

Effectiveness and Tolerability of a Synbiotic Vaginal Suppository for the Treatment of Bacterial Vaginosis

Andreas Schwiertz^{1*}, Marina Knaut², Ute Pohl², Brunhilde Hackel³ and Hans-Jorg Mueller⁴

¹Institute of Microecology, Auf den Lueppen 8, Herborn, Hesse, Germany

²Analyze and Realize GmbH, Waldseeweg 6, Berlin, Germany

³Pflanzgartenstr, Berlin, Germany

⁴SymbioPharm GmbH, Auf den Luppen 10, Herborn-Hörsbach, Germany

*Corresponding author: Andreas Schwiertz, Institute of Microecology, Auf den Lueppen 8, Herborn, Hesse, Germany, Tel: 00492772981139; E-mail: andreas.schwiertz@mikrooek.de

Received date: 02 Feb, 2015; Accepted date: 18 Feb, 2015; Published date: 20 Feb, 2015

Copyright: © 2015 Schwiertz A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Objective: Aim of this clinical study was the evaluation of the medical device SymbioVag® on bacterial vaginosis (BV) and its tolerability.

Method: An open-label study was conducted on 103 female subjects aged 18 to 56 years with manifested BV. Enrolled subjects were treated for 10 successive days with the synbiotic vaginal suppository SymbioVag® containing *Lactobacillus acidophilus* and *Lactobacillus gasseri* in combination with the prebiotic substance inulin. BV was diagnosed using the Amsel criteria. In addition, bacterial counts of *lactobacilli*, *Gardnerella vaginalis*, *Atopobium vaginae* and total anaerobes were detected. Symptoms associated with bacterial vaginosis were rated by the patients before and after the use of SymbioVag®.

Results: Treatment with SymbioVag® reduced vaginal pH in more than 80% of the patients. The concentration of *lactobacilli* increased significantly. A decrease in pathogen organisms was observed in nearly 60% of the patients for both marker organisms, *G. vaginalis* and *A. vaginae*, as well as for one out of four measured anaerobes. 80-90% of the patients rated the symptoms associated with bacterial vaginosis as being improved. Microscopic examination and test for the presence of amines showed an improvement in more than half of the patients. The evaluation of tolerability, together with the observed adverse events, attests the very good safety profile of the vaginal suppository.

Conclusion: The presented study showed that the synbiotic vaginal suppository, a combination of *Lactobacillus acidophilus*, *Lactobacillus gasseri* and the prebiotic substance inulin, is well tolerated, and confirms a general improvement of symptoms, a decrease in vaginal pH, and an improvement of the vaginal flora. Hence, the trial provides clear evidence for the justification of SymbioVag® being applied in patients suffering from bacterial vaginosis.

Keywords: Bacterial vaginosis; *Lactobacilli*; Probiotics; Synbiotic; Vaginal pH; *G. vaginalis*; *A. vaginae*

Introduction

A healthy vaginal microbiota is important for preventing lower genital tract diseases and vaginal discomfort. The most common vaginal discomfort occurring in women of reproductive age is bacterial vaginosis (BV), which is characterized by a depletion of beneficial *lactobacilli* and the occurrence of mainly anaerobic bacteria [1]. The present study was conducted to substantiate the impact of *Lactobacillus acidophilus* and *Lactobacillus gasseri*, together with the prebiotic substance inulin as SymbioVag® on the treatment of bacterial vaginosis.

The vaginal tract of fertile, premenopausal, healthy women is normally colonized by *lactobacilli*. The presence of different *lactobacillus* species within the vaginal tract plays an important role in maintaining the natural barrier and thereby preventing colonization

by anaerobic, Gram-negative bacteria and pathogenic microorganisms. Some lactobacillus species produce anti-microbial substances such as H₂O₂ and bacteriocins, which are known to be toxic for several bacteria [2]. Due to the fact that *lactobacilli* keep the vaginal pH below 4.5 by fermentation of glucose to lactic acid, an overgrowth by anaerobic bacteria is prevented [3]. It is assumed that a change in the vaginal microbiota, from *lactobacilli* to anaerobes, causes BV [4]. Alterations in vaginal flora are characterized by depletion of *lactobacilli* and a 100 – 1000 fold overgrowth by anaerobic bacteria, especially *Gardnerella vaginalis* and *Atopobium vaginae* [5]. Furthermore, it is assumed that other anaerobes, such as *bifidobacteria* and *Bacterioides spp*, colonize the vagina of women showing symptoms of BV [6]. Vaginal discharge, itching and a “fishy” odor are symptoms in women suffering from BV and are used in addition to an increased pH for the diagnosis of BV [7,8]. BV however, is not only associated with discomfort, but it also seems to facilitate infection with sexually transmitted diseases such as HIV, HSV-2, and Chlamydia trachomatis, as well as urinary tract infections, poor pregnancy outcomes, and severe obstetrical problems like preterm delivery and

low birth weight [7,9-11]. Over the last years it became evident, that in addition to the Amsel [8] and Nugent criteria [12] the presence of *G. vaginalis*, *A. vaginae* and the depletion of *lactobacilli* may be an indicator for the presence of BV [13].

Conventionally, symptoms of BV are treated with antibiotics such as metronidazole or clindamycin, with cure rates between 50% and 80% and a relapse rate of about 50%-75% within six months following treatment [7,14,15]. Studies showed that *G. vaginalis* and *A. vaginae* exist predominantly in dense biofilms on the vaginal epithelium. Since *A. vaginae* is resistant to metronidazole the high relapse rates may be attributed to these biofilms [16-19]. An alternative treatment option is the restoration of the microbiota by the use of *lactobacilli*. Initial clinical data have shown that probiotics have a positive effect on BV. Different lactobacillus species used alone or in combination with antibiotics resulted in positive outcomes on the treatment of BV [14]. Saunders et al. reported that *lactobacilli* disrupt biofilms and interfere with *G. vaginalis* [20].

Material and Methods

Study design

This clinical trial was conducted as a multi-centric, open-label, one-arm study on female subjects with manifested BV in Berlin (Germany) from March 2012 to November 2013. The subjects were enrolled at the practices of five gynecologists. The tolerability and the efficacy of SymbioVag® on the alteration of vaginal pH and changes in colonization of the vaginal microbiota were determined. This trial was approved by the Ethic Committee of the Charité, Berlin, Germany (EA1/046/12) and performed according to the Declaration of Helsinki and the ICH-GCP (E6) guidelines. Furthermore, this study was conducted according to DIN EN ISO 14155:2012-01 for medical devices.

Study population

One hundred and three women were enrolled in this clinical trial. The included subjects met the following main inclusion criteria: age \geq 18, vaginal pH \geq 4.7, signed informed consent for participation, BV (manifestation based on Amstel criteria: pH $>$ 4.5 and two more symptoms such as homogeneous grey-white vaginal discharge, positive amine test, and microscopic evidence of clue cells).

In order to ensure that menstrual bleeding does not interrupt treatment, a regular menstrual period was required. The main exclusion criteria were as follows: a manifested *Candida* infection or a positive microbial detection in case of suspected *Candida* infection, atrophic vaginitis and/or lack of oestrogen, $>$ 1 year of menopause, trichomoniasis, use of preparations that may influence the study outcome (eg antibiotics, probiotics), incidence of alcohol abuse, medication, or drug abuse, pregnancy or nursing, severe organic or systemic disorders, postoperative lesions in the vaginal area, known sensibility to one of the ingredients of the study product, participation in a clinical trial within the last 30 days.

All enrolled participants met the inclusion criteria and did not violate the exclusion criteria. During the study, 16 participants were excluded from the full analysis set (FAS) due to missing data at the second visit, or adverse events that led to the termination of the study 16 participants were excluded from the valid case analysis set (VCAS) due to protocol violations.

Intervention

This clinical trial was performed with the medical device SymbioVag®. It is a synbiotic vaginal suppository containing *Lactobacillus acidophilus* and *Lactobacillus gasseri* with at least 1×10^9 viable units per strain per gram, together with inulin, calcium lactate and hydrogenated fat. Inulin is a prebiotic that enhances growth of *lactobacilli*. Study participants were instructed to apply the vaginal suppository once daily over a period of 10 days. The vaginal suppository had to be inserted deeply into the vagina every evening before bedtime. The participants had to stop SymbioVag® application during menstrual bleeding and were accordingly withdrawn from the study, if menstruation started. Each study participant had to attend two regular visits in the practices of one of the five participating investigators: Visit 1 (V1) at baseline, and visit 2 (V2) 3-7 days after termination of the treatment.

Measurements/Objectives

Primary outcome

The primary outcome of this open-label study was a decrease in vaginal pH of at least 0.5 compared to the baseline pH after a 10-day treatment.

Secondary outcomes

Secondary outcomes were alteration of vaginal pH, changes in bacterial count in the vaginal microbiota, and tolerability of SymbioVag®.

Changes of vaginal pH were subdivided into a determination of percentages of subjects, who 1) reached a vaginal pH not higher than 5, and who 2) showed a lowered vaginal pH for at least 0.5, reaching a pH not higher than 5.

Changes in colonization of vaginal microbiota were subdivided into:

1) Changes in total number of H₂O₂ producing *lactobacilli*, and a determination of percentage of subjects whose count of *lactobacilli* increased by at least 1 order of magnitude;

2) Changes in the total number of anaerobic bacteria (*Bacteroides spp.*, *Bifidobacteria spp.*), including a determination of percentages of subjects whose count of pathogen organisms decreased by at least 0.5 orders of magnitude;

3) Appearance of pathogenic marker organisms (*G. vaginalis* and *A. vaginae*) including determination of percentages of subjects in whom the presence of both decreased by at least 0.5 orders of magnitude.

Adverse events were documented and judged by the investigators in order to prove safety and tolerability of SymbioVag®. In addition, in a global evaluation by both investigators and subjects, the tolerability was rated to be "very good", "good", "moderate" or "poor" at the end of the study.

Concurrent variable

The participants evaluated subjective symptoms such as vaginal discharge, itching and amine odor during the study as "none", "poor", "moderate" or "strong" before and after the treatment.

In addition, microscopic analysis of the vaginal discharge (smear tests) was performed together with a test for amines and a determination of the concentration of clue cells. Finally, a global

evaluation of efficacy by both investigators and subjects was performed at the end of the study. The rating scale was defined as “very good”, “good”, “moderate” or “poor”.

Microbiological examination

Collection of samples for analysis

All participating physicians used the same test system (Vaginal Status, IFM, Herborn, Germany), which contained one swab. Samples were taken in the in-between periodic bleedings. The swab (pre-weighed) was placed into Portagerm-Amies-Agar transport tubes (bioMérieux) and was used for the culturing of *lactobacilli*, anaerobic bacteria and the enumeration of *G. vaginalis* and *A. vaginae* by PCR. The VaginalStatus-test system was sent to the laboratory for further analysis.

Identification and enumeration of microorganisms

Vaginal samples were plated on selective agar and subsequently incubated under aerobic or anoxic conditions at 37°C for two days. The bacteria were submitted to Gram staining and the colony morphologies recorded. Additionally, identifications were performed by the API®- and VITEK®-systems (bioMérieux). All counts were listed as the numbers of log10 colony forming units (CFU) per ml of sample.

Detection of H₂O₂-production

Following identification, *lactobacilli* were tested for hydrogen peroxide production as described previously [21]. Colonies that produced H₂O₂ on the agar appeared dark blue Colonies, which did not produce H₂O₂, were colourless.

Detection of *Atopobium vaginae* and *Gardnerella vaginalis*

For the detection of *G. vaginalis* and *A. vaginae* the PCR-methods described by Ferris et al. and Sha et al. were used [22,23].

Statistical methods

All variables were analyzed in an explorative way and presented descriptively using statistical key data or frequency distribution. Alterations in outcome parameters were determined using non-parametric methods (Wilcoxon test, Chi2 test). For comparisons of independent subgroups, non-parametric sample testing was used. To determine the influence of baseline values on the outcome, relative changes were examined. In addition, parametric tests (t-test) were used for quantitative characterization. All statistic analyses were carried out on the FAS population, while primary target values were analyzed additionally in the VCAS population using SPSS® P-values ≤ 0.05 were considered significant, and the confidence interval was defined at 95%.

Results

Changes in vaginal pH

The use of SymbioVag® reduced the vaginal pH-value in 81.6% of the FAS population and in 84.5% of the VCAS population, respectively. These changes were highly significant (p<0.001; Tables 1 and 2).

On average, the vaginal pH decreased in the FAS population by 0.64 ± 0.61 (pt=0.040), and in the VCAS population by 0.65 ± 0.62 (pt=0.040).

At the beginning of the study, a pH-value below 5.0 was reported for 37.9% of the FAS population, while at the end of the study 81.6% of the patients showed a vaginal pH below 5.0 These findings are statistically highly significant (pChi<0.001).

	All subjects n=103	FAS population n=87	VCAS population n=71
Age (year)	34.6 ± 11.0	34.1 ± 11.1	34.1 ± 11.6
BMI (kg/m ²)	24.7 ± 5.8	24.8 ± 5.6	24.9 ± 5.5

Table 1: Mean baseline characteristics (+SD) of study subjects

pH Changes compared to baseline	FAS		VCAS	
	Number of patients	%	Number of patients	%
Lower	71	81.6	60	84.5
unchanged	10	11.5	6	8.5
higher	6	6.9	5	7
p ^a		<0.001		<0.001

p^a: p-value of intra-individual comparison obtained by Wilcoxon test

Table 2: Qualitative changes of vaginal pH (V1-V2, FAS and VCAS population)

A total of 48 participants of the FAS population showed a lowered pH-value for at least 0.5 and reached a vaginal pH-value not higher than 5.0 at the end of the study. No statistical difference in lowering of the vaginal pH was detected considering variation in time of V2 (3-7 days after the last treatment).

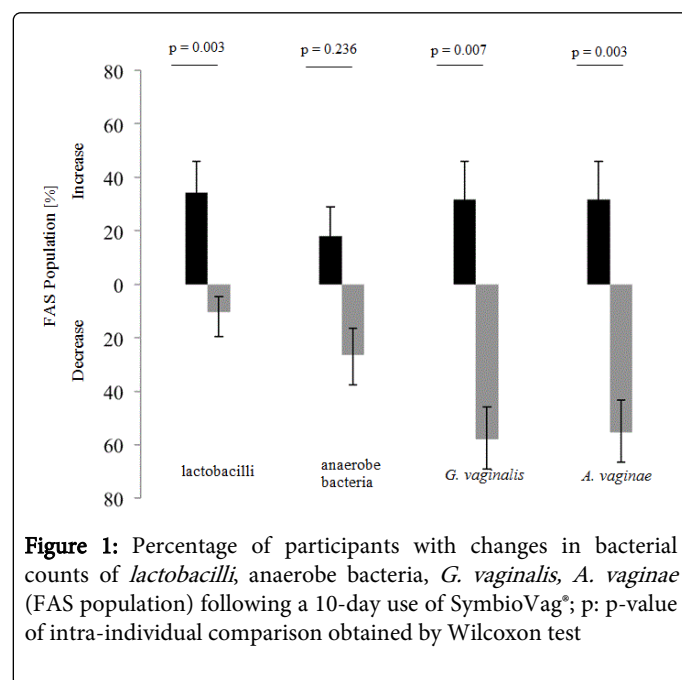


Figure 1: Percentage of participants with changes in bacterial counts of *lactobacilli*, anaerobe bacteria, *G. vaginalis*, *A. vaginae* (FAS population) following a 10-day use of SymbioVag®; p: p-value of intra-individual comparison obtained by Wilcoxon test

Microbiological examination

Ten days of treatment with SymbioVag® led to a significant increase of *lactobacilli* in the vaginal flora of FAS participants (Figure 1 and Table 3). For the FAS population, an increase in the total number of *lactobacilli* was determined in 34.2% of the patients, while an increase of at least one order of magnitude was observed in 31.6%. However, in 55.3% of the FAS population, no changes of the total number of *lactobacilli* could be determined due to the non-detectability of *lactobacilli* at both time points.

The counts of anaerobes such as bacteroides and bifidobacteria decreased in 26.4% of the FAS population, while an increase was observed in 18.1%. These findings were not significant (Figure 1 and Table 3). Considering pathogen organisms associated with BV, a significant reduction in the concentration of *G. vaginalis* and *A. vaginae* could be observed in 57.9% and 55.3% of the participants, while changes for 0.5 orders of magnitude were found in 40.8% and 47.4%, respectively (Figure 1 and Table 3).

Safety evaluation

The tolerability of SymbioVag® was rated as “very good” or “good” by 88.7% of the participants and for 91.8% of the participants by the investigators (Tables 4 and 5). All measured clinical parameters, ie body weight, temperature, heart rate, and blood pressure remained constant during the treatment with SymbioVag®. During this clinical trial, 14 adverse events were documented; all of them were considered as “not severe”. Three of these (pain and burning, itching and burning, vaginal pain) were considered as “possible” and two (both vulvovaginal burning) as “probable” being related to the application of the study product.

Bacterial count of	Increase [%]	No change [%]	Decrease [%]	p ^a	% of patients with orders of magnitude changes
H ₂ O ₂ producing <i>lactobacilli</i>	34.2	55.3	10.5	0.003	In 31.6% (CI ^b : 21.3%-43.3%) count decreased ≥ 1 order of magnitude
Anaerobic bacteria	18.1	55.6	26.4	0.236	In 26.4% (CI ^b : 16.6%-38.2%) count decreased ≥ 0.5 order of magnitude
<i>G. vaginalis</i>	31.6	10.5	57.9	0.007	In 40.8% (CI ^b : 29.6%-52.7%) count decreased ≥ 0.5 order of magnitude
<i>A. vaginae</i>	31.6	13.2	55.3	0.003	In 47.4%; (CI ^b : 35.7%-59.2%) count decreased ≥ 0.5 order of magnitude

p^a: p-value of intra-individual comparison obtained by Wilcoxon test; CI^b: Confidence interval (95%)

Table 3: Percentage of participants with changes in bacterial counts (*lactobacilli*, *anaerobe bacteria*, *G. vaginalis* and *A. vaginae*) following 10-day use of SymbioVag®

Microscopic findings

Since the presence of clue cells and a positive amine test were inclusion criteria, all enrolled subjects showed positive results in the

respective examinations at the beginning of the study. At V2, no clue cells were found in 54% of the participants, and the amine test was negative in 58.6% of cases. These findings were statistically highly significant (p_{wil}<0.001 and p_{wil}<0.001, respectively).

Evaluation of clinical findings

Participants rated BV associated symptoms such as itching, vaginal discharge, and amine odor using a subjective scale (“none”, “poor”, “moderate” and “strong”) before starting and following the treatment with SymbioVag® (V1,V2). All three symptoms improved significantly following treatment with SymbioVag® in subjects presenting these symptoms (Table 4). Among participants that did not suffer from any symptoms at V1, no worsening of symptoms was detected (Data not shown).

Alteration of symptoms	Itching (n=37)		Vaginal discharge (n=77)		Amine odor (n=78)	
improved	30	81.10%	67	87.00%	72	92.30%
unchanged	7	18.90%	9	11.70%	5	6.40%
worsened	0	0, 0%	1	1.30%	1	1.30%
p ^a	<0.001		<0.001		<0.001	

p^a: p-value of intra-individual comparison obtained by exact Wilcoxon test

Table 4: Changes of BV associated symptoms in self-assessment following SymbioVag® use (FAS population; V1-V2)

Global evaluation of efficacy

At the end of the study, a global evaluation of efficacy was performed 81.6% of the participants rated the efficacy of SymbioVag® as “very good” or “good”, while the investigators rated the efficacy for 47.7% of the subjects as “very good” or “good”.

	Evaluation [%]				p ^a
	“Very good”	“Good”	“Moderate”	“Poor”	
By investigator	53.4	38.4	5.7	4.5	0.096
By participants	48.9	39.8	5.7	5.7	

p^a: p-value obtained by exact Wilcoxon test

Table 5: Evaluation of Tolerability in self-assessment and by investigators

Discussion

In this open-label clinical trial, the effect and the tolerability of the synbiotic vaginal suppository SymbioVag® were investigated. Following a 10-day treatment with SymbioVag®, the impact on vaginal pH, changes in bacterial counts, and BV associated symptoms were evaluated.

Deducing from the results of this clinical trial, SymbioVag® has a positive effect on BV. In 82% of the FAS and 85% of the VCAS populations, vaginal pH was significantly reduced following the use of SymbioVag®. On average, a reduction of 0.6 was observed. Vaginal pH has a huge influence on bacterial colonization of the lower genital tract

and is therefore directly related to the maintenance of the healthy vaginal ecosystem consisting mainly of *Lactobacilli* [24]. *Lactobacillus spp.* are beneficial organisms that conserve the normal microbiota of the lower genital tract and prevent overgrowth by pathogen anaerobes through competition for nutrients, and through production of lactic acid, H₂O₂ and bacteriocins [25]. Both *L. acidophilus* and *L. gasseri*, which are the active components of SymbioVag®, are known to produce H₂O₂. Therefore, enhancement of H₂O₂ producing *Lactobacilli* was a secondary outcome of this clinical trial. In 30% of patients, an increased number of *Lactobacilli* was observed. Colonization by *Lactobacilli* and their adherence to the vaginal epithelium depend on many circumstances. SymbioVag® provides calcium lactate to acidify the vaginal mucosa, and inulin as a substrate for *Lactobacilli* growth. Additional, aspects like estrogen level, glycogen concentration, and lifestyle risks such as smoking have a major impact on *Lactobacilli* colonization [16,26]. To reach a comprehensive understanding on how and to which extent *Lactobacilli* colonization is influenced by SymbioVag®, further aspects like hormonal contraception, the respective state of menstrual cycle in which the treatment takes place, and lifestyle issues should be taken into account in further investigations.

A shift in the vaginal microbiota was observed following the treatment of SymbioVag®. The concentration of BV marker organisms, *G. vaginalis* and *A. vaginae*, was reduced following a 10-day treatment with SymbioVag® in 58% and 55% of the participants, respectively. A reduction in bacterial counts of both pathogenic organisms may be directly related to curing BV. A significant reduction of both leads to the conclusion that ingredients of SymbioVag® influence the depletion of pathogenic organisms. However, it is not clear if the reduced vaginal pH in general or the numerical increase of *Lactobacilli* in conjunction with the production of substances, such as H₂O₂, is responsible for the decrease of *G. vaginalis* and *A. vaginae*.

The current diagnosis of BV is based on the Amstel criteria: increased vaginal pH (≥ 4.5), the presence of clue cells, a positive amine test, and vaginal discharge [8]. Clue cells were detected in all participants before the trial, while no clue cells were observed in 54% following treatment with SymbioVag®. The number of negative amine tests increased from 0% to 58.6%. Beyond that, subjective rating of the symptoms itching, vaginal discharge and “fishy” odor improved in 80-90% of the FAS population. These findings substantiate the positive effect of SymbioVag® on healing BV and confirm results of previously performed trials using probiotics [14,27].

Finally, the safety of the medical device SymbioVag® could be shown as no severe side effects were observed. This is in line with previous studies [14,16]. The risk-benefit analysis of this clinical trial justifies the application of SymbioVag® in patients suffering from BV.

This study was conducted as an open-label study without randomization or control; therefore the conclusions drawn are limited. Additional studies with a suitable control should be performed and further parameters such as reinfections due to sexual intercourse, risky behavior like smoking, alcohol abuse, contraceptive methods and hormonal changes during the menstrual cycle should be included [28,29].

In conclusion, this open labeled study with patients suffering from BV confirms the assumption that an intra-vaginal application of *Lactobacilli* in combination with prebiotic substances, given as a vaginal suppository, lowers vaginal pH, decreases the total number of

pathogen organisms, improves symptoms of BV, and therefore promotes healing of BV.

References

1. Turovskiy Y, Sutyak Noll K, Chikindas ML (2011) The aetiology of bacterial vaginosis. *J Appl Microbiol* 110: 1105-1128.
2. Lepargneur JP, Rousseau V (2002) [Protective role of the Doderlein flora]. *Journal de gynécologie, obstétrique et biologie de la reproduction* 31: 485-494.
3. O'Hanlon DE, Moench TR, Cone RA (2011) In vaginal fluid, bacteria associated with bacterial vaginosis can be suppressed with lactic acid but not hydrogen peroxide. *BMC infectious diseases* 11: 200.
4. Vasquez A, Jakobsson T, Ahrne S, Forsum U, Molin G (2002) Vaginal lactobacillus flora of healthy Swedish women. *J Clin Microbiol* 40: 2746-2749.
5. Shipitsyna E, Roos A, Dacru R, Hallen A, Fredlund H, et al. (2013) Composition of the vaginal microbiota in women of reproductive age--sensitive and specific molecular diagnosis of bacterial vaginosis is possible? *PLoS One* 8: e60670.
6. Schwiertz A, Taras D, Rusch K, Rusch V (2006) Throwing the dice for the diagnosis of vaginal complaints? *Annals of clinical microbiology and antimicrobials* 5: 4.
7. Martin DH (2012) The microbiota of the vagina and its influence on women's health and disease. *Am J Med Sci* 343: 2-9.
8. Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, et al. (1983) Nonspecific vaginitis Diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 74: 14-22.
9. Schwebke JR, Desmond R (2007) A randomized trial of metronidazole in asymptomatic bacterial vaginosis to prevent the acquisition of sexually transmitted diseases. *American journal of obstetrics and gynecology* 196: e511-516.
10. van de Wijgert JH, Borgdorff H, Verhelst R, Crucitti T, Francis S, et al. (2014) The vaginal microbiota: what have we learned after a decade of molecular characterization? *PLoS One* 9: e105998.
11. Marrs CN, Knobel SM, Zhu WQ, Sweet SD, Chaudhry AR, et al. (2012) Evidence for Gardnerella vaginalis uptake and internalization by squamous vaginal epithelial cells: implications for the pathogenesis of bacterial vaginosis. *Microbes Infect* 14: 500-508.
12. Nugent RP, Krohn MA, Hillier SL (1991) Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol* 29: 297-301.
13. Wang KD, Su JR (2014) Quantification of Atopobium vaginae loads may be a new method for the diagnosis of bacterial vaginosis. *Clinical laboratory* 60: 1501-1508.
14. Mastromarino P, Vitali B, Mosca L (2013) Bacterial vaginosis: a review on clinical trials with probiotics. *New Microbiol* 36: 229-238.
15. Ngugi BM, Hemmerling A, Bukusi EA, Kikvi G, Gikunju J, et al. (2011) Effects of bacterial vaginosis-associated bacteria and sexual intercourse on vaginal colonization with the probiotic *Lactobacillus crispatus* CTV-05. *Sexually transmitted diseases* 38: 1020-1027.
16. Cribby S, Taylor M, Reid G (2008) Vaginal microbiota and the use of probiotics. *Interdisciplinary perspectives on infectious diseases* 2008: 256490.
17. Swidsinski A, Mendling W, Loening-Baucke V, Swidsinski S, Dorffel Y, et al. (2008) An adherent Gardnerella vaginalis biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. *American journal of obstetrics and gynecology* 198: e91-e96.
18. Swidsinski A, Mendling W, Loening-Baucke V, Ladhoff A, Swidsinski S, et al. (2005) Adherent biofilms in bacterial vaginosis. *Obstet Gynecol* 106: 1013-1023.
19. Ferris MJ, Maszta A, Aldridge KE, Fortenberry JD, Fidel PL, et al. (2004) Association of Atopobium vaginae, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. *BMC infectious diseases* 4: 5.

20. Saunders S, Bocking A, Challis J, Reid G (2007) Effect of *Lactobacillus* challenge on *Gardnerella vaginalis* biofilms. *Colloids and surfaces B, Biointerfaces* 55: 138-142.
21. Eschenbach DA, Davick PR, Williams BL, Klebanoff SJ, Young-Smith K, et al. (1989) Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women with bacterial vaginosis. *J Clin Microbiol* 27: 251-256.
22. Ferris MJ, Masztal A, Martin DH (2004) Use of species-directed 16S rRNA gene PCR primers for detection of *Atopobium vaginae* in patients with bacterial vaginosis. *J Clin Microbiol* 42: 5892-5894.
23. Sha BE, Chen HY, Wang QJ, Zariffard MR, Cohen MH, et al. (2005) Utility of Amsel criteria, Nugent score, and quantitative PCR for *Gardnerella vaginalis*, *Mycoplasma hominis*, and *Lactobacillus* spp for diagnosis of bacterial vaginosis in human immunodeficiency virus-infected women. *J Clin Microbiol* 43: 4607-4612.
24. McLean NW, Rosenstein IJ (2000) Characterisation and selection of a *Lactobacillus* species to re-colonise the vagina of women with recurrent bacterial vaginosis. *J Med Microbiol* 49: 543-552.
25. Motevaseli E, Shirzad M, Raoofian R, Hasheminasab SM, Hatami M, et al. (2013) Differences in vaginal lactobacilli composition of Iranian healthy and bacterial vaginosis infected women: a comparative analysis of their cytotoxic effects with commercial vaginal probiotics. *Iranian Red Crescent medical journal* 15: 199-206.
26. Brotman RM, He X, Gajer P, Fadrosh D, Sharma E, et al. (2014) Association between cigarette smoking and the vaginal microbiota: a pilot study. *BMC infectious diseases* 14: 471.
27. Vicariotto F, Mogna L, Del Piano M (2014) 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. *Journal of clinical gastroenterology* 48: S106-S112.
28. Bradshaw CS, Morton AN, Garland SM, Morris MB, Moss LM, et al. (2005) Higher-risk behavioral practices associated with bacterial vaginosis compared with vaginal candidiasis. *Obstet Gynecol* 106: 105-114.
29. Vodstrcil LA, Hocking JS, Law M, Walker S, Tabrizi SN, et al. (2013) Hormonal contraception is associated with a reduced risk of bacterial vaginosis: a systematic review and meta-analysis. *PLoS One* 8: e73055.