A RANDOMISED, DOUBLE BLIND, COMPARATIVE, PROSPECTIVE, PARALLEL GROUP STUDY OF ORAL PROBIOTICS IN FEMALE PATIENTS WITH UROGENITAL INFECTIONS

Dissertation submitted to

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DOCTOR OF MEDICINE

IN

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CERTIFICATE

This is to certify that the dissertation entitled, **"A RANDOMISED, DOUBLE BLIND, COMPARATIVE, PROSPECTIVE, PARALLEL GROUP STUDY OF ORAL PROBIOTICS IN FEMALE PATIENTS WITH UROGENITAL INFECTIONS"** submitted by **Dr.T.Praveena Pearl,** in partial fulfillment for the award of the degree of Doctor of Medicine in Pharmacology by the Tamilnadu Dr.M.G.R. Medical University, Chennai is a bonafide record of the work done by her in the Institute of Pharmacology, Madras Medical College, during the academic year 2004 – 2007.

DEAN
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CONTENTS

INTRODUCTION

INTRODUCTION

The urogenital microflora of a healthy woman comprises approximately 50 species of organisms, [1] that differs in composition according to the reproductive stages and exposure to several factors, including antibiotics[2] and spermicides.[3] Urogenital infections include those that affect the urethra, urinary bladder, vagina and cervix and constitute a worldwide problem that affects more than 300 million women/year.

Common urogenital infections include bacterial vaginosis (BV), vulvovaginal candidiasis (VVC/yeast vaginitis), and urinary tract infections (UTI). At the time of infection in the bladder and vagina, the urogenital flora is often dominated by the infecting pathogens, in contrast with healthy phases when indigenous organisms dominate. Although antimicrobial therapy is generally effective at eradicating these infections, there is still a high incidence of recurrence. The patient's quality of life is affected and there is a cycle of repeated antimicrobial agents whose effectiveness is diminishing due to increasing development of microbial resistance.

There is now growing evidence that certain species and strains present in the healthy urogenital tract protect the host against infection by pathogenic microorganisms. The dominant presence of lactobacilli in the urogenital microflora of healthy women and the obliteration of lactobacilli in patients who develop UTI, [4,5] BV, and many other genital infections has led to a focus on these bacteria.

Lactobacilli are gram-positive rods, primarily facultative or strict anaerobes that generally have a fastidious growth requirement. They prefer an acidic environment and help create one by producing lactic and other acids. In general, lactobacilli have not been associated with disease and for more than 100 years have been regarded as nonpathogenic members of the intestinal and urogenital floras. Premenopausal women have a flora of mostly lactobacilli, and certain properties of these strains, including adhesive ability, production of acids, bacteriocins, hydrogen peroxide, and biosurfactants appear important in conferring protection to the host.

The term probiotic was derived from the Greek, meaning “for life.” An expert panel commissioned by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) defined probiotics as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host.” [6] Ingestion of Probiotics beneficially affects the host by –

1) Replenishing the depleted gut microflora, and
2) Improving the properties of the indigenous microflora.

Two Lactobacilli strains - *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 are clinically documented and they possess antipathogenic properties and colonize in the intestine[7] and in the urogenital tract,[8] conferring health benefits to women.

We conducted this study to evaluate the efficacy of probiotic Lactobacilli strains - *Lactobacillus rhamnosus GR-1* and *Lactobacillus reuteri RC-14* as integral therapy with conventional treatment of female urogenital infections namely -

- Bacterial vaginosis (BV),
- Vulvovaginal candidiasis (VVC/yeast vaginitis) and
- Urinary tract infections (UTI).

REVIEW OF LITERATURE

REVIEW OF LITERATURE

In great number and diversity, microbes inhabit the intestinal tract, skin and urogenital tract, oral and nasal cavities and, in short, any part of the human body that is exposed to the outside world and in which conditions are favorable for bacterial survival. Hundreds of species have been identified as human commensals. Bacterial concentrations reach $\mathbf{10^{13}}$ cells on the human body.[9]

Studies from germ-free animals have proven that animals do not require microbial colonization for survival, but germ-free animals, compared with their conventional counterparts, demonstrate many physiologic and biochemical differences and are more susceptible to infection.[10] This is attributed to a poorly primed immune system and perhaps the absence of what has been termed "competitive colonization" .[11]Competitive colonization is a term describing the interference of virulence of invading pathogens by commensal microbes. The differences between conventional and germ-free animals have provided a basis for the belief that microbial colonization has important health implications for humans.

HISTORICAL PERSPECTIVE

The age-old quote, *“Let food be thy medicine and medicine be thy food*” is certainly the tenet of today. There is a long history of health claims concerning living microorganisms in food, particularly lactic acid bacteria. In 76 BC, a Roman historian recommended the administration of fermented milk

products for treating gastroenteritis.[12] Administration of bifidobacteria was found to be effective in infants suffering from diarrhoea.[13]A Russian Immunologist linked the bacterium *Lactobacillus bulgaricus* in yoghurt to the longevity of Bulgarians who ate large quantities of yoghurt.[14]

PROBIOTICS

In 1965, the term 'probiotic' was used to describe growth promoting factors produced by microorganisms.[15] The term *probiotic* was derived from the Greek word ”pro bios” meaning “for life.” In 2002, an expert panel commissioned by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) defined probiotics as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host.” [6]

MICROORGANISMS USED AS PROBIOTICS

The most common probiotic microorganisms that have been found to be clinically useful include members of the genera Lactobacillus and Bifidobacterium and the species of most interest for efficacy testing include *Lactobacillus acidophilus, L. johnsonii, L. casei, L. gasseri, L. plantarum, L. rhamnosus, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium bifidum and Bifidobacterium infantis*.[16] Table 1 lists down the microorganisms used as probiotics.[17]

TABLE – 1

Microorganisms Used As Probiotics [17]			
A. BACTERIA			
Lactobacillus species	**Bifidobacterium species**	**Other lactic acid bacteria**	**Non-lactic acid bacteria**
L. acidophilus L. amylovorus L. casei L. crispatus L.delbrueckii bulgaricus L. gallinarum L. gasseri L. johnsonii L. paracasei L. plantarum L. reuteri L. rhamnosus	B. adolescentis B. animalis B. bifidum B. breve B. infantis B. lactis B. longum	Enterococcus faecalis Enterococcus faecium Lactococcus lactis Leuconstoc mesenteroides Pediococcus acidilactici Sporolactobacillus inulinus Streptococcus thermophilus Streptococcus lactis Streptococcus intermedius	Bacillus cereus var. toyoi Escherichia coli strain nissle Propionibacterium freudenreichii
B. YEAST AND MOULDS			
Aspergillus cerevisiae, Aspergillus niger, Aspergillus oryzue, Candida intolopesii, Sacharomyces boulardii.			

THE IDEAL PROBIOTIC SUPPLEMENT

A summary of conventional criteria that are used for the selection of microbial strains to be used as probiotics includes the following properties.[18]

1) ***Strain origin*** - Those isolated from the same species as the intended use have an enhanced chance of survival.
2) ***Strain count*** - The supplement should have adequate number of colony forming units (CFUs) to confer a health benefit.[19]To achieve health benefits, probiotic bacteria must be viable and available at a high concentration, typically $\mathbf{10^6–10^7}$ CFUs/gm of product.[20]
3) ***Strain taxonomy*** - The probiotic strain should be identified by an accurate taxonomy. [18]
4) ***Inhabitation*** - Should be a normal inhabitant of the human species.
5) ***Biosafety*** - Probiotics are generally recognized as safe. They should be non-toxic and non-pathogenic.
6) ***Survivability*** - Both in the formulation and after digestion, strains that have improved resistance to acid, bile and adherence to the gut epithelium have better survival characteristics. [21]
7) ***Production characteristics and processing*** - Amenable to production processing: adequate growth in bulk culture, recovery, concentration, freezing, dehydration, storage and distribution.

8) ***Effect on the consumer*** –Should be able to exert one or more clinically documented health benefits. Adverse side effects such as bloating and effects on gut transit should not occur.
9) ***Genetic stability*** – Should be genetically stable.
10) ***Viability*** – Should be viable at high populations during storage and use. [21]
11) ***Produce antimicrobial substances*** – Should be able to produce anti-microbial substances including bacteriocins, H_2O_2 and organic acids.

Lactobacillus rhamnosus strain GG meets most of the above criteria. [21]

PROBIOTICS AS THERAPEUTIC TOOLS

A. Infectious Diarrhoea - Resistance To Enteric Pathogens

Lactobacillus *rhamnosus* GG and *Bifidobacterium lactis* BB-12 are used for the prevention [22] and treatment [23] of acute diarrhea caused by rotaviruses and other enteropathogens. [24] The postulated mechanisms are-

1) Secretory immune effect. [22]
2) Alteration of intestinal conditions to be less favorable for pathogenicity. (pH, short chain fatty acids, bacteriocins)
3) Adherence to intestinal mucosa, interfering with pathogen adherence.[25]
4) Upregulation of intestinal mucin production, interfering with pathogen attachment to intestinal epithelial cells. [24]

B. Anti-Microbial Agent Associated Diarrhoea

A major problem associated with antibiotic treatment is the appearance of diarrhoea, often caused by *Clostridium difficile*. Probiotics have proved useful as a prophylactic regimen, and are used to alleviate the signs and symptoms of antibiotic induced diarrhea.[26]

C. Helicobacter Pylori Infection

In vitro and animal data indicate that lactic acid bacteria inhibit the growth of the H.pylori and decrease the urease enzyme activity necessary for the pathogen to remain in the acidic environment of the stomach.[27]

D. Lactose intolerance

Probiotic bacterial lactase causes hydrolysis of lactose and is used to alleviate the signs and symptoms of lactose intolerance.

E. Immune System Modulation

B. lactis HN019 and *L. rhamnosus* HN001 produce measurable enhancement of immune responses by activating macrophages and by increasing the level and activity of cytokines, natural killer cells and secretory immunoglobulin A. [28]

F. Hepatic Encephalopathy

Probiotics produce inhibition of urease-producing gut flora.

G. Anti Cancer Effect

There is some evidence that cancer recurrences at urinary bladder can be reduced by intestinal instillation of probiotics like *L. casei shirota*[29] which

act by- mutagen binding, carcinogen (nitrosamine) deactivation and inhibition of carcinogen-producing enzymes of colonic microbes. [29]

H. Allergy

Probiotic microorganisms like L*. rhamnosus* GG modulate the immune response and prevent the onset of allergic diseases.[30]

I. Inflammatory Diseases And Bowel Syndromes

Probiotic strains have a potential role in the therapy and prophylaxis of irritable bowel syndrome and inflammatory bowel diseases, such as pouchitis and Crohn's disease. [31]

J. Effects on blood lipids

The proposed hypocholesterolemic action of probiotic strains include assimilation of cholesterol within bacterial cell, antioxidative effect and increased excretion of bile salts due to deconjugation by bile salt hydrolase.[32]

K. Antihypertensive Effect

Bacterial peptidase action yields tripeptides, which function as angiotensin-converting enzyme inhibitors mediating a mild antihypertensive effect.[33]

DYNAMICS OF UROGENITAL MICROFLORA

The microbiological flora of the lower female genital tract is a dynamic, complex example of microbial colonization and what constitutes a pathogen is dependent not only on the type of offending microorganism and its intrinsic virulence but, also, on the species complexity of the flora.

Lactobacilli are both the predominant bacteria in the vaginal tract and the regulator of normal vaginal flora.[34] Lactobacilli by producing lactic acid, maintain the normal vaginal pH of 3.8 to 4.5, and inhibit the adherence of bacteria to vaginal epithelial cells. Estrogen improves lactobacilli colonization by enhancing vaginal epithelial-cell production of glycogen, which breaks down into glucose and acts as a substrate for the bacteria.

Quantitative studies have reported that vaginal washings contain approximately $\mathbf{10^7}$ lactobacilli per gram of secretion. The most common *Lactobacillus* species include *L. acidophilus* and *L. fermentum;* less common are *L. plantarum, L. brevis, L. jensenii, L. casei, L. delbrueckii,* and *L. salivarius.* More than one species may be present in an individual.[34] Although lactobacilli are the dominant bacteria, other bacteria are also present in the vagina, including streptococcal species, gram-negative bacteria, *Gardnerella vaginalis,* and anaerobes. *Candida albicans* is found in normal flora as a commensal agent in 10 to 25% of asymptomatic women.[35]

Scientific studies showed a significant correlation between the absence of hydrogen peroxide producing lactobacilli and vaginal colonization by *G. vaginalis, Bacteroides* species, *Peptostreptococcus* species, and *Mycoplasma hominis.*[36] Inhibitory proteins have been isolated from strains of *Lactobacillus acidophilus*.[37]

The uropathogens and non-pathogenic lactobacilli originate from the host microbial flora and most commonly from the faecal flora [38] as depicted in figure 1.

Figure 1 Bacteria emerging from the colon to colonise the vagina.

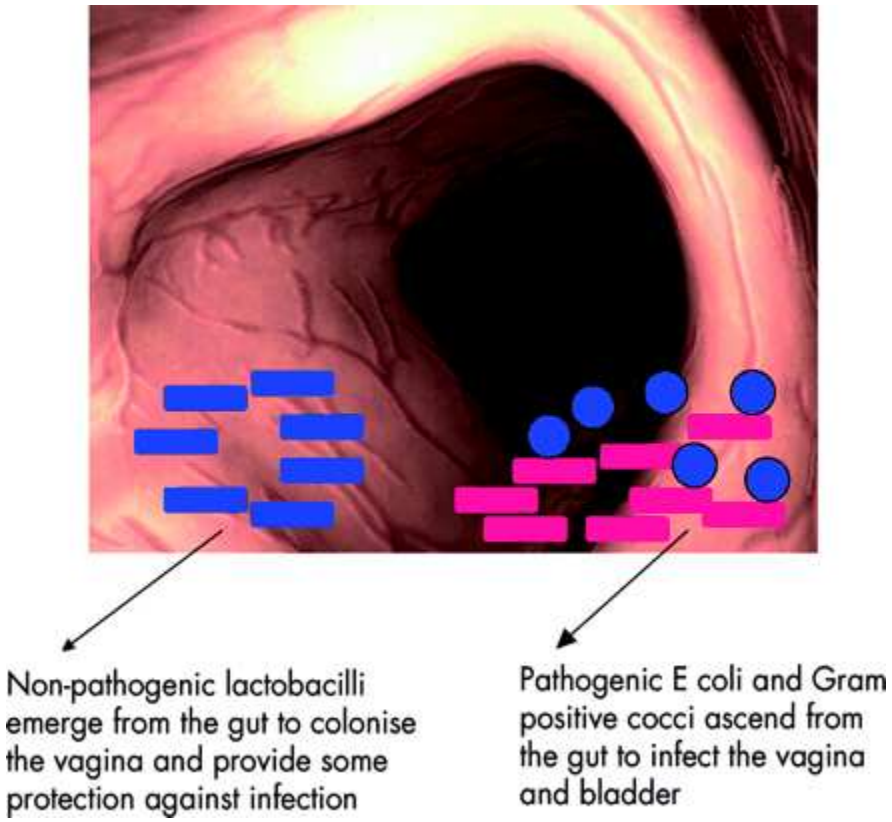


Figure 2 Byproducts of lactobacillus metabolism

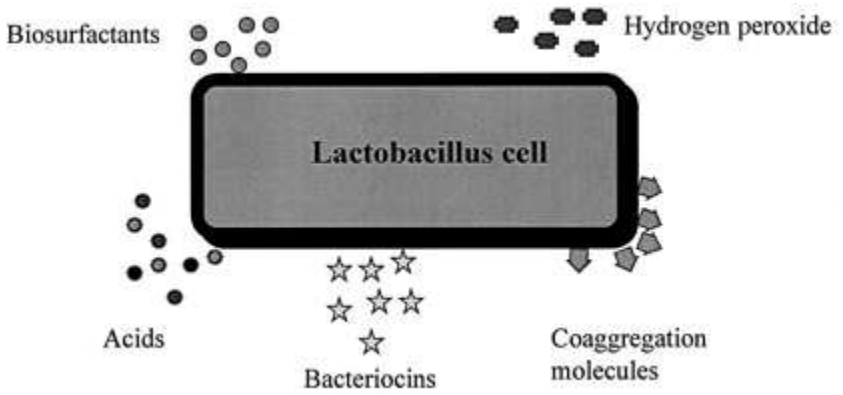


Figure 2 depicts the various byproducts of lactobacillus metabolism like *biosurfactants, acids, bacteriocins, hydrogen peroxide* and *coaggregation* molecules that have an antagonistic effect against urinary and vaginal pathogens. The *biosurfactants* inhibit adhesion; the *acids, bacteriocins, and hydrogen peroxide* inhibit growth; and the *coaggregation* molecules block the spread of the pathogens.[38]

L. rhamnosus GR-1 and *L. reuteri* RC-14 are extensively characterized urogenital isolates which possess a number of properties considered important for urogenital probiotics; both strains adhere to uroepithelial cells and inhibit the growth and adhesion of uropathogens, while GR-1 is resistant to the spermicide nonoxynol 9 and RC-14 produces hydrogen peroxide.[39] Furthermore, studies with humans have shown that these strains are efficacious in the prevention and treatment of urogenital infections in women.[40, 41]

UROGENITAL INFECTIONS

Urogenital infections include those that affect the urethra, urinary bladder, vagina and cervix and constitute a worldwide problem that affects more than 300 million women/year. Common urogenital infections include bacterial vaginosis (BV), vulvovaginal candidiasis (VVC/yeast vaginitis), and urinary tract infections (UTI).

BACTERIAL VAGINOSIS

Bacterial vaginosis previously known as nonspecific vaginitis or Gardnerella vaginitis is the most common cause of acute vaginitis and accounts

for 15 to 50% of the cases in symptomatic women.[42] It is characterized by a disequilibrium in vaginal microflora in which the normally predominant hydrogen peroxide–producing strains of lactobacilli are overgrown by facultative and anaerobic vaginal microorganisms.[43] BV is associated with significant complications like cervicitis[44], endometritis, [45] HIV infection[46] and preterm labour.[47]

The diagnosis of BV is made by the use of clinical criteria or Gram stain. At least three of the following four elements must be present to fulfill the clinical criteria of Amsel et al. for bacterial vaginosis[48]:

- thin, homogeneous, milky vaginal discharge;
- vaginal-fluid pH greater than 4.5;
- a positive whiff test (i.e., production of a fishy odor when 10 percent potassium hydroxide is added to a slide containing vaginal-fluid); and
- clue cells (>20 percent of epithelial cells with adherent bacteria) on microscopic examination of vaginal fluid.

An alternative diagnostic approach is Gram's staining of vaginal fluid to distinguish normal vaginal flora (i.e., gram-positive rods and lactobacilli) from bacterial vaginosis flora according to the Nugent score.[49] The specificity and sensitivity of the Gram stain for diagnosis of BV are 83% and 89% respectively.[50]

Figure 3 Normal vaginal smear: Lactobacillus dominant.

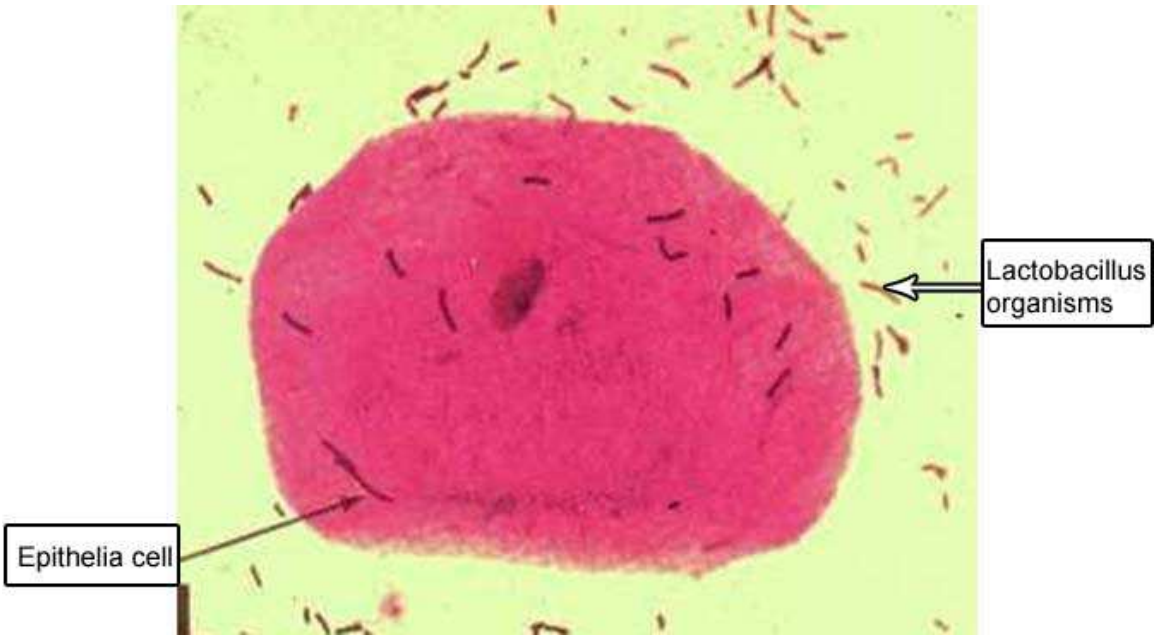


Figure 4 Vaginal smear in bacterial vaginosis

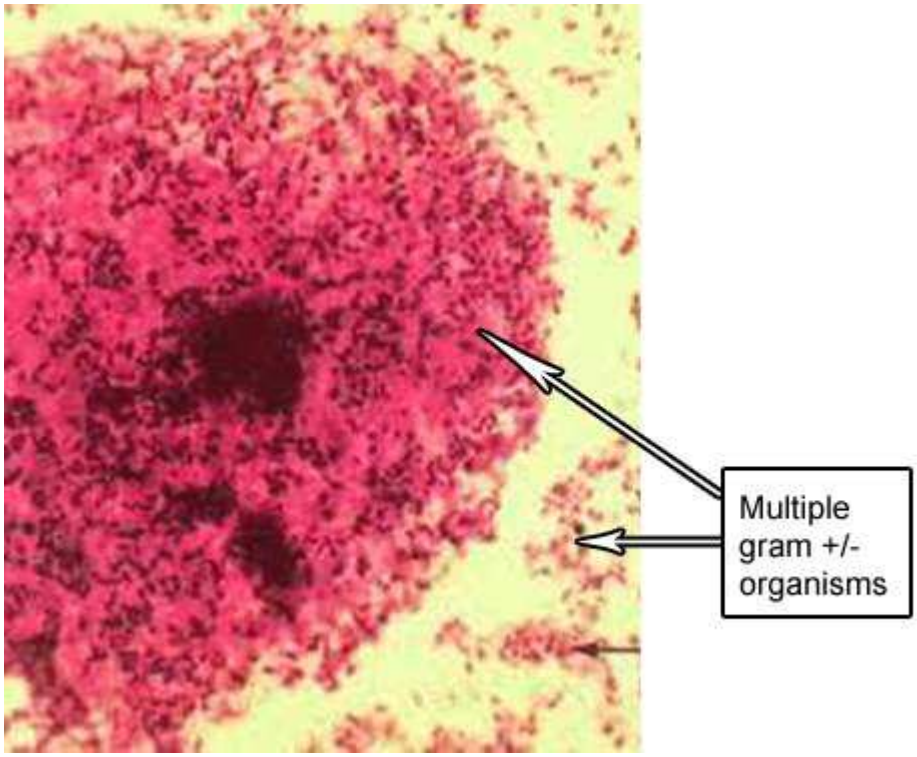


Figure 3 shows the normal vaginal Gram stained smear with dominant lactobacillus (long Gram positive rods). Figure 4 shows the vaginal smear in bacterial vaginosis in which there is few or absent lactobacilli (long Gram positive rods) and greatly increased number of small Gram negative or Gram variable rods or both.

NUGENT'S DIAGNOSTIC CRITERIA FOR BACTERIAL VAGINOSIS[49]

Scoring system (zero to 7+)* is a weighted combination of the following bacterial morphotypes:

A. *Lactobacillus acidophilus* (large gram-positive rods)

B. *Gardnerella vaginalis* and Bacteroides species (small gram-variable or gram-negative rods)

C. Mobiluncus species (curved gram-variable rods)

The total score is the sum of the weighted quantity of the three bacterial morphotypes.

Scoring for each of the above bacterial morphotypes:

Zero = No morphotypes per oil-immersion field

1+ = Less than one morphotype per oil-immersion field

2+ = One to four morphotypes per oil-immersion field

3+ = Five to 30 morphotypes per oil-immersion field

4+ = More than 30 morphotypes per oil-immersion field

For the combined score (A + B + C), zero to 3 represents normal flora, 4 to 6 represents indeterminate, and 7 or higher is diagnostic of bacterial vaginosis

Currently, metronidazole is the agent of choice for the treatment of bacterial vaginosis. This therapy is moderately effective against *G vaginalis* and *Mobiluncus spp* and is inactive against *Mycoplasma hominis* but its metabolites are highly active against anaerobes. There are two recommended dosage regimens for oral metronidazole: 500 mg twice daily for seven days or 2 gm given in a single oral dose.[51] Unfortunately, metronidazole is often poorly tolerated due to side effects, including gastrointestinal upset, alcohol intolerance, metallic taste, and infrequently neurological and or hematological adverse reactions. In addition, cure rates associated with this treatment are low (as low as 61% one month after therapy) [51] and there is a high incidence of overgrowth of pathogenic bacteria after treatment.

The loss of vaginal lactobacilli appears to be the major factor in the cascade of changes leading to bacterial vaginosis[52] and relapses are associated with failure to establish healthy lactobacilli dominated vaginal flora. The ways in which vaginal lactobacillus flora can be optimized are by using pessaries to deliver lactobacillus directly in to the vagina or oral supplementation with lactobacillus probiotics which can colonize in the vagina.

Several studies have been conducted to evaluate the efficacy of different probiotic lactobacillus strains in the treatment of bacterial vaginosis.

A study showed that vaginal douching with yoghurt was ineffective in the treatment of bacterial vaginosis as the lactobacilli in yoghurt failed to colonise in the vagina.[53]

In a double blind, placebo-controlled study, 60 women with bacterial vaginosis were randomised to receive vaginal suppositories of either lyophilized *Lactobacillus acidophilus* or placebo. 16 out of 28 women who were treated with lactobacilli had normal vaginal wet smear results, in comparison to none of the 29 women treated with placebo. Only three of the women who received the Lactobacillus suppository were free of bacterial vaginosis after the subsequent menstruation.[54]

In a clinical trial conducted in 32 women with BV in the first trimester of pregnancy, intravaginal application of yoghurt was found to be effective in the treatment and prevention of bacterial vaginosis at two month follow up.[55]This study concluded that probiotics may be used as an alternative to antimicrobials to resolve BV in pregnancy.

L. rhamnosus GR-1 and *L. reuteri* RC-14 was found to adhere to uroepithelial cells to inhibit the growth and adhesion of uropathogens. [39]These two strains were the first oral probiotic supplementation for restoration and maintenance of a healthy vaginal flora.

In a study conducted in 10 women with recurrent yeast vaginitis, bacterial vaginosis and urinary tract infections, strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 suspended in skim milk were given twice daily for 14 days, were recovered from the vagina and identified by morphology and molecular typing within 1 week of commencement of therapy. Six cases of BV were resolved within 1 week of therapy. [56]

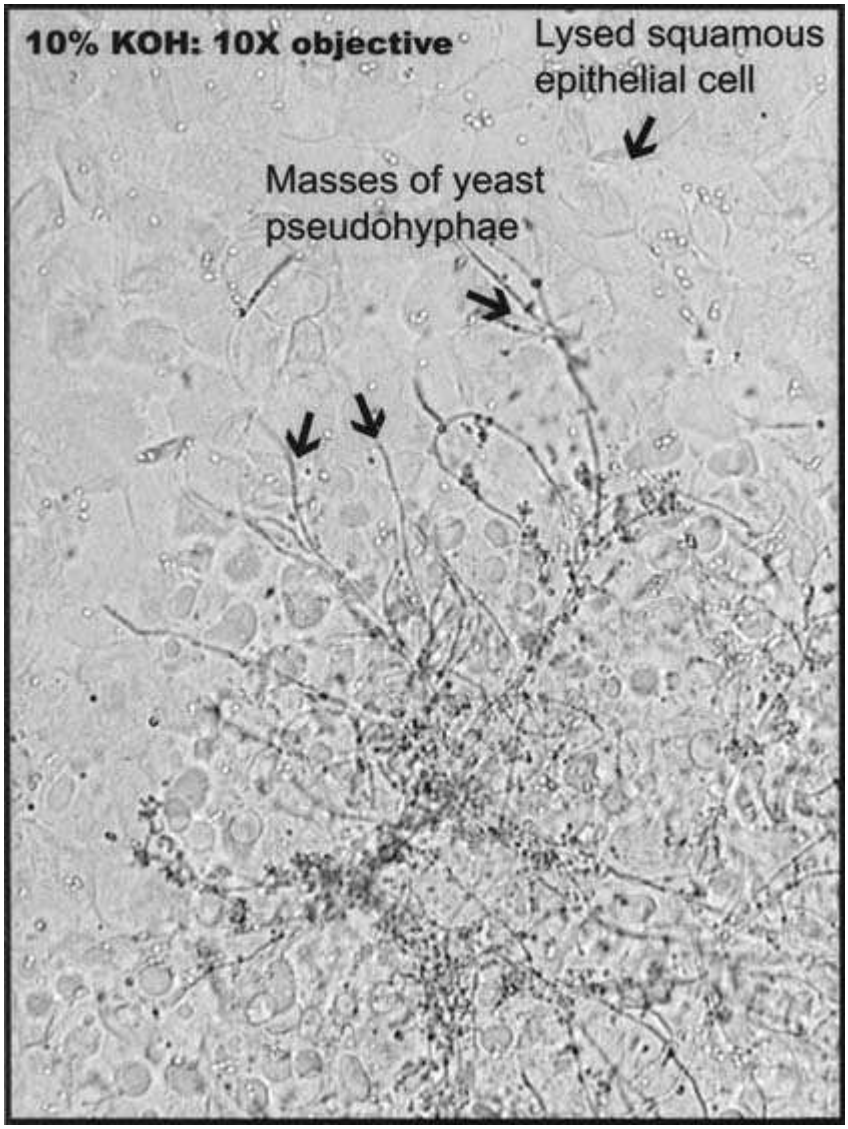
A randomised, placebo-controlled trial of 64 healthy women given daily oral capsules of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 for 60 days showed no adverse effects. Microscopy analysis showed restoration from asymptomatic bacterial vaginosis microflora to a normal lactobacilli colonized microflora in 37% women during lactobacilli treatment compared to 13% on placebo. Lactobacilli were detected in more women in the lactobacilli-treated group than in the placebo group at 28 day and 60-day. The combination of probiotic L. rhamnosus GR-1 and L. fermentum RC-14 reduced the colonization of the vagina by potential pathogenic bacteria and yeast .[57]

VULVOVAGINAL CANDIDIASIS

Approximately 75% of women are diagnosed to have vulvovaginal candidiasis at least once, and of those, about 50% have a recurrence.[58] *Candida* yeasts reside in the mouth, gastrointestinal tract, and vagina without causing symptoms. Symptoms develop only when *Candida* becomes overgrown in these sites. Rarely, *Candida* is transmitted from person to person through sexual intercourse. [58]

Candida albicans is responsible for 80 to 92 percent of episodes of vulvovaginal candidiasis.[59]Recently, an increased frequency of other candida species, particularly *C. glabrata,* has been reported, [60] possibly due to widespread use of over-the-counter drugs, long-term use of suppressive azoles, and the use of short courses of antifungal drugs. Sporadic attacks of vulvovaginal

Figure-5 -- 10% Potassium hydroxide preparation of vaginal smear.



candidiasis usually occur without an identifiable precipitating factor, except in patients with uncontrolled diabetes. Patients with vulvovaginal candidiasis have vaginal discharge, which is classically white, creamy, and curdy (cottage cheese-like) in appearance, adherent to the epithelium and associated with itching, burning, irritation, edema, erythema and or excoriation of the vagina or vulva. [61]
A screening 10% Potassium hydroxide preparation from the inflamed vaginal mucosa reveals yeast forms (hyphae/pseudohyphae) or budding yeasts as shown in figure-5. Topical azole and oral fluconazole are equally efficacious in the management of vulvovaginal candidiasis. [61]

There is little evidence that probiotics can effectively cure a symptomatic yeast vaginitis.

A crossover one year study in which 33 patients with a history of recurrent yeast vaginitis (≥5 per year) were given eight ounces of *L acidophilus* supplemented yoghurt daily for six months then switched to a yoghurt-free diet, resulted in 0.4 breakthrough infections compared with 2.5 per study term.[62]

The significant reduction in number of positive cultures for candida was consistent with the use of *L rhamnosus* GR-1 and *L fermentum* RC-14 in lyophilized capsule form, which showed up to one log fewer yeast recovered from the vagina during treatment compared with baseline.[57]

URINARY TRACT INFECTION

Worldwide, every year it is estimated that several hundred million women suffer from UTI especially from lower UTI, which is the infection of the urethra and urinary bladder. Urinary tract infections are defined by the presence of viable microorganisms within the urinary tract and are usually caused by Gram-negative aerobic organisms originating from the gut flora.[63]

Symptoms of urinary tract infection (UTI) include fever, frequency and urgency of micturition, dysuria, foul-smelling urine, supra pubic pain, vomiting, irritability and scalding pain in the urethra during micturition. The conventional treatment for UTI is antibiotics. Unfortunately, the infection usually recurs (27-48%) once the antibiotic regimen has ended, as bacterial adherence of uropathogens is not altered by the antibiotics. [38]

Extensive studies of various lactobacilli strains and the properties believed to be important for protecting the host, led to selection of a two-strain combination for vaginal use. This comprised of distal urethral isolate *L rhamnosus* GR-1, selected primarily for its anti-Gram negative activities and resistance to spermicide, and *L fermentum* B-54, replaced more recently by RC-14, for anti-Gram positive cocci activities and hydrogen peroxide production. In order to optimise a consistent dose with a good shelf life in a formulation preferred by patients, the organisms were freeze dried and placed in gelatin capsules, with dosage at $\mathbf{10^9}$ per capsule.[64]

Results from various studies indicate that the recurrence rate of UTI was significantly reduced after using one or two capsules vaginally per week for one year, with no side effects or yeast infections.[64] The rate of infection was the same as those found in studies using daily antibiotics for one year.[65]

After going through these references, we decided to evaluate the efficacy and tolerability of two-strain combination of oral probiotics - *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 as integral therapy with conventional treatment in the following female urogenital infections -

- Bacterial vaginosis (BV),
- Vulvovaginal candidiasis (VVC or yeast vaginitis), and
- Urinary tract infection (UTI).

STUDY OBJECTIVES

STUDY OBJECTIVES

- To evaluate the **efficacy** of probiotics as integral therapy with conventional treatment in the following female urogenital infections

  - **Bacterial vaginosis (BV)**,
  - **Vulvovaginal candidiasis (VVC or yeast vaginitis),** and
  - **Urinary tract infection (UTI).**

- To evaluate the **tolerability** of probiotics.

METHODOLOGY

STUDY METHODOLOGY

Study Centre

Department of Female Urology and Urogynaecology,

Government Kasturba Gandhi Hospital for Women and Children, Triplicane, Chennai -5.

Study Design

Randomised, Double blind, Comparative,

Prospective, Parallel group study.

Study Phase

Phase - IV- Clinical Study.

Study Duration

Eight weeks.

Study Period

September 2005 to August 2006.

Study Population

Patients attending the outpatient clinic of the Department of Female Urology and Urogynaecology, Government Kasturba Gandhi Hospital for Women and Children with urinary tract infections, bacterial vaginosis or vulvo vaginal candidiasis fulfilling the recruitment criteria were considered for the study.

Ethical Requirement

The study was performed in accordance with the principles stated in the Declaration of Helsinki. Ethical approval of the study protocol was obtained from the Ethics Committee at the Institution before the study was undertaken.

Informed Consent

Written informed consent was obtained from each patient in the prescribed format prior to performance of any study related procedures: before physical examination, laboratory screening or any other investigational procedure and before administration of any study related medication. The patients were given full information about the nature, procedure and importance of the study. If the patient was illiterate, an impartial witness (a person, who was independent of the trial and who cannot be unfairly influenced by people involved in the trial) attended the informed consent process and read out and explained the procedure to the patient in a language known to the patient.

Inclusion Criteria

- Females in the reproductive age group from 18 to 45 years.
- Patients with history of symptoms consistent with diagnosis of bacterial vaginosis like abnormal, profuse, white and homogenous vaginal discharge confirmed by an elevated vaginal pH >4.5 and Gram staining of vaginal discharge showing few or absent lactobacilli (long Gram positive rods) with greatly increased number of small Gram negative or Gram variable rods or both.

- Patients with history of symptoms consistent with diagnosis of vulvovaginal candidiasis like itching, burning, irritation, edema and or excoriation of the vagina or vulva with a white, creamy and curdy (cottage cheese like) vaginal discharge confirmed by 10% KOH preparation revealing yeast forms (Hyphae or pseudohyphae or budding yeasts).
- Patients with history of symptoms consistent with diagnosis of lower urinary tract infections (UTI) like fever, frequency and urgency of micturition, dysuria, foul-smelling urine, supra pubic pain, vomiting, irritability and scalding pain in the urethra during micturition and the clinical diagnosis confirmed by microscopic examination of urine and mid stream urine culture positive for pathogenic bacteria.

Exclusion Criteria

- Pregnant and lactating women.
- Patients with history of antibiotic administration in the past one month.
- Patients with history of probiotic or synbiotic administration in the past three months.
- Patients with history of vaginal discharge due to other causes like stricture, fistula, congenital abnormality and malignancy.

- ❖ Patients with clinically significant renal, hepatic, cardiovascular, haematopoetic, pulmonary, gastrointestinal, nervous or endocrine disorders except uncomplicated diabetes mellitus.
- ❖ Patients with history of surgery in urogenital system except episiotomy and tubectomy.
- ❖ Patients with vaginal or cervical smear negative for pathogenic organisms.

Laboratory Investigations

- **Complete Haemogram:** Haemoglobin, Total count, Differential count, Platelet count and Erythrocyte sedimentation rate.

- **Routine Urine Analysis:** Albumin, sugar and microscopic examination of urine for white blood cells, red blood cells and casts.

- **Midstream Urine Colony count and Culture-Sensitivity**: for patients with urinary tract infection.
- **Vaginal or cervical swab or smear examination**:

  GRAM STAIN -- NUGENT'S SCORE

  10% Potassium hydroxide vaginal smear preparation.

- **Vaginal pH monitoring:**

  A cotton-tipped swab was touched to the sidewall of the vagina midway between the introitus and the cervix and brought in contact with a pH paper (expanded in the range of 3.6 to 6.1 pH). The colour developed in the pH paper was compared with a standard pH paper indicator chart.

  Vaginal **pH >4.5** is consistent with BV.

- **Blood Chemistry**:

  Blood Sugar, Blood Urea, Serum Creatinine, Serum bilirubin, SGOT, SGPT, Serum alkaline phosphatase, Serum total proteins.

- **Ultrasound Abdomen**

Study Procedures

Visit I -Screening

Patients attending the outpatient clinic of the Department of Female Urology and Urogynaecology, Government Kasturba Gandhi Hospital for Women and Children with symptoms of urinary tract infections, bacterial vaginosis or vulvovaginal candidiasis were screened. The patients were given the informed consent form and were asked to read it completely. If they were satisfied, they were asked to sign in the informed consent form. During screening, a complete examination was done which included detailed medical history and physical examination. All patients underwent urogenital examination

in which examination of vagina, cervix and external urethra was done. Vaginal or cervical smear was taken and sent for laboratory analysis. Vaginal pH was monitored. Nugent score for bacterial vaginosis was done. In vulvovaginal candidiasis, clinical assessment was based on *composite signs/symptoms score* in which each evaluated sign and/or symptom like itching, burning, irritation, edema, erythema and/or excoriation of the vagina/vulva were given a numerical rating based on severity (absent = 0; mild = 1; moderate = 2; severe = 3). Complete haemogram and blood chemistry was done for all the patients. Midstream urine routine analysis, colony count and culture-sensitivity were done. Ultrasound abdomen was done for all patients to rule out kidney, urinary bladder, uterine and ovarian abnormalities (congenital or pathological). The patients were instructed to return after 2 days.

Visit- II- Recruitment

The laboratory results were analysed with the clinical features and physical examination. Those who fulfilled the inclusion criteria were recruited for the study. Demographic details like address and contact number were recorded. Patients were divided in to three groups based on the type of urogenital infection.

Patients with confirmed diagnosis of bacterial vaginosis were assigned to Group I. Patients with confirmed diagnosis of vulvovaginal candidiasis were assigned to Group II. Patients with confirmed diagnosis of urinary tract infection were assigned to Group III.

- *Randomisation to probiotic or placebo*

Patients of each group (Group I- BV; Group II – VVC; Group III –UTI) were randomly assigned to receive either of the two study therapies –either integral therapy of probiotics with conventional treatment or placebo with conventional treatment.

- Patients with bacterial vaginosis (Group I) received metronidazole 400 mg orally twice daily for one week and randomly assigned to receive probiotic or placebo 150 mg capsules twice daily for eight weeks.
- Patients with vulvovaginal candidiasis (Group II) received clotrimazole 1% vaginal cream applied 5 gm intravaginally per day for one week and randomly assigned to receive probiotic or placebo 150 mg capsules twice daily for eight weeks.
- Patients with urinary tract infection (Group III) received the appropriate sensitive antibacterial for one week and randomly assigned to receive probiotic or placebo 150 mg capsules twice daily for eight weeks.

The study drugs were supplied by Tablets India Limited. The probiotic and placebo capsules were matched for size, shape and volume of contents. The placebo was microcrystalline cellulose. The study was a double blind comparative study. No patient was entered more than once in the study. Each patient was randomised after ensuring that recruitment criteria are met.

Study medication according to the randomised schedule was issued for 2 weeks. Patients were instructed to report to the outpatient clinic of the Department of Female Urology and Urogynaecology, Government Kasturba Gandhi Hospital for women and children after 2 weeks along with the empty medication bottle.

Follow up

Urogenital examination and clinical assessment of symptoms were done at the end of week 2, week 4, week 6 and at the end of week 8. Study medication according to the randomised schedule was issued every 2 weeks. Adverse event if any, observed or reported by the patients were recorded.

Apart from routine urogynaecological examination and symptom assessment, the following were done at the end of 4^{th} and 8^{th} week.

- For patients with Bacterial vaginosis (Group I), vaginal pH monitoring and Gram staining of vaginal smear for Nugent scoring were done.
- For patients with vulvovaginal candidiasis (Group II), vaginal pH monitoring, 10% KOH preparation of vaginal smear and composite sign/symptom score were done.
- For patients with urinary tract infection (Group III), routine urine analysis, colony count and culture-sensitivity were done.

Complete blood count, blood sugar, blood urea, serum creatinine, serum bilirubin, SGOT, SGPT, serum alkaline phosphatase and serum total proteins were done at the end of eighth week in all the treatment groups. Patients were advised to report to the outpatient clinic of the study centre if they had recurrence of symptoms after completion of study.

Withdrawal and dropouts

Patients were withdrawn from the study by the physician if any adverse effect was observed. For all patients who dropped out of the study, efforts were made to ascertain the reason for dropout.

Decoding was done at the end of the study.

Statistical report

Data were analysed using SPSS 11.5. Descriptive analysis for non-parametric variables was expressed in *proportion* and parametric variables in *mean* and *standard deviation*. The treatment difference was assessed using *t test* for independent samples for parametric variables and by *Chi square test* for non-parametric variables. Statistical significance was assessed using p at 0.05 cut off or 95% confidence interval. (95% CI).

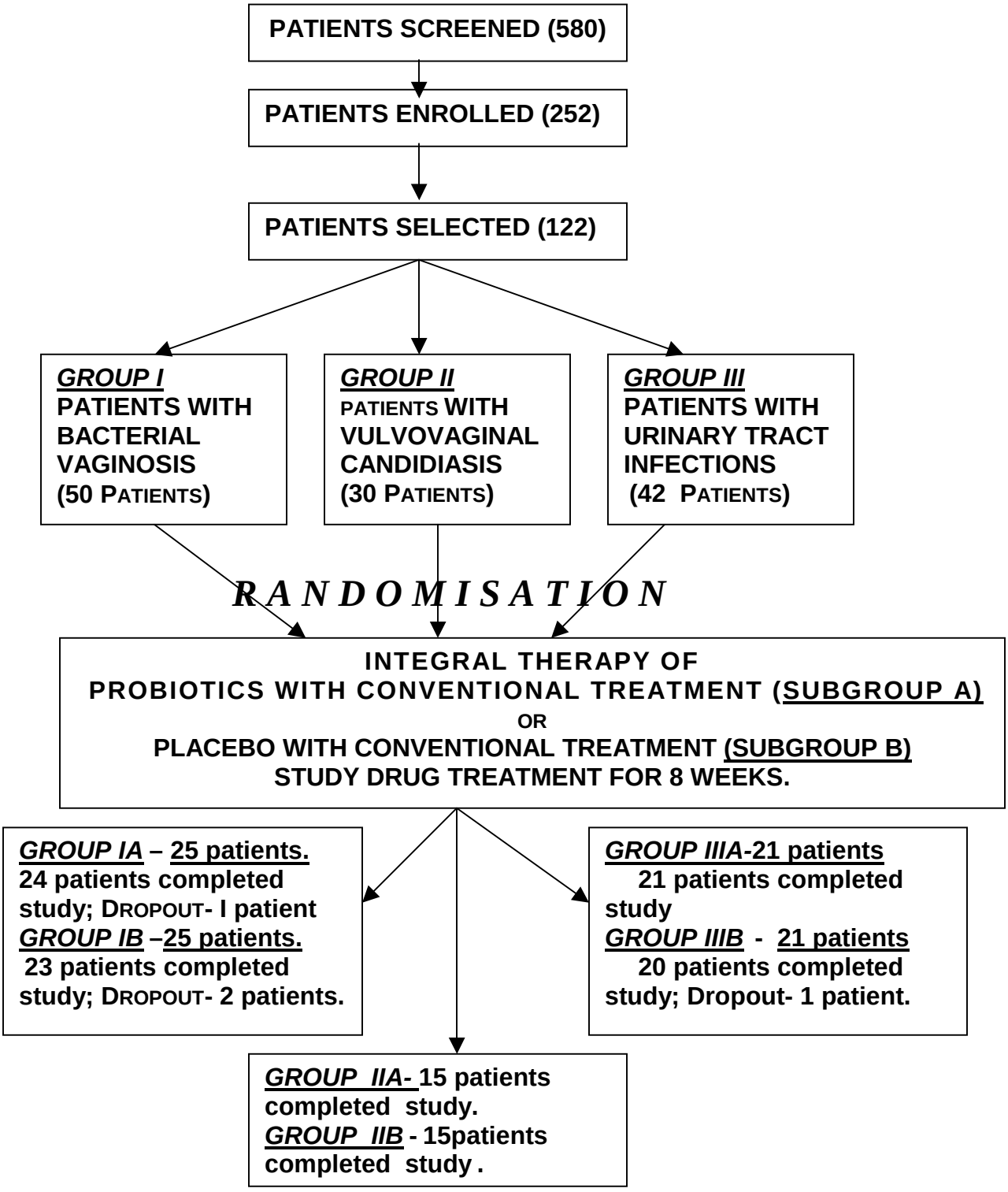
RESULTS

RESULTS

A total of **580** patients were screened for the study. Among them, **252** patients were enrolled. Only **122** patients fulfilled the inclusion criteria and were divided in to three groups based on the type of urogenital infection -

- **GROUP I**: Patients with bacterial vaginosis (50 patients),
- **GROUP II**: Patients with vulvovaginal candidiasis (30 patients),
- **GROUP III**: Patients with urinary tract infection (42 patients).

Patients of each group were randomly allocated to subgroups A or B to receive either of the two study therapies –either integral therapy of probiotics with conventional treatment (**subgroup A**) or placebo with conventional treatment (**subgroup B**). Each 150mg capsule of the probiotic mixture had a minimum of 5 billion CFU of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 in equal proportions. The placebo was microcrystalline cellulose. Twenty-four patients of group IA and twenty-three patients of group IB completed the study. Fifteen patients of group II A and fifteen patients of group II B completed the study. Twenty-one patients of group III A and twenty patients of group III B completed the study. There were four-drop outs (1 patient in group IA, 2 patients in group I B and 1 patient in group III B). Drop out patients of group I A, III B and one patient of group I B refused to take the medication and did not return after the second visit. One patient of group I B had intercurrent illness and discontinued from the intervention. The following flow chart explains the progress of participants through the trial.


PATIENTS SCREENED (580)
PATIENTS ENROLLED (252)
PATIENTS SELECTED (122)
GROUP I
PATIENTS WITH BACTERIAL VAGINOSIS
(50 PATIENTS)
GROUP II
PATIENTS WITH VULVOVAGINAL CANDIDIASIS
(30 PATIENTS)
GROUP III
PATIENTS WITH URINARY TRACT INFECTIONS
(42 PATIENTS)
RANDOMISATION
INTEGRAL THERAPY OF
PROBIOTICS WITH CONVENTIONAL TREATMENT (SUBGROUP A)
OR
PLACEBO WITH CONVENTIONAL TREATMENT (SUBGROUP B)
STUDY DRUG TREATMENT FOR 8 WEEKS.
GROUP IA – 25 patients.
24 patients completed study; DROPOUT- I patient
GROUP IB –25 patients.
23 patients completed study; DROPOUT- 2 patients.
GROUP IIIA-21 patients
21 patients completed study
GROUP IIIB - 21 patients
20 patients completed study; Dropout- 1 patient.
GROUP IIA- 15 patients completed study.
GROUP IIB - 15patients completed study .

GROUP-I-BACTERIAL VAGINOSIS

DEMOGRAPHIC FEATURES

Table 2- Age distribution

Drug Group	No. of patients	Mean Age	Std. Deviation	P	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Probiotic Group IA	24	32.7	6.4	0.52	1.14	-2.37	4.66
Placebo Group IB	23	31.6	5.5				

Figure 6- Mean age distribution

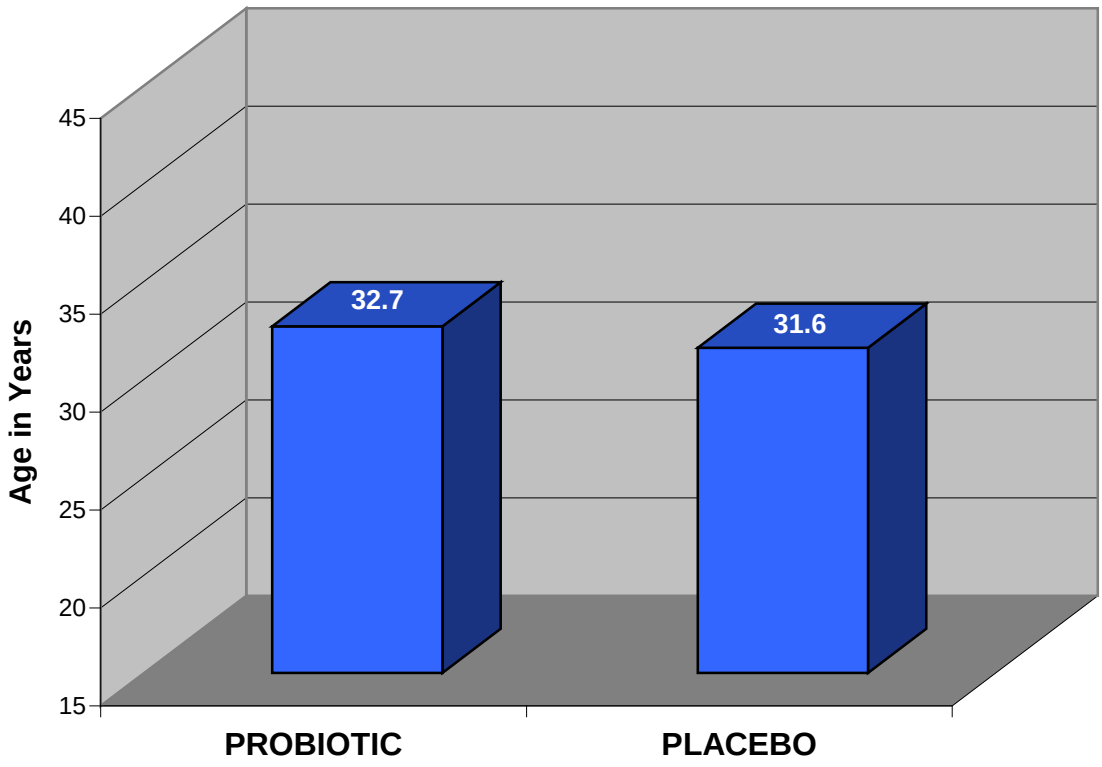


Table-2 shows

- The number of bacterial vaginosis patients (group I) in probiotic (group IA) and placebo subgroups (group I B).
- The mean age of patient in probiotic (group IA) and placebo subgroup (group I B).
- The mean age of patient in probiotic (group IA) subgroup was 32.7
- The mean age of patient in placebo subgroup (group I B) was 31.6
- The difference between the probiotic (group IA) and placebo subgroups (group I B) was not statistically significant (95% confidence interval, -2.37 to 4.66; p= 0.52).

Figure-6 is the diagrammatic representation of the mean age distribution in the probiotic and placebo groups.

Table 3 –Comparison of mean vaginal pH

Visits	Drug Group	Mean vaginal pH	Std. Deviation	p	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Baseline	Probiotic	5.4	0.4	0.36	0.09	-0.11	0.29
	Placebo	5.3	0.3				
After 4 weeks	Probiotic	5.1	0.5	0.64	-0.07	-0.36	0.23
	Placebo	5.1	0.5				
After 8 weeks	Probiotic	4.8	0.4	0.00	-0.36	-0.58	-0.14
	Placebo	5.1	0.4				

Figure 7–Comparison of mean vaginal pH

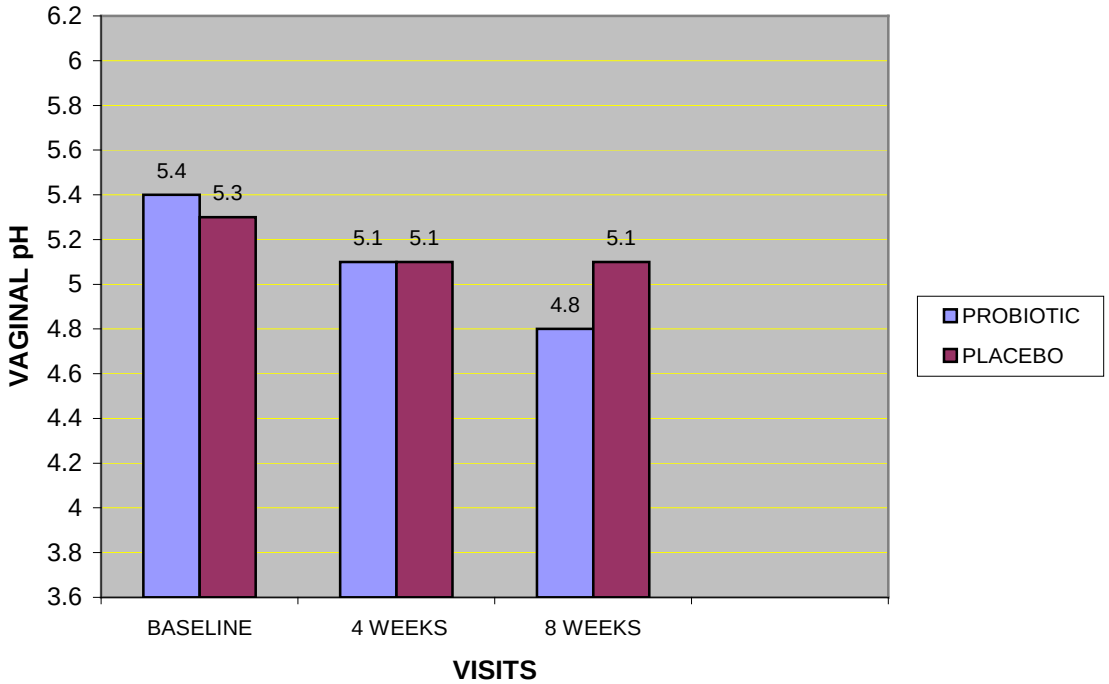


Table 3 –shows

- The mean vaginal pH in the probiotic and placebo groups at baseline and after 4 weeks and 8 weeks of study therapy.
- The difference in mean vaginal pH between the probiotic and placebo groups at the baseline was not statistically significant (95% confidence interval**, -**0.11 to 0.29; p= 0.36).
- The difference in mean vaginal pH between the probiotic and placebo groups after 4 weeks of study therapy was not statistically significant (95% confidence interval**,** -0.36 to 0.23; p= 0. 64).
- The difference in mean vaginal pH between the probiotic and placebo groups after 8 weeks of study therapy was statistically significant (95% confidence interval**,** -0.58 to 0.14; p= 0. 00).

Figure 7 – is the diagrammatic representation of the mean vaginal pH in the probiotic and placebo groups at baseline and after 4 weeks and 8 weeks of study therapy.

Table-4 - Comparison of mean Nugent score

Visits	Drug Group	Mean Nugent score	Std. Deviation	p	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Baseline	Probiotic	8.5	1.1	0.95	-0.02	-0.66	0.62
	Placebo	8.5	1.1				
After 4 weeks	Probiotic	5.5	1.4	0.00	-1.15	-1.88	-0.42
	Placebo	6.6	1.0				
After 8 weeks	Probiotic	4.6	1.1	0.03	-0.81	-1.56	-0.06
	Placebo	5.4	1.4				

Figure-8 - Comparison of mean Nugent score

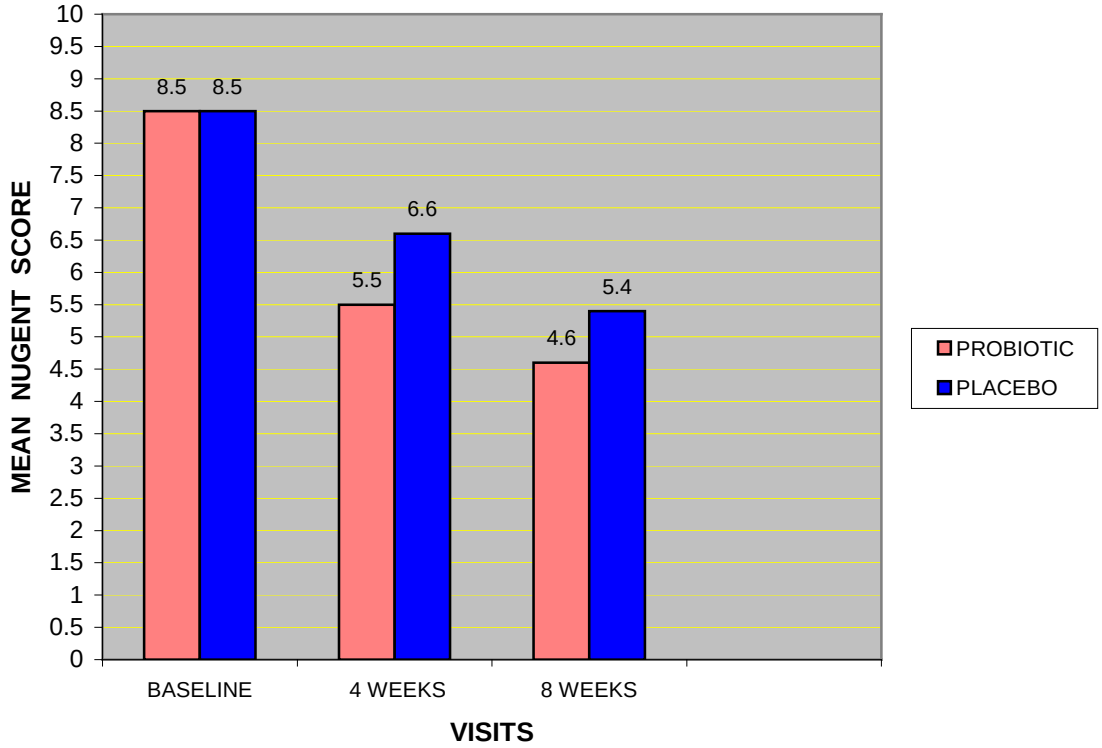


Table 4 –shows

- The mean Nugent score in the probiotic and placebo groups at baseline and after 4 weeks and 8 weeks of study therapy.
- The difference in mean Nugent score between the probiotic and placebo groups at the baseline was not statistically significant (95% confidence interval**, -**0.66 to 0.62; p= 0.95).
- The difference in mean Nugent score between the probiotic and placebo groups after 4 weeks of study therapy was statistically significant (95% confidence interval**,** -1.88 to -0.42; p= 0. 00).
- The difference in mean Nugent score between the probiotic and placebo groups after 8 weeks of study therapy was statistically significant (95% confidence interval**, -**1.56 to –0.06; p= 0. 03).

Figure 8 – is the diagrammatic representation of the mean Nugent score in the probiotic and placebo groups at baseline and after 4 weeks and 8 weeks of study therapy.

Table 5 – Comparison of frequency of vaginal discharge characteristic of bacterial vaginosis.

Variable		Group				Total	Chi Square	p
		Probiotic		Placebo				
		No of subjects	%	No of subjects	%			
Baseline	Present	24	100%	23	100%	47		
	Absent	0	0%	0	0%	0	NA	NA
4 Weeks	Present	6	25%	11	47.8%	17		
	Absent	18	75%	12	52.2%	30	2.65	0.1
8 Weeks	Present	3	12.5%	11	47.8%	14		
	Absent	21	87.5%	12	52.2%	33	7.01	0.008

Figure 9-Comparison of percentage of patients with characteristic vaginal discharge of bacterial vaginosis.

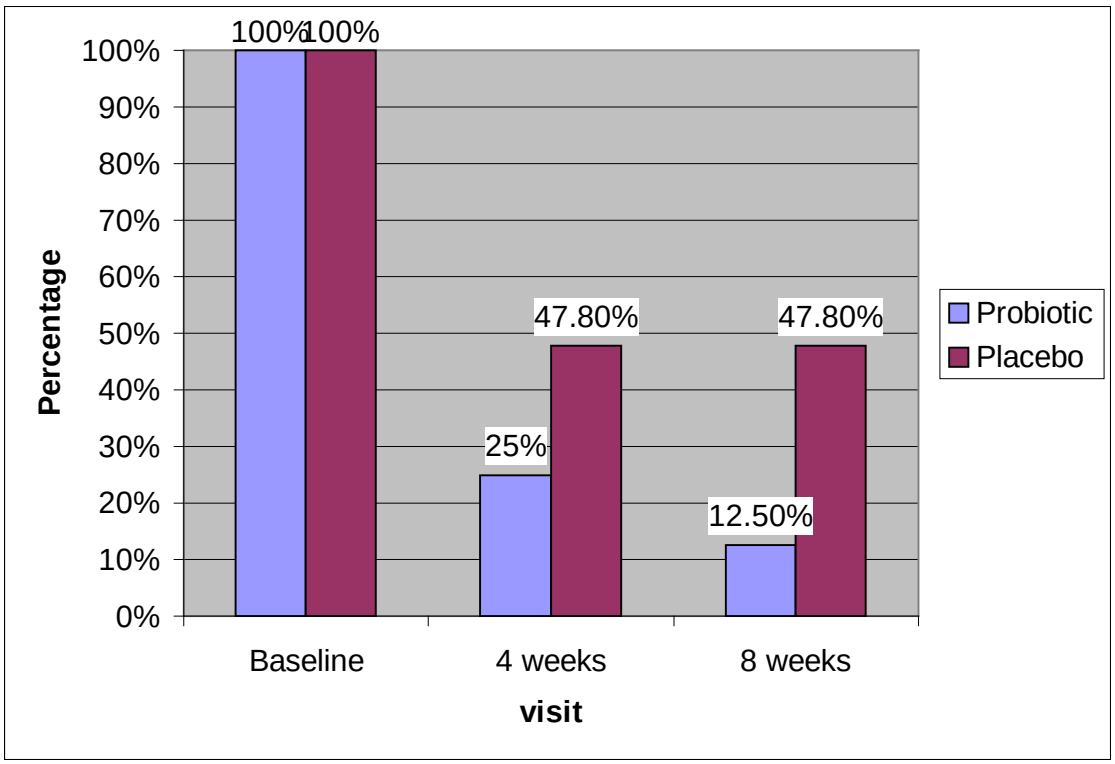


Table 5 shows

- The number and percentage of patients in probiotic and placebo groups with characteristic vaginal discharge of bacterial vaginosis.
- The treatment difference was assessed using Chi square test.
- At baseline, all the patients of probiotic and placebo group had vaginal discharge characteristic of bacterial vaginosis.
- There was no significant difference in the frequency of vaginal discharge characteristic of bacterial vaginosis between the probiotic and placebo groups at 4 weeks of study therapy (p=0.1).
- There was a statistically significant difference in the frequency of vaginal discharge characteristic of bacterial vaginosis between the probiotic and placebo groups at 8 weeks of study therapy (p=0.008).

Figure 9- represents a diagrammatic representation of comparison of percentage of observed characteristic vaginal discharge of bacterial vaginosis in probiotic and placebo groups at baseline, and after 4 weeks and 8 weeks of study therapy.

Table 6 – Comparison of frequency of foul smelling odor of vaginal discharge.

Variable		Group				Total	Chi Square	p
		Probiotic		Placebo				
		No of subjects	%	No of subjects	%			
Baseline	Present	24	100%	23	100%	47		
	Absent	0	0%	0	0%	0	NA	NA
4 Weeks	Present	4	16.7%	7	30.4%	11		
	Absent	20	83.3%	16	69.6%	36	1.24	0.26
8 Weeks	Present	2	8.3%	7	30.4%	9		
	Absent	22	91.7%	16	69.6%	38	3.706	0.054

Figure 10-Comparison of percentage of patients with characteristic foul-smelling odor of vaginal discharge in the study groups.

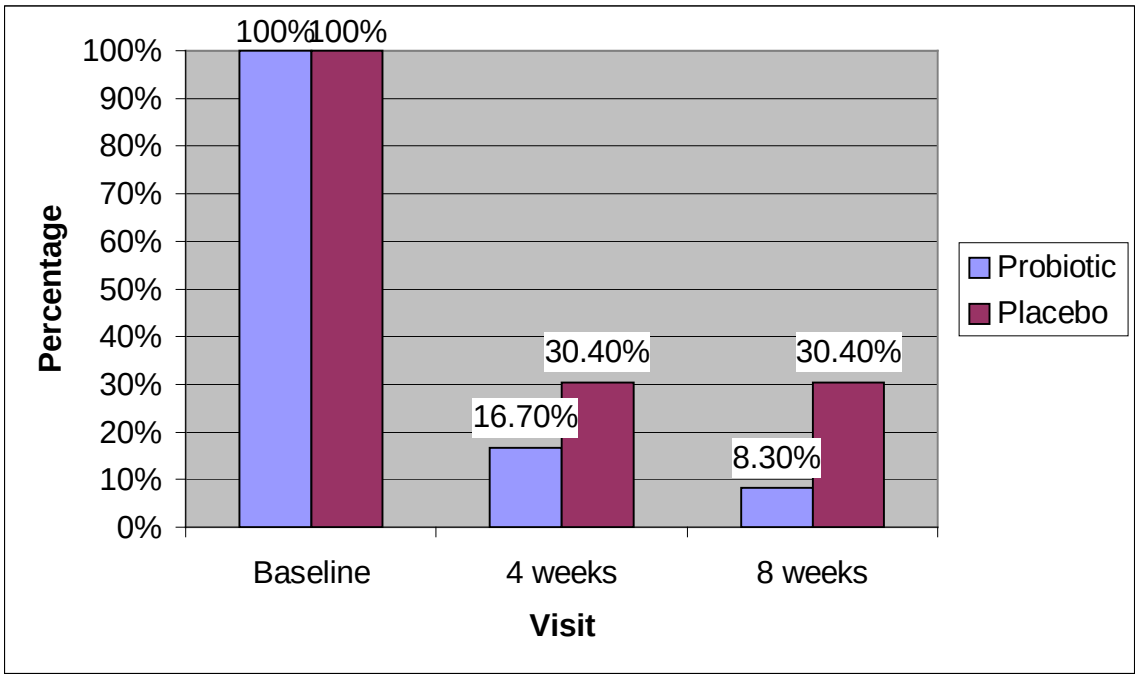


Table 6 shows

- The number and percentage of patients in probiotic and placebo groups with foul smelling odor of vaginal discharge.
- The treatment difference was assessed using Chi square test.
- At baseline, all the patients of probiotic and placebo group had foul smelling odor of vaginal discharge.
- There was no significant difference in the frequency of foul smelling odor of vaginal discharge between the probiotic and placebo groups at 4 weeks of study therapy (p=0.26).
- The difference in the frequency of foul smelling odor of vaginal discharge between the probiotic and placebo groups at 8 weeks of study therapy was not significant (p=0.054).

Figure 10- represents a diagrammatic representation of comparison of percentage of patients with foul smelling odor of vaginal discharge in probiotic and placebo groups at baseline, and after 4 weeks and 8 weeks of study therapy.

Table 7

Variables	Group	Mean	Std. Deviation	p
Blood Urea Baseline	Probiotic	25.5	2.9	**0.98**
	Placebo	25.5	2.8	
Blood Urea 8 Wks	Probiotic	24.9	2.9	
	Placebo	24.7	2.3	**0.82**
Blood Sugar Baseline	Probiotic	96.8	15.4	
	Placebo	99.5	15.0	**0.54**
Blood Sugar 8 Wks	Probiotic	95.67	15.16	
	Placebo	104.20	15.74	**0.71**
SrCreatinine Baseline	Probiotic	0.7	0.1	
	Placebo	0.8	0.1	**0.57**
SrCreatinine 8 Wks	Probiotic	0.8	0.2	**0.83**
	Placebo	0.8	0.1	
SGOT Baseline	Probiotic	18.9	2.7	**0.55**
	Placebo	18.4	2.8	
SGOT 8 Wks	Probiotic	19.0	2.3	**0.24**
	Placebo	18.2	2.2	
SGPT Baseline	Probiotic	13.3	2.5	**0.54**
	Placebo	12.8	2.6	
SGPT 8 Wks	Probiotic	13.3	2.3	**0.56**
	Placebo	12.9	2.1	
TotProt Baseline	Probiotic	6.0	0.2	**0.20**
	Placebo	5.9	0.1	
TotProt 8 Wks	Probiotic	6.0	0.2	**0.29**
	Placebo	5.9	0.2	

The above table depicts the difference between probiotic and placebo groups in blood urea, blood sugar, serum creatinine, SGOT, SGPT and total proteins taken at baseline and end of 8 weeks. By independent samples t test, the difference between the treatment groups was not statistically significant (p value > 0.05).

GROUP-II-VULVOVAGINAL CANDIDIASIS

Demographic features

Table 8- Mean age distribution

Drug Group	No. of patients	Mean Age	Std. Deviation	P	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Probiotic	15	29.73	5.98	0.82	-0.53	-5.30	4.23
Placebo	15	30.27	6.73				

Figure 11- Mean age distribution

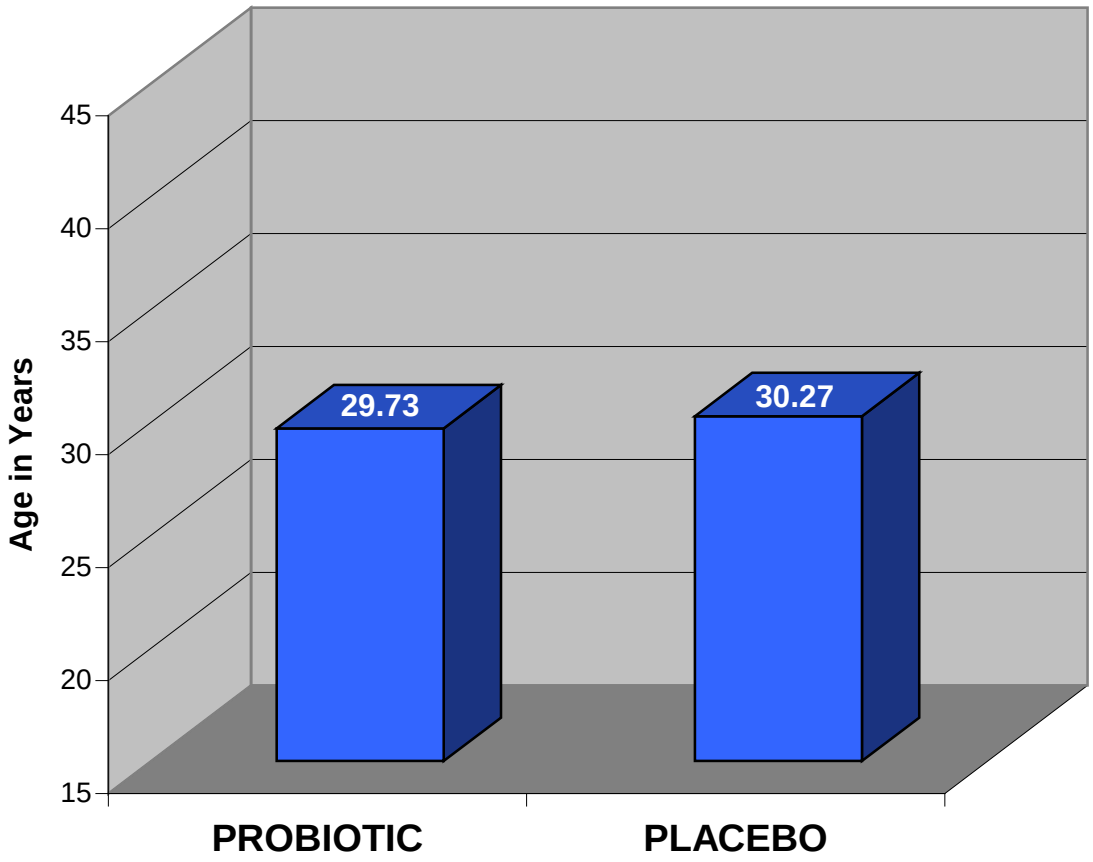


Table-8 shows

- The number of patients with vulvovaginal candidiasis (group II) in probiotic (group IIA) and placebo subgroups (group II B).
- The mean age of patient in probiotic (group IIA) and placebo subgroup (group II B).
- The mean age of patient in probiotic (group IIA) subgroup was 29.73
- The mean age of patient in placebo subgroup (group II B) was 30.27
- The difference between the probiotic (group IIA) and placebo subgroups (group II B) was not statistically significant (95% confidence interval, -5.30 to 4.23; p= 0.82).

Figure-11 is the diagrammatic representation of the mean age distribution in the probiotic and placebo groups

Table 9 –Comparison of mean vaginal pH

Visits	Drug Group	Mean vaginal pH	Std. Deviation	p	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Baseline	Probiotic	4.17	0.24	0.72	-0.03	-0.22	0.15
	Placebo	4.20	0.25				
After 4 weeks	Probiotic	4.17	0.24	0.72	-0.03	-0.22	0.15
	Placebo	4.20	0.25				
After 8 weeks	Probiotic	4.23	0.26	0.07	0.07	-0.12	0.25
	Placebo	4.17	0.24				

Figure 12 –Comparison of mean vaginal pH

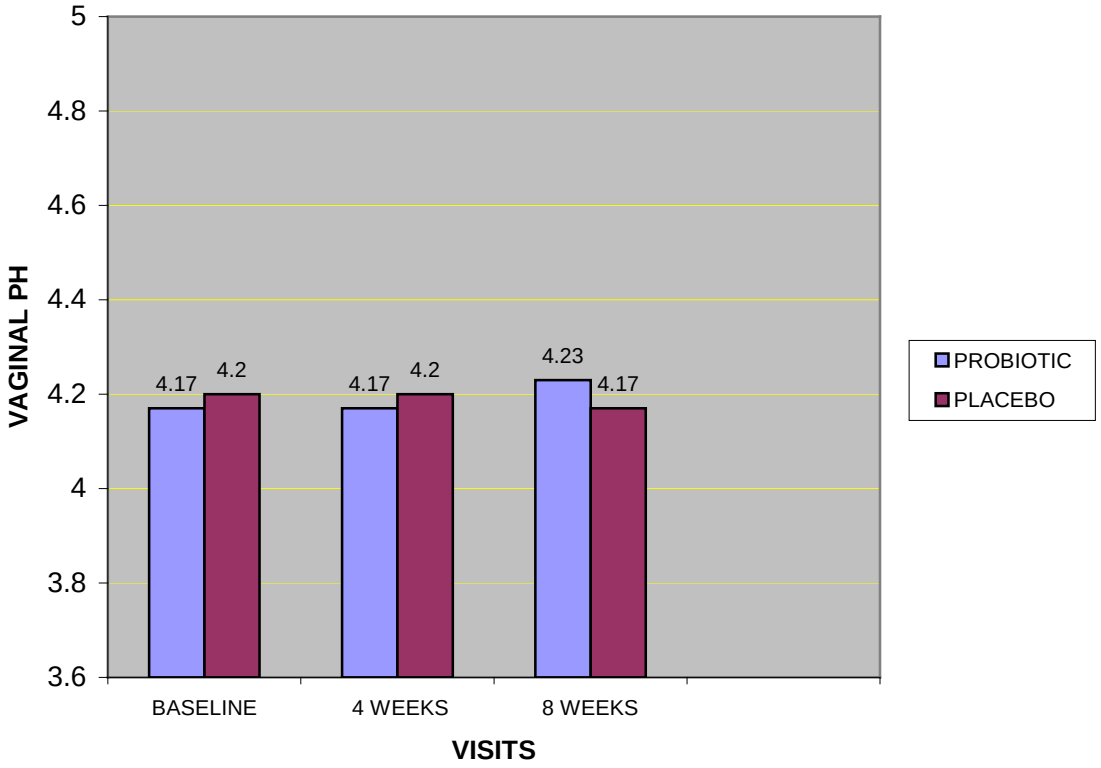


Table 9 –shows

- The mean vaginal pH in the probiotic and placebo groups at baseline and after 4 weeks and 8 weeks of study therapy.
- The difference in mean vaginal pH between the probiotic and placebo groups at the baseline was not statistically significant (95% confidence interval**, -**0.22 to 0.15; p= 0.72).
- The difference in mean vaginal pH between the probiotic and placebo groups after 4 weeks of study therapy was not statistically significant (95% confidence interval**,** -0.22 to 0.15; p= 0. 72).
- The difference in mean vaginal pH between the probiotic and placebo groups after 8 weeks of study therapy was not statistically significant (95% confidence interval, -0.12 to 0.25; p= 0. 07).

Figure 12 – is the diagrammatic representation of the mean vaginal pH in the probiotic and placebo groups at baseline and after 4 weeks and 8 weeks of study therapy.

Table 10- Comparison of mean composite sign/ symptom score in VVC.

Visits	Drug Group	Mean	Std. Deviation	P	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Baseline	Probiotic	9.20	1.37	0.39	0.47	-0.62	1.56
	Placebo	8.73	1.53				
After 4 weeks	Probiotic	3.80	1.74	0.72	0.20	-0.91	1.31
	Placebo	3.60	1.18				
After 8 weeks	Probiotic	1.80	1.26	0.72	-0.20	-1.32	0.92
	Placebo	2.00	1.69				

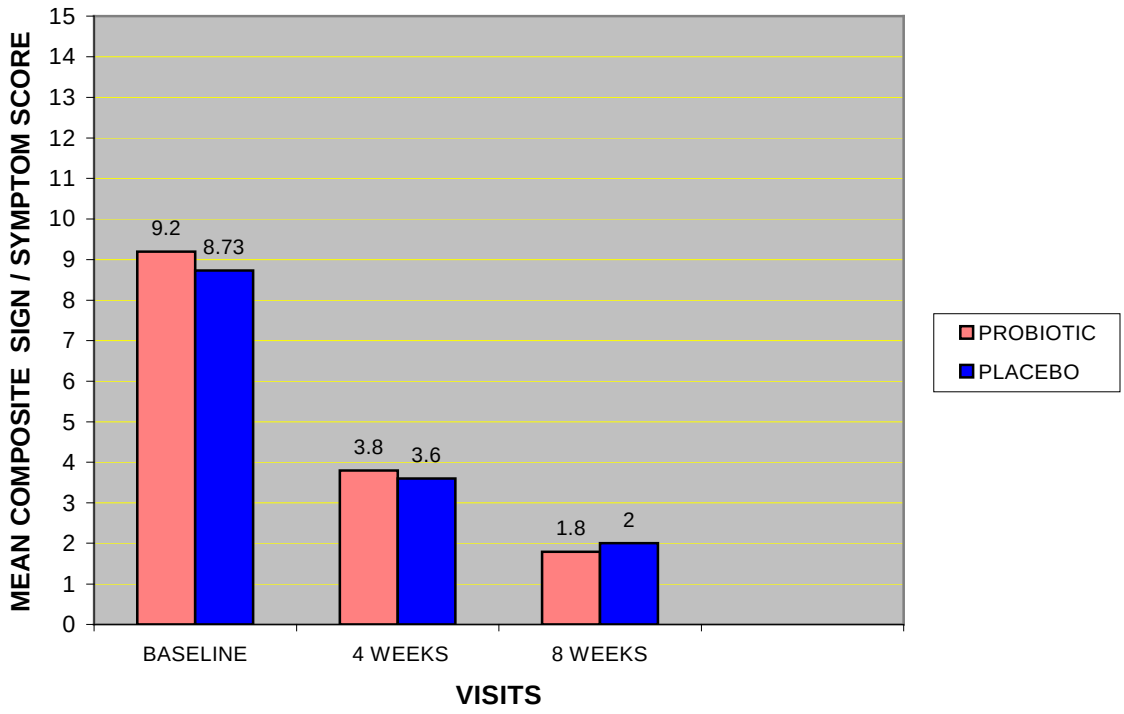


Figure 13 - Comparison of mean composite sign/ symptom score in VVC.

Table 10–shows

- The mean composite sign/ symptom score in the probiotic and placebo groups at baseline and after 4 weeks and 8 weeks of study therapy.
- The difference in mean composite sign/ symptom score between the probiotic and placebo groups at the baseline was not statistically significant (95% confidence interval**, -**0.62 to 1.56; p= 0.39).
- The difference in mean composite sign/ symptom score between the probiotic and placebo groups after 4 weeks of study therapy was not statistically significant (95% confidence interval**,** -0.91 to 1.31; p= 0. 72).
- The difference in mean composite sign/ symptom score between the probiotic and placebo groups after 8 weeks of study therapy was not statistically significant (95% confidence interval**, -**1.32 to –0.92; p= 0. 72).

Figure 13 – is the diagrammatic representation of the mean composite sign/ symptom score in the probiotic and placebo groups at baseline and after 4 weeks and 8 weeks of study therapy.

Table 11- Comparison of frequency of potassium hydroxide (KOH) preparation of vaginal smear

Variable	Group	KOH				Total	Chi-Square	P
		Probiotic		Placebo				
		Count	%	Count	%			
Baseline	Positive	15	100 %	15	100 %	30	0	Not sig
	Negative	0	0	0	0	0		
KOH 4 wks	Positive	7	46.7%	9	60 %	16	0.54	Not sig
	Negative	8	53.3%	6	40 %	14		
KOH 8 wks	Positive	7	46.7%	9	60 %	16	0.54	Not sig
	Negative	8	53.3%	6	40 %	14		

Figure 14- Comparison of percentage of patients with KOH preparation of vaginal smear positive for Candida.

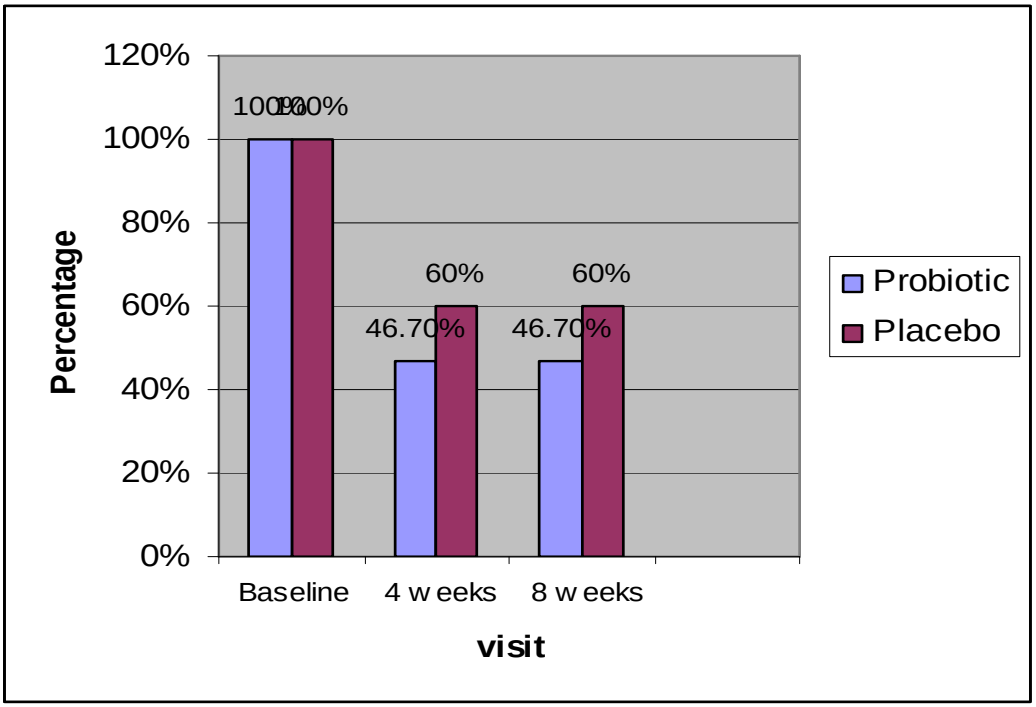


Table 11 shows

- The number and percentage of patients in probiotic and placebo groups with KOH preparation of vaginal smear positive or negative for candida.
- The treatment difference was assessed using Chi square test.
- At baseline, all the patients of probiotic and placebo group had KOH preparation of vaginal smear positive for candida.
- There was no significant difference in the frequency of KOH preparation of vaginal smear positive for candida between the probiotic and placebo groups at 4 weeks of study therapy (p=0.54).
- There was no significant difference in the frequency of KOH preparation of vaginal smear positive for candida between the probiotic and placebo groups at 8 weeks of study therapy (p=0.54).

Figure 14 - represents a diagrammatic representation of comparison of percentage of patients in probiotic and placebo groups with KOH preparation of vaginal smear positive for candida at baseline, and after 4 weeks and 8 weeks of study therapy.

Table- 12

Variables	Group	Mean	Std. Deviation	p
Blood Urea Baseline	Probiotic	24.53	2.20	**0.13**
	Placebo	23.33	2.02	
Blood Urea 8 Wks	Probiotic	24.27	1.83	**0.60**
	Placebo	23.87	2.33	
Blood Sugar Baseline	Probiotic	95.67	15.16	**0.14**
	Placebo	104.20	15.74	
Blood Sugar 8 Wks	Probiotic	99.73	9.76	**0.71**
	Placebo	101.07	9.65	
SrCreatinine Baseline	Probiotic	0.69	0.09	**0.70**
	Placebo	0.71	0.10	
SrCreatinine 8 Wks	Probiotic	0.69	0.10	**1.00**
	Placebo	0.69	0.07	
SGOT Baseline	Probiotic	18.9	2.7	**0.55**
	Placebo	18.4	2.8	
SGOT 8 Wks	Probiotic	19.0	2.3	**0.24**
	Placebo	18.2	2.2	
SGPT Baseline	Probiotic	13.3	2.5	**0.54**
	Placebo	12.8	2.6	
SGPT 8 Wks	Probiotic	13.3	2.3	**0.56**
	Placebo	12.9	2.1	
TotProt Baseline	Probiotic	5.97	0.15	**0.70**
	Placebo	5.95	0.13	
TotProt 8 Wks	Probiotic	5.94	0.12	**0.47**
	Placebo	5.91	0.13	

The above table depicts the difference between probiotic and placebo groups in blood urea, blood sugar, serum creatinine, SGOT, SGPT and total proteins taken at baseline and end of 8 weeks. By independent samples t test, the difference between the treatment groups was not statistically significant (p value > 0.05).

GROUP-III URINARY TRACT INFECTION

Demographic features

Table 13- Mean age distribution

Drug Group	No. of patients	Mean Age	Std. Deviation	P	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
Probiotic	21	35.0	6.9	0.57	-1.10	-5.02	2.83
Placebo	20	36.1	5.4				

Figure 15- Mean age distribution

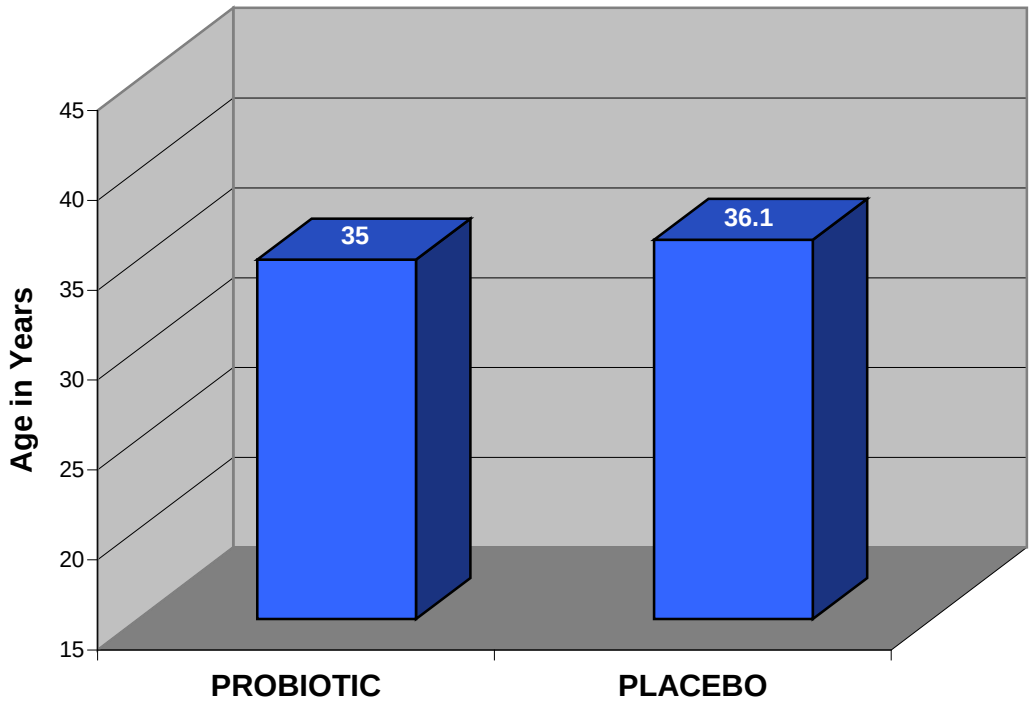


Table-13 shows

- The number of patients with urinary tract infection (group III) in probiotic (group IIIA) and placebo subgroups (group III B).
- The mean age of patient in probiotic (group IIIA) and placebo subgroup (group III B).
- The mean age of patient in probiotic (group IIIA) subgroup was 35.0
- The mean age of patient in placebo subgroup (group III B) was 36.1
- The difference between the probiotic (group IIIA) and placebo subgroups (group III B) was not statistically significant (95% confidence interval, -5.02 to 2.83; p= 0.57).

Figure-15 is the diagrammatic representation of the mean age distribution in the probiotic and placebo groups.

Table 14: Comparison of frequency of occurrence of fever

Variable		Group				Total	Chi Square	p
		Probiotic		Placebo				
		Number	%	Number	%			
Baseline	Present	21	100.0%	20	100.0%	41	NA	NA
	Absent	0	0.0%	0	0.0%	0		
4 Weeks	Present	1	4.8%	1	5.0%	2	0.001	0.97
	Absent	20	95.2%	19	95.0%	39		
8 Weeks	Present	0	0.0%	0	0.0%	0	NA	NA
	Absent	21	100.0%	20	100.0%	41		

Figure 16: Comparison of percentage of patients with fever

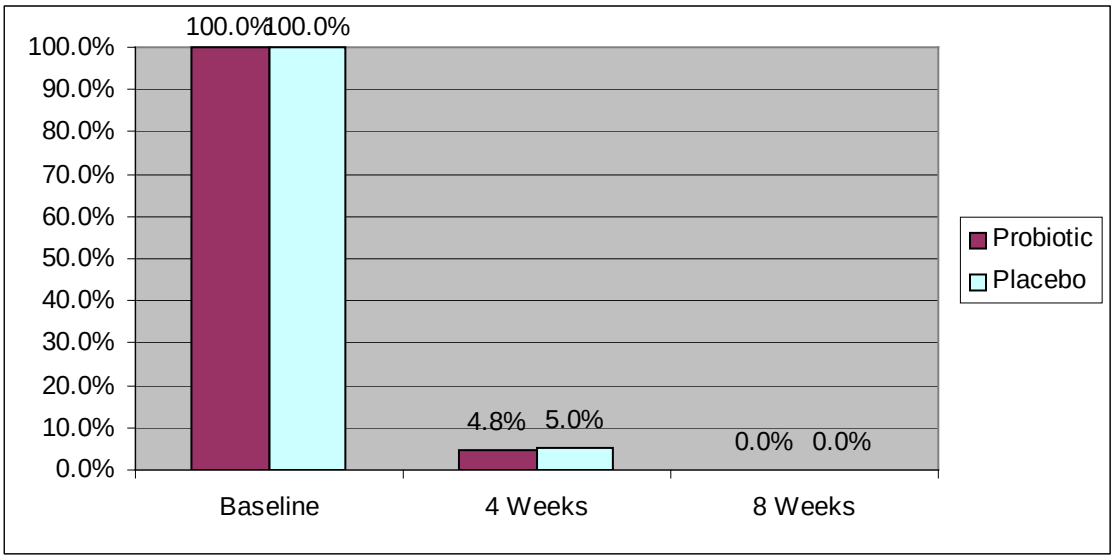


Table 14 shows

- The number and percentage of patients in probiotic and placebo groups with fever.
- The treatment difference was assessed using Chi square test.
- At baseline, all the patients of probiotic and placebo group had fever.
- There was no significant difference in the frequency of fever between the probiotic and placebo groups at 4 weeks of study therapy (p=0.97).
- After 8 weeks of study therapy, there was no report of fever by any patient in probiotic or placebo group.

Figure 16- represents a diagrammatic representation of percentage of patients with fever in probiotic and placebo groups at baseline, and after 4 weeks and 8 weeks of study therapy.

Table 15: Comparison of frequency of occurrence of dysuria.

Variable		Group				Total	Chi Square	p
		Probiotic		Placebo				
		Number	%	Number	%			
Baseline	Present	21	100.0%	20	100.0%	41	NA	NA
	Absent	0	0.0%	0	0.0%	0		
4 Weeks	Present	4	19.0%	7	35.0%	11	1.33	0.25
	Absent	17	81.0%	13	65.0%	30		
8 Weeks	Present	1	4.8%	4	20.0%	5	2.22	0.14
	Absent	20	95.2%	16	80.0%	36		

Figure 17: Comparison of percentage of patients with dysuria.

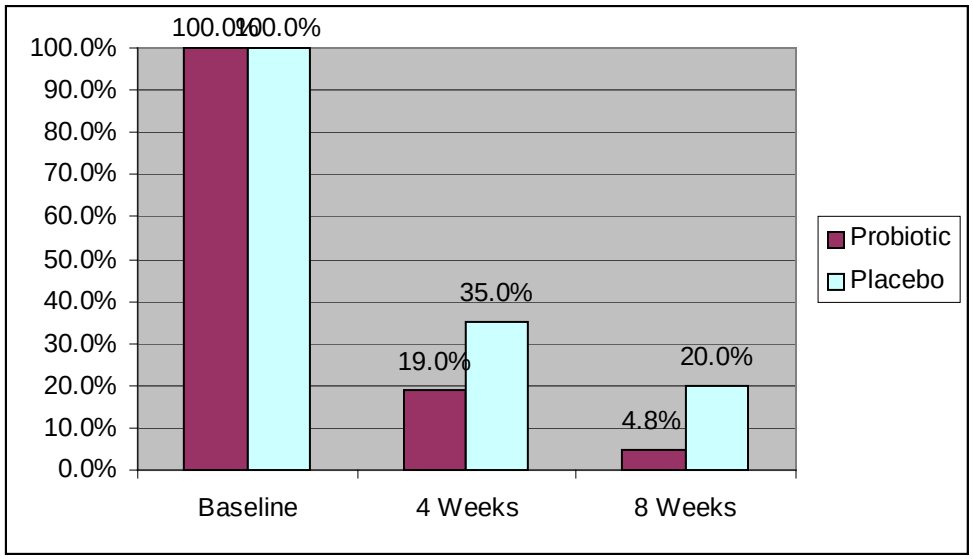


Table 15 shows

- The number and percentage of patients in probiotic and placebo groups with dysuria.
- The treatment difference was assessed using Chi square test.
- At baseline, all the patients of probiotic and placebo group had dysuria.
- There was no significant difference in the frequency of dysuria between the probiotic and placebo groups at 4 weeks of study therapy (p=0.25).
- There was no significant difference in the frequency of dysuria between the probiotic and placebo groups at 8 weeks of study therapy (p=0.14).

Figure 17- represents a diagrammatic representation of comparison of percentage of patients with dysuria in probiotic and placebo groups at baseline, and after 4 weeks and 8 weeks of study therapy

Table 16: Comparison of frequency of occurrence of suprapubic pain.

Variable		Group				Total	Chi Square	p
		Probiotic		Placebo				
		Number	%	Number	%			
Baseline	Present	11	52.4%	4	20.0%	15	4.63	0.03
	Absent	10	47.6%	16	80.0%	26		
4 Weeks	Present	0	0.0%	1	5.0%	1	1.1	0.3
	Absent	21	100.0%	19	95.0%	40		
8 Weeks	Present	0	0.0%	0	0.0%	0	NA	NA
	Absent	21	100.0%	20	100.0%	41		

Figure 18: Comparison of percentage of patients with suprapubic pain.

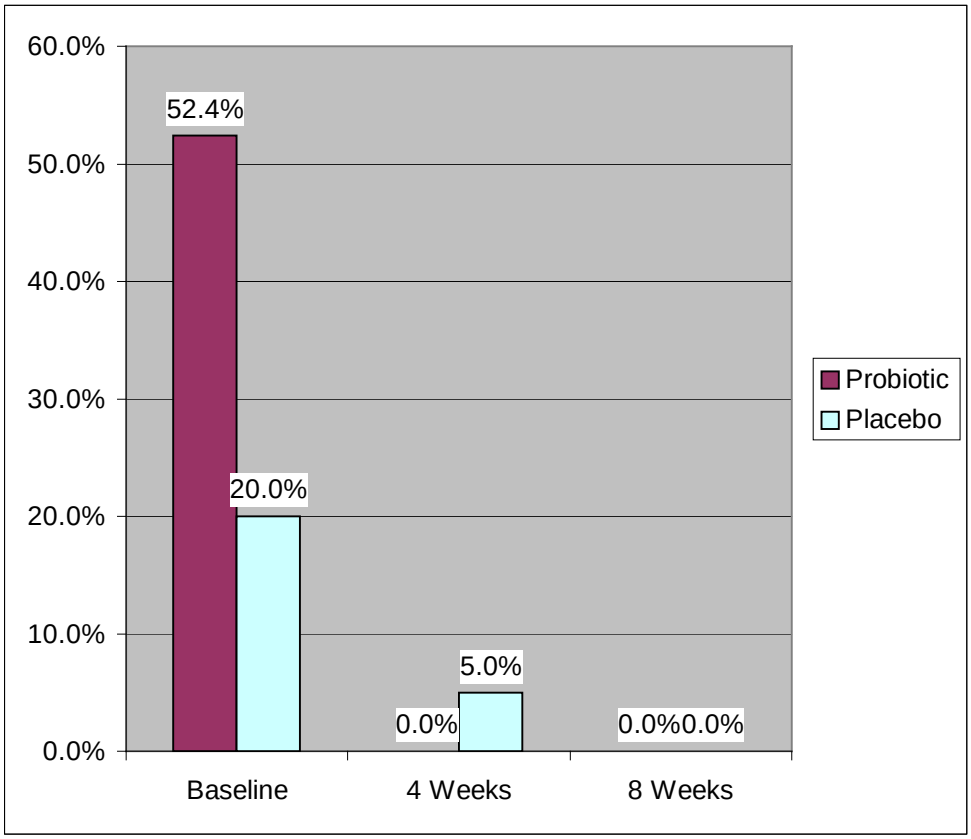


Table 16 shows

- The number and percentage of patients in probiotic and placebo groups with suprapubic pain.
- The treatment difference was assessed using Chi square test.
- At baseline, there was significant difference in the frequency of suprapubic pain between the probiotic and placebo groups (p=0.03).
- There was no significant difference in the frequency of suprapubic pain between the probiotic and placebo groups at 4 weeks of study therapy (p=0.3).
- After 8 weeks of study therapy, there was no report of suprapubic pain by any patient in probiotic or placebo group.

Figure 18- represents a diagrammatic representation of comparison of percentage of patients with suprapubic pain in probiotic and placebo groups at baseline, and after 4 weeks and 8 weeks of study therapy

Table 17: Comparison of frequency of occurrence of positive urine culture

VISIT	Urine culture	Group				Total	Chi Square	p
		Probiotic		Placebo				
		Number	%	Number	%			
Baseline	Positive	21	100.0%	20	100.0%	41	NA	NA
	Negative	0	0.0%	0	0.0%	0		
4 Weeks	Positive	3	14.3%	8	40.0%	11	0.034	0.85
	Negative	18	85.7%	12	60.0%	30		
8 Weeks	Positive	1	4.8%	4	20.0%	5	0.05	0.82
	Negative	20	95.2%	16	80.0%	36		

Figure 19:Comparison of percentage of patients with positive urine culture.

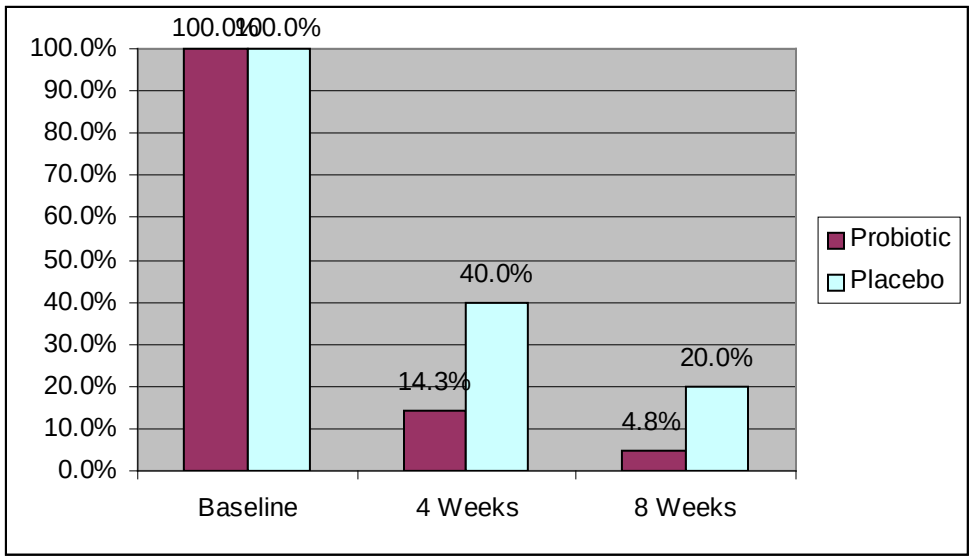


Table 17 shows

- The number and percentage of patients in probiotic and placebo groups with urine culture report.
- The treatment difference was assessed using Chi square test.
- At baseline, all the patients of probiotic and placebo group had positive urine culture.
- There was no significant difference in the frequency of positive urine culture between the probiotic and placebo groups at 4 weeks of study therapy (p=0.85).
- There was no significant difference in the frequency of positive urine culture between the probiotic and placebo groups at 8 weeks of study therapy (p=0.82).

Figure 19- represents a diagrammatic representation of comparison of percentage of patients with positive urine culture in probiotic and placebo groups at baseline, and after 4 weeks and 8 weeks of study therapy

Table-18

Variables	Group	Mean	Std. Deviation	p
Blood Urea Baseline	Probiotic	24.7	2.6	**0.76**
	Placebo	24.5	2.9	
Blood Urea 8 Wks	Probiotic	24.6	1.6	**0.07**
	Placebo	23.4	2.4	
Blood Sugar Baseline	Probiotic	100.5	14.5	**0.05**
	Placebo	108.7	11.0	
Blood Sugar 8 Wks	Probiotic	99.73	9.76	**0.71**
	Placebo	101.07	9.65	
SrCreatinine Baseline	Probiotic	0.7	0.1	**0.57**
	Placebo	0.8	0.1	
SrCreatinine 8 Wks	Probiotic	0.7	0.1	**0.60**
	Placebo	0.7	0.1	
SGOT Baseline	Probiotic	14.9	3.7	**0.54**
	Placebo	15.5	2.2	
SGOT 8 Wks	Probiotic	15.4	2.4	**0.73**
	Placebo	15.2	1.7	
SGPT Baseline	Probiotic	13.3	2.5	**0.54**
	Placebo	12.8	2.6	
SGPT 8 Wks	Probiotic	13.3	2.3	**0.24**
	Placebo	12.9	2.1	
TotProt Baseline	Probiotic	5.9	0.1	**0.95**
	Placebo	5.9	0.2	
TotProt 8 Wks	Probiotic	6.0	0.1	**0.44**
	Placebo	6.0	0.1	

The above table depicts the difference between probiotic and placebo groups in blood urea, blood sugar, serum creatinine, SGOT, SGPT and total proteins taken at baseline and end of 8 weeks. By independent samples t test, the difference between the treatment groups was not statistically significant (p value > 0.05).

DISCUSSION

Urogenital infections include those that affect the urethra, urinary bladder, vagina and cervix and constitute a worldwide problem that affects more than 300 million women/year. Common urogenital infections include bacterial vaginosis (BV), vulvovaginal candidiasis (VVC/yeast vaginitis), and urinary tract infection (UTI). There is now growing evidence that certain species and strains present in the healthy urogenital tract protect the host against infection by pathogenic microorganisms.

Probiotics are “Live microorganisms which when administered in adequate amounts confer a health benefit on the host.” [6] *L. rhamnosus* GR-1 and *L. reuteri* RC-14 are extensively characterized urogenital isolates which possess a number of properties considered important for urogenital probiotics.[39] Furthermore, studies with humans have shown that these strains are efficacious in the prevention and treatment of urogenital infections in women.[40, 41]

So far, no study of urogenital probiotic strains - *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 were carried out in our population .So we conducted a randomised, double blind, comparative, prospective, parallel group study of two-strain combination of oral probiotics - *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 as integral therapy with conventional treatment in the following female urogenital infections - bacterial vaginosis (BV), vulvovaginal candidiasis (VVC or yeast vaginitis) and urinary tract infection (UTI).

The study was approved by the Ethical committee and was conducted in the outpatient clinic of the Department of female Urology and Urogynaecology, Government Kasturba Gandhi Hospital for Women and Children. **122** patients fulfilled the inclusion criteria and were divided in to three groups based on the type of urogenital infection -

- **GROUP I**: Patients with bacterial vaginosis (50 patients),
- **GROUP II**: Patients with vulvovaginal candidiasis (30 patients),
- **GROUP III**: Patients with urinary tract infection (42 patients).

Patients of each group were randomly allocated to subgroups A or B to receive either of the two study therapies –either integral therapy of probiotics with conventional treatment (**subgroup A**) or placebo with conventional treatment (**subgroup B**). Each 150mg capsule of the probiotic mixture had a minimum of 5 billion CFU of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 in equal proportions and the dosage recommended was two capsules per day for eight weeks. The placebo was microcrystalline cellulose. Patients were assessed clinically once in two weeks and they were instructed to report to the physician in case of any unwanted drug effect. The results were collected and statistically analyzed.

GROUP-I-BACTERIAL VAGINOSIS

- **Demographic profile**

Age

The mean age of patient in probiotic subgroup was 32.7 years. The mean age of patient in placebo subgroup was 31.6 years. The difference in age group between the probiotic and placebo subgroups was not statistically significant. Our sample reflected the widely published trial conducted in Canada in which the mean age of selected patients with bacterial vaginosis was 35 years.[66]

- **Vaginal pH**

The difference in mean vaginal pH between the probiotic and placebo groups at the baseline (p= 0.36) and at 4 weeks (p= 0. 64) were not statistically significant. The difference in mean vaginal pH between the probiotic and placebo groups after 8 weeks of study therapy was statistically significant (p= 0. 00) which indicate that the probiotic lactobacillus strains confer protection to the host by producing an acidic environment. [38] The mean vaginal pH of the probiotic group at the end of 4 weeks and 8 weeks were 5.1 and 4.8 which fulfill one of the clinical criteria of Amsel et al. for bacterial vaginosis[48]- vaginal-fluid pH greater than 4.5.Many studies have shown that vaginal pH is non-specific and can be altered by BV, semen, blood, cervical secretions and lubricating jelly.[67]

- **Nugent Score**

The difference in mean Nugent score between the probiotic and placebo groups at the baseline (p= 0.95) was not statistically significant. The difference in mean Nugent score between the probiotic and placebo groups after 4 weeks (p= 0. 00) and 8 weeks (p= 0. 03) of study therapy was statistically significant. Our study results are comparable to a randomised, placebo-controlled trial of 64 healthy women which showed restoration from asymptomatic bacterial vaginosis microflora to a normal lactobacilli colonized microflora in 37% women during lactobacilli treatment compared to 13% on placebo. [57] Another study showed a similar result of a significant increase in the degree of purity of the vaginal flora.[68]

- **Vaginal Discharge**

At baseline, all the patients of probiotic and placebo group had vaginal discharge characteristic of bacterial vaginosis. There was no significant difference in the frequency of vaginal discharge characteristic of bacterial vaginosis between the probiotic and placebo groups at 4 weeks of study therapy (p=0.1). There was a statistically significant difference in the frequency of vaginal discharge characteristic of bacterial vaginosis between the probiotic and placebo groups at 8 weeks of study therapy (p=0.008). The patients of probiotic group reported subjective sense of well-being.

- **Foul Smelling Odor**

At baseline, all the patients of probiotic and placebo group had foul smelling odor of vaginal discharge. There was no significant difference in the frequency of foul smelling odor of vaginal discharge between the probiotic and placebo groups at 4 weeks (p=0.26) and 8 weeks (p=0.054) of study therapy.

- **Hematological parameters**

The total count, differential count, erythrocyte sedimentation rate and hemoglobin were within the normal physiological range in both groups.

- **Biochemical Parameters**

All the biochemical parameters, blood sugar, blood urea, serum creatinine, SGOT, SGPT, serum bilirubin, serum alkaline phosphatase, serum proteins and urine routine evaluated during the study period were within the normal physiological range.

- **Adverse drug reaction**

One patient in probiotic group complained of constipation and flatulence. No other significant adverse drug reaction was found in our study. No medical intervention was required. Similarly in literature also no side effects were reported with the use of probiotics.

GROUP II VULVOVAGINAL CANDIDIASIS

- **Demographic profile**

Age

The mean age of patient in probiotic subgroup was 29.73 years. The mean age of patient in placebo subgroup was 30.27 years. The difference between the probiotic and placebo was not statistically significant (p= 0.82).

- **Vaginal pH**

The difference in mean vaginal pH between the probiotic and placebo groups at the baseline (p= 0.72), 4 weeks (p= 0.72) and 8 weeks (p= 0. 07) were not statistically significant. Our results are similar to the results of a meta-analysis, which also showed no difference in mean vaginal pH with study therapy.[69]

- **Composite Sign/ Symptom Score**

The difference in mean composite sign/ symptom score between the probiotic and placebo groups at the baseline (p= 0.39), 4weeks(p= 0.72) and 8 weeks (p= 0.72) were not statistically significant.

- **Microscopic analysis with potassium hydroxide preparation**

At baseline, all the patients of probiotic and placebo group had KOH preparation of vaginal smear positive for candida. There was no significant difference in the frequency of KOH preparation of vaginal smear positive for candida between the probiotic and placebo groups at 4 weeks (p=0.54) and 8 weeks (p=0.54) of study therapy.

A randomised, placebo-controlled trial recorded significant reduction in number of positive cultures for candida consistent with the use of *L rhamnosus* GR-1 and *L fermentum* RC-14 in lyophilized capsule form, which showed up to one log fewer yeast recovered from the vagina during treatment compared with baseline.[57]In our study, candida culture was not done. Further studies are needed to assess the efficacy of probiotic strains in VVC.

- **Hematological parameters**

The total count, differential count, erythrocyte sedimentation rate and hemoglobin were within the normal physiological range in both groups.

- **Biochemical Parameters**

All the biochemical parameters, blood sugar, blood urea, serum creatinine, SGOT, SGPT, serum bilirubin, serum alkaline phosphatase, serum proteins and urine routine evaluated during the study period were within the normal physiological range.

- **Adverse drug reaction**

No significant adverse drug reaction was found in our study. No medical intervention was required. Similarly in literature also no side effects were reported with the use of probiotics.

GROUP-III URINARY TRACT INFECTION

- **Demographic profile**

Age

The mean age of patient in probiotic subgroup was 35years. The mean age of patient in placebo subgroup was 36.1years.The difference between the mean age of probiotic and placebo subgroups was not statistically significant (p= 0.57).Our sample reflected the widely published trial in which the mean age of selected patients with bacterial vaginosis was 30.3 years. [70]

- There was no significant difference in the frequency of dysuria, fever and suprapubic pain between the probiotic and placebo groups.

- **Urine culture**

At baseline, all the patients of probiotic and placebo group had positive urine culture. There was no significant difference in the frequency of positive urine culture between the probiotic and placebo groups at 4 weeks (p=0.85) and 8 weeks (p=0.82) of study therapy. Results from various studies indicate that the recurrence rate of UTI was significantly reduced after using one or two capsules vaginally per week for one year, with no side effects or yeast infections.[64]In our study, the patients were followed up only for a period of 8 weeks. Further studies are needed to assess the efficacy of probiotic strains in reducing recurrence rate of UTI per year.

- **Hematological parameters**

The total count, differential count, Erythrocyte Sedimentation Rate and Hemoglobin were within the normal physiological range in both groups.

- **Biochemical Parameters**

All the biochemical parameters, blood sugar, blood urea, serum creatinine, SGOT, SGPT, serum bilirubin, serum alkaline phosphatase, serum proteins and urine routine evaluated during the study period were within the normal physiological range.

- **Adverse drug reaction**

No significant adverse drug reaction was found in our study. No medical intervention was required. Similarly in literature also no side effects were reported with the use of these probiotic lactobacilli strains.

In a climate of the trend toward reduced antibiotic use, awareness of disease resulting directly from micro ecosystem disruption, emergence of pathogens with enhanced virulence, clinical conditions refractory to conventional treatment, and awareness that some infections lead to serious sequelae, probiotic bacteria may add a low-cost, low risk layer of protection from infection and disease.

CONCLUSION

The eight week, randomised, double blind, comparative, prospective, parallel group study of two-strain combination of oral probiotics - *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 as integral therapy with conventional treatment in female urogenital infections showed –

- Significant therapeutic effect in the management of Bacterial vaginosis (BV),

- The combination therapy was not significant in the management of Vulvovaginal candidiasis (VVC or yeast vaginitis) and Urinary tract infection (UTI).

- Oral urogenital probiotic strains - *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 were found to be safe and well tolerated.

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APPENDICES

Appendix 1

ABBREVIATIONS

BV	**Bacterial Vaginosis**
CFUs	**Colony Forming Units**
CFUs/gm	**Colony Forming Units per gram**
CI	**Confidence Interval**
FAO	**Food and Agriculture Organization**
HIV	**Human Immunodeficiency Virus**
H_2O_2	**Hydrogen peroxide**
KOH	**Potassium Hydroxide**
L.	**Lactobacillus**
SGOT	**Serum Glutamic Oxaloacetic Transaminase**
SGPT	**Serum Glutamic Pyruvic Transaminase**
Std.Deviation	**Standard Deviation**
Tot.Prot.	**Total Protein**
UTI	**Urinary Tract Infection**
VVC	**VulvoVaginal Candidiasis**
WHO	**World Health Organization**

Appendix 2

Informed Consent Form-English

Study Title:

"A Randomised, Double Blind, Comparative, Prospective, Parallel Group Study Of Oral Probiotics In Female Patients With Urogenital Infections"

Name: _____ O.P.No. : _____

Address : _____ Enroll No.: _____

_____ Age : _____

_____ Sex : ☐ Female

_____

I, _____ Age _____ Yrs, exercising my free power of choice, hereby give my consent to be included as a patient in the clinical study "A Randomised, Double Blind, Comparative, Prospective, Parallel Group Study of Oral Probiotics In Female Patients with Urogenital Infections"

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study, nature of the drug treatment, follow up visits and study procedures including investigations, to monitor and to safeguard my body function.
- I understand that the laboratory investigations will require the withdrawal of blood sample in required amount at follow up visits.
- I agree to co-operate fully and to inform the doctor immediately if I suffer any unusual symptom.
- I have informed the doctor, about all medications that I have taken in the recent past and those I am currently taking. I shall not take any medications without the agreement of the supervising doctor.
- I understand that study doctor may stop my participation in the study for any reasons. I am also aware of my right to opt out of the study at any time during the study duration without giving any reason.
- I hereby give permission to use my medical records for research purpose. I am told that study doctor and study institution will keep my identity close.

_____	_____	_____
Name of the Patient	Signature/Thumb impression	Date
_____	_____	_____
Name of Impartial Witness	Signature	Date
_____	_____	_____
Name of the Investigator	Signature	Date

Consent form - Tamil

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PROFORMA

"A Randomised, Double Blind, Comparative, Prospective, Parallel Group Study Of Oral Probiotics In Female Patients With Urogenital Infections"

1. Name: Date
2. Age:
3. Address:
4. OP No. :
5. Randomization No: _____

Body Measurement

Height: _____ mts Weight: _____ Kg

BMI: _____ Kg/m2

Demography

Age:
Sex: Female

Vital Signs

Blood Pressure: _____ / _____ mmHg
Pulse: _____ Beats Per minute

Medical History

Concomitant Illness:

Concomitant Medications:

UROGENITAL EXAMINATION:

INVESTIGATIONS: Following basal investigation were done for all the patients

Complete blood count

Hb g%	TC	DC	ESR

Routine Urine Analysis :

Albumin g/dL	Sugar g/dL	WBC/ RBC	Casts

Midstream Urine Colony count &Culture-Sensitivity:

Vaginal /Cervical **--- swab/smear. examination**

1.Vaginal smear for Gram staining [Nugent's Diagnostic Method]

Nugent Scores of Gram Stain : _____

2) Vaginal smear for 10% KOH mount: - Budding Yeast ☐ Yes ☐ No

Vaginal pH monitoring:

Ultrasound Abdomen ---

Blood Chemistry –
Blood Sugar: ---------- mg/dL

Liver Function Tests and Renal Function Tests:

SGOT U/L	SGPT U/L	Bilirubin mg/dL	Alkaline Phosphatase U/L	Urea mg/dL	Creatinine mg/dL

Clinical Response Evaluation:

INVESTIGATIONS DONE AT THE END OF 4 WEEKS

Bacterial Vaginosis:
1.Vaginal smear for Gram staining [Nugent's Diagnostic Method]

Nugent Scores of Gram Stain : _____
2) pH of Vaginal Fluid _____

Vulvovaginal Candidiasis:
1) Vaginal smear for 10% KOH mount: - Budding Yeast ☐ Yes ☐ No
2) pH of Vaginal Fluid _____

Urinary Tract Infection:
1) Routine Urine analysis**:**

Albumin g/dL	Sugar g/dL	WBC/ RBC	Casts

2) Midstream Urine Colony count &Culture-Sensitivity

Clinical Response Evaluation:

INVESTIGATIONS DONE AT THE END OF 8 WEEKS

Bacterial Vaginosis:
1.Vaginal smear for Gram staining [Nugent's Diagnostic Method]

Nugent Scores of Gram Stain : _____
2) pH of Vaginal Fluid _____

Vulvovaginal Candidiasis:
1) Vaginal smear for 10% KOH mount: - Budding Yeast ☐ Yes ☐ No
2) pH of Vaginal Fluid _____

Urinary Tract Infection:
1) Routine Urine analysis:

Albumin g/dL	Sugar g/dL	WBC/ RBC	Casts

2) Midstream Urine Colony count &Culture-Sensitivity

Other Investigations:

Blood Routine:

Hb g%	TC	DC	ESR

Blood Sugar : ---------- mg/dL

Liver Function Tests and Renal Function Tests:

SGOT U/L	SGPT U/L	Bilirubin mg/dL	Alkaline Phosphatase U/L	Urea mg/dL	Creatinine mg/dL

Clinical Response Evaluation: