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# Effect of Lactobacilli Oral Supplement on the Vaginal Microflora of Antibiotic Treated Patients: Randomized, Placebo-Controlled Study

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## Abstract

**Many antibiotic monographs cite the induction of vaginal infections as a possible side effect. Invariably, this is believed to be due to *Candida albicans*, and empirical therapy is given. However, recent studies raise the question of the extent to which yeast do infect the host after antibiotic use. A double-blind, randomized, placebo-controlled study was undertaken on female patients to determine how many yeast infections occurred following 10 days antibiotic use. In addition, the study was designed to examine whether oral use of probiotic lactobacilli can reduce the risk of vaginal infection. Twenty four patients diagnosed with respiratory, oral or throat infections received one of several types of antibiotic for 10 days, and two capsules containing $10^9$ dried *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 from the day of commencement of antibiotic therapy for 21 days. The most commonly prescribed antibiotic was biaxin (clarithromycin). All but one patient had lactobacilli in the vagina upon entry to the study, and none developed yeast vaginitis or diarrhea during treatment or 20 days after completion of antibiotics. The mean Nugent score was higher in the placebo than the lactobacilli group (4.1 versus 2.4), and three cases of bacterial vaginosis arose (25% incidence compared to 0% in the lactobacilli group) in the placebo group (2 receiving cefuroxime, 1 on biaxin). The study suggested that current antibiotic use is not necessarily associated with either diarrhea or yeast infection, as is often surmised. Nevertheless, daily use of probiotics was safe and could potentially reduce the risk of patients developing bacterial vaginosis after antibiotic use.**

**Key words:** antibiotics, probiotics, lactobacilli, yeast, bacterial vaginosis, randomized controlled trial

## INTRODUCTION

A considerable number of women, estimated to be over 15 million in the USA, suffer from yeast vaginitis each year. This ailment often recurs and interferes with sexual health and general well-being of the patient (1). Several factors influence the pathogenesis, including recent exposure to antibiotics. However, not all the evidence supports the antibiotic induction concept. In otherwise healthy women not already colonized in the vagina by yeast, the candidiasis rates may not be as high as perceived. Indeed, presumed yeast vaginitis may actually be bacterial vaginosis (BV) in many women, as the latter has a similar clinical presentation and is not easy to diagnose. This is illustrated in a study of 71 patients who presented with vaginal discharge, itching and/or pain and a presumed diagnosis of candidiasis, yet only 23 (32.4%) had positive yeast cultures (2). In many cases, empirical and self-treatment management means that vaginal cultures are not routinely done, and as yeast can be recovered from the vagina of healthy women, the true nature of post-antibiotic 'infections' is unclear.

While intravaginal antibiotics, such as metronidazole to treat bacterial vaginosis (BV) significantly increase the risk of candidiasis (3), the rate of infection is highest in patients colonized by yeast at the time of treatment (4). The explanation for this may be related to the vaginal microflora. In the case of BV, lactobacilli are lacking or severely depleted, thus the environment for the growth of yeast could become enhanced with eradication of the remaining Gram negative anaerobes. Clearly some antibiotics significantly impact the vaginal microflora (5). However, in patients with urinary tract infection (UTI), the ability of antibiotics to target the vaginal pathogens can be important as this mucosa is the nidus from which infections occur.

The concept of creating or strengthening a bacterial barrier population in the vagina through oral and vaginal ap-

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plication of probiotic lactobacilli has been tested with some success in preventing recurrence of infection including candidiasis (6-9). In order to optimise probiotic therapy after antibiotic use, the impact of these drugs on the vaginal microflora needs to be better understood.

The aim of the present study was to investigate a group of women who were receiving antibiotics for respiratory, mouth or throat infections, and to determine the rate of yeast and BV occurrences and the effect of the drugs on the vaginal flora, with and without lactobacilli supplementation.

## MATERIALS AND METHODS

### Human subjects and protocol

Fifty eight female patients with oral, throat, or respiratory infections requiring treatment with a 10 day therapeutic dose of antibiotic were reviewed for the study. Twenty seven patients (mean age $39 \pm 9$) were recruited and of those twenty four (12 per group) provided samples suitable for analysis. All but four subjects reported a previous experience of presumed yeast vaginitis following antibiotic use. Each patient received either a penicillin (amoxicillin 500 mg TID, penicillin V 300 mg TID), macrolides (azithromycin 500 mg one dose then 250 mg QD, clarithromycin 250 mg BID), doxycycline (100 mg BID) or cephalosporin (cefuroxime 250 mg BID) antibiotic. The initial point of contact was a physician or dentist. Each patient read a Letter of Information and signed an Informed Consent approved by the Ethics Review Board of the University of Western Ontario.

The subjects were randomized to receive orally two capsules of lactobacilli or placebo once daily for 21 days, commencing on the same day that antibiotic therapy began. The probiotics were taken one hour prior or two hours post antibiotic ingestion. Prior to taking the first dose of antibiotic and lactobacilli/placebo, the subjects provided two vaginal swabs to test for yeast infection, BV or a 'normal' microflora dominated by lactobacilli, as tested by standard microbiology culture and Gram stain Nugent scoring system. The Nugent test comprised scoring the cell population as normal (0 to 3) and dominated by *Lactobacillus* rods, intermediate (4 to 6) with colonization by small gram-negative or gram-variable rods (*Bacteroides* or *Gardnerella*) and curved gram-variable rods (*Mobiluncus*), and BV (7 to 10) with domination by pathogens and absence of lactobacilli. Another two vaginal swabs were collected one month from this date and at any time if the patient reported symptoms and signs of yeast vaginitis. Clinical diagnosis for yeast vaginitis included pruritus, caseous discharge, perineal edema or erythema, and patient self-diagnosis of what they perceived to be a yeast infection (1). The patients were asked to report any loose stools during the study. No subject used over-the-counter yeast medication or another form of probiotic during the trial.

### Probiotic organisms

The probiotic strains *L. rhamnosus* GR-1 and *L. fermentum* RC-14 were prepared under European Pharmacopia Good Manufacturing Practices by Chr Hansen, Horsholm, Denmark. They were prepared as dried organisms in gelatin capsules at a concentration of $> 10^9$ per capsule dose. Both organisms were found to inhibit the growth of *Candida albicans in vitro* using an agar overlay test.

## RESULTS

No cases of diarrhea or yeast vaginitis or other adverse events were recorded for any of the 24 subjects. In only patient #47 were a few yeast cells seen by microscopy. There were no side effects attributed to the lactobacilli or placebo capsule therapy. Lactobacilli were present in the vaginal microflora of all but one of the 24 subjects at day 0. In the lactobacilli treated group, the mean Nugent scores did not increase from day 0 to 30 and no patients developed BV or UTI (Table 1). Also, 9/12 had a 'normal' flora upon entry and this was retained at day 30. In the placebo group, three subjects developed bacterial vaginosis by day 30 (incidence of 25% compared to 0% in the lactobacilli group) following use of cefuroxime and clarithromycin. The mean Nugent score in the placebo group increased from 2.7 to 4.1, although not quite reaching statistically significant levels (Wilcoxon two-sample test, p=.39). The mean Nugent score for the placebo was higher than the lactobacilli treated group (4.1 versus 2.4; p=.16).

## DISCUSSION

This clinical study showed that yeast infections post-antibiotic therapy are not as common as once perceived. Indeed, no cases of candidiasis were found in the 24 patients, even although 20 of the women claimed to have had post-antibiotic vaginitis following previous antibiotic treatment. The present findings might reflect the use of newer drug types that have less impact on the indigenous microflora. This is in agreement with a previous study (11) which showed that antibiotics, including doxycycline, azithromycin and amoxicillin, used here, did not decrease vaginal lactobacilli. However, a more plausible explanation could be that the subjects entered the study with a relatively normal vaginal flora and this helped to protect against yeast and bacterial superinfections in the vagina. In a recent randomized, placebo-controlled trial, control subjects had significantly increased yeast and bacterial

**Table 1.** Nugent scores for vaginal swabs obtained from patients before (day 0) ten days antibiotic therapy plus 21 days' supplementation with placebo or *L. rhamnosus* GR-1 and *L. fermentum* RC-14, then at day 30 (20 days after completion of antibiotic and 9 days after completion of probiotic therapy)

| Lactobacillus treated | | | | Placebo treated | | | |
|---|---|---|---|---|---|---|---|
| Nugent scores | | | | Nugent scores | | | |
| Patient | Antibiotic | Day 0 | Day 30 | Patient | Antibiotic | Day 0 | Day 30 |
| 1 | clarithromycin | 1 | 2 | 2 | amoxacillin | 0 | 0 |
| 4 | clarithromycin | 0 | 3 | 7 | azithromycin | 1 | 1 |
| 5 | azithromycin | 1 | 1 | 8 | penicillin V | 4 | 3 |
| 6 | clarithromycin | 3 | 3 | 10 | cefuroxime | 1 | 8 |
| 9 | azithromycin | 5 | 5 | 13 | clarithromycin | 7 | 6 |
| 11 | amoxicillin | 1 | 0 | 21x | clarithromycin | 1 | 2 |
| 29 | amoxicillin | 1 | 1 | 27 | amoxicillin | 5 | 2 |
| 40 | amoxicillin | 5 | 2 | 37 | azithromycin | 5 | 5 |
| 43 | clarithromycin | 3 | 2 | 39 | penicillin V | 5 | 2 |
| 44 | clarithromycin | 6 | 5 | 41 | clarithromycin | 1 | 0 |
| 47 | tetracycline | 1 | 4 | 42 | clarithromycin | 2 | 10 |
| 58 | clarithromycin | 1* | 1 | 57 | cefuroxime | 0 | 10 |
| | | 2.4 | 2.4 | | | 2.7 | 4.1 |

The Nugent scores were normal (0 to 3), intermediate (4 to 6) and BV (7 to 10).
*Nugent slide not available but lab result reported normal flora.

pathogen numbers in the vagina when lactobacilli numbers were reduced (8). In the present study, three subjects (25%) had a dramatic alteration in the vaginal microflora resulting in BV at day 30, representing a higher rate than in fertile (9.8%), perimenopausal (11%) or postmenopausal (6%) women (12). Although this BV score was asymptomatic, such conditions significantly increase the risk of the development of symptomatic urogenital infections (13,14), and therefore represent an unwanted clinical condition.

The sample size is too small to confirm that *L. rhamnosus* GR-1 and *L. fermentum* RC-14 prevented symptomatic urogenital infection from occurring, but the preliminary evidence suggests it is possible. Previous studies have shown that these probiotic organisms can colonize the vagina following oral use (7,8) and displace *Gardnerella vaginalis*, the main cause of BV (15). Furthermore, use of these strains in women highly susceptible to recurrent urogenital infections has never resulted in yeast vaginitis.

The findings raise the question of whether women develop true yeast vaginitis after antibiotic use, or if indeed it is BV, a syndrome which can have similar clinical presentation. The findings of this study should not be interpreted to show that yeast vaginitis does not occur after antibiotic use, as there are many cases in clinical practice where this indeed happens. Nevertheless, yeast vaginitis may not always be the cause of vaginal symptoms. Large amounts of money are spent each year on self-treatment of suspected candidiasis (16,17), but in a portion of the patients therapeutic failures and so-called recurrences may be due to anaerobic or aerobic Gram negative pathogens and not yeast (18-20).

No cases of diarrhea were reported in this study. This is not surprising given the product monographs of newer antibiotics such as azithromycin and clarithromycin show low occurrences (4~6%) of diarrhea as a side effect. This raises the question of whether yogurt containing viable bacteria, or products comprising dried probiotic strains, are necessary as an adjunct to antibiotics. Certain probiotic strains clearly can prevent or reduce the duration of diarrhea (21), but that alone is not sufficient for recommended use, given the apparently low risk of diarrhea. In terms of reducing the risk of urogenital infections, the organisms used to ferment milk (*S. thermophilus* and *Lactobacillus delbruekii* var *bulgaricus*) while possibly surviving passage through the adverse stomach and bile conditions, have not been shown to prevent yeast vaginitis or BV. If yogurt is supplemented with a probiotic organism, some effect may occur. In two studies, yogurt supplemented with an *L. acidophilus* seemed to reduce the risk of yeast vaginitis and BV, but only 7 and 13 patients, respectively, completed the study (22,23). A recent Finnish study in which *L. rhamnosus* GG was taken as a daily dietary supplement, showed reduced recurrence of UTI, thereby supporting the concept of probiotic foods for health maintenance (24). The most convincing data comes from studies using *L. rhamnosus* GR-1 and *L. fermentum* RC-14 which when ingested daily in dried form have been shown to reduce yeast and bacterial pathogen colonization in the vagina (8). In patients without indigenous lactobacilli, this daily therapy can help maintain a normal vaginal microflora (7).

In summary, antibiotic therapy can disrupt the vaginal microflora in a portion of women, but BV appears to be

a more likely outcome than yeast vaginitis, according to the antibiotics tested in this study. The similtaneous use of probiotics during antibiotic treatment may have a role to play in reducing the risk of urogenital infection especially if the indigenous vaginal lactobacilli have been depleted. As an abnormal vaginal microflora is associated with inflammation (higher cervical IL-1 beta and IL-8 cytokine levels) (25) and an increased risk of sexually transmitted diseases including HIV (26), the use of safe probiotic supplements could still be beneficial to women taking antibiotics.

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## REFERENCES

1. Abbott J. 1995. Clinical and microscopic diagnosis of vaginal yeast infection: a prospective analysis. *Ann Emerg Med* 25: 587-591.
2. Daus AD, Hafez ES. 1975. *Candida albicans* in women. *Nurs Res* 24: 430-433.
3. Ferris DG, Litaker MS, Woodward L, Mathis D, Hendrich J. 1995. Treatment of bacterial vaginosis: a comparison of oral metronidazole, metronidazole vaginal gel, and clindamycin vaginal cream. *J Fam Pract* 41: 443-449.
4. Glover DD, Larsen B. 1998. Longitudinal investigation of candida vaginitis in pregnancy: role of superimposed antibiotic use. *Obstet Gynecol* 91: 115-118.
5. Reid G, Bruce AW, Cook RL, Llano M. 1990. Effect on the urogenital flora of antibiotic therapy for urinary tract infection. *Scand J Infect Dis* 22: 43-47.
6. Reid G, Bruce AW, Taylor M. 1995. Instillation of *Lactobacillus* and stimulation of indigenous organisms to prevent recurrence of urinary tract infections. *Microecol Ther* 23: 32-45.
7. Reid G, Beuerman D, Heinemann C, Bruce AW. 2001. Probiotic *Lactobacillus* dose required to restore and maintain a normal vaginal flora. *FEMS Immunol Med Microbiol* 32: 37-41.
8. Reid G, Charbonneau D, Erb J, Kochanowski B, Beuerman D, Poehner R, Bruce AW. 2003. Oral use of *Lactobacillus rhamnosus* GR-1 and *L. fermentum* RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immunol Med Microbiol* 35: 131-134.
9. Cadieux P, Burton J, Gardiner G, Braunstein I, Bruce AW, Kang CY, Reid G. 2002. *Lactobacillus* strains and vaginal ecology. *JAMA* 287: 1940-1941.
10. Nugent RP, Krohn MA, Hillier SL. 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardization method of Gram stain interpretation. *J Clin Microbiol* 29: 297-301.
11. Agnew KJ, Hillier SL. 1995. The effect of treatment regimens for vaginitis and cervicitis on vaginal colonization by lactobacilli. *Sex Transm Dis* 22: 269-273.
12. Cauci S, Driussi S, De Santo D, Penacchioni P, Iannicelli T, Lanzafame P, De Seta F, Quadrifoglio F, de Aloysio D, Guaschino S. 2002. Prevalence of bacterial vaginosis and vaginal flora changes in peri- and postmenopausal women. *J Clin Microbiol* 40: 2147-2152.
13. Geerlings SE, Stolk RP, Camps MJ, Netten PM, Collet TJ, Hoepelman AI. 2000. Diabetes Women Asymptomatic Bacteriuria Utrecht Study Group. Risk factors for symptomatic urinary tract infection in women with diabetes. *Diabetes Care* 23: 1737-1741.
14. Hillebrand L, Harmanli OH, Whiteman V, Khandelwal M. 2002. Urinary tract infections in pregnant women with bacterial vaginosis. *Am J Obstet Gynecol* 186: 916-917.
15. Burton JP, Cadieux P, Reid G. 2003. Improved understanding of the bacterial vaginal microbiota of women before and after probiotic instillation. *Appl Environ Microbiol* 69: 97-101.
16. Foxman B, Barlow R, D'Arcy H. 2000. *Candida* vaginitis: self-reported incidence and associated costs. *Sex Trans Dis* 27: 230-225.
17. Sobel JD. 1997. Vaginitis. *N Engl J Med* 337: 1896-1903.
18. Lamont RF, Morgan DJ, Wilden SD, Taylor-Robinson D. 2000. Prevalence of bacterial vaginosis in women attending one of three general practices for routine cervical cytology. *Int J STD AIDS* 11: 495-498.
19. Ferris DG, Nyirjesy P, Sobel JD, Soper D, Pavletic A, Litaker MS. 2002. Over-the-counter antifungal drug misuse associated with patient-diagnosed vulvovaginal candidiasis. *Obstet Gynecol* 99: 419-425.
20. Donder GG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Spitz B. 2002. Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. *BJOG* 109: 34-43.
21. FAO/WHO. 2001. Evaluation of health and nutritional properties of powder milk and live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation Report. http://www.fao.org/es/ESN/Probio/probio.htm.
22. Shalev E, Battino S, Weiner E, Colodner R, Keness Y. 1996. Ingestion of yogurt containing *Lactobacillus acidophilus* compared with pasteurized yogurt as prophylaxis for recurrent candidal vaginitis and bacterial vaginosis. *Arch Fam Med* 5: 593-596.
23. Hilton E, Isenberg HD, Alperstein P, France K, Borenstein MT. 1992. Ingestion of yogurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. *Ann Intern Med* 116: 353-357.
24. Kontiokari T, Laitinen J, Jarvi L, Pokka T, Sundqvist K, Uhari M. 2003. Dietary factors protecting women from urinary tract infection. *Am J Clin Nutr* 77: 600-604.
25. Spandorfer SD, Neuer A, Giraldo PC, Rosenwaks Z, Witkin SS. 2001. Relationship of abnormal vaginal flora, proinflammatory cytokines and idiopathic infertility in women undergoing IVF. *J Reprod Med* 46: 806-810.
26. Sewankambo N, Gray RH, Wawer MJ. 1997. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. *Lancet* 350: 546-550.

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