Clinical Scenario:

An 84-year-old woman with severe repeated episodes of vulvovaginitis that have not responded to several treatment regimens of fluconazole orally, clotrimazole topically, and topical miconazole.

Clinical Question:

In women with vaginosis, what is the efficacy of an "azole" antifungal in combination with a Lactobacillus probiotic versus an "azole" antifungal alone with regard to treatment outcome?

Articles:

Martinez, R. C. R., Franceschini, S. A., Patta, M. C., Quintana, S. M., Candido, R. C., Ferreira, J. C.,...Reid, G. (2009). Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14. *Letters in Applied Microbiology, 48*, 269-274.

Martinez, R. C., Franceschini, S. A., Patta, M. C., Quintana, S. M., Gomes, B. C., De Martinis, E. C., & Reid, G. (2009). Improved cure of bacterial vaginosis with single dose of tinidazole (2g), Lactobacillus rhamnosus GR-1, and Lactobacillus reuteri RC-14: A randomized, double-blind, placebo-controlled trial. *Canadian Journal of Microbiology, 55*, 133-138.

Critical Review of Study/Appraisal of Key Evidence:

Study Design:

Both study designs were randomized, double-blind, placebo-controlled trials qualifying as a level of evidence Ia. In the study by Martinez, Franceschini, Patta, Quintana, Gomes, et al. (2009), half of the women received a single dose of tinidazole (2g) in addition to 2 placebo capsules while half received tinidazole (2g) supplemented with 2 capsules containing *L. rhamnosus GR-1* and *L. reuteri RC-14*. Martinez, Franceschini, Patta, Quintana, Candido, et al. (2009) administered fluconazole (150 mg) with 2 oral capsules of *L. rhamnosus GR-1* and *L. reuteri RC-14* to 29 subjects while 26 subjects were treated with fluconazole and placebo capsules. The medication regimens in both trials were taken every morning for 28 days starting on the day of "azole" antifungal use.

Sample:

Both studies appointed gynecologists at four institutions to determine subject eligibility. In the Martinez, Franceschini, Patta, Quintana, Gomes, et al. (2009) study, 64 subjects were voluntarily enrolled in the study after being diagnosed with bacterial vaginosis (BV). Exclusion

criteria included immunosuppression, concomitant vulvovaginal candidiasis and/or trichomoniasis, use of systemic or intravaginal antibiotic or antifungal agents presently or within the past 2 weeks, current menstruation, or imidazole drug allergy.

The inclusion criteria for the 55 subjects in the research performed by Martinez, Franceschini, Patta, Quintana, Candido, et al. (2009) included women suffering from vaginal discharge associated with any one of the following: vaginal itching and burning, dyspareunia, and/or dysuria. In addition, vaginal discharge samples collected were required to be positive for Candida spp. by a standardized culture method. Exclusion criteria included pregnancy, HIV positive status, BV infection, trichomoniasis infection, current or recent (within the last two weeks) use of systemic or intravaginal antibiotic or anti-fungal agents, present menstruation, and fluconazole drug allergy.

Procedure:

In the study conducted by Martinez, Franceschini, Patta, Quintana, Gomes, et al. (2009), evaluation was performed implementing two standardized scoring systems of three vaginal samples in addition to a Gram-stained vaginal smear. The objective and consistent methodology implored provided reliability and validity to the study. At the end of the 28 days, subjects were examined in the exact fashion they were on the initial visit. Investigators remained blinded until all analysis had commenced.

Procedural methodology by Martinez, Franceschini, Patta, Quintana, Candido, et al. (2009) implemented measurements of vaginal pH, Gram-staining, wet mount preparation, and swab collection for seeding, isolation, and identification of Candida microorganisms. The objective and consistent methodology implored provided reliability and validity to the study. At the end of the 28 days, presence of vaginal discharge and any of the aforementioned symptoms and signs were evaluated. Investigators remained blinded until all analysis had commenced.

Outcome(s) Measured:

Outcomes measured in the data collected by Martinez, Franceschini, Patta, Quintana, Gomes, et al. (2009) included a positive Amsel test, vaginal pH > 4.5, white and/or homogenous and/or aqueous vaginal discharge, positive "Whiff" test, and bad vaginal odor (self-reported).

Outcomes measured in the data collected by Martinez, Franceschini, Patta, Quintana, Candido, et al. (2009) included vaginal discharge associated with at least one of the symptoms (itching and burning vaginal feeling, dyspareunia, and/or dysuria), presence of Candida by culture method, positive "Whiff" test, vaginal pH > 4.5, and Nugent BV score.

Results:

Martinez, Franceschini, Patta, Quintana, Gomes, et al. (2009) found an 87.5% cure rate of BV versus 50.0% in the placebo group with a statistical significance of $p < 0.05$. Moreover, 75.0% of the probiotic group was found to have "normal" vaginal flora versus 34.4% in the placebo group, with a statistical significance of $p < 0.05$. No statistical significance was found in

subjects with self-reported recurrent BV. Cure rate for the probiotic group was 75.0% compared to a 57.1% for the placebo group with a statistical significance of $p > 0.05$.

Martinez, Franceschini, Patta, Quintana, Candido, et al. (2009) found an 89.7% cure rate in the probiotic group for VVC versus 61.5% in the placebo group with a statistical significance of $p < 0.05$. In addition, cure rates for recurrent VVC were 81.8% for the probiotic group and 20% for the placebo group with a statistical significance of $p < 0.05$. There was no statistical significance in remission of symptoms between the two groups with regard to a positive "Whiff" test, vaginal pH >4.5, and Nugent BV score.

Weaknesses:

Both studies failed to implement objective measurements for some of the criteria measured, which leads to a decrease in reliability of the research. Subjects were simply asked for measurements with regard to recurrent BV infections and adherence to treatment protocols. In addition, one of the researchers in both studies holds a patent for lactobacilli, however, he was not included in accumulation of data and was blinded to results until the cessation of the study.

The research conducted by Martinez, Franceschini, Patta, Quintana, Candido, et al. (2009) is the first research completed on the use of probiotics with regard to VVC and, therefore, comparative evidence is lacking. As a result, the strength of the outcomes identified lack merit.

Clinical Bottom Line:

Vaginosis affects a large majority of women across the world with approximately 75% of sexually active women exhibiting signs and symptoms throughout their lifetime and 50% developing a second infection (Martinez, Franceschini, Patta, Quintana, Candido, et al., 2009). Vulvovaginal candidiasis and bacterial vaginosis are the two predominant causes of vaginal infections in women today. Lactobacilli probiotics in combination with "azole" antifungals have proven to demonstrate superior efficacy in the management of these disease entities when compared to a placebo. Lack of treatment options and successful long-term therapy has been problematic in the treatment of both VVC and BV. Moreover, the increasing amount of microbial resistance encountered throughout healthcare today poses a challenge. Research such as this opens the opportunity for complementary and alternative methodologies to be considered as adjunctive treatment that provide significant results in patient outcomes.