Original Article

Oral use of probiotics as an adjunctive therapy to fluconazole in the treatment of yeast vaginitis: A study of Nigerian women in an outdoor clinic

Kingsley C. Anukam1,3,5,6, Martin U. Duru2,4, Clinton C. Eze2, Johnbull Egharevba2, Alfred Aiyebelihin2, Andrew Bruce1 & Gregor Reid1,3

1 Canadian Research & Development Centre for Probiotics, Lawson Health Research Institute, London, ON, Canada, 2 Faith Mediplex, Benin City, Nigeria, 3 Department of Microbiology and Immunology, University of Western Ontario, Canada, 4 Department of Microbiology, Faculty of Life Sciences, University of Benin, 5 Department of Microbiology, Faculty of Basic and Applied Sciences, Benson Idahosa University, and 6 Department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medicine, University of Benin, Nigeria

Abstract

Background: Vulvovaginal candidiasis (VVC) is one of the major urogenital infections for which women seek medical treatment or use self-prescribed antifungals. The objective of this study was to investigate whether probiotic lactobacilli can be used as an adjunctive treatment in the management of VVC in Nigerian women.

Patients and methods: Fifty-nine premenopausal women attending health clinics were diagnosed with vaginal yeast infection by both clinical assessment and standard laboratory culture techniques. After informed consent, they were randomized blindly to receive a conventional single oral dose of fluconazole (150 mg) and a daily probiotic capsule containing Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 or placebo for 3 months (90 days).

Results: At day 7, 47 of the 59 patients attended for follow-up. Seven of the 33 on probiotics (23%) and 2 of 14 on placebo (14%) had evidence of yeast infection by culture on day 7 (p = 0.1), indicating a cure rate of approximately 80% with single dose fluconazole. However, of the 26 subjects who reported at day 90, 79% who received probiotics were free of yeast infection compared with 43% on placebo (p = 0.1490). PCR confirmed this finding, as 75% in the probiotics group were negative for heat shock protein specific for Candida albicans at day 90. A PCR primer set specific for L. rhamnosus GR-1 and L. reuteri RC-14 revealed the presence of the Lactobacillus strains on day 90 in 25% of the subjects who took probiotics. All the patients in the placebo group (100%) had two or three recurrences during the 90 day follow-up, while 53% of the probiotics group had one to two recurrences (p = 0.05).

Conclusion: The study shows that adjunctive treatment of VVC with probiotic L. rhamnosus GR-1 and L. reuteri RC-14 did not impact the cure rate at day 7, but did lead to fewer vulvovaginitis recurrences. Problems with patients returning for follow-up appointments suggest the need for a more active education programme in Nigeria on clinical trials per se and probiotics in particular. Clinical Trial Registration Number: NCT00479947.

Key words: yeast infection, probiotics, lactobacilli, fluconazole

Introduction

Vulvovaginal candidiasis (VVC) remains a common cause of morbidity, with three-quarters of women affected during their lifetime. Recurrent VVC is defined as four or more episodes of symptomatic acute candidiasis in a 12 month period. Use of antibiotics is an acknowledged trigger for VVC, which adversely affects women’s physical and emotional health (1). Following antibiotic treatment, VVC is a major concern for many women, as this may affect their compliance with prescribed antibiotic treatments (2).

In Nigeria, women with secondary infertility (couples who have been pregnant at least once, but have not been able to achieve a pregnancy again) have a higher rate of carriage of Candida albicans as compared with women with primary infertility (couples who have never been able to achieve a...
pregnancy after at least 1 year of unprotected sex) (3). Among asymptomatic pregnant women, prevalence of \textit{C. albicans} has been reported in excess of 65\%. (4). Clinicians rely on antifungals as the main form of treatment, and in most cases the rate of recurrent vaginal candidiasis is between 70 and 80\%. The inappropriate use of antibiotics, propelled by their availability as over-the-counter products, self-medication and outpatient prescribing by physicians (5), exacerbates proliferation of candida infections in Nigeria.

The vaginal microbiota, particularly lactobacilli, has long been known to help protect the vagina against infection, but the perception that these organisms can cure VVC is not based upon solid clinical data. On the other hand, a combination of antifungal therapy plus probiotics might help to improve the cure of VVC and reduce the risk of recurrences. A recent study has shown that probiotic lactobacilli can augment metronidazole in the treatment of bacterial vaginosis (6). Therefore, a study was conceived to examine whether probiotics could improve the efficacy of fluconazole, an antifungal agent that achieves rapid penetration and high concentration in the vaginal tissue above the minimum inhibitory concentration for a period of 96 h. The probiotics used in this study were selected because of their documented ability to populate the vagina after oral administration (7) and reduce transfer of candida from the rectum to the vagina (8).

\textbf{Patients and methods}

\textit{Study participants}

Premenopausal women presenting for urogenital health care at Faith Mediplex Medical Center, Benin City, Nigeria were recruited for the study. The ethics committee of the hospital gave approval for the study. Participation was voluntary and did not deviate from the approved treatment of candidiasis. Each volunteer provided signed consent after thorough explanation of the protocol by the treating clinician and study nurse.

\textit{Inclusion/exclusion criteria}

The inclusion criteria were as follows: women between 18 and 50 years of age; clinical history of acute or chronic yeast vaginitis with three or more episodes of infection over the preceding 12 months; symptomatic at presentation (abnormal, odourless vaginal discharge (cheese-like), dyspareunia, dysuria, localized irritation or discomfort in the vulvo-vaginal area); and negative for evidence of other urogenital infections. Pregnant women were excluded.

\textbf{Diagnosis}

The presence of hyphae and mycelia on microscopic examination of the vaginal secretion in 10% KOH and confirmation by culture using Sabouraud agar were used to diagnose VVC.

\textbf{Randomization and treatment protocol}

Premenopausal women (n=59) presenting with acute VVC were randomized for age and previous history of VVC and treated with one oral dose of fluconazole (150 mg), plus a daily placebo capsule (group A) or a daily capsule of \textit{L. rhamnosus} GR-1 and \textit{L. reuteri} RC-14 (5 billion live organisms per dose) (group B) for 3 months. The probiotics and placebo capsules were supplied by Chr Hansen, Denmark. The pharmacy at the hospital generated the randomization numbers and both the clinicians recruiting the patients and the laboratory scientists performing the microscopy and culture were blinded to the study product.

All subjects were followed at 7 days for proof of cure, and at 1 month, 2 months and 3 months following enrolment. At all follow-up visits, the subjects were checked for symptoms and examined physically. Vaginal swabs (two per visit) were collected at day 0 (before treatment), day 7 (proof of cure for the two groups), and at 1, 2 and 3 month (90 days) follow-up. The swabs were tested for yeast microscopically and by culture. The swabs collected at day 90 were also packaged and placed in ice packs and transported by courier to the Lawson Health Research Institute, London, Canada, for bacterial/fungal DNA extraction and PCR.

\textit{Confirmation of \textit{C. albicans}, presence of \textit{Lactobacillus} species and \textit{Lactobacillus} GR-1/RC-14 at day 90 using species-specific primers for PCR extraction of bacterial/fungal DNA from vaginal swabs}

Bacterial DNA was extracted from the vaginal swabs using Instagene Matrix (Bio-Rad Laboratories) according to the manufacturer’s instructions. Briefly, swabs were vigorously agitated in 1 ml of PBS (phosphate-buffered saline, pH 7.1) to dislodge cells. The cells were pelleted by centrifugation (Eppendorf, Digital Centrifuge 5417C) at 10 000 \textsuperscript{g} for 3 min. The pellets were resuspended in 200 \textmu l Instagene Matrix and incubated for 20–30 min in a water bath (Isotemp®, Fisher Scientific, USA) at 55°C. The sample was vortex mixed for 10 s and boiled at 100°C (Tekstir® Hot plate) for 8 min. The sample was vortex mixed for 10 s and centrifuged at 13 000 \textsuperscript{g} for 3 min. The supernatant containing the DNA was stored at \(-20\)°C.
PCR amplification of the DNA template/sample

The amplification reactions of the DNA template/sample were carried out in 0.2 ml PCR single tube-RNase/DNase/pyrogen-free (Diamed, Lab Supplies, Mississauga, ON, Canada) with hinged flat cap in a thermocycler (Eppendorf Mastercycler). Each PCR consisted of 5.0 μl of 10× buffer (no MgCl₂), 10 mM Tris-HCl and 50 mM KCl, 2.5 μl of MgCl₂ (50 mM), 1.0 μl dNTPs (5 mM each), 1.25 μl of glycerol (80%; Sigma), 4.0 μl of bovine serum albumin (BSA; 10 mg/ml; Sigma), 50 pmoles/μl of each primer for lactobacilli (9) (LGC-1-5′-AGC AGT AGG GAA TCT TCC A-3′ and LGC-2-GC with the sequence; 5′-CGC CCG GGG CGG CCC GCG GGC GGC CGG GGA CAT TYC ACC GCT ACA C-3′) (Invitrogen; Life Technologies), 0.2 μl of Platinum® Taq DNA polymerase (5 U/μl; Invitrogen™, Life Technologies), 2.0 μl of the DNA template/sample, and sterile water (Fluka H₂O) to a volume of 50 μl. Species-specific primers for C. albicans were used to confirm the presence of infection at day 90. The primers based on sequence of the heat shock protein gene (HSP90) specific for C. albicans are as follows; HSP90 forward primer (GAC ACCCTATGCTCTT CTTAC) and HSP90 reverse primer (GCAGATTCCGCAGCTGTTTCGTC) (10). For Lactobacillus GR/RC-14, a new primer set (forward-GAGAAGACGTGCGTGAG and reverse-ATGTGAAAGCCTTCGCTT) developed by Anukam and Reid (11) that is able to identify both organisms was used.

The PCR amplification followed the LACTO program in the Mastercycler, with initial DNA denaturation at 95°C for 2 min, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 1 min and elongation at 72°C for 1 min, which was followed by a final extension at 72°C for 10 min. To confirm amplicon production, the mixture (5 μl PCR product and 2 μl of loading buffer) was analysed by electrophoresis (Bio-Rad) in 1.5% Ultrapure™ agarose gel (Invitrogen, Life Technologies), at 100 V for 45 min, followed by staining with 1% solution of ethidium bromide (50 μl/L) and de-staining with 1× TAE for 10 min. Gels were visualized by UV transillumination and recorded with Polaroid 667 instant film.

Patients’ perceptions of the impact of treatment at 7, 30, 60 and 90 days

A structured questionnaire was designed to obtain any change in the condition of the infection from each patient’s perception. All were asked to indicate the day (day 1 to day 7), when they obtained relief of symptoms and to report any side effects or recurrences of infection during the 90 day period.

Primary outcome

The primary outcome of the study was reduction in the incidence of yeast infection as determined by culture and PCR at day 90 for the two groups of participants.

Statistical analysis

A non-parametric χ² test and two-sided Fisher’s exact test were used for significant associations between two categorical variables in 2 by 2 contingency tables. Differences were considered statistically significant if the p value was ≤0.05.

In the placebo group of patients, it was anticipated that at the 3 month period, a recurrence rate of 50% would occur, whereas in the treatment arm a reduction in recurrence to 25% would be achieved. The study was designed to have a power of 80% and to detect a reduction in recurrence at the two-sided 5% significance level.

Results

Fifty-nine women who met all the inclusion criteria were recruited. At day 7, 47 patients (80%) attended for follow-up, while 26 patients (44%) attended on days 30 and 90. Data analysis was only performed on patients who returned for follow-up. This is a population that has day-to-day challenges that despite our best efforts did not make it possible for them to return to the clinic. The randomization scheme had been created with a view to enrolling 90 subjects, but due to poor patient compliance – which is usually one of the limitations of conducting clinical trials in developing countries – funding limits and clinicians’ tight working schedules, enrollment was stopped at 59 patients. Of these, 39 received probiotics while 20 received placebo. Lack of full compliance from the patients (mainly due to poor educational background in grasping the importance of participation in clinical trials) affected the power of the study. This was evident with the drop-outs after day 7. The traditional reason for patients not returning for follow-up is that they feel well and have no recurrence of their initial symptoms. This finding was confirmed in several patients who did not return but who were traced by the clinical staff.

At day 7, 7/33 subjects on probiotics (21%) and 2/14 on placebo (14%) had evidence of yeast infection on culture, within the expected cure rate with fluconazole at this time of follow-up (Table I). At
day 90, 15/19 (79%) in the probiotic group B were free of yeast infection on culture as compared with 3/7 in the placebo group A (43%) \((p=0.1490)\). PCR with heat shock protein (HSP90) primers recovered an additional positive finding for one of the infection-free subjects who received probiotics. To confirm the presence of *Lactobacillus* species in the subjects who provided vaginal swabs at day 90, PCR with LGC primers (universal primers specific for *Lactobacillus*) showed that all 16 (100%) of subjects on probiotics were colonized by lactobacilli, as were the 5 (100%) on placebo. A newly designed primer set specific for *L. rhamnosus* GR-1 and *L. reuteri* RC-14 confirmed the presence of these strains in the vaginal swabs of 4/16 in group B and 0/7 in group A subjects.

### Table I. Yeast and *Lactobacillus* status of patients who completed the trial at various time points.

| Patient no. | Group | Yeast culture (days) | *C. albicans* PCR (day 90) | Lacto PCR (day 90) | GR-1/RC-14 PCR (day 90) |
|-------------|-------|----------------------|-----------------------------|-------------------|------------------------|
| 1           | B     | − − − −              | −                           | +ve               | +ve                    |
| 2           | B     | − − − −              | −                           | +ve               | −                      |
| 3           | B     | − − − −              | −                           | +ve               | −                      |
| 4           | B     | + + + −              | +ve                         | +ve               | −                      |
| 5           | A     | − − − +              | −                           | +ve               | −                      |
| 6           | B     | − − − −              | −                           | +ve               | +ve                    |
| 7           | A     | − + − +              | −                           | +ve               | −                      |
| 8           | B     | − − − −              | −                           | +ve               | −                      |
| 9           | B     | − − − −              | −                           | +ve               | −                      |
| 10          | A     | − − − −              | −                           | +ve               | −                      |
| 11          | B     | − − − −              | −                           | +ve               | −                      |
| 12          | B     | − + + +              | −                           | +ve               | −                      |
| 13          | A     | + + + +              | +ve                         | +ve               | −                      |
| 14          | B     | − − + −              | −                           | +ve               | −                      |
| 15          | B     | − − − −              | +ve                         | +ve               | −                      |
| 16          | B     | − − − −              | +ve                         | +ve               | −                      |
| 17          | B     | − + + +              | −                           | +ve               | −                      |
| 18          | B     | + − + +              | +ve                         | +ve               | −                      |
| 19          | A     | − − − −              | +ve                         | +ve               | −                      |
| 20          | B     | − − − −              | +ve                         | +ve               | −                      |
| 21          | B     | + + + +              | +ve                         | +ve               | +ve                    |
| 22          | A     | − + + +              | +ve                         | +ve               | +ve                    |
| 23          | B     | − − + −              | NA                          | NA                | NA                     |
| 24          | A     | − − − −              | NA                          | NA                | NA                     |
| 25          | B     | − + + +              | NA                          | NA                | NA                     |
| 26          | B     | − − − −              | NA                          | NA                | NA                     |
| 27          | A     | − − NA               | −                           | +ve               | −                      |
| 28          | B     | − − NA               | −                           | +ve               | −                      |
| 29          | B     | − − NA               | −                           | +ve               | −                      |
| 30          | B     | + − NA               | −                           | +ve               | −                      |
| 31          | A     | − − NA               | −                           | +ve               | −                      |
| 32          | A     | − − NA               | −                           | +ve               | −                      |
| 33          | B     | − − NA               | −                           | +ve               | −                      |
| 34          | A     | − − − −              | −                           | +ve               | −                      |
| 35          | B     | − − − −              | −                           | +ve               | −                      |
| 36          | B     | − − − −              | −                           | +ve               | −                      |
| 37          | B     | − − NA               | −                           | +ve               | −                      |
| 38          | A     | − − − −              | −                           | +ve               | −                      |
| 39          | B     | − − − −              | −                           | +ve               | −                      |
| 40          | B     | − + − −              | −                           | +ve               | −                      |
| 41          | B     | − − − −              | −                           | +ve               | −                      |
| 42          | B     | − − − −              | −                           | +ve               | −                      |
| 43          | B     | − − − −              | −                           | +ve               | −                      |
| 44          | B     | − + − −              | −                           | +ve               | −                      |
| 45          | B     | − + − −              | −                           | +ve               | −                      |
| 46          | B     | − − − −              | −                           | +ve               | −                      |
| 47          | A     | − − − −              | −                           | +ve               | −                      |

Group A, placebo group; group B, probiotics group; −, negative for yeast; +, positive for yeast; +ve, PCR positive; NA, not available.
Nine of 19 (47%) patients receiving probiotics reported resolution of symptoms by day 7 compared with 1 of 7 (14%) on placebo \((p = 0.2794)\) (Table II). At day 90, the percentages were 58% and 43%, respectively. As noted above, it was difficult to get the subjects to come to the study centre for follow-up. Thus, we relied on the patients reporting episodes of VVC in between visits to the centre (Table III). All the subjects in the placebo group (100%) had two to three recurrences within the 90 day study period, while 53% of the probiotics group had one to two recurrences \((p = 0.057)\). These subjects were treated conventionally by their physician. Only a few subjects reported side effects of single episodes of headache and nausea during the 90 day period, and none could be correlated to the probiotic or placebo treatment.

**Discussion**

The primary end point of this study was recurrences of VVC at day 90, and 79% of subjects treated with probiotics had fewer recurrences of VVC and were free of infection, compared with only 43% being free of infection on placebo. While the sample size of returning subjects in the placebo group was too small to reach significance, the trend certainly suggests that probiotics had a beneficial effect.

PCR confirmed this finding, with 75% in the probiotics group B negative for HSP specific for *C. albicans* at day 90. Recurrence of VVC is still a major problem and occurs mostly 1–2 months after stopping antifungal therapy, even fluconazole (150 mg) given weekly for 6 months (12).

The confirmation that the probiotic strains were recovered in 25% of the vaginal swabs provided further confirmation of the ability to deliver vaginal probiotics via oral consumption and ascent from the rectum to the vagina (7,13). In another study performed in Brazilian women, the strains were recovered in 20/66 (30%) vaginal samples (11). While the relatively low presence may in part be due to limitations in sampling (we cannot definitively state that the probiotics were absent, just that they were not detected by the collected swab), the overall goal of such a therapeutic approach is not necessarily to deliver the probiotic strains, but to reduce pathogen ascent and increase the overall lactobacilli load, including recovery of indigenous species.

Thus, the bottom line is clinical outcome, and in this study there were more women \((p = 0.2794)\) in the probiotic group who reported complete resolution of VVC symptoms by day 7 compared with those on fluconazole and placebo. The mechanisms were not investigated, but immune modulation may play a role, as inflammation is associated with discharge and itching, and strains GR-1 and RC-14 have immunomodulatory properties (14–16).

In terms of correlating symptoms and signs of VVC and presence of yeast, an interesting finding emerged from this study. When only 25% subjects showed the

### Table II. Questionnaire responses from the patients (probiotics and placebo groups) on how they felt during the study.

| Day | Probiotic group \((n = 19)\) | Placebo group \((n = 7)\) |
|-----|-----------------------------|------------------------|
|     | Got relief within days/weeks |                        |
|     | Day 1 = 0 Week 1 = 9        | Day 1 = 0 Week 1 = 1   |
|     | Day 2 = 3 Week 2 = 1        | Day 2 = 0 Week 2 = 3   |
|     | Day 3 = 2 Week 3 = 3 Week 4 = 6 | Day 3 = 0 Week 3 = 0 Week 4 = 4 |
|     | Day 4 = 6 Week 4 = 3 Week 5 = 1 Week 9 = 7 | Day 4 = 3 Week 4 = 0 Week 5 = 1 Week 9 = 2 |
|     | Day 5 = 2 – Week 6 = 6 Week 10 = 2 | Day 5 = 0 – Week 6 = 2 Week 10 = 1 |
|     | Day 6 = 1 – Week 7 = 2 Week 11 = 5 | Day 6 = 0 – Week 7 = 0 Week 11 = 1 |
|     | Day 7 = 1 – Week 8 = 3 Week 12 = 0 | Day 7 = 2 – Week 8 = 0 Week 12 = 0 |
|     | No relief 4/19 (21%)        | 2/7 (29%) 3 0 3 (43%)  |
|     | Days/weeks completely resolved |                        |
|     | Day 1 = 0 Week 1 = 2        | Day 1 = 0 Week 1 = 0   |
|     | Day 2 = 0 Week 2 = 3        | Day 2 = 0 Week 2 = 2   |
|     | Day 3 = 1 Week 3 = 2 Week 4 = 3 | Day 3 = 0 Week 3 = 0 Week 4 = 2 |
|     | Day 4 = 5 Week 4 = 0 Week 5 = 1 Week 9 = 6 | Day 4 = 0 Week 4 = 0 Week 5 = 1 Week 9 = 2 |
|     | Day 5 = 2 – Week 6 = 1 Week 10 = 1 | Day 5 = 0 – Week 6 = 1 Week 10 = 1 |
|     | Day 6 = 0 – Week 7 = 2 Week 11 = 4 | Day 6 = 0 – Week 7 = 0 Week 11 = 0 |
|     | Day 7 = 2 – Week 8 = 0 Week 12 = 0 | Day 7 = 1 – Week 8 = 0 Week 12 = 0 |
|     | Did not resolve 10/19 (52.6%) | 12/19 (63.1%) (63.1%)  |

This represents the number (%) of participants responding to questions (‘Has the condition changed since you started the treatment given to you in this study?’) indicating when they got relief within the first 7 days. Patients also indicated the day or week for questions such as ‘On which day did you feel that the problem was completely resolved?’.
Table III. Number of patients reporting recurrence of yeast infections within the 90 day study period.

| Recurrence | Probiotic group (n=19) | Placebo group (n=7) |
|------------|------------------------|---------------------|
| None       | 9                      | 0                   |
| One        | 6                      | 0                   |
| Two        | 4                      | 3                   |
| Three      | 0                      | 4                   |

In conclusion, there was evidence of fewer recurrences of VVC with long-term use of probiotic *L. rhamnosus* GR-1 and *L. reuteri* RC-14 in patients with a history of chronic infection. At present in Nigeria, neither these probiotics nor any other are available for women to use as an adjunct to urogenital care. Even if probiotics were introduced, many of the population might find their purchase beyond their financial means. Thus, there are challenges with respect to conducting clinical trials in Nigeria and bringing novel therapies from the developed world to women who need them in poor countries.

Acknowledgements

The support of Faith Mediplex Hospital board and other staffs is highly appreciated. Dr Gregor Reid held patents for *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14, but he has recently transferred ownership to Chr Hansen so as to free him from any further conflicts and these two strains have been licensed to Chr Hansen. He was not involved in the clinical study per se or acquisition of data. Funding support from Chr Hansen and Urex Biotech Inc. is highly appreciated.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Chapple A, Hassell K, Nicolson M, Cantrill J. ‘You don’t really feel you can function normally’: women’s perceptions and personal management of vaginal thrush. J Reprod Infant Psychol. 2000;18:309–19.
2. Pirotta M, Gunn J, Chondros P. “Not thrush again!” Women’s experience of post-antibiotic vulvovaginitis. Med J Aust. 2003;179:43-6.
3. Okonofua FE, Ako-Nai KA, Dightoghi MD. Lower genital tract infections in infertile Nigerian women compared with controls. Genitourin Med. 1995;71:163-8.
4. Akerele J, Abbulimen P, Okonofua F. Prevalence of asymptomatic genital infection among pregnant women in Benin City, Nigeria. Afr J Reprod Health. 2002;6:93–7.
5. Obaseiki-Ebor EE, Akerele JO, Ebea PO. A survey of antibiotic outpatient prescribing and antibiotic self-medication. J Antimicrob Chemother. 1987;20:759–63.
6. Anukam K, Osazuwa A, Ahonkhai I, Ngwu M, Osemene G, Bruce AW, et al. Augmentation of antimicrobial metronidazole therapy of bacterial vaginosis with oral probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14: randomized, double-blind, placebo controlled trial. Microbes Infect. 2006;8:1450-4.
7. Reid G, Bruce AW, Fraser N, Heinemann C, Owen J, Henning B. Oral probiotics can resolve urogenital infections. FEMS Immunol Med Microbiol. 2001;30:49-52.
8. Reid G, Charbonneau D, Erb J, Kochanowski B, Beuerman D, Poehner R, et al. 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. 2003;35:131–4.
9. Walter J, Hertel C, Tannock GW, Lis CM, Munro K, Hammes WP. Detection of Lactobacillus, Pediococcus, Leuconostoc, and Weissella species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 2001;67:2578–85.
10. Swoboda RK, Bertram G, Budge S, Gooday GW, Gow NA, Brown AJ. Structure and regulation of the HSP90 gene from *Candida albicans*. Infect Immun. 1995;63:4506–14.
11. Anukam KC, Reid G. A two species-specific primers to identify *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 after probiotic use. Int J Probiotics Prebiotics 2008 (in press).
12. Sobel JD, Wiesenfeld HC, Martens M, Danna P, Hooton TM, Rompalo A, et al. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. N Engl J Med. 2004;351:876–83.
13. Morelli L, Zonenschain D, Del Piano M, Cognin P. Utilization of the intestinal tract as a delivery system for urogenital probiotics. J Clin Gastroenterol. 2004;38(Suppl 6):S107–S110.
14. Kim SO, Sheikh HI, Ha SD, Martins A, Reid G. G-CSF-mediated inhibition of JNK is a key mechanism for *Lactobacillus rhamnosus*-induced suppression of TNF production in macrophages. Cell Microbiol. 2006;8:1958–71.
15. Lorea Baroja M, Kirjavainen PV, Hekmat S, Reid G. Anti-inflammatory effects of probiotic yogurt in inflammatory bowel disease patients. Clin Exp Immunol. 2007;149:470–9.
16. Kirjavainen PV, Laine RM, Carter DE, Hammond J, Reid G. Expression of antimicrobial defense factors in vaginal mucosa following exposure to *Lactobacillus rhamnosus* GR-1. Int J Probiotics Prebiotics. 2008;3:99–106.