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Review Article

Rennin and lecithin to treat vaginosis in a lesbian and bisex woman

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ABSTRACT

Vaginosis seems to be more severe, fastidious and afflictive in lesbians, since they are accustomed to employ sexual toys and use to douche more frequently that women who lead a life more correct and regular (even having multiple sex encounters during the week). Many are the OTC remedies, especially acid syndets and detergents, or many infusions made with particular herbs (Aloe barbadensis, coconut milk, garlic decoction, tea tree oil, mauve or rhodiola extracts), even if they yield to relaps too often. Many Researchers have proposed the usage of amphoteric tensides for this scope of treating vaginosis, even there is no hitherto reliable sources in the international literature. The AA have made up their mind to create a cosmetic cocktail containg a biological protease that acts as an amphoteric surfactant (presenting the two hydrophilic and lypophilic moyeties) and the soy lecithin. Results appear to be interesting and satisfying.

Key words: Gardnerella vaginalis; Organic amphoteric surface active agents; Mauve; Aloe barbadensis; Rhodiola

INTRODUCTION

Bacterial vaginosis is a type of vaginal inflammation caused by the overgrowth of bacteria naturally found in the vagina, which upsets the natural balance.

Women in their reproductive years are most likely to get bacterial vaginosis, but it can affect women of any age. The cause isn't completely understood, but certain activities, such as unprotected sex or frequent douching, increase the woman's risk.

Bacterial vaginosis signs and symptoms may include: Thin, gray, white or green vaginal discharge; Foul-smelling "fishy" vaginal odor; Vaginal itching.

Burning during urination; Many women with bacterial vaginosis have no signs or symptoms.

It is suggested to call at the doctor when vaginal discharge appears new and not associated with an odor or fever. The physician can help determine the cause and identify signs and symptoms., if no vaginal infections had manifested before, but the color and consistency of discharge seems different this time.,if the woman has sex partners or a recent new partner. Sometimes, the signs and symptoms of a sexually transmitted infection are similar to those of bacterial vaginosis.

Bacterial vaginosis results from overgrowth of one of several bacteria naturally found in the vagina. Usually, "good" bacteria (lactobacilli) outnumber "bad" bacteria (anaerobes). But if there are too many anaerobic bacteria, they upset the natural balance of microorganisms in the vagina and cause bacterial vaginosis.

Risk factors for bacterial vaginosis include:
Having multiple sex partners or a new sex partner. Doctors don›t fully understand the link between sexual activity and bacterial vaginosis, but the condition occurs more often in women who have multiple sex partners or a new sex partner. Bacterial

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vaginosis also occurs more frequently in women who have sex with women., since they are accustomed to help to join orgasm using objects, as godemichés or dildos or sex toys.

Douching. The practice of rinsing out your vagina with water or a cleansing agent (douching) upsets the natural balance of the vagina. This can lead to an overgrowth of anaerobic bacteria, and cause bacterial vaginosis. Since the vagina is self-cleaning, douching isn't necessary.

Natural lack of lactobacilli bacteria. If the natural vaginal environment doesn›t produce enough of the good lactobacilli bacteria, a woman or girl is more prone likely to develop bacterial vaginosis.

Bacterial vaginosis doesn't generally cause complications. Sometimes, having bacterial vaginosis may lead to:

Preterm birth. In pregnant women, bacterial vaginosis is linked to premature deliveries and low birth weight babies.

Sexually transmitted infections. Having bacterial vaginosis makes women more susceptible to sexually transmitted infections, such as HIV, herpes simplex virus, chlamydia or gonorrhea. If you have HIV, bacterial vaginosis increases the odds that you'll pass the virus on to your partner.

Infection risk after gynecologic surgery. Having bacterial vaginosis may increase the risk of developing a post-surgical infection after procedures such as hysterectomy or dilation and curettage (D&C).

Pelvic inflammatory disease (PID). Bacterial vaginosis can sometimes cause PID, an infection of the uterus and the fallopian tubes that can increase the risk of infertility.

To help prevent bacterial vaginosis:
Minimize vaginal irritation. Use mild, nondeodorant soaps and unscented tampons or pads.

Don't douche. Your vagina doesn›t require cleansing other than normal bathing. Frequent douching disrupts the vaginal balance and may increase your risk of vaginal infection. Douching won›t clear up a vaginal infection.

Avoid a sexually transmitted infection. Use a male latex condom, limit your number of sex partners or abstain from intercourse to minimize your risk of a sexually transmitted infection.

Here a digest of the most suggested medications:
Metronidazole (Flagyl, Metrogel-Vaginal, others). This medicine may be taken as a pill by mouth (orally). Metronidazole is also available as a topical gel that you insert into your vagina. To reduce the risk of stomach upset, abdominal pain or nausea while using this medication, avoid alcohol during treatment and for at least one day after completing treatment — check the instructions on the product.

Clindamycin (Cleocin, Clindesse, others). This medicine is available as a cream that you insert into your vagina. Clindamycin cream may weaken latex condoms during treatment and for at least three days after you stop using the cream.

Tinidazole (Tindamax). This medication is taken orally. Tinidazole has the same potential for stomach upset and nausea as oral metronidazole does, so avoid alcohol during treatment and for at least three days after completing treatment.

Secnidazole (Solosec). This is an antibiotic you take orally in one dose. The medication comes as a packet of granules that you sprinkle onto a soft food, such as applesauce, pudding or yogurt. You eat the mixture within 30 minutes, being careful not to crunch or chew the granules.

It's generally not necessary to treat an infected woman's male sexual partner, but bacterial vaginosis can spread between female sexual partners. Female partners should seek testing and may need treatment. It's especially important for pregnant women with symptoms to be treated to help decrease the risk of premature delivery or low birth weight.

It's common for bacterial vaginosis to recur within three to 12 months, despite treatment. Researchers are exploring treatments for recurrent bacterial vaginosis. One option may be extended-use metronidazole therapy.

A self-help approach is lactobacillus colonization therapy — which attempts to boost the number of good bacteria in the vagina and re-establish a balanced vaginal environment — possibly accomplished by eating certain types of yogurt or other foods containing lactobacilli. While current research shows there may be some benefit to probiotic therapy, more research is needed on the subject.

Bacterial vaginosis (BV) is a common infection in reproductive age woman and is characterized by dysbiosis of the healthy vaginal flora which is dominated by Lactobacilli, followed by growth of bacteria like *Gardnerella vaginalis*. The ability of *G. vaginalis* to form biofilms contributes to the high rates of recurrence that are typical for BV and which unfortunately make repeated antibiotic therapy inevitable. Here we developed a biofilm model for *G. vaginalis* and screened a large spectrum of compounds for their ability to prevent biofilm formation and to resolve an existing *G. vaginalis* biofilm. The antibiotics metronidazole and tobramycin were highly effective in preventing biofilm formation, but had no effect on an established biofilm. The application of the amphoteric tenside sodium cocoamphoacetate (SCAA) led to disintegration of existing biofilms, reducing biomass by 51% and viability by 61% and it was able to increase the effect of metronidazole by 40% (biomass) and 61% (viability). Our data show that attacking the biofilm and the bacterial cells by the combination of an amphoteric tenside with the antibiotic metronidazole might be a useful strategy against BV.

In nature, bacteria rarely live in suspensions, but are frequently attached to surfaces as biofilms. In such a way they seek protection in a community where sharing of nutrients, genetic exchange and protection, e.g. from antimicrobials, is ensured. This is true for bacteria living in ponds or water distribution systems as well as for bacteria residing in humans [1]. Since biofilms offer a stable mode of existence, biofilm forming bacteria can cause large health problems in the human body, e.g. when they are persisting in catheters and chronic wounds, develop on implants or are causative of chronic diseases, such as rhinosinusitis or osteomyelitis [2]. It can be extremely challenging to erase pathogenic biofilms that have formed on human tissues. Therefore strategies to attack them are diverse and include antibiotics alone or in combination with bioactive molecules or bacteriophages [3,4].

One of those challenges is bacterial vaginosis (BV), a vaginal infection which might be associated with biofilm formation and persistence with a prevalence of 10–50% in women worldwide [5–7]. It is characterized by a change in bacterial diversity from a uniform flora dominated by Lactobacilli such as *Lactobacillus crispatus*, *L. gasseri*, *L. iners* or *L. jensenii* to a flora that is highly diverse and mostly anaerobic [8,9]. Although its etiology is still unclear, *Gardnerella vaginalis*, but also *Atopobium vaginae*, *Prevotella sp.*, *Sneathia sp.*, *Mobiluncus sp.* and many others were frequently identified in women with BV [10]. BV causes malodorous vaginal discharge and can also lead to miscarriage, preterm birth and an increased risk of acquiring sexually transmitted infections such as HIV [11]. One of the criteria used for diagnosis ("Amsel" criteria) is the presence of at least 20% clue cells [12]. Clue cells can be identified microscopically and are vaginal epithelial cells covered with a layer of bacteria [13,14]. Although Amsel criteria were established already in 1983 it took more than 20 years until Swidsinski et al., using fluorescent in situ hybridization (FISH), were able to show that those clue cells are frequently covered by a biofilm consisting mainly of the facultative anaerobe *G. vaginalis* [15] which was only recently confirmed by Hardy *et al.* using peptide nucleic acid (PNA) probes [16]. There are now many culture based and non-culture based studies that identified *G. vaginalis* only as part of a multispecies biofilm [10,17,18]. However, some scientific reports have shown *G. vaginalis* as the big rascal of BV, since the majority of virulence factors it possesses is important for disease development. One publication directly tested *G. vaginalis* against the other bacterial vaginosis associated species in terms of adherence, biofilm formation and cytotoxicity and found that *G. vaginalis* had the strongest virulence potential [19]. But *G. vaginalis* also bears a great challenge. *In vivo* studies, for example, revealed that after successful therapy with oral metronidazole, which is currently the treatment of choice for BV, patches of biofilms consisting of *G. vaginalis* and *A. vaginae* persisted on epithelial cells [15,20]. The high rate of recurrence of up to 60% within 12 months of treatment may therefore be due to the lack of effectiveness of metronidazole against biofilms. Moreover, antibiotic treatment, especially when it occurs repeatedly, supports the development of resistant bacteria [21]. Therefore, developing strategies to destroy biofilms of *G. vaginalis* and possibly other biofilm associated bacteria might be a first step to develop a more sustainable way to treat BV and its recurrences. Various approaches concerning the effect of different substances on *G. vaginalis* have already been pursued: The antiseptic octenidine dihydrochloride was initially very effective against *G. vaginalis in vivo* but resulted in a high rate of resistance after a short period [22]. Another clinical trial showed that treatment with glycerol monolaurate kept *Lactobacillus species* intact and was able to inhibit growth of *G. vaginalis* [23]. The antimicrobial peptide

Retrocyclin inhibited biofilm formation but not planktonic growth of *G. vaginalis* [24] and Thymol was able to inhibit formation of new *G. vaginalis* biofilms as well as destroy mature ones *in vitro* [25]. So far those substances have not been applied *in vivo* and therefore their efficiency has not been tested in women.

There are approaches with substances that are attacking the extracellular polymeric substance (EPS) that forms around biofilms, rather than attacking the bacteria, in order to make them susceptible to antibiotic treatment. DNase or the tenside lauramide arginine ethyl ester showed synergistic effects with antibiotics *in vitro* but have not been tested in vivo and are therefore not yet feasible for therapy [26,27]. A clinical study investigated whether boric acid, which is commonly administered against candidiasis, could disturb the biofilm in BV, but although the results looked promising after 2–3 months, the rate of recurrence after 38 weeks was unchanged [28]. An *in vitro* study showed that *G. vaginalis* can be displaced by *L. reuteri* and clinical trials that use different Lactobacillus species as probiotics in combination with antibiotics or alone showed potential [29,30].

BV is a multifactorial disease with a different flora and different problems in every affected woman. Therefore there is a need for medically applicable compounds that could be used either alone or in combination with antibiotics to treat BV and the physiological conditions which lead to BV recurrences. In our approach, as a first step, we analyzed different substances for their effectiveness against *G. vaginalis* biofilms. With the objective to use substances that have already proven successful in other antimicrobial settings, we tested four different categories of compounds on a *G. vaginalis* biofilm model of newly forming and already established biofilms, with the aim to identify new substances that could prevent, weaken or even destroy *G. vaginalis* biofilms. Those four categories were (1) antibiotics, (2) antibacterial enzymes and peptides, (3) antiseptics and (4) tensides.

Two antibiotics were tested: Tobramycin (TOB) is usually applied as treatment of *Pseudomonas aeruginosa* biofilms and has not yet been used against *G. vaginalis* biofilms. It blocks the bacterial protein synthesis. Metronidazole (MET) inhibits nucleic acid synthesis and is the current treatment of choice for BV. Its impact on *G. vaginalis* has already been investigated in previous studies either alone or in combination with additional substances [26,27,29,31].

We tested enzymes and peptides because extracellular proteins are important components of biofilms and they might help to degrade the biofilm matrix [32]. Two antibacterial hydrolases were selected: Lysozyme (LYS) that disrupts cell walls of bacteria and proteinase K (PRO) which degrades proteins [32,33]. Both are frequently used in the laboratory, but have not been applied as anti-biofilm treatments. As an antibacterial peptide, OP-145 (OP1) was tested. It interacts with membrane phospholipids and induces membrane thinning in bacteria and has been effective in the treatment of chronic middle ear infections [34] but was not tested against *G. vaginalis* biofilms before.

Antiseptics are commonly defined as substances that kill (bactericidal) or inhibit (bacteriostatic) the growth of bacteria [35]. The antimicrobial preservative chlorocresol (CLC, 4-chloro-3-methylphenol), the detergent cetylpyridinium chloride (CPC, 1-hexadecylpyridinium chloride) which can reduce gingivitis and was previously able to prevent dental plaque [36,37] and polyaminopropyl biguanide (PBI), also known as polyhexamethylene biguanide, that has been shown to be effective against *Staphylococcus aureus* amongst others and is used as disinfectant in swimming pools [38,39], were tested here because they have known antimicrobial properties and are already commercially used. Therefore obtaining approval for a new application would be simplified.

The fourth category of compounds studied here are surface-active agents (tensides) such as the emulsifier lecithin (LEC), which in combination with silver has been shown to be effective against biofilms on catheters due to its hydrophilic properties [40] and the amphoteric tenside sodium cocoamphoacetate (SCAA) that is frequently used in cosmetics and pharmaceutical products, but little has been published about it [41,42]. Due to their hydrophilic and hydrophobic moieties, amphoteric tensides have antibacterial properties. Their effectiveness against biofilms has not been studied yet [42,43].

MATERIALS AND METHODS

For this reason the AA have decided to prepare a mix containing rennin and lecithin to cure vaginosis, that in lesbians produces very malodorous discharges., even if the cosmetic cocktail does not forecast the addiction of any perfum or fragrance.

Rennin is a protease found in rennet. It is an aspartic endopeptidase belonging to MEROPS A1 family. It is produced by newborn ruminant animals in the lining of the abomasum to curdle the milk they ingest, allowing a longer residence in the bowels and better absorption. It is widely used in the production of cheese. Bovine chymosin is now produced recombinantly in E. coli, Aspergillus niger var awamori, and K. lactis as alternative resource.

Chemically speaking this protease is assimilable to an organic amphoteric surface agent item.

The AA have recruited a mulâtre, 24 y. old, who loves to have sex performances both with women and men too.

The volunteer's symptoms were severe itching and malodour and paineven during urination.

Her discharge was accurately analysed and showed a strong amount of Gardnerella vaginalis.

The AA created a combinaison of soy lecithin and rennin (25/75) and prayed the girl to spread in the inner vagina all the nights before to go to bed for one week.

The mix should have been applied without rinsing with water and let it stay overnight in situ.

The experiment lasted one entire week.

RESULTS

After the entire week of treatment, the girl was examined and interrogated about her real symptoms and she utterly declared not to feel pain during urination, no pelvic discomfort or inconvenience after the 4th day and vaginal discharges ceased to erupt.

She did not appreciate any odour at all both in her urine and eventual residual leakage.

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