



**ORIGINAL RESEARCH PAPER**

**Gynecology**

**PREVALENCE OF BACTERIAL VAGINOSIS AND VAGINAL FLORA CHANGES IN PERI- AND POSTMENOPAUSAL WOMEN**

**KEY WORDS:** Vaginosis , Bacterial flora, Urogenital tract ,Polymicrobial

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**ABSTRACT**

**Objectives** To study colonization of bacteria in vagina and perianal region in elderly women and correlation of colonization in vaginal region as well as possible variations with hormone levels.  
**Methodology** A total of 147 smears from women aged 40 to 79 years were examined. Women were classified as follows: fertile (regular cycles) (n = 32), perimenopausal (irregular cycles) (n = 23), and postmenopausal (n = 92), including 33 women on estroprogestinic hormone replacement therapy (HRT)  
**Results** The prevalences of bacterial vaginosis (assessed as a Nugent score of ≥7) in fertile (9.8%) and perimenopausal (11.0%) women were not statistically different, whereas the prevalence was significantly lower overall in postmenopausal women (6.0%) (P = 0.02). Specifically, 6.3% of postmenopausal women without HRT and 5.4% of postmenopausal women with HRT were positive for bacterial vaginosis  
**Conclusion.** The Nugent score system was not adequate for evaluating the normal and intermediate vaginal flora in women over the age of 40 years. High numbers of peri- and postmenopausal women had no lactobacilli and no bacterial-vaginosis-associated microorganisms.

**INTRODUCTION**

The ordinary vaginal bacterial flora of healthy premenopausal women comprises primarily of Lactobacillus spp. The urogenital tract is protected against infection by pathogens which are supposed to cooperate by a defensive role. Many women with these infections are asymptomatic, necessitating both a high degree of clinical awareness and adequate screening.

Bacterial vaginosis (BV) is a polymicrobial disorder characterized by an increase in the vaginal pH over 4.5, a reduction in or absence of lactobacillus colonization, and overgrowth of several facultatively and obligately anaerobic bacteria . BV is associated with adverse pregnancy outcomes, upper genital tract infections such as pelvic inflammatory disease, endometritis, post-gynecologic-surgery infections, cervicitis, urinary tract infections, cervical intraepithelial neoplasia, and increased risk of sexual acquisition of human immunodeficiency virus infection

The physiological reduction in lactobacillus colonization of the vagina in postmenopausal women does not cause an increase in bacterial-vaginosis prevalence. Reversion of lactobacillus flora to premenopausal levels due to HRT does not increase the prevalence of bacterial vaginosis in postmenopausal women.

BV is usually diagnosed by the clinical Amsel's criteria or by the Nugent method of Gram stain interpretation. In fact, in postmenopausal women BV cannot be clinically diagnosed by Amsel's criteria because one of these criteria, the vaginal pH value, is constitutively elevated ; moreover, the scarcity of vaginal discharge makes it difficult to judge the kind of secretion. The Nugent method is based on the assumption that normal women have full vaginal colonization by lactobacilli ; this is valid for pregnant and fertile women but not for postmenopausal women . The present study is a cohort study to determine the changes in vaginal flora and the prevalence of BV by standardized evaluation of Gram-stained smears for women 40 years old or older as a function of reproductive condition: fertile, perimenopausal, or postmenopausal with or without HRT.

**METHODOLOGY**

**Study population.** Nonpregnant women aged 40 to 79 years were consecutively recruited during routine gynecologic examinations .Women were enrolled after giving informed consent according to local Ethics Committee guidelines, and clinical research was conducted in accordance with guidelines for human experimentation. As a routine practice, at the time of

scheduling of the Pap test, patients were requested to refrain from sexual intercourse and from any vaginal treatment for 3 days prior to their checkups.

All women included were eligible for the Pap test (no bleeding or major vaginal inflammatory signs) and had no malignancies or severe medical illnesses. None had overt yeast vaginitis, and none was positive for Neisseria gonorrhoeae infection.

Further exclusion criteria were partial or total hysterectomy, menopause induced by drug treatments, HRT terminated less than 6 months earlier, HRT taken for less than 3 months, vaginal estrogenic treatment, progestinic treatment in perimenopause, and tamoxifen or analogous antiestrogen drug therapy. When interviewed at the times of their visits, women included in the study said that they had not had sexual intercourse or engaged in any vaginal practice (such as douching or using vaginal suppositories) in the past 3 days and had not used antibiotics in the past 2 weeks.

Among eligible women over the age of 40, 7 were excluded for reasons such as an incomplete questionnaire, positive cervical intraepithelial neoplasia discovered as a result of the Pap test performed at the time of the visit, or an inadequate vaginal secretion smear, leaving a study base of participants for analysis. The mean age ± standard deviation of the 147 women enrolled was 53.4 ± 7.0 years.

From the study base of 147 women, **four different groups** of participants were identified. The first group consisted of 32 fertile women who had regular menses. The mean age of this group was 45.3 ± 3.7 years (range, 40 to 55 years);The second group consisted of 23 perimenopausal women who had irregular menses, or absence of menstruation for less than 12 months . The mean age was 50.1 ± 3.0 years (range, 40 to 58 years);The third group consisted of 59 postmenopausal women who had not received HRT in the past 6 months (95% had never received HRT). The mean age was 58.1 ± 6.0 years (range, 42 to 79 years); the mean age of onset of menopausal status was 50.2 ± 3.6 years (range, 33 to 59 years); the average number of postmenopausal years was 7.9 ± 6.1 (range, 1 to 34 years);The fourth group consisted of 33 postmenopausal women currently receiving oral or transdermal estroprogestinic replacement therapy. The mean age was 55.4 ± 4.4 years (range, 42 to 69 years); the mean onset of menopausal status was 49.3 ± 4.1 years (range, 32 to 58 years); the average number of postmenopausal years was 6.1 ± 3.5

(range, 1 to 26 years). The average time of HRT was 3.9 ± 2.6 years. One hundred ninety-eight of these women were receiving transdermal estrogenic treatment, and 133 were on oral treatment; 9 of those on oral treatment were receiving tibolone. Progestinic treatment was transdermal in 25 cases, vaginal in 2 cases, and oral in the remaining cases. Two hundred sixty-eight protocols were sequential; the rest were combined estrogen-progestinic protocols.

Overall, 92 women were postmenopausal, with a physiological termination of menstruation. Their mean age was 57.1 ± 5.6 years (range, 42 to 79 years); the mean age of onset of menopausal status was 49.9 ± 3.8 years (range, 32 to 59 years); the average number of postmenopausal years was 7.3 ± 5.4 (range, 1 to 34 years).

**Vaginal sample collection.** Samples were collected from the posterior fornix or lateral vaginal wall of nonbleeding women with an Ayre's spatula using a nonlubricated speculum to perform a smear on a glass slide. The smear was then stained according to the Gram procedure. Clue cells and vaginal flora were evaluated on the Gram-stained smear.

**Gram-stained-smear evaluation.** The vaginal flora was determined by evaluation of five different fields under oil immersion (magnification, ×1,000). For the first analysis we adopted the Nugent score method. The sum of the points assigned to each of the three morphotypes is the final Nugent score of the patient, which is classified as normal (a Nugent score from 0 to 3), intermediate altered flora (a Nugent score from 4 to 6), or full BV (a Nugent score from 7 to 10).

In a more-refined analysis, the absence of lactobacilli, of *Gardnerella*-like bacteria, and of *Mobiluncus* spp., which is very frequent in peri- or postmenopausal women, was considered apart from the group with intermediate flora (which denotes a partially pathological change in the vaginal flora). For most of these women only epithelial host cells are visible in the smear; occasionally gram-positive cocci and/or enterobacteriaceae are detectable. In fact, the decrease in lactobacilli is a commonly observed phenomenon in healthy postmenopausal women. Since such a physiological condition characterizes a subgroup of women with a Nugent score of 4, we refer to it below as score 4\*. None of the women with score 4\* had clue cells. Women with Nugent scores from 4 to 10 who were positive for clue cells were considered to have abnormal anaerobic vaginal flora.

Full lactobacillus colonization was defined as more than 30 lactobacilli observed in all the five (magnification, ×1,000) fields examined. All the Gram smear evaluations were performed by two independent investigators, with more than 90% agreement; discrepant readings were reexamined, and a third investigator was consulted in case of persistent disagreement.

**OBSERVATION TABLES**

**TABLE 1. PREVALENCE OF NORMAL, INTERMEDIATE, AND FULL BV VAGINAL FLORAA AND OF ABNORMAL ANAEROBIC FLORAB IN SPECIFIC SUBGROUPS WOMEN OVER THE AGE OF 40 YEARS**

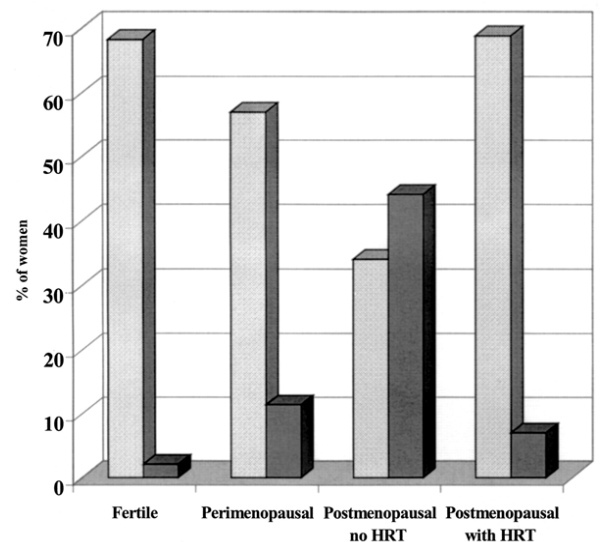
| Group              | Total no. of patients | % (no.) of women with a Nugent score of: |                          |                      |   |
|--------------------|-----------------------|--|--------------------------|----------------------|---|
|                    |                       | 0-3 (normal flora)                       | 4-6 (intermediate flora) | 7-10 (full BV flora) | ≥4 plus clue cells (abnormal anaerobic flora) |
| All aged ≥40 yr    | 1,486                 | 67.8 (1,008)                             | 24.6 (365)               | 7.6 (113)            | 9.9 (147)                                     |
| Fertile            | 328                   | 84.1 (276)                               | 6.1 (20)                 | 9.8 (32)             | 12.5 (41)                                     |
| Perimenopausal     | 237                   | 75.1 (178) <b>c</b>                      | 13.9 (33) <b>c</b>       | 11.0 (26)            | 13.1 (31)                                     |
| All postmenopausal | 921                   | 60.2 (554) <b>c</b>                      | 33.9 (312)               | 6.0 (55) <b>d</b>    | 8.1 (75) <b>d</b>                             |

|                            |     |                     |                     |                   |                   |
|----------------------------|-----|---------------------|---------------------|-------------------|-------------------|
| Postmenopausal without HRT | 590 | 46.3 (273) <b>c</b> | 47.5 (280) <b>c</b> | 6.3 (37) <b>d</b> | 8.5 (50) <b>d</b> |
| Postmenopausal with HRT    | 331 | 84.9 (281)          | 9.7 (32)            | 5.4 (18) <b>d</b> | 7.6 (25) <b>d</b> |

**TABLE 2. PREVALENCE OF WOMEN WITH SCORE 4\* AND REDEFINED INTERMEDIATE AND NORMAL FLORA GROUPS IN SPECIFIC SUBGROUPS OF WOMEN OVER THE AGE OF 40 YEARS**

| Group                      | % (no.) of women with: |                              |                        |
|----------------------------|------------------------|------------------------------|------------------------|
|                            | Score 4*               | Intermediate flora* <b>b</b> | Normal flora* <b>c</b> |
| All aged ≥40 yr            | 21.3 (317)             | 3.2 (48)                     | 89.2 (1,325)           |
| Fertile                    | 2.1 (7)                | 4.0 (13)                     | 86.3 (283)             |
| Perimenopausal             | 11.4 (27) <b>d</b>     | 2.5 (6)                      | 86.5 (205)             |
| All postmenopausal         | 30.7 (283) <b>d</b>    | 3.1 (29)                     | 90.9 (837) <b>e</b>    |
| Postmenopausal without HRT | 44.1 (260) <b>d</b>    | 3.4 (20)                     | 90.3 (533)             |
| Postmenopausal with HRT    | 6.9 (23) <b>d</b>      | 2.7 (9)                      | 91.8 (304) <b>e</b>    |

**Profile of full lactobacillus colonization (stippled bars) and of score 4\* (physiological absence of lactobacilli) (dark shaded bars) in subgroups of women over the age of 40 years.**



**RESULTS**

*Lactobacillus crispatus* was more often found in the vaginal flora of fertile women than in that of postmenopausal (p = 0.036). Fifteen of 20 fertile women had lactobacilli in their rectal smears compared to 10 postmenopausal women (p = 0.071). There was no correlation between the number of bacteria in vagina and rectum, or between the number of bacteria and hormonal levels. Neither could any association between the presence of rectal lactobacilli and hormonal levels be found.

**STATISTICAL ANALYSIS-**

Data was analyzed using SPSS 20 statistical package. A descriptive analysis was done on all variables to obtain a frequency distribution. The mean + SD and ranges were calculated for quantitative variables. Continuous variables were compared by the Student t test. Proportions were analyzed with the chi-square test. A P value of 0.05 or less was considered statistically significant.

**DISCUSSION**

The purpose of study was to investigate the relationship between vaginal and rectal *Lactobacillus* flora in healthy women, and possible variations with sex hormonal levels. Several studies have shown that the vaginal microbial flora varies in relationship to hormonal levels. However, no studies have been performed to investigate the correlation between hormonal levels and lactobacilli in the gut. Therefore, we analyzed vaginal and rectal smears from fertile and postmenopausal women. In the fertile women, smears were collected both in day 7 and day 21 of the

menstrual cycle.

Gustafsson RJ, Ahnrén S et al studied the *Lactobacillus* flora in vagina and rectum of fertile and postmenopausal healthy Swedish women. The aim of the study was to investigate the relationship in healthy women between vaginal and rectal *Lactobacillus* flora, as well as possible variations with sex hormone levels with special references to changes in menstrual phases as well as in the menopause. *Lactobacillus crispatus* was more prevalent in the vaginal flora of fertile women, whereas the *Lactobacillus* flora of rectum did not correlate to the vaginal flora nor to hormonal levels. They did not find any correlation between the overall levels of *Lactobacillus* species in vagina and rectum, and variations in sex hormone levels. However, *L. crispatus* was more often found in the vaginal flora of fertile women than in that of postmenopausal women. [1]

Heinemann C, Reid G et al studied vaginal microbial diversity among postmenopausal women with and without hormone replacement therapy. Urogenital infections in postmenopausal women remain problematic. The use of estrogen replacement therapy has been shown to lower these infection rates, corresponding to increasing colonization by *Lactobacillus* species. Despite the gut's 500 microbial species and the proximity of the anus to the vagina, only a relatively few microbial strains appear to be able to colonize the urogenital area. In the present study, the sparsity of microbes in the vagina was confirmed by denaturing gradient gel electrophoresis analysis of swabs taken at time zero and monthly for 3 months from 40 postmenopausal subjects receiving Premarin (conjugated equine estrogen in combination with progesterone) hormone replacement therapy (HRT) and 20 who were not on HRT.

*Lactobacilli* were recovered from the vagina of 95% or more women in both groups, but in the HRT group, *Lactobacillus* were more often the dominant and only colonizers and significantly fewer bacteria with pathogenic potential were found. The incidence of bacterial vaginosis was significantly lower in the HRT group than in the non-HRT-treated women (5.6% versus 31%). The use of HRTs has recently come under criticism. The ability of drugs such as Premarin to help recover the *Lactobacilli* vaginal microbiota appears to be at least one benefit of HRT use. In women not using HRTs, use of probiotics may be the only way to restore a nonpathogen-dominated flora. [2]

Burton JP, Reid G et al did evaluation of the bacterial vaginal flora of 20 postmenopausal women by direct (Nugent score) and molecular (polymerase chain reaction and denaturing gradient gel electrophoresis) techniques. To monitor the bacterial vaginal microflora of postmenopausal women, several approaches were used: (1) Nugent scoring of Gram-stained smears from vaginal swabs; (2) testing for bacteria, using specific polymerase chain reaction (PCR) primers; (3) PCR-denaturing gradient gel electrophoresis (DGGE) analysis of total bacterial DNA, amplified using eubacterial and *Lactobacillus*-specific primers; and (4) sequence analysis of amplified DNA fragments of interest from denaturing gradient gels. Of 20 women studied, 70% had either intermediate-grade bacterial colonization or bacterial vaginosis (BV), as defined by Nugent criteria, at the first sampling point. Subjects with normal Nugent scores had  $\geq 1$  species of *Lactobacillus* detected by sequencing of dominant DNA fragments from denaturing gradient gels generated using eubacterial primers. Samples from women with Nugent scores that indicated BV had varied bacterial DGGE profiles, which emphasizes the diverse nature of such infections. This study indicates that asymptomatic BV appears to be much more common than is currently perceived; these findings may have implications for the health of postmenopausal women. [3]

Cauci S, Driussi S et al evaluated prevalence of bacterial vaginosis and vaginal flora changes in peri- and postmenopausal women. A number of trends emerged from the present study. They found that in the group of all premenopausal women over the age of 40, the prevalence of BV was 10.3% and that of abnormal anaerobic flora was 12.7%. The presence of regular (fertile women) or

irregular (perimenopausal women) menses did not statistically affect the percentages of full BV and abnormal anaerobic flora. In the group of all postmenopausal women, the prevalence of BV was 6.0% and that of abnormal anaerobic flora was 8.1%, both statistically lower than those found in premenopausal women.

Estroprogestinic treatment of postmenopausal women had no statistically significant effect on either full BV (prevalence, 5.4%) or abnormal anaerobic flora (prevalence, 7.6%). To the best of our knowledge, no previous study has evaluated the effects of HRT on the prevalence of BV or abnormal anaerobic flora. The absence of *Lactobacilli* without detectable BV-associated microorganisms is a rather rare event in women over the age of 40 who still have regular menses (2.1%), whereas this condition increases markedly in perimenopausal women (11.4%) and is present in as many as 44.1% of postmenopausal women without HRT. We defined this condition as score 4 because it constitutes a subset of women with a Nugent score of 4. It appears that this distinction has to be made especially for peri- and postmenopausal women, because the absence of *Lactobacillus* colonization in these women appears to be a normal physiologic condition rather than an intermediate abnormal flora. In fact, in healthy fertile women, full vaginal colonization by *Lactobacilli* is properly considered the normal physiologic condition. However, the mere absence of *Lactobacillus* colonization (without any evidence of *Gardnerella*-like or *Mobiluncus* microorganisms) should be distinguished from other conditions resulting in a Nugent score of 4, which derive from a partial decrease in *Lactobacillus* colonization and a limited increase in growth of BV-associated bacteria.

This kind of mixed flora, in our opinion, should be more properly defined as intermediate abnormal flora. On this basis, we propose a new scoring system, a refinement of the Nugent score method, that distinguishes score 4 among women having a Nugent score of 4. We consider it advisable that our method be adopted for all women regardless of hormonal status. In summary, our data reveal that postmenopausal status involves major vaginal changes that are not confined to the glycogen content of epithelial cells, which can be modulated by HRT. Other, still unclarified subtle differences occur in the vaginas of postmenopausal women, and their future identification could contribute to understanding of the general mechanisms of colonization by BV-associated microorganisms. Our data show that the absence of *Lactobacilli* by itself is not related to an elevated prevalence of BV, as observed in peri- and postmenopausal women. This finding suggests that the decrease in *Lactobacillus* colonization is more likely a consequence than a leading cause of the anaerobic alteration of the vaginal ecology. [4]

On the other hand, Raz R, Stamm WE et al did a controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. Their results support the hypothesis that estrogen deficiency is a major contributor to the pathogenesis of recurrent urinary tract infections in postmenopausal women and show that prolonged estrogen replacement with a topically applied vaginal cream safely and effectively prevents urinary tract infections in these patients. This preventive approach can be considered an alternative to the use of long-term low-dose antibiotics such as nitrofurantoin, co-trimoxazole, trimethoprim, cephalexin, or more recently, the fluoroquinolones. These regimens are effective; however, topical estrogen may be particularly useful in patients in whom the prolonged use of antibiotics induces side effects, allergic reactions, drug interactions, or the emergence of multidrug-resistant microorganisms.

The results of two previous open studies and one small controlled trial also suggest that orally administered estriol may prevent recurrent urinary infections in postmenopausal women. However, in a case-control study of more than 23,000 older women attending general practice clinics in England, Orlander and associates found that estrogen use was associated with a twofold increase in the risk of a first episode of urinary tract infection. Thus, the effects of oral estrogen use on initial and recurrent episodes of urinary tract infection in postmenopausal women require further evaluation. [5]

Hillier SL, Lau RJ et al did a study on vaginal microflora in 73 postmenopausal women who have not received estrogen replacement therapy. The median age of these women was 67 years, and none of them had bacterial vaginosis. Lactobacilli were detected in 36 (49%) of these women at a mean concentration of  $10^{5.7}$  cfu/g of vaginal fluid.  $H_2O_2$ -producing lactobacilli were recovered from 38% of the women. Some of the other organisms that were recovered, including *Gardnerella vaginalis* (27% of the women), *Ureaplasma urealyticum* (13%), *Candida albicans* (1%), and *Prevotella bivia* (33%), were less frequently isolated from postmenopausal women than from women of reproductive age, while coliforms (41%) were recovered at higher frequencies. Lactobacilli, yeasts, and bacterial vaginosis-associated bacteria are less commonly part of the vaginal microflora in postmenopausal women than in women of reproductive age, which may explain the decrease in the incidence of bacterial vaginosis and yeast vaginitis among these women.[6]

Petricic L, Unger FM et al did a randomized, double-blind, placebo-controlled study of oral lactobacilli to improve the vaginal flora of postmenopausal women. The purpose of this study was to evaluate the influence of the orally administered probiotic strains *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 on the quality of the vaginal flora in postmenopausal women. Postmenopausal women with Nugent scores between 4 and 6 in initial vaginal swab, were randomized into two groups. Women in the intervention group received probiotic capsules containing  $2.5 \times 10^9$  CFU (colony forming units) each of lyophilized *L. rhamnosus* GR-1 and *L. reuteri* RC-14 and women in the control group received an oral placebo once daily, in both groups for 14 days. Final vaginal swabs were taken 1 day after the last administration of the medication.

The primary efficacy variable was a change in the Nugent score between baseline and the end of the study of at least two grades in each individual patient. Seventy two women were recruited in the study, 35 assigned to the intervention group and 37 to the control group. Twenty-one of the 35 subjects (60%) in the intervention group and 6 of the 37 subjects (16%) in the control group showed a reduction in the Nugent score by at least two grades. The difference in the number of patients with improvement was highly significant ( $p = 0.0001$ ). The median difference in Nugent scores between baseline and the end of the study was 3 in the intervention group and 0 in the control group ( $p = 0.0001$ ). Our results provide evidence for an alternative modality to restore the normal vaginal flora using specific probiotic strains administered orally.[7]

#### CONCLUSION:

The Nugent score system was not adequate for evaluating the normal and intermediate vaginal flora in women over the age of 40 years. High numbers of peri- and postmenopausal women had no lactobacilli and no bacterial-vaginosis-associated microorganisms.

#### REFERENCES

1. Gustafsson RJ, Ahm  S, Jeppsson B, Benoni C, Olsson C, Stjernquist M, Ohlsson B. The Lactobacillus flora in vagina and rectum of fertile and postmenopausal healthy Swedish women. BMC women's health. 2011 May 25;11(1):17.
2. Heinemann C, Reid G. Vaginal microbial diversity among postmenopausal women with and without hormone replacement therapy. Canadian Journal of Microbiology. 2005 Sep 1;51(9):777-81.
3. Burton JP, Reid G. Evaluation of the bacterial vaginal flora of 20 postmenopausal women by direct (Nugent score) and molecular (polymerase chain reaction and denaturing gradient gel electrophoresis) techniques. The Journal of infectious diseases. 2002 Dec 15;186(12):1770-80.
4. Cauci S, Driussi S, De Santo D, Penacchioni P, Iannicelli T, Lanzafame P, De Seta F, Quadrioglio F, de Aloisio D, Guaschino S. Prevalence of bacterial vaginosis and vaginal flora changes in peri- and postmenopausal women. Journal of clinical microbiology. 2002 Jun 1;40(6):2147-52.
5. Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with recurrent urinary tract infections. N Engl J Med. 1993 Sep 9;329(329):753-6.
6. Hillier SL, Lau RJ. Vaginal microflora in postmenopausal women who have not received estrogen replacement therapy. Clinical infectious diseases. 1997 Sep 1;25(Supplement\_2):S123-6.
7. Petricic L, Unger FM, Viernstein H, Kiss H. Randomized, double-blind, placebo-controlled study of oral lactobacilli to improve the vaginal flora of postmenopausal women. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2008 Nov 30;141(1):54-7.