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Polymicrobial Gardnerella biofilm resists repeated intravaginal antiseptic treatment in a subset of women with bacterial vaginosis: a preliminary report

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Abstract
Purpose Bacterial vaginosis is a recalcitrant polymicrobial biofilm infection that often resists standard antibiotic treatment. We therefore considered repeated treatment with octenidine, a local antiseptic that has previously been shown to be highly effective in several biofilm-associated infections.
Methods Twenty-four patients with recurrent BV were treated with a 7-day course of octenidine (octenidine dihydrochloride spray application with the commercial product Octenisept®). In case of treatment failure or relapse within 6 months, patients were re-treated with a 28-day course of octenidine. In case of recurrence within 6 months after the second treatment course, patients were treated again with a 28-day course followed by weekly applications for 2 months. Treatment effect was evaluated by assessment of the presence of the biofilm on voided vaginal epithelial cells through fluorescence in situ hybridisation.
Results The initial cure rate following a 7-day course of octenidine was as high as 87.5 %. The 6-month relapse rate was, however, as high as 66.6 %. Repeated treatment for 28 days led to an overall cure rate of 75.0 %; however, it was also associated with emergence of complete resistance to octenidine in a subset of women. The overall cure rate after three treatment courses with 1-year follow-up was 62.5 %, with 37.5 % of the patients showing complete resistance to octenidine.
Conclusion Our preliminary results showed that octenidine dihydrochloride was initially highly effective, but the efficacy of repeated and prolonged treatment dropped quickly as challenge with the antiseptic rapidly led to bacterial resistance in a considerable subset of women.

Keywords Bacterial vaginosis · Biofilm · *Gardnerella vaginalis* · Octenidine · Antiseptics

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Introduction

Bacterial vaginosis (BV) is a pervasive polymicrobial infestation of the vagina that affects millions of women worldwide. As such, bacterial vaginosis is considered a nuisance condition causing vulvovaginal discomfort, including vaginal discharge, malodour and vulvar irritation [1] A vast body of evidence has associated bacterial vaginosis, however, with adverse pregnancy outcomes including late foetal loss [2] and preterm birth [3], presumably involving an ascending genital tract infection pathway, though the precise mechanisms involved remain elusive. In addition, bacterial vaginosis predisposes to the

acquisition of sexually transmitted diseases, including the HSV [4], HPV [5], and HIV-1 [6] viruses.

Treatment of BV has been a longstanding challenge. Standard regimens with metronidazole or clindamycin as recommend by the US Centers for Disease Control and Prevention (CDC) are associated with fairly good short-term cure rates and however also with high recurrence rates in the long run [1]. We have previously reported that a polymicrobial *Gardnerella* dominated bacterial biofilm is a characteristic feature of BV [7] and that antibiotics, such as metronidazole or moxifloxacin, do not eradicate the biofilm in the majority of BV cases, but only temporarily suppress it [8, 9]. It has been acknowledged indeed that biofilms pose a major challenge to effective treatment of numerous recalcitrant clinical infections [10] with a number of mechanisms of antimicrobial resistance in biofilms having been identified [11].

An alternative in this respect for systemic antibiotics might be local therapy with antiseptics. Treatment with a local antiseptic can be prolonged or repeated, making local antiseptic therapy especially promising in the treatment of recalcitrant polymicrobial biofilm-associated infections. One such antiseptic, is octenidine dihydrochloride, a well-tolerated broad-spectrum antimicrobial agent that has previously been shown to be highly effective in defeating oral biofilms [12], biofilms growing on orthopaedic steel implants [13, 14], and biofilms involved in wound infections [15].

In the present study, we investigated whether repeated treatment with octenidine dihydrochloride is effective in the eradication of the BV biofilm.

Methods and materials

Patients and clinical procedures

Consecutive patients with symptomatic BV were enrolled at the Charité outpatient clinic for Gynaecology through written and oral informed consent. Ethical clearance had previously been obtained from the Institutional Review Board. Assessment of the presence or absence of bacterial vaginosis was based on FISH analysis of bacteria attached to desquamated vaginal epithelial cells in urine sediments [16]. All women had to have at least 3 months urine samples positive for *Gardnerella* biofilm prior to treatment. The effects of therapy on biofilm occurrence was monitored weekly over the duration of the therapy, at the last day of the therapy and then in monthly intervals following treatment cessation for at least 6 months. Twenty-four premenopausal women with BV (range 22–44 years of age) were included. Fifteen of them had received previously antibiotic therapy for BV, and however continued to show BV relapses.

Study design

The initial therapy consisted of daily octenidine dihydrochloride spray application with the commercial product Octenisept® (Schülke & Mayr GmbH, Norderstedt, Germany) for 7 days according to the recommendations of the manufacturer. We aimed, however, to prolong the therapy as long as the overall response rates remained higher than 70 % and no better therapy regimen is available. Accordingly, in cases of relapse after the initial 7 days of therapy, we treated again for 28 days starting in the middle of the menstrual cycle. Women effectively treated with 28 days of Octenisept® but who relapsed thereafter were treated again for 28 days followed by weekly applications of Octenisept® for a 2-month duration. The Octenisept® spray was renewed weekly in all cases of longer lasting treatment, to exclude the occlusion of the opening of the spray nozzle. No further Octenisept® treatment was considered if *Gardnerella* biofilm was still detectable after the second 28-day treatment course with Octenisept®. Partners of the women were not investigated, but the women were asked to treat them in the same manner or to avoid sexual contacts over the observation period.

Desquamated epithelial cells of the vagina in urine sediments

The urine samples were collected and fixated by the patients. The women received 15 mL Falcon tubes filled with 8 mL of Carnoy solution. They were asked not to wash the genital region the evening before sampling and to use the first portion of the morning urine. Two mL of urine was added to 8 mL Carnoy solution and the Falcon tubes were labelled. The fixated samples were collected and delivered to the Molecular Genetic Laboratory for Polymicrobial Infections and Bacterial Pathogens at the Charité Hospital, Berlin, Germany.

An aliquot of 1.5 mL urine/Carnoy mix, prepared as described above, was centrifuged in a 1.5 mL Eppendorf tube for 6 min at 6,000 G. The sediment was decanted; the tube was filled with 1 mL of Carnoy solution and left at room temperature. After 1–5 min, the sediment was centrifuged once more (6 min/9,000 G), decanted, 50 µL Carnoy solution was added, and then stored at 4 °C.

Fixation

Fixation was performed with modified non-aqueous Carnoy solution (6/6/1 vol. ethanol/glacial acetic acid/

chloroform). The fixated material could be stored in Carnoy at room temperature up to 6 months.

Analysis of urine sediments

A 5 × 5 mm quadrant area of hybridization was marked with a PAP Pen on a SuperFrost plus glass slide. The Carnoy-fixated urine sediment was vortexed; 5 µL aliquots were pipetted within the area of hybridization and dried for 30 min at 50 °C just prior to the hybridization. Five mL of the final aliquot was used for single hybridizations and represented 30 µL of the initial urine volume. Epithelial cells within the 5 × 5 mm area of hybridization (30 µL of sample volume) were counted and converted to numbers of epithelial cells per ml of urine. The maximal and mean numbers of adherent bacteria per epithelial cell were determined. The overall concentrations of adherent bacteria in the urine resulted from multiplication of the mean number of bacteria per epithelial cell with the concentration of epithelial cells per mL of urine.

Adherent *Gardnerella* biofilms were recognized on their typical appearance of structured *Gardnerella* dominated polymicrobial adherent biofilms attached to desquamated vaginal epithelial cells (see Fig. 1), which we showed previously to be specific for bacterial vaginosis.

Fish

Bacteria were assessed in a multi-colour analysis using a mix of specific and universal FISH probes stained with Cy3 (orange fluorescence), FITC (green fluorescence), Cy5 (dark red fluorescence) and DAPI counter stain (blue fluorescence) according to previously described protocols [7, 8]. The

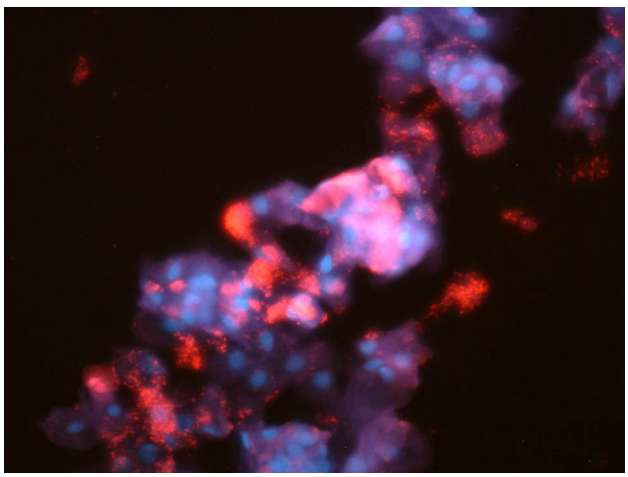

Fig. 1 Urine sediment from women with BV previous to therapy hybridized with a Gard V C5 probe (*dark red*) and counterstained with DAPI (*blue* fluorescence) ×400. Adherent *Gardnerella* biofilm can be recognized on its typical structured adherence to desquamated vaginal epithelial cells well seen in DAPI

GardV, *Ato*, *Lab*, *Bac 303*, *Ebac* and *Eub 338* probes were applied to each sample thereby targeting *Gardnerella*, *Atopobium*, *Lactobacillus, Bacteroides/Prevotella, Enterobacteriaceae,* and bacteria from the *Eubacteria* cluster.

Nikon e600 fluorescence microscope, Nikon DXM1200F camera and accompanying software (Nikon, Tokyo, Japan) were used. The enumeration of bacteria was performed when hybridization signals were clear and morphologically distinguishable as bacterial cells by at least triple colour identification with universal and group-specific FISH probes and DAPI stain, with absence of cross-hybridization with taxonomically unrelated probes [7, 8].

Results

Tolerability

All women ($n = 24$) reported a feeling of vaginal burning and dryness after application of Octenisept® for a duration of 5–15 min after the application. None of the women stopped the application for this reason. In cases of repeated treatment courses, we recommended that study participants to either use Relactagel® (Kora Corporation Ltd, Dublin, Republic of Ireland) to ease the vaginal dryness or to stop the therapy as soon as the side effects became intolerable. Eight women regularly used Relactagel® and reported a better tolerance of Octenisept® treatment, and none discontinued the therapy.

Cure rates as assessed by the effect on the presence of a Gardnerella biofilm

Following the initial 7-day treatment course, the structured polymicrobial *Gardnerella* biofilm was undetectable in 21 out of the 24 patients, corresponding to a cure rate of 87.5 % (Table 1). In three out of the 24 women, the biofilm persisted after the initial 7-day therapy. In 14 out of the 21 women with an initial complete response (Fig. 2), the structured polymicrobial *Gardnerella* biofilm was again detectable within one to 6 months after the end of therapy, lowering the overall cure rate to 29.2 % after 6 months (7/24).

The three women without response to the 7-day therapy and the 14 women with relapse were again treated with Octenisept® this time for 28 days. After treatment cessation, the structured polymicrobial *Gardnerella* biofilm disappeared from the urine in 11/17 women, corresponding to an overall cure rate after two treatment cycles of 75.0 % (18/24). So, no response was observed to octenidine in six women and the vaginal epithelial cells of the former were covered with a prolific *Gardnerella* biofilm despite continuation of the Octenisept® treatment (Fig. 3), indicating complete resistance to the antiseptic.

Table 1 Cure rates as assessed by the effect on the presence of a *Gardnerella* biofilm

	Response	Relapse within 6 months	No response	Cumulative non-response rate (%)
Initial therapy for 7 days (n = 24)	21 (87.5 %)	14	3	12.5
Therapy repeated (2nd) for 28 days (n = 17)	11 (64.7 %)	4	6[a]	25.0
Therapy repeated (3rd) for 28 days followed weekly applications for 2 months (n = 4)	1 (25.0 %)	–	3[a]	37.5

[a] Patients were not treated further after failure/persistence of the biofilm

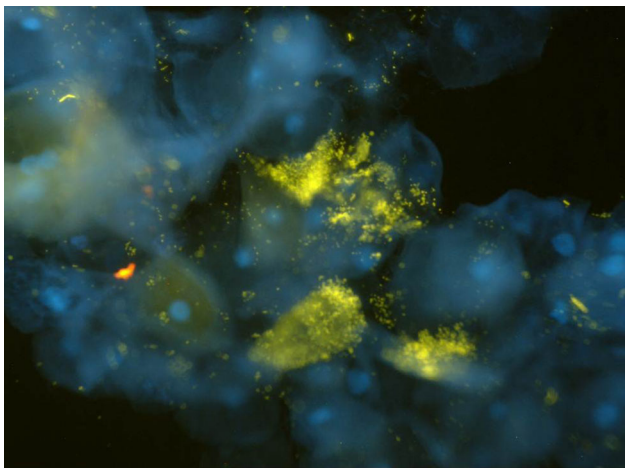

Fig. 2 Urine sediment of BV women successfully treated with octenidine, 3 months after the end of the therapy. Sediment is hybridized with Gard V C5 (*dark red*), Lab C3 (*yellow*) and counterstained with DAPI (*blue* fluorescence) ×400. No *Gardnerella* bacteria are seen. The *red* inclusions are non-bacterial biases, since they have no form of bacteria and do not counterstain with DAPI. Mainly lactobacilli are present (*yellow* fluorescence)

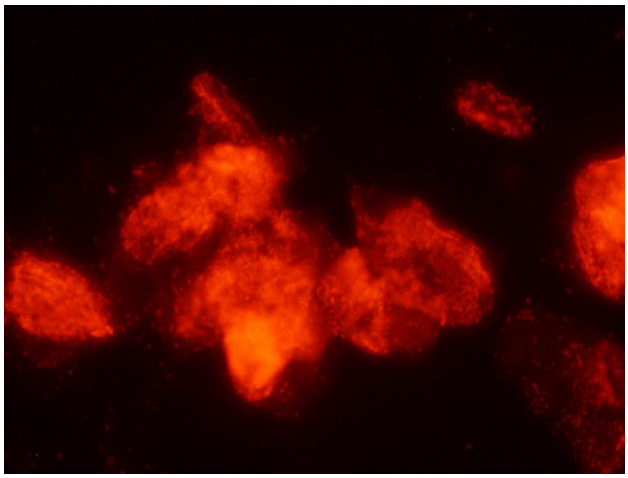

Fig. 3 Women with repeated octenidine treatment (third cyclus, day 20.) Hybridization with Gard V c5 probe (*dark red* fluorescence), ×100. Prolific Gardnerella biofilm can be seen despite ongoing octenidine therapy

Four women who were treated twice showed a relapse within the following 6 months, corresponding to an overall cure rate of 58.3 % after 1 year (14/24). The four women with relapse after the second round of therapy were treated with another 28-day course of daily Octenisept® applications followed by weekly applications for another 2 months. Only one of these women responded. So the overall cure rate following the above treatment scheme was 62.5 % (15/24) (Table 1).

The high non-response rates accompanied by the persistence of a structured *Gardnerella* biofilm despite continuation of the Octenisept® treatment (Fig. 3) in a subset of women moved us to discontinue the study.

Discussion

In the present study, we evaluated the efficacy of repeated courses of local treatment with octenidine dihydrochloride in eradicating the vaginal polymicrobial *Gardnerella* biofilm associated with BV. Although octenidine dihydrochloride was initially highly effective, the cure rate being as high as 87.5 %, the relapse rate was also high (66.6 %). It was also found that the efficacy of repeated and prolonged treatment dropped quickly as challenge with the antiseptic rapidly led to bacterial resistance in a considerable subset of women (37.5 %).

The high initial response rate is in accordance with the results that were recently obtained in large randomized controlled trial in which patients were treated with a local antiseptic spray (octenidine hydrochloride/phenoxyethanol), either with locally applied metronidazole [17], although the authors in the latter study did not specify how cure was defined [18].

Since no biopsies were taken in this study, we cannot say, whether the episodes of relapse of BV were due to transition of the biofilm from the active to the dormant state as we previously described for metronidazole [8]. However, in over one-third of the women, the use of octenidine dihydrochloride led to complete resistance of the polymicrobial *Gardnerella* biofilm, indicating that the biofilm may flourish under octenidine even without down

regulating of its metabolic activity. The emergence of antibiotic resistance has previously been described in relation to challenge of biofilms with antibiotics, as recently reviewed [19].

Our preliminary study clearly illustrates that antimicrobial susceptibility tests of individual BV-associated species in planktonic cultures are of very limited value. In this respect, it would be most helpful if we could dispose of a proper ex vivo model of the polymicrobial BV biofilm.

Conflict of interest None.

References

1. Verstraelen H, Verhelst R (2009) Bacterial vaginosis: an update on diagnosis and treatment. Expert Rev Anti Infect Ther 7:1109–1124
2. Oakeshott P, Hay P, Hay S, Steinke F, Rink E, Kerry S (2002) Association between bacterial vaginosis or chlamydial infection and miscarriage before 16 weeks' gestation: prospective community based cohort study. BMJ 325:1334
3. Leitich H, Kiss H (2007) Asymptomatic bacterial vaginosis and intermediate flora as risk factors for adverse pregnancy outcome. Best Pract Res Clin Obstet Gynaecol 21:375–390
4. Kirakoya-Samadoulougou F, Nagot N, Defer MC, Yaro S, Fao P, Ilboudo F, Langani Y, Meda N, Robert A (2011) Epidemiology of herpes simplex virus type 2 infection in rural and urban Burkina Faso. Sex Transm Dis 38:117–123
5. Gillet E, Meys JF, Verstraelen H, Bosire C, De Sutter P, Temmerman M, Broeck DV (2011) Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. BMC Infect Dis 11:10
6. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS (2008) Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. AIDS 22:1493–1501
7. Swidsinski A, Mendling W, Loening-Baucke V, Ladhoff A, Swidsinski S, Hale LP, Lochs H (2005) Adherent biofilms in bacterial vaginosis. Obstet Gynecol 106:1013–1023
8. Swidsinski A, Mendling W, Loening-Baucke V, Swidsinski S, Dörffel Y, Scholze J, Lochs H, Verstraelen H (2008) An adherent Gardnerella vaginalis biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. Am J Obstet Gynecol 198:97.e1-6.1
9. Swidsinski A, Dörffel Y, Loening-Baucke V, Schilling J, Mendling W (2011) Response of Gardnerella vaginalis biofilm to 5 days of moxifloxacin treatment. FEMS Immunol Med Microbiol 61:41–46
10. Cos P, Toté K, Horemans T, Maes L (2010) Biofilms: an extra hurdle for effective antimicrobial therapy. Curr Pharm Des 16:2279–2295
11. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O (2010) Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 35:322–332
12. Rupf S, Balkenhol M, Sahrhage TO, Baum A, Chromik JN, Ruppert K, Wissenbach DK, Maurer HH, Hannig M (2012) Biofilm inhibition by an experimental dental resin composite containing octenidine dihydrochloride. Dent Mater 28:974–984
13. Bartoszewicz M, Rygiel A, Krzemiński M, Przondo-Mordarska A (2007) Penetration of a selected antibiotic and antiseptic into a biofilm formed on orthopedic steel implants. Ortop Traumatol Rehabil 9:310–318
14. Sennhenn-Kirchner S, Wolff N, Klaue S, Mergeryan H, Borg-von Zepelin M (2009) Decontamination efficacy of antiseptic agents on in vivo grown biofilms on rough titanium surfaces. Quintessence Int 40:e80–e88
15. Hübner NO, Siebert J, Kramer A (2010) Octenidine dihydrochloride, a modern antiseptic for skin, mucous membranes and wounds. Skin Pharmacol Physiol 23:244–258
16. Swidsinski A, Doerffel Y, Loening-Baucke V, Swidsinski S, Verstraelen H, Vaneechoutte M, Lemm V, Schilling J, Mendling W (2010) Gardnerella biofilm involves females and males and is transmitted sexually. Gynecol Obstet Invest 70:256–263
17. Novakov Mikic A, Budakov D (2010) Comparison of local metronidazole and a local antiseptic in the treatment of bacterial vaginosis. Arch Gynecol Obstet 282:43–47
18. Verstraelen H, Verhelst R, Roelens K, Temmerman M (2012) Antiseptics and disinfectants for the treatment of bacterial vaginosis: a systematic review. BMC Infect Dis 12:148
19. Cantón R, Morosini MI (2011) Emergence and spread of antibiotic resistance following exposure to antibiotics. FEMS Microbiol Rev 35:977–991