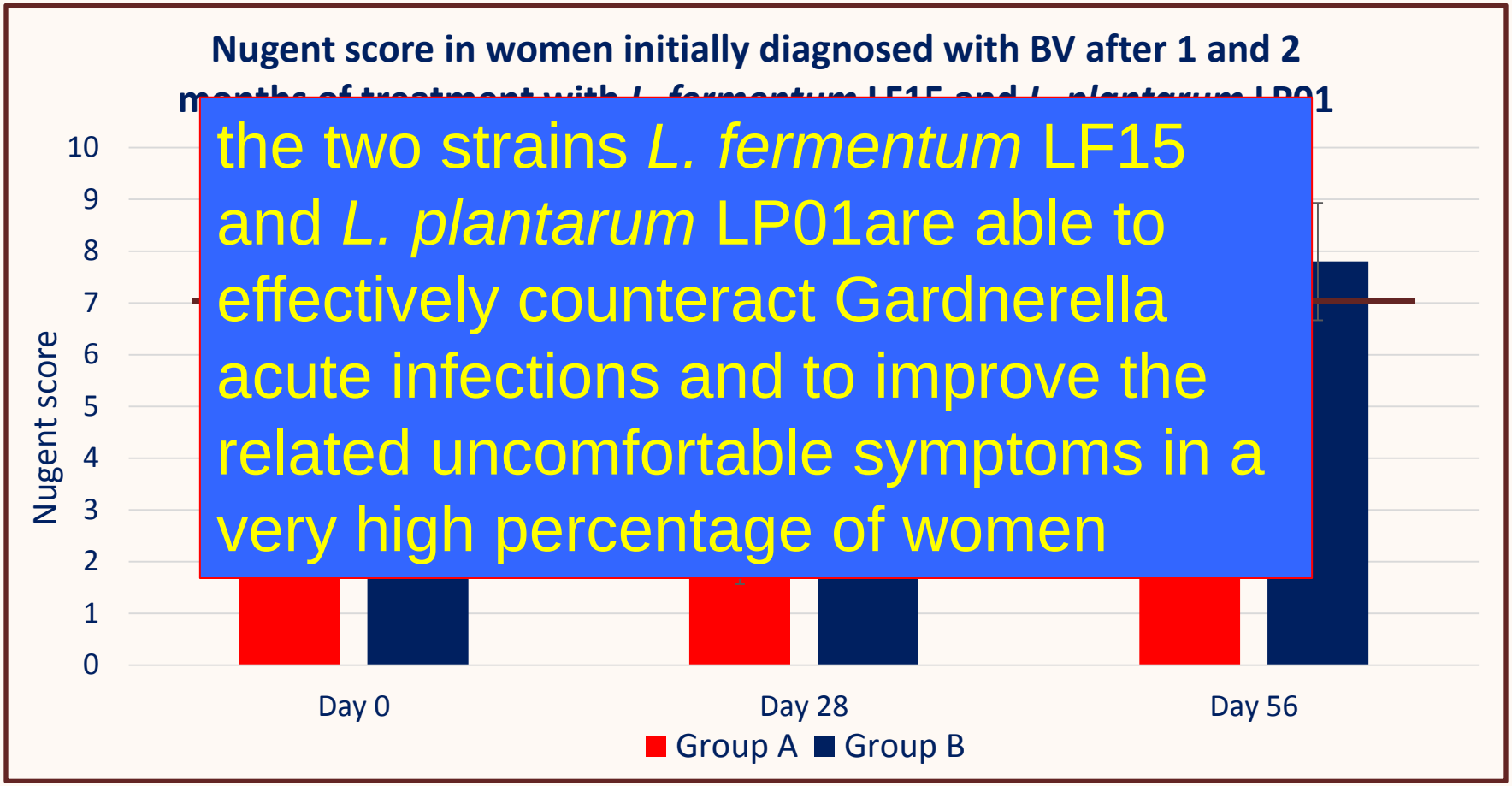
SCORE*	Lactobacillus morphotypes	*Gardnerella/Bacteroides* spp. Morphotypes	Curved Gram-variable rods
0	**4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

* 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), healthy vaginal microbiota (≤3).

The mean Nugent score (BV diagnosis)



At the baseline all the subjects recorded a score higher than 7, with a mean equal to 8.54 in Group A and 7.90 in the placebo (p=0.137).

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

Urinary Tract Infection (UTI)

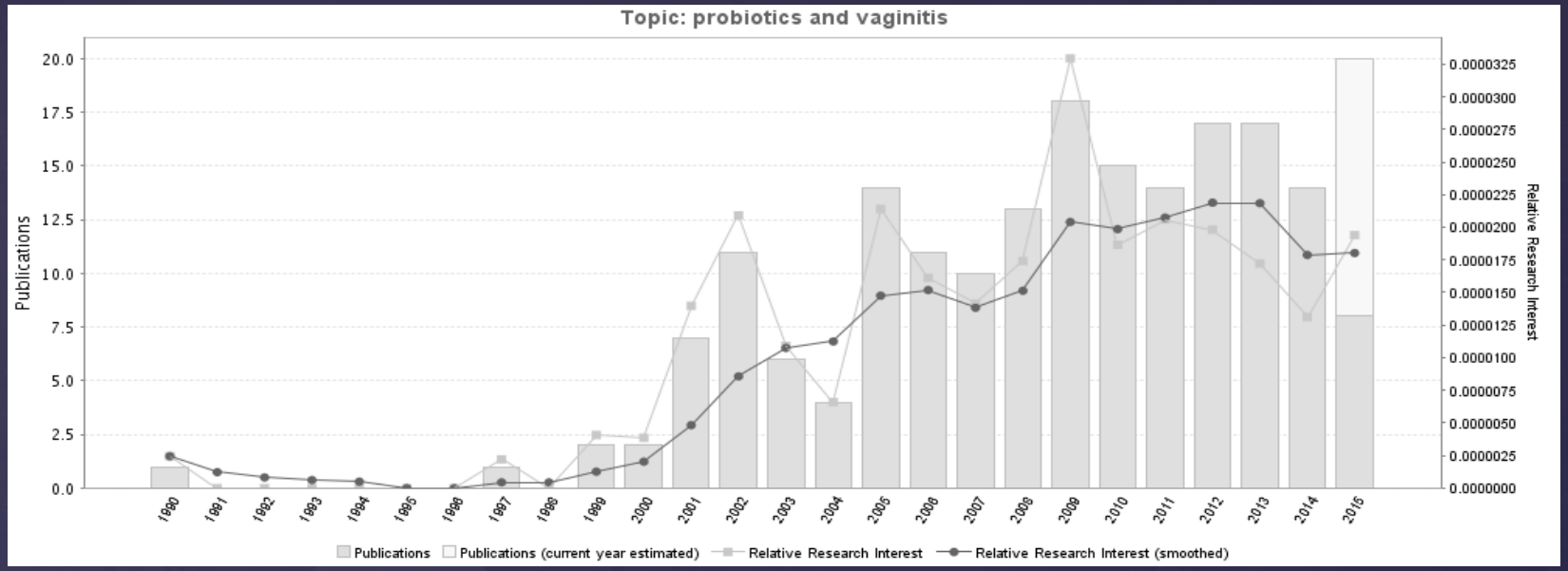
Vicariotto F. Effectiveness of an association of a cranberry dry extract, D-mannose, and the two microorganisms Lactobacillus plantarum LP01 and Lactobacillus paracasei LPC09 in women affected by cystitis: a pilot study. J Clin Gastroenterol. 2014

Urinary tract infection (UTI) is one of the most incident bacterial infections .

More than 50% of the women will have an episode of UTI throughout life. Up to 15% of women develop UTIs every year and at least 25% of them will have one or more recurrences.

Of the pathogens involved in cystitis, Escherichia coli is the most frequent one, with 74.6% of the cases.

Cystitis is the infection limited to the lower urinary tract with symptoms such as dysuria, polyuria and, eventually, suprapubic pain.
Four symptoms and a sign (including dysuria, frequency, hematuria, back pain, costovertebral angle pain) significantly increase the probability of UTI.



TOPICS :PROBIOTICS AND VAGINITIS

THANK YOU