

Life in the littoral zone: lactobacilli losing the plot

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Sex Transm Infect 2005;81:100–102. doi: 10.1136/sti.2003.007161

Recurrent bacterial vaginosis is a challenge for those affected by it, and their physicians. Our inability to prevent relapse after treatment, may be because of the flawed approach of using antibiotics to treat a condition that is an imbalance rather than an infection. The maintenance of a healthy lactobacillus population offers an approach to preventing relapse: the problem is how best to do this. Physiological approaches such as the use of hydrogen peroxide, lactic acid, and exogenous lactobacilli need to be explored further. The role of bacterial vaginosis as a risk factor for acquisition of HIV and other STIs is a further impetus to attempting to prevent bacterial vaginosis from recurring.

The aetiology of bacterial vaginosis (BV) continues to be debated more than 20 years after the name was adopted, and nearly 50 years after Gardner and Dukes first described clue cells.¹ Some behavioural studies have associated risk taking sexual behaviour with BV. For instance, a Swedish study identified risk factors for BV as being similar to those for chlamydia.² A recent Australian study associated BV with having three or more male partners in the preceding year, at least one female sexual partner in the past 12 months, as well as a past pregnancy and smoking.³

The most compelling evidence against BV being exclusively a sexually transmitted infection (STI) is its prevalence in virgin women. Bump and Buesching found clue cells in 15% of 68 sexually active adolescents and 12% of 52 virgin adolescents in a survey conducted in a high school.⁴ More recently, a study of female army recruits in the United States detected BV in 28% of sexually experienced women and 18% of non-sexually experienced women.⁵

The reported point prevalence of BV varies widely between different populations, with more than 50% women in rural Uganda having BV in the Rakai study.⁶ Lifetime incidence has not been studied. Given the high frequency with which asymptomatic BV and intermediate flora developed in studies of volunteers⁷ it seems likely that most women will develop BV at some point. The point prevalence therefore reflects the balance of occurrence, persistence and spontaneous clearance in a population. It is important that we learn how to prevent relapse/incidence of BV to help the symptomatic women who experience considerable distress from recurrent BV, as well as aiming for a potential public health impact to reduce BV

associated adverse pregnancy outcomes and HIV transmission.

Rather than viewing BV as an overgrowth of anaerobic bacteria, some authors have described it as a deficiency of lactobacilli. We know little about what influences the lactobacillus flora that establishes itself as the predominant vaginal organism at menarche in “healthy women.” At this time, under the influence of increasing levels of oestrogen the vaginal epithelium becomes stratified and the pH of vaginal fluid falls. The vaginal flora becomes dominated by lactobacilli; often more than one subspecies is present. *Lactobacillus crispatus* and *L. gensenii* have been identified as common high hydrogen peroxide producing vaginal strains.⁹ The subspecies that thrive in the vagina are different from those in the gut, and we know little about their origin, and the mechanisms that support their persistence in the vagina. In the gut, production of IgA specific to commensal species helps to prevent an inflammatory response when the organisms come into contact with the mucosal surface. Is there a similar tolerance in the vagina? And how is such tolerance established? If this is organism specific, using exogenous hydrogen peroxide producing lactobacilli to recolonise the vagina of women with recurrent BV may not be a successful strategy. A recent study of adolescent women reported a predominance of transforming growth factor beta (TGF- β) and interleukin (IL) 10 production by peripheral blood mononuclear cells in response to *L. crispatus* antigens.¹⁰ The authors comment that TGF- β , together with IL-10, counteracts the effects of proinflammatory cytokines, thereby creating a condition of tolerance. They did not know how species specific such a response is. The lactobacilli maintain their dominance through a combination of acidity, hydrogen peroxide, lactocins, and other bacteriocins to inhibit the growth of other bacteria. One key factor is that the organisms associated with BV can overcome such inhibition at a pH >4.5¹¹ and have a mutually symbiotic relationship.^{12,13} These mechanisms were reviewed recently by Janet Wilson in this journal.¹⁴ Additional mechanisms might include the production of biosurfactants and collagen binding proteins that inhibit pathogen adhesion and to some extent displace pathogens, as has been shown in the gut.¹⁵

The vagina, however, is not a steady state ecosystem. As the menstrual cycle becomes established there are marked cyclical changes

Abbreviations: BV, bacterial vaginosis; IL, interleukin; TGF- β , transforming growth factor beta; STI, sexually transmitted infections; WSW, women who have sex with women

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Accepted for publication
5 August 2004

in the environment, which can be compared to life in the littoral zone of the seashore. Levels of oestrogen and progesterone alter, changing the endogenous environment for lactobacilli by influencing cell surface receptor expression,¹⁶ levels of glycogen and glucose as substrate, and levels of vaginal pH. Additionally, factors exogenous to the vagina, such as quantity of cervical mucus and menstrual products with a pH of 7–8, all ebb and flow. The influence of hormones over the course of the menstrual cycle have been studied little in the aetiology of BV.^{17–18} In a mouse model of chlamydia infection progesterone is required to make the animal susceptible to chlamydia.^{19–20} This is associated with a dramatic change in the vaginal flora, with an overgrowth of many organisms that appears similar to BV. This is an area requiring further study. Oestrogen may be protective in women as the prevalence of BV has been lower in women using combined oral contraception⁵ and tends to decrease during pregnancy.²¹

In addition to inescapable physiological changes that occur over the course of a menstrual cycle, there are multiple behavioural factors that can challenge the ecological niche of the lactobacilli. Washing practices will have a variable effect on the flora. Douching is generally associated with BV, but prospective studies investigating the potential benefit of stopping douching have not been done. An American study of 250 adolescent women confirmed associations between BV and multiple partners, recent sexual intercourse and gonorrhoea.²² However, douching after menses was the factor most strongly associated with BV. The authors comment that we still do not know if the douching is causal, or a response to symptoms of BV. As a young woman becomes sexually active additional environmental stresses may come into play. Semen has a pH >7.0. It has been estimated that a normal level of lactobacilli will take several hours to reacidify the vagina following one episode of unprotected intercourse.²³ If condoms are used the spermicide may disrupt vaginal epithelium more than unprotected intercourse does. If they are not used there is the risk of acquiring STIs and the secondary effects that they have on the vaginal milieu. Gardner and Duker were able to induce BV in women by inoculating them with fresh vaginal discharge from another woman with BV, while vaginal flora cultured in vitro for even 12 hours was less likely to induce BV.²⁴ Inoculation of organisms from a male or female partner may therefore be one means of initiating BV. Women who have sex with women (WSW) have been found to have a high prevalence of BV in several studies.^{25–27} A high concordance rate among couples to have either BV or normal flora has been found, with 95% concordance in one study.²⁶ Further support for the hypothesis of a sexually transmissible agent between women was provided by the association between BV and a higher lifetime number of female sex partners, failure to always clean an insertive sex toy before use, and oral-anal sex with female partners.

Bacteria from the gut, such as *Escherichia coli* and group B streptococci commonly colonise the vagina. Little is known about how much this affects the bacterial balance of the vagina. Is there an influence of gut lactobacilli? Lactobacilli ingested from yoghurt might harbour phages, potentially toxic to vaginal lactobacilli in up to 25% of cases.^{28–29} This hypothesis has been expanded to include the potential role of smoking in inducing a lytic phase of phage infection to explain the association between BV and smoking.²⁹ BV is associated with STIs including gonorrhoea, chlamydia, and HIV. There are some in vitro data supporting the hypothesis that BV increases susceptibility to STIs through mechanisms such as cleavage of IgA and mucins³⁰ and lack of hydrogen peroxide.¹¹ In a clinical study of female partners of men with urethral infections, BV was strongly predictive of both

gonorrhoea (odds ratio (OR), 4.1; 95% confidence interval (CI) 1.7 to 9.7) and chlamydia (OR, 3.4; 95% CI, 1.5 to 7.8).³¹ This association does not exclude the possibility that the BV is arising as a consequence of the STI, rather than inducing susceptibility to STI acquisition. It is likely that the STIs alter vaginal physiology by inducing inflammation, increasing production of pus, mucus, and sometimes blood from the cervix. This is likely to alter vaginal pH and make the environment less conducive to survival of lactobacilli and promote the development of BV.

Our increasing understanding of the factors controlling the microbial interactions in the vaginal environment has not yet led to successful interventions to prevent relapse of BV. The use of exogenous lactobacilli as a probiotic has not so far proved successful. However, understanding the influence of cyclical changes should enable us to focus on supporting endogenous lactobacilli when they are most vulnerable. The use of repeated courses of antibiotics is not an attractive long term proposition as inevitably bacterial resistance will develop, and the antibiotics may induce unwanted side effects for those treated. A more physiological approach would be to use acidification and/or hydrogen peroxide to assist the lactobacilli during menstruation or after unprotected sexual intercourse. There are uncontrolled studies showing that hydrogen peroxide is successful.^{32–33} Such an approach warrants further study in randomised controlled trials. Given the dramatic environmental changes that occur over the course of a menstrual cycle, with additional exogenous factors, it would be surprising if lactobacilli were able to survive every environmental insult that comes their way in such a littoral zone, and don't occasionally lose their plot in the ecosystem.

Conflict of interest statement: The author has received payment for lectures and consultancy from Osmetech, which is developing a diagnostic test for bacterial vaginosis; 3M, which manufactures 0.75% metronidazole vaginal gel; and Pharmacia and Upjohn, which manufacture 2% clindamycin vaginal cream and clindamycin capsules. He has conducted clinical trials for which his unit has received reimbursement from Osmetech; 3M; Pharmacia and Upjohn, and he has received financial support to attend conferences from these companies.

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