

A Safe Wide Spectrum Polyherbal Microbicide and Three Meritorious Strains of Probiotics for Regressing Infections and Restoration of Vaginal Health (Regression of Vaginosis with BASANT and Probiotics)

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Abstract

This article reviews the findings of the Phase II clinical trial conducted in 80 women suffering from recurrent episodes of Vaginosis whose vaginal pH was above 5.0. Intravaginal intake every night for seven days of easily dispersible capsules containing either the Polyherbal wide spectrum action microbicide BASANT, or 3×10^9 bacilli of 3 selected meritorious Probiotics cured Vaginosis in 65%-70% of women. However, the intake of the Combination of the two was remarkably effective in curing 95% (19 out of 20) women, restoring fully healthy vagina.

Introduction

Vaginosis is a widely prevalent syndrome. According to a study conducted by the Indian Council of Medical Research (ICMR), the prevalence of this syndrome is about 30% in women attending the antenatal clinics in urban Delhi [1]. Bang reported in Lancet its incidence to be 50% in rural Maharashtra [2]. Its incidence was 80% in slums of Delhi in a survey conducted by us along with Safdarjung Hospital and Population Foundation of India [3]. It is reported as a public health problem of concern in USA [4]. Its prevalence in the United States is estimated to be 29.2% in women of 14-49 age [5]. Vaginosis is expressed as abnormal vaginal discharge, fishy odour and the pH of vagina above 5.0 with absence of normally dwelling Lactobacilli. The present treatment for Bacterial vaginosis employs various antibiotics which have different spectrum of activity with a cure rate at 4 weeks of 60%-70% [6]. The antibiotic Metronidazole usually prescribed is not pleasant to take for many women who experience nausea, dizziness, drowsiness, vomiting, headache and metallic taste [7]. The recurrence of vaginosis is frequent in women treated with antibiotics.

Development of a Polyherbal Microbicide

As alternate to antibiotics, we considered making a formulation employing traditionally used herbs of age old known properties. This polyherbal microbicide named as BASANT was formulated with 95% pure diferulolymethane {(1E, 6E)-1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6- heptadiene-3, 5-Dione} (Curcumin) from *Curcuma longa*, purified extracts of *Emblica officinalis*, Neem (*Azadirachta indica*) leaves, and Aloe vera (*Aloe barbadensis*). These ingredients were dispensed along with pharmacopoeially approved excipients: citric acid, sorbitol, microcrystalline cellulose, sodium starchglycolate, starlac, Crospovidones and sodium alginate as a lubricating agent.

BASANT was formulated as both a cream and as powder encapsulated in easily dispersible cellulose capsules. It was tested & found effective against WHO strains and clinical isolates of *Neisseria gonorrhoeae*, including those resistant to penicillin, tetracycline, nalidixic acid and ciprofloxacin [8]. BASANT has also pronounced inhibitory action against *Candida glabrata*, *Candida albicans* and *Candida tropicalis* isolated from women with vulvovaginal candidiasis, including three isolates resistant to azole drugs and amphotericin. Table 1 Summarizes the variety of genital pathogens against which this microbicide was tested & found to exercise inhibitory action. At

1. Bacteria	
<i>Neisseria gonorrhoeae</i>	<i>N. gonorrhoeae</i> WHO-C, <i>N. gonorrhoeae</i> WHO-G, <i>N. gonorrhoeae</i> WHO-K, <i>N. gonorrhoeae</i> WHO-L6 <i>N. gonorrhoeae</i> 1586, <i>N. gonorrhoeae</i> 1669, <i>N. gonorrhoeae</i> 1794, <i>N. gonorrhoeae</i> 2182, <i>N. gonorrhoeae</i> 2436, <i>N. gonorrhoeae</i> 2482, <i>N. gonorrhoeae</i> 267 [6], and all strains isolated from patients at the STD centre, New Delhi.
<i>Chlamydia trachomati</i>	CT231, CT233, CT239, CT272, CT279, CT244, tested free and in infected cells [7].
2. Fungi	
	<i>Candida</i> - <i>C. glabrata</i> , <i>C. glabrata</i> (7 clinical isolates), <i>C. glabrata</i> (2 clinical isolates), <i>C. albicans</i> ATCC 36082, <i>C. albicans</i> (5 clinical isolates) <i>C. albicans</i> , <i>C. tropicalis</i> (2 clinical isolates) [6].
3. Viruses	
Human Immunodeficiency Virus (HIV)	Inhibition of virus production by BASANT in HIV-1 NL4.3- infected CEM-GFP and P4 cells [6] Both CCR5 and CXCR4 tropic HIV-1 lab-adapted strains and primary isolates from different clades [6]
Human Papilloma Virus (HPV)	Inhibition of entry of HPV-16 in Hela Cells [6]

Table 1: BASANT inhibits the following genital pathogens.

the National Cancer Institute, National Institutes of Health (NIH), Dr. John Schiller observed that BASANT inhibits the entry of HPV16 in Hela cells [6]. Prof. Rajyashree Sharma Head of the Department of Obstetrics & Gynecology at Medical College, Aligarh found that 30 daily intravaginal intakes of BASANT capsules caused the elimination

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of HPV 16/18 from the infected cervical cells of women at early stages of Carcinoma of Cervix patients. Their Pap smear also became normal. At the National Institute of Pathology, New Delhi, Prof. Aruna Mittal found that BASANT exercised an inhibitory action on Chlamydia trachomatis whether tested against the free bacteria or lodged in cells [9]. More recently, studies carried out at the University of Oregon, USA by Prof. Manoj Pастey reported BASANT to be effective against both CCR5 and CCR4 tropic HIV 1 lab-adapted strains and primary isolates belonging to different clades [10]. Dr. Smita Kulkarni at the National Institute of Virology Pune has found that BASANT inhibits various HIV-1 subtypes at non cytotoxic concentration. It was also found to inhibit HIV-1 replication in the Epi Vaginal explants model. Thus this Polyherbal microbicide has a wide spectrum action on a variety of genital pathogens, including HIV.

Isolation of Probiotic Lactobacilli from Healthy Vagina

Human female vagina has some interesting characteristics differing from the rest of the body. Healthy vagina has an acidic pH of about 4.5 whereas the normal pH of the rest of the body is 7.4. Acidic pH is generally protective to discourage growth and colonization of a large number of microorganisms. The acidic pH of vagina is caused and maintained by Lactobacilli strains which co-exist along with the endometrial cells of healthy vagina. To learn more about these indwelling bacilli which confer an important health benefit, we isolated from 80 healthy women with their written consent, Lactobacilli resident in the vagina. These were characterized on the basis of Genus, Group and Species specific PCR's along with Random Amplified Polymorphic DNA (RAPD) and 16s rDNA analysis [11]. The most frequent Lactobacilli strains found in vagina of women in Delhi belong to Group IV (*L.reuteri*, *L.fermentum*, *L.salivarius*, *L. plantarum*) in contrast to Group II (*L.crispatus*, *L.jensenii*, *L.gasseri*, *L.acidophilus*) Lactobacilli strains which are predominant in Europe, USA & Canada. Group III (*L casei*, *L.paracasei*, *L.rhamnosus*) were present in only 6% of women in Delhi. It was further found out that all strains of even the same species are not high secretors of lactic acid on the basis of their ability to make and secrete large amounts of lactic acid [12]. On the basis of their ability to make large quantities of lactic acid, positivity for making H₂O₂, high hydrophobicity and presence of arginine deiminase which prevents the formation of foul odour derivatives, we shortlisted three strains of Lactobacilli: *L. salivarius* TRF#30, *L. fermentum* TRF#36, and *L. gasseri* TRF#8. Figure 1 gives the species specific PCR profile of the three selected strains along with few others. (Table 2) summarizes the metabolic properties of the selected three strains of Probiotics. These meritorious Lactobacilli have been deposited in the Microbial Type Culture Collection and Gene Bank (MTCC), an International Depository Authority who have assigned the following numbers for these strains, *L. gasseri* (MTCC 5615), *L. salivarius* (MTCC5616) *L. fermentum* (MTCC 5617).

After individually testing these bacilli for their merit for restoration of healthy vagina, these three strains namely, *L. salivarius* TRF#30, *L. fermentum* TRF#36 and *L. gasseri* TRF#8 (hereafter denoted as Pro-Vag-Health) were selected for Phase II clinical trials.

Evaluation of BASANT and Pro-Vag-Health Probiotics for Treatment of Vaginosis

After obtaining permission of The Drugs Controller General of India and approval of the Institutional Ethics Committees of the All India Institute of Medical Sciences (AIIMS) and Sir Ganga Ram Hospital New Delhi, 80 women suffering from recurring episodes of Vaginosis were enrolled for Phase II trials.

The criteria for enrolment of subjects were:

Enrollment criteria

- (a) Women between age groups of 18-45 years with regular cycles.
- (b) Clinical history of chronic and/ or recurring episodes of abnormal vaginal discharge. On pelvic examination, they are seen to have either excessive or unhealthy vaginal discharge.
- (c) Willing to participate in the study after having been explained the nature of the study.
- (d) Willing to return for follow up after 7 days of completion of treatment and thereafter on fortnightly basis for 2 months.
- (e) Women enrolled should have telephone for recording by phone the discomfort, if any, after insertion of the capsules in the vagina.

Exclusion criteria

- (a) Known case of diabetes mellitus.
- (b) Suspected/confirmed pregnancy.

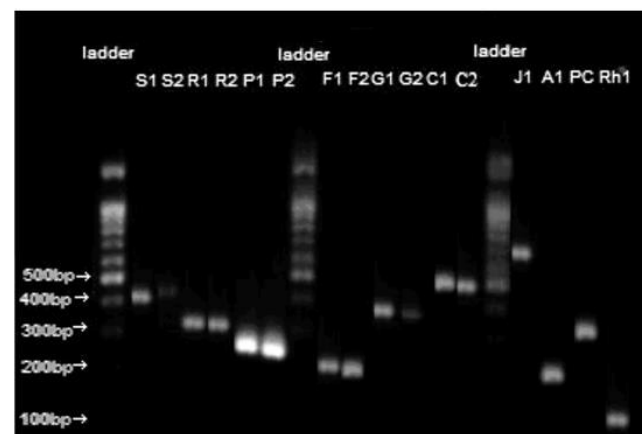


Figure 1: Species specific PCR products of the 10 species of Lactobacilli isolated from healthy vagina. This representative figure shows the profiles of 2 isolates of each of the 6 species and 1 isolate of the rest of 4 species as viewed on 2% agarose gel. S1 and S2, *L. salivarius* strains, 400 bp amplicon; R1 and R2, *L. reuteri* strains, 300 bp amplicon; P1 and P2, *L. plantarum* strains, 250 bp amplicon; F1 and F2, *L. fermentum* strains, 200 bp amplicon; G1 and G2, *L. gasseri* strains, 350bp product; C1 and C2, *L. crispatus* strains, 500bp PCR amplicon; J1, *L. jensenii*, 700 bp amplicon; A1, *L. acidophilus*, 200 bp PCR amplicon; PC, *L. paracasei*, 300 bp amplicon; Rh1 *L. rhamnosus*, 100 bp amplicon [9].

Isolate	D- Lactic acid (in mM)	L-Lactic acid (in mM)	H2O2 (in mM)	Arginine deiminase activity (in mM)	Hydrophobicity (in %)
<i>L. gasseri</i> TRF# 8	11.6	6.1	55	0.63	61.2
<i>L. fermentum</i> TRF # 36	8.2	5.4	60	0.65	30.2
<i>L. salivarius</i> TRF # 30	11.9	6.2	0	0.52	17.4

Table 2: Metabolic properties of the 3 meritorious strains of lactobacilli isolated from healthy vagina.

- (c) Suspected or known malignancy of reproductive tract.
- (d) Less than 6 weeks since last delivery or abortion since last delivery or abortion.
- (e) H/O severe allergic reaction.
- (f) Abnormalities of vaginal anatomy which will interfere with placement of drug.
- (g) Known systemic diseases immune-compromised states.
- (i) Adnexal mass or tenderness.
- (j) Having received a course of antibiotic therapy, less than 14 days prior to enrollment.
- (k) Dysfunctional uterine bleeding.
- (l) Vaginal polyp.

The women enrolled were clinically examined and considered as suffering from Vaginosis on basis of thin white homogeneous abnormal vaginal discharge, presence of Clue cells indicative of microorganisms on epithelial cells of the vagina, pH of vaginal fluid above 5.0 and release of fishy odour on addition of 10% KOH (Whiff's test).

They were divided into four groups of 20 each. Twenty women in Group I was given the Pro-vag-Health Probiotics capsules. They were

asked to take one capsule containing 3×10^9 lyophilized stabilized lactobacilli every night for seven days. Group II women were given BASANT capsules 250 mg, one capsule to be taken every night for seven days. Group III of 20 women were given a Combination of Pro-vag-Health and BASANT with the instruction to use one capsule of each every night for seven days. Group IV of 20 women were given placebo empty capsules to gauge the extent of spontaneous regression of Vaginosis over the observation time. The trials were randomized and women were distributed in these four groups with the help of a Statistician.

The efficacy of treatment with Polyherbal microbicide BASANT, Pro-vag-Health Probiotics and the Combination of the two hereafter named NAUROZ were gauged by:

- (a) Absence of abnormal vaginal discharge.
- (b) Absence of clue cells.
- (c) Presence of lactobacilli.
- (d) Absence of fishy odour.
- (e) PH returning to below 5.0.

Figure 2 represents the typical findings of an effective treatment.

Pro-vag-Health Probiotics used by Group I women cured Vaginosis in 13 out of 20 women. BASANT was effective in 14 out of 20 women.

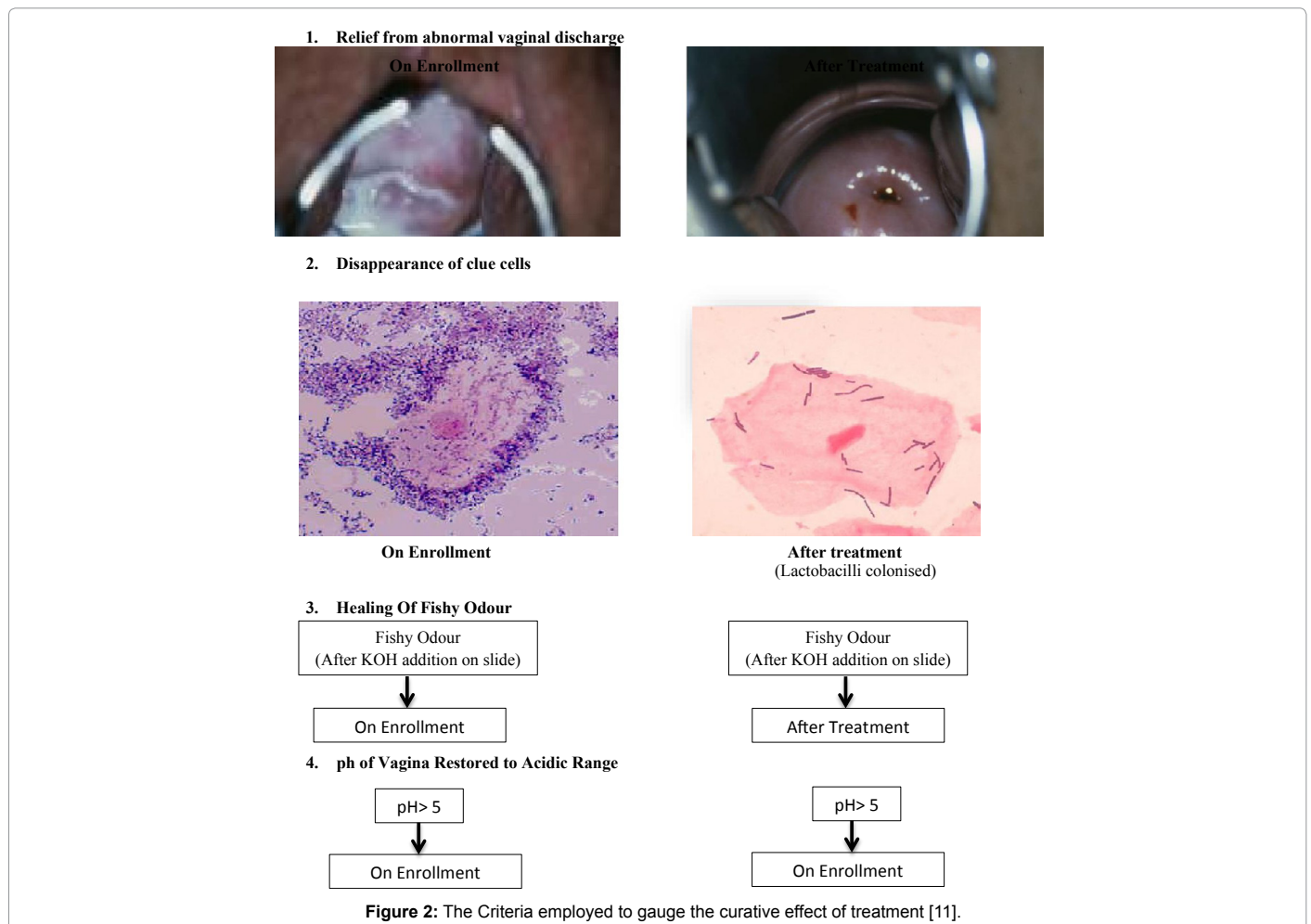


Table 3 gives the findings in women treated effectively by BASANT. Table 4 represents a case, where Pro-vag-Health alone was unable to regress Vaginosis. Impressively the combination of the two, NAUROZ was effective in nearly every woman, 19 out of 20 women. Table 5 is a representative record of a patient using NAUROZ. Women were fully satisfied with the treatment. The subjects stopped coming to the clinic for follow- up even

before completion of 2 months telling us on telephone that they do not feel the necessity of coming to us when they have no problem. In the fourth group of women, given Placebo capsules, only one woman out of 20 showed amelioration of her symptoms, while 19 others continued to suffer over the observation period [13]. Table 6 summarizes the overall findings of the trials. None of the women complained of any side effects.

Patient ID BV-A-107	Initial On Enrollment	1st Follow Up (Day 10)	2nd Follow Up (Day 31)	3rd Follow Up (Day 66)	4th Follow Up (Day 83)
Date	22/03/12	02/04/12	23/04/12	28/05/12	14/06/12
Discharge Amount	+++	+	-	-	-
pH	5.5	4.5	4.0	4.0	4.0
Odour	+	-	-	-	-
Itching/burning	-	-	-	-	-
Clue Cells	+	-	-	-	-
Lactobacilli growth	-	+	+	+	+
Prescription	BASANT (7 capsules)	BASANT (2 capsules) one each week	BASANT (2 capsules) One each week	BASANT (2 capsules) One each week	BASANT(2 capsules) One each week
Side effects	None	None	None	None	None
Subjective Relief	-	95% Relief	100% Relief	100% Relief	Patient Satisfied

"+" Present "-" Absent

Table 3: Representative record of the patient BV-A-107, who experienced regression of Vaginosis by intravaginal use of BASANT.

Patient ID BV-A- 100	Initial On Enrollment	1st Follow Up (Day 14)	2nd Follow Up (Day 31)
Date	8/3/2012	22/03/12	9/3/2012
Discharge Amount	+++	++	++
pH	5.5	5	5
Odour	+	+	+
Clue Cells	+	+	+
Itching/burning	-	-	-
Lactobacilli growth	-	-	-
Prescription	Pro-vag-Health (7 capsules)	Pro-vag-Health (2 capsules) One each week	Pro-vag-Health (2 capsules) One each week
Side effects	None	None	None
Subjective Relief	-	20% Relief	Patient not satisfied

"+" Present, "-" Absent

Table 4: Representative record of the Patient BV-A-100, who did not respond to the intake of (Pro-vag-Health) Probiotics.

Patient ID BV-A-127	Initial On Enrollment	1st Follow Up (Day 10)	2nd Follow Up (Day 28)	3rd Follow Up (Day 43)	4th Follow Up (Day 55)
Date	11/12/12	21/12/12	08/01/13	24/01/13	05/02/13
Discharge Amount	+++	+	-	-	-
pH	5.5	4.5	4.5	4.5	4.5
Odour	+	-	-	-	-
Itching/burning	-	-	-	-	-
Clue Cells	+	-	-	-	-
Lactobacilli growth	-	+	+	+	+
Prescription	BASANT + Pro-vag-Health (7 capsules)	BASANT + Pro-vag-Health (2 capsules) One each week	BASANT + Pro-vag-Health (2 capsules) One each week	BASANT + Pro-vag-Health (2 capsules) One each week	BASANT + Pro-vag-Health (2 capsules) One each week
Side effects	None	None	None	None	None
Subjective Relief	-	95% Relief	100% Relief	100% Relief	Patient Fully Satisfied

"+" Present, "-" Absent

Table 5: Representative record of the patient BV-A-127/II B, who experienced regression of Vaginosis by intravaginal use of BASANT+Pro-vag-Health.

Treatment given N=20	Improved (%)	P value Comparison with Placebo	P value Comparison with BASANT+Probiotics
Probiotics	13 (65%)	P<0.001	P = 0.04
BASANT	14 (70%)	P<0.001	P = 0.09
BASANT + Probiotics	19 (95%)	P<0.001	-
Placebo	1 (5%)	-	P<0.001

Table 6: Summary of Results of treatment given to the four groups of women suffering from recurrent Vaginosis.

Concluding Comments

These trials lay the basis of treating Vaginosis by a Combination of the Polyherbal microbicide BASANT along with 3 meritorious strains of Probiotics. These were highly acceptable, fully safe, low cost, natural components. Being given that these patients come to the Clinics laden with aerobic and anaerobic microorganisms causing the syndrome, the efficacy of BASANT as an alternate to the antibiotics for elimination of the infecting microorganisms is noteworthy. Lactobacilli are present in the healthy vagina and perform the important task of bringing down the pH. Pro-vag-Health Probiotics are of human origin meritorious strains of Lactobacilli resident in healthy vagina. Their use recolonizes vagina with Lactobacilli to restore the acidic pH, hence these form part of the treatment in the Combination NAUROZ.

Before NAUROZ can be made available to the public, it is necessary to conduct Phase III trials which we plan to conduct in 120 women employing Metronidazole as comparator. Application has been filed to the Drugs Controller General of India for permission to conduct these trials. Meanwhile an established company HLL Life care (currently making Condoms) will make and supply BASANT capsules. The three meritorious strains of Lactobacilli, grown on a large scale, lyophilized along with stabilizers, will be supplied by M/s Microbax, Hyderabad.

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