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Clinical and personal lubricants impact growth of vaginal *Lactobacillus* species and colonization of vaginal epithelial cells: an *in vitro* study

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Abstract

Background: Vaginal lubricants are commonly used during gynecological exams, sexual activities or to alleviate vaginal dryness. Many lubricants contain potentially bacteriostatic or bactericidal agents (parabens, chlorhexidine gluconate, nonoxynol-9). Our objective was to evaluate the impact of lubricants that vary in formulation, on the growth and viability of vaginal *Lactobacillus* species and vaginal epithelial cell (VEC) colonization in an *in vitro* model.

Methods: Growth curve, disk diffusion and minimal inhibitory assays were used to determine impact of lubricants or excipients on the growth of *L. crispatus*, *L. gasseri*, *L. jensenii* and *L. iners*. Two *L. crispatus* strains were utilized in VEC colonization assays. Statistical differences were determined by ANOVA.

Results: Lubricants containing chlorhexidine gluconate or nonoxynol-9 (Conceptrol®, K-Y™ Jelly, and Surgilube®) significantly inhibited *Lactobacillus* spp. growth ($P<0.05$). In contrast, other clinical lubricants (E-Z Lubricating Jelly, McKesson Lubricating) and personal lubricants (Astroglide® Liquid, Good Clean Love Almost Naked, K-Y™ Warming Jelly) did not exhibit this effect. Chlorhexidine gluconate had a detrimental effect on *Lactobacillus* growth and exhibited stronger antimicrobial activity compared to methylparaben and propylparaben ($P<0.0001$). There were lubricants that did not induce cytotoxicity in VEC (Good Clean Love Almost Naked, E-Z Lubricating Jelly, McKesson Lubricating Jelly), but these products did substantially decrease attachment of *Lactobacillus* to VEC, particularly when VEC were pre-exposed to lubricants prior to inoculation with bacteria ($P<0.0001$).

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Authors' contributions

P.Ł., R.M.B. and M.M.H.-K. contributed to the conception and design of the study. P.Ł., K.A.O. and M.K. performed experiments and subsequent analyses. P.Ł. and K.A.O. drafted the manuscript. R.M.B and M.M.H.-K. critically reviewed and edited the manuscript. All authors approved the final version of the manuscript.

Potential conflicts of interest

The authors declare no conflict of interest.

Conclusions: This *in vitro* model indicates that select vaginal lubricants, particularly those with chlorhexidine gluconate, have potentially adverse effects on women's health by reducing growth and re-colonization of vaginal *Lactobacillus* species.

Short summary

An *in vitro* study found that vaginal lubricants containing chlorhexidine gluconate can deleteriously affect protective *Lactobacillus* species, which may lead to increased risk for vaginitis or other adverse gynecologic outcomes.

Keywords

chlorhexidine gluconate; host–microbe interactions; paraben; vaginal microbiota; women's health

Introduction

Women commonly report use of personal lubricants in sexual practices and to help alleviate vaginal dryness and the genitourinary syndrome of menopause.[1] In addition, clinicians frequently use vaginal lubricants in the conduct of gynecologic exams and recommend them to patients with gynecologic cancer to help mitigate adverse effects of treatments, which cause vulvovaginal atrophy and an overall reduction in quality of life.[2] Yet, the effect of lubricants on the cervicovaginal microenvironment, including local microbiota, has not been comprehensively studied. In the majority of healthy, reproductive-age women, the vaginal microbiota is dominated by one or few *Lactobacillus* species, such as *L. crispatus*, *L. gasseri*, *L. jensenii* or *L. iners*.[3] However, in postmenopausal women, as a result of hormonal changes, the vaginal microbiota frequently lacks *Lactobacillus* dominance.[3] Colonization of the vagina with lactic-acid producing *Lactobacillus* species has broadly been associated with vaginal health, since the acidic microenvironment consequently protects the host from invading pathogens, including sexually transmitted infections (STIs).[3]

Studies investigating the impact of lubricants on the human mucous membranes are limited. Two reports, utilizing *in vitro* biomimetic models, demonstrated that lubricants with high osmolality reduce epithelial barrier integrity, cause cellular damage and alter inflammatory responses.[4,5] Relative to other body sites, hyperosmolar lubricants have been shown to cause rectal epithelial cell damage or denudation.[6] Furthermore, consistent use of hyperosmolar lubricants during anal intercourse has been associated with higher prevalence of STIs among men who have sex with men.[7] The World Health Organization (WHO) guidelines published in 2012 state that lubricants' osmolality should not exceed 1200 mOsm/kg and by comparison, but the majority of lubricants on the market exceed this osmolality recommendation and the osmolality of vaginal secretions is 260–290 mOsm/kg.[8]

Most clinical and personal lubricants also contain excipients with antimicrobial properties, such as parabens and chlorhexidine gluconate (CHG). Parabens are commonly used preservatives with a broad spectrum activity against fungi and bacteria.[9] CHG is a broad spectrum antiseptic effective against gram-positive and gram-negative bacteria, as well as fungi.[9] The mechanism of parabens' and CHG's action relates to the damage of cell membrane and wall integrity.[9] Intriguingly, CHG has been banned by the United States

Food and Drug Administration (FDA) from use in over-the-counter healthcare antiseptics and hand sanitizers in 2018 and 2020, respectively.[10,11] Ultimately, the effect of lubricants containing these excipients on the healthy constituents of the vaginal microbiota is unknown. In this study, we aimed to determine the effects of a broad range of personal and clinical lubricants on the growth of vaginal *Lactobacillus* species and investigated the impact of lubricants on *Lactobacillus* colonization of vaginal epithelial cells (VEC).

Materials and Methods

Lubricants and bacterial strains

Personal lubricants were obtained from a local drugstore and clinical lubricants were obtained from clinics located in Phoenix, AZ and Baltimore, MD (Table 1). All lubricants were used prior to the expiration date. Bacterial strains were obtained from the American Type Culture Collection (ATCC) or the Biodefense and Emerging Infections (BEI) Research Resources Repository and included five vaginal *Lactobacillus* strains: *L. crispatus* JV-V01, *L. gasseri* JV-V03, *L. jensenii* JV-V16 and *L. iners* AB107, as well as *L. crispatus* type strain VPI 3199 (the latter isolated from eye). *L. crispatus*, *L. gasseri*, and *L. jensenii* were grown on de Man, Rogosa and Sharpe agar or in MRS broth at 37 °C under 5% CO_2. *L. iners* was grown on tryptic soy agar (TSA) supplemented with 5% defibrinated sheep blood (Quad Five, Ryegate, MT) or in tryptic soy broth (TSB) supplemented with 5% horse serum at 37 °C under anaerobic conditions, generated with a GasPak EZ Anaerobe Container System. All bacterial culture media and supplements were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ).

Growth curve assay.—To determine the bacteriostatic and/or bactericidal effect of lubricants on vaginal *Lactobacillus* spp., the growth of bacteria in liquid media with or without lubricants, was analyzed. Bacteria were inoculated into MRS or supplemented TSB liquid media and cultured overnight at 37 °C under 5% CO_2 or anaerobic conditions. The broths containing 10% (v/v) lubricants were inoculated with *Lactobacillus* spp. at the final optical density at 600 nm (OD_{600}) of 0.5 and cultured as described earlier. The broth without any lubricant was used as a positive control. The growth of bacteria with or without lubricants was determined after 4 and 24 h by measuring the OD_{600} of bacterial cultures and the standard plating assay. For the standard plating assay, bacterial cultures were serially diluted in phosphate-buffered saline (PBS) and spotted on MRS or supplemented TSA plates. The agar plates were incubated at 37 °C for 48–72 hours for enumeration of colony-forming units (CFU). The concentration of viable bacterial cells in each culture was calculated and presented as CFU/mL.

Disk diffusion assay.—The disk diffusion assay was used to determine antimicrobial properties of select excipients, such as parabens and chlorhexidine gluconate, on vaginal *Lactobacillus* spp. MRS or supplemented TSA plates were inoculated with 1×10^7 CFU of bacteria. Under aseptic conditions, 6 mm Whatman™ filter disks (GE Healthcare, Chicago, IL) were impregnated with 20 µl of 20% (w/v) solution of chlorhexidine gluconate in water (Tokyo Chemical Industry, Tokyo, Japan) or 20% (w/v) solutions of methylparaben or propylparaben in ethanol (Tokyo Chemical Industry). Bleach and ethanol were used as

positive and negative controls, respectively. Disks were placed onto inoculated agar plates and incubated at 37 °C under 5% CO_2 (for *L. crispatus*, *L. gasseri* and *L. jensenii*) or anaerobic conditions (for *L. iners*). Following 24 h incubation, zones of inhibitions were measured in millimeters.

Minimal inhibitory concentration assay.—Minimal inhibitory concentrations (MIC) of parabens and chlorhexidine gluconate were assessed using the broth microdilution method. *Lactobacillus* spp. were grown overnight on MRS or TSA plates and resuspended in sterile PBS. Bacterial suspensions were adjusted to the OD_{600} of 1.0 and diluted in MRS or supplemented TSB liquid media. Two-fold serial dilutions of excipients (methylparaben, propylparaben and chlorhexidine gluconate) in appropriate broths (50 μl) were aliquoted into respective wells in a sterile 96-well microtiter plate. 50 μl aliquots of bacterial inoculum containing 5×10^5 CFU were added to wells with excipient dilutions. Broth without added excipients or bacterial inoculum were used as a growth and sterility controls, respectively. The inoculated microtiter plate was incubated at 37 °C under 5% CO_2 or anaerobic conditions. Following 24 h incubation, the OD600 was recorded using a Safire II Multi-Mode Microplate Reader (Tecan, Männedorf, Switzerland). The MIC was defined as the lowest concentration of the excipient that inhibits the visible growth of the tested bacteria.

Colonization assays.—Human vaginal epithelial (V19I) cells were cultured as monolayers in 1:1 (v/v) mixture of EpiLife and Keratinocyte Serum-Free Media (Life Technologies, Carlsbad, CA) at 37 °C under 5% CO_2. For experimental manipulations, cells were quantified using trypan blue (0.25%; v/v) exclusion staining and seeded into 24-well tissue culture-treated plates at a density of 2×10^5 cells/mL. *L. crispatus* strain JV-V01 was used for colonization assays. Bacteria were grown for 16–18 h on MRS agar plates, resuspended in sterile Dulbecco's PBS and used for *in vitro* colonization of VEC at a multiplicity of infection (MOI) of 10. The impact of lubricants on *L. crispatus* colonization was tested using pre- and post-exposure approaches. For pre-exposure assays, VEC were pre-exposed to 10% (v/v) solution of select lubricants in cell culture media following immediate inoculation with bacteria. After adding bacterial inoculum to the cells, plates were centrifuged for 10 min at 900 × *g* to ensure bacterial inoculum reached the epithelial monolayers and incubated under standard conditions. For post-exposure assays, VEC were pre-colonized with bacteria prior to exposure to lubricants. VEC were infected for 2 h, washed three times with Dulbecco's PBS to remove bacteria not attached to the cells and then treated with 10% (v/v) solution of lubricants. Dulbecco's PBS or 10% (v/v) solution of glycerol was used as a negative treatment control. Following 4 or 24 h incubation, VEC were washed three times with Dulbecco's PBS, trypsinized and resuspended in Dulbecco's PBS. Suspensions of bacteria and VEC were vortexed for 5 min and serially diluted, plated on MRS agar and incubated for 48–72 h for bacterial quantification. All incubations were performed at 37 °C under 5% CO_2.

Statistical analysis

All experiments were performed at least in triplicate. The statistical differences were determined using an analysis of variance (ANOVA) with Dunnett or Bonferroni adjustments for multiple comparisons. *P* values <0.05 were considered significant.

Results

Herein, we tested three clinical lubricants and five commercially available personal lubricants, which differed in osmolality and formulation. Six out of eight lubricants contained an antimicrobial, such as methylparaben, propylparaben, polyquaternium 15 or CHG (Table 1). The impact of each lubricant on the growth of the four most predominant vaginal *Lactobacillus* spp. (*L. crispatus*, *L. gasseri*, *L. jensenii* and *L. iners*) was evaluated. Initially, we assessed the bacterial growth by measuring optical densities of bacterial cultures containing lubricants. The presence of Conceptrol®, K-Y™ Jelly or Surgilube® in liquid media significantly inhibited the growth of *L. crispatus, L. gasseri*, *L. jensenii* and *L. iners* following 24 h exposure when compared to controls (*P* ranging from <0.05 to <0.0001) (Fig. 1). Then, we quantified viable bacteria following 4 and 24 h exposure to each lubricant using standard plating assay. In the presence of K-Y™ Jelly or Surgilube®, concentrations of viable *Lactobacillus* spp. were significantly lower compared to cultures without lubricant (*P* ranging from <0.05 to <0.001) (Fig. 2). However, these numbers did not significantly decline relative to the numbers of viable bacteria at the time of inoculation, suggesting a bacteriostatic effect of lubricants on the *Lactobacillus* growth. In contrast, GCL Almost Naked, McKesson Lubricating Jelly and Astroglide® Liquid had minor or no effect on the viability of tested vaginal *Lactobacillus* spp. (Fig. 2). Notably, the lubricants that significantly inhibited the growth of *Lactobacillus* spp., i.e. Conceptrol®, K-Y™ Jelly and Surgilube®, were not distinguished by the highest osmolality; however, all contained antimicrobial agents, such as parabens or CHG.

To test the antimicrobial properties of excipients listed as key components of lubricants (Table 1) against vaginal *Lactobacillus* spp., we performed disk diffusion assays. We did not test polyquaternium 15 since this compound is not commercially available. In addition, lack of bacterial growth inhibition following exposure to Astroglide® Liquid, containing polyquaternium 15, indicates that this compound does not impact the growth of *Lactobacillus* spp. (at least at the concentration present in the tested lubricant). The growth of *L. crispatus*, *L. gasseri* and *L. jensenii* on agar plates had significant zones of inhibition from all excipients tested when compared to the negative control (*P* ranging from 0.0007 to <0.0001) (Fig. 3A). For *L. iners*, CHG caused significant ($P<0.001$) growth inhibition, whereas zones of inhibition from parabens did not reach statistical significance. Furthermore, CHG inhibited *Lactobacillus* spp. at a significantly higher magnitude (19–24 mm) compared to parabens (8–12 mm) ($P<0.0001$) (Fig. 3A).

To better delineate the differences in antimicrobial potentials of parabens and CHG against vaginal *Lactobacillus* spp., we determined the minimal inhibitory concentrations (MIC) for these compounds. Both parabens inhibited bacterial growth at 8,000 mg/L for all tested *Lactobacillus* spp. except *L. iners*, which was inhibited at 2,000 mg/L and 4,000 mg/L of methylparaben and ethylparaben, respectively (Fig. 3B). In contrast, CHG inhibited growth

of *Lactobacillus* spp. in a species-specific manner at a concentration ranging from 1.25 to 10 mg/L, which are 200–6400 times (2.3–3.8 log) lower than parabens.

To assess the effect of lubricants on the colonization of the vaginal epithelium with *Lactobacillus* associated with optimal vaginal health, we exposed human vaginal epithelial cells (VEC), grown as monolayers, to lubricants and infected the *in vitro* VEC cultures with *L. crispatus*. We were able to test only three lubricants: GCL Almost Naked, E-Z Lubricating Jelly and McKesson Lubricating Jelly. The other lubricants used in this study induce substantial cytotoxicity, including condensation of chromatin and a loss of intercellular connections in the *in vitro* VEC model[5], which precluded testing the colonization. Glycerol was used as an additional control to mimic the viscosity of lubricants in the event that this property impacted colonization alone. We also used two different approaches to determine the impact of lubricants on bacterial colonization. First, we tested the effect of lubricants on VEC that were pre-colonized with *L. crispatus* prior to exposure lubricants. Four-hour exposure to lubricants did not significantly impact the colonization levels; however, 24 h exposure to E-Z Lubricating Jelly or McKesson Lubricating Jelly significantly decreased *L. crispatus* colonization levels by 0.9 log ($P<0.05$) (Fig. 4A). In contrast, GCL Almost Naked did not impact the colonization at any tested time points compared to untreated or glycerol controls (Fig. 4A). Second, we tested the effect of lubricants on *L. crispatus* colonization levels when VEC were pre-exposed to lubricants following infection with *L. crispatus*. Pre-exposure to all tested lubricants for 4 h or 24 h significantly impacted the VEC colonization by *L. crispatus* compared to negative controls (VEC cultures without lubricants or with glycerol) ($P<0.0001$ and <0.001, respectively). The colonization levels were reduced by 1.7–2.3 logs at 4 h and 1.3–1.4 logs at 24 h when compared to untreated controls (Fig. 4B).

Discussion

The vaginal microbiota play a critical role in women's health and disease.[3,12] Particularly, *Lactobacillus*-dominant communities contribute to homeostasis and protect the cervicovaginal microenvironment from invading pathogens.[3] In contrast, the depletion of *Lactobacillus* spp. and the overgrowth of diverse anaerobes (characteristic of bacterial vaginosis (BV)) can lead to numerous gynecologic and obstetric sequalae, including increased risk of STIs, preterm birth, spontaneous miscarriages, pelvic inflammatory disease and gynecologic cancer.[12,13] Multiple factors have been shown to affect the vaginal microbiome, including sexual practices and use of lubricants, sex toys, and feminine hygiene products.[14] Indeed, lubricants, which frequently contain antimicrobial preservatives, may directly impact bacterial communities in the cervicovaginal microenvironment.

The available epidemiological data suggest that the use of lubricants is associated with increased risk of vaginal dysbiosis or BV.[15–17] However, mechanistic *in vitro* studies investigating vaginal lubricants or feminine hygiene products are still very limited. Previously, we demonstrated the detrimental effect of hyperosmolar lubricants on the vaginal epithelium using *in vitro* models.[5] Herein, we sought to determine the impact of personal and clinical lubricants, which varied in osmolality and formulations, on health-associated vaginal *Lactobacillus* spp. We examined lubricants routinely used in clinics for vaginal

ultrasounds and pelvic exams (E-Z Lubricating Jelly, McKesson Lubricating Jelly, K-Y™ Jelly, Surgilube®), as well as, over-the-counter (OTC) personal lubricants used for sexual practices, alleviating vaginal dryness (Astroglide® Liquid, GCL Almost Naked, K-Y™ Jelly, K-Y™ Warming Jelly) or prevention of pregnancy (Conceptrol®) (Table 1).

In this study, we demonstrated that certain lubricants, such as K-Y™ Jelly, and Conceptrol®, exhibited antimicrobial properties against vaginal *Lactobacillus* spp. *in vitro*, despite a lack of association to high osmolality, which relates to the concentration of glycols and glycerin (Table 1). These antimicrobial properties could potentially be due to their excipients i.e. CHG, parabens, polyquaternium-15 or a known spermicide, nonoxynol-9 (N-9). Intriguingly, lubricants containing CHG (K-Y™ Jelly, Surgilube®) or N-9 (Conceptrol®) inhibited the most bacterial growth, whereas lubricants containing parabens or polyquartenium-15 (Astroglide® Liquid, E-Z Lubricating Jelly) did not have this effect (Fig. 1, 2) in our *in vitro* systems. These findings strongly suggest that CHG or N-9 in these lubricants are responsible for the bacterial growth inhibition. The disk diffusion and MIC assays performed in this study confirmed that CHG exhibit stronger antimicrobial properties against vaginal *Lactobacillus* spp. compared to parabens (Fig. 3).

CHG is a broad-spectrum microbicide, which can be found in a variety of products, including OTC antiseptic mouthwashes, creams, wipes, toothpastes, deodorants, sunscreens, eye drops, hair conditioners and more.[9] In the context of the oral cavity, previous clinical studies demonstrated that use of mouthwash containing CHG has been linked to major shifts in the microbiota composition.[18] Studies on the impact of CHG on skin microbiota have shown conflicting findings. On one hand, a minimal effect of CHG on the skin microbiota has been demonstrated, suggesting high stability and resilience of bacterial communities,[19] whereas other studies showed decreased bacterial density on CHG-treated skin.[20]

Data presented in this *in vitro* study suggests that intravaginal use of products containing CHG may also have a detrimental effect on the vaginal microbiota by decreasing the overall bacterial load, including health-associated *Lactobacillus* spp. This might allow BV-associated bacteria to colonize the vagina, leading to vaginal dysbiosis or BV. Our findings are in accordance with a previous report showing that CHG inhibit growth not only of genital pathogens (such as *Neisseria gonorrhoeae* or *Trichomonas vaginalis*), but also vaginal *Lactobacillus* spp.[21] A 2010 study also demonstrated that use of CHG-based Surgilube® during pelvic examination decreased the detection of group B *Streptococcus* (GBS), a common vaginal opportunistic pathogen.[22] Intriguingly, despite the links to anaphylaxis and recent FDA ban of CHG in healthcare antiseptics and classification as ‘not generally recognized as safe and effective for use’[10], the American College of Obstetricians and Gynecologists (ACOG) approved the off-label use of CHG for surgical vaginal preparation, as there is no other FDA-approved alternative to povidone-iodine.[23] This approval comes from reports demonstrating that CHG is more effective than povidone-iodine at killing vaginal bacteria[24] and thus far CHG has not been linked to signs of vaginal irritation.[25] The discordance in recommendations from different bodies highlights the urgent need for better safety screening of female hygiene products, for example, utilizing *in vitro* 3-D biomimetic models and larger epidemiologic studies and trials.[5]

The other antimicrobial excipient, N-9, is a surfactant spermicide and an active ingredient in Conceptrol®. It has been shown in clinical and *in vitro* studies to cause genital inflammation and barrier breach,[26] and consequently has been associated with increased HIV-1 transmission in high-risk women.[27] Furthermore, the adverse effect of N-9 on vaginal *Lactobacillus* spp. were well characterized by several studies in the 1990s.[28,29] Adverse findings on N-9 were also validated in a 2012 study, utilizing 16S rRNA sequencing, which demonstrated shifts in composition from *Lactobacillus*-dominant communities to communities dominated by anaerobes associated with BV, as well as *Streptococcus*, *Enterococcus*, and *Escherichia*, following twice-daily vaginal application of 4% N-9.[30] In accordance to previous reports, Conceptrol®, containing 4% N-9, also reduced growth of vaginal *Lactobacillus* species in our study.

Our *in vitro* evidence suggest that lubricants might impact attachment of *Lactobacillus* to the vaginal epithelium (Fig. 4). A limited number of lubricants were tested for bacterial colonization, since the majority of products induce substantial damage to human epithelial cells due to high osmolality.[5] The viscosity of the tested lubricants could have been a limitation in experiments. However, the viscosity of the lubricants does not explain the effect of tested lubricants on colonization of VEC with bacteria. This was confirmed by the use of glycerol as a viscosity control, which did not elicit any substantial decrease in *Lactobacillus* colonization of epithelial cells. Therefore, the reason for the reduction of *L. crispatus* colonization on VEC was potentially due to specific ingredients or other physical properties of lubricants than the viscosity.

Overall, this *in vitro* study suggests that personal and clinical lubricants containing N-9 or CHG might exhibit adverse effects on the growth of vaginal microbiota species and highlights the need for consumers and clinicians to utilize these lubricants with caution. Future clinical studies, particularly with longitudinal study designs, are needed to show whether the use of these vaginally applied products have a long-lasting effect on the microbiota *in vivo* and to confirm the clinical relevance of our *in vitro* findings. Notably, the WHO warned consumers to avoid certain ingredients that are commonly found in OTC lubricants (e.g., N-9, polyquaternium, etc.) as these may increase the risk of HIV infection.[6,8] However, currently, they have made no comment of CHG regarding consumer products. Our data suggest that feminine hygiene products containing CHG could impact vaginal *Lactobacillus* growth and potentially re-colonization of the vagina. Ultimately, additional clinical studies and mechanistic *in vitro* studies, are required to investigate the impact of vaginal lubricants and other feminine products, including moisturizers, washes, wipes, creams, sprays, powders, douches, as well as probiotics/pharmaceutical vehicles and other intravaginal practices, on vaginal *Lactobacillus* species and cervicovaginal epithelium.

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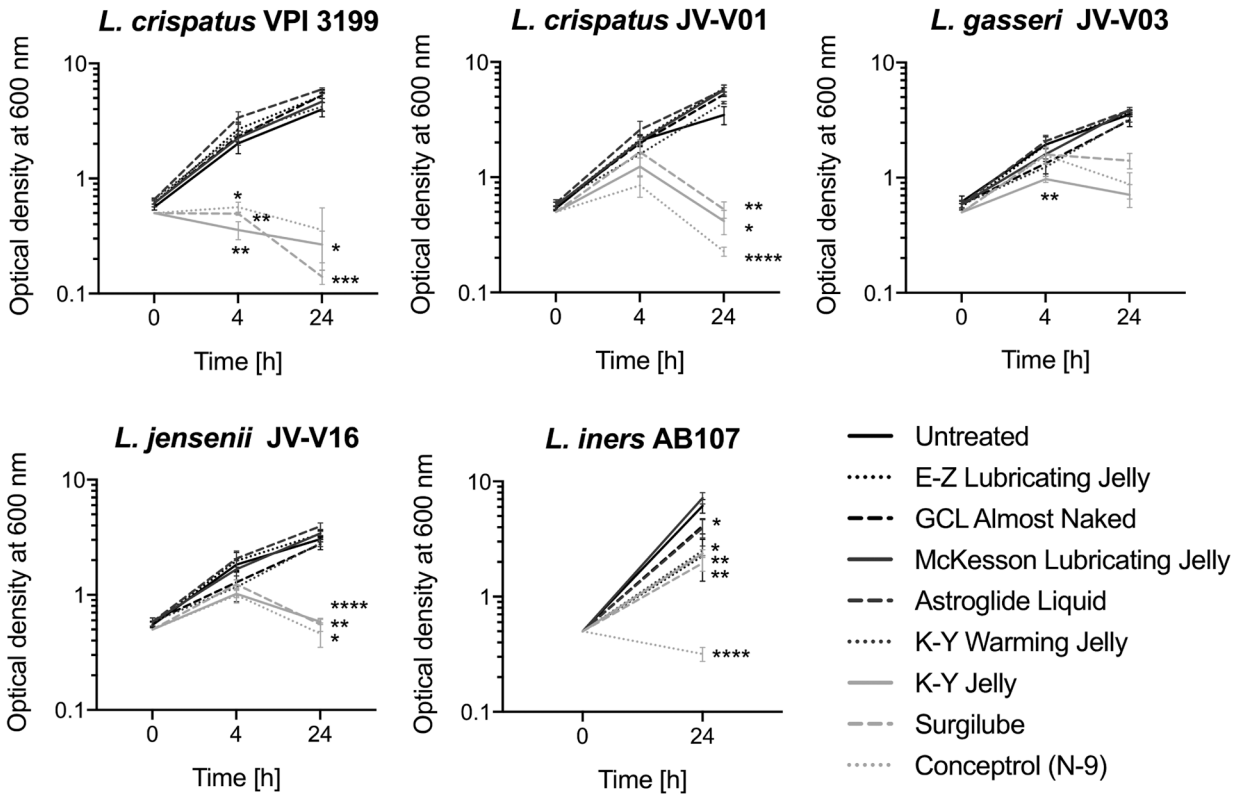

Figure 1. Select lubricants (K-Y™ Jelly, Surgilube® and Conceptrol®) inhibit the growth of vaginal *Lactobacillus* spp.

L. crispatus strain VPI 3199, *L. crispatus* strain JV-V01, *L. gasseri* strain JV-V03, *L. jensenii* strain JV-V16 and *L. iners* strain AB107 were grown in MRS or supplemented TSB broth with 10% (v/v) lubricants at 37°C under 5% CO_2 or anaerobic conditions. Bacterial growth was assessed by measuring optical densities at 600 nm (OD_{600}) at 4 h and 24 h post inoculation. The broth without any lubricant was used as a positive control. OD_{600} measurements of each culture with lubricants were compared to cultures without lubricants at respective time points. Data are shown as means ± SE from at least three independent experiments. *P* values were calculated using one-way ANOVA with Dunnett post-test (* $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$).

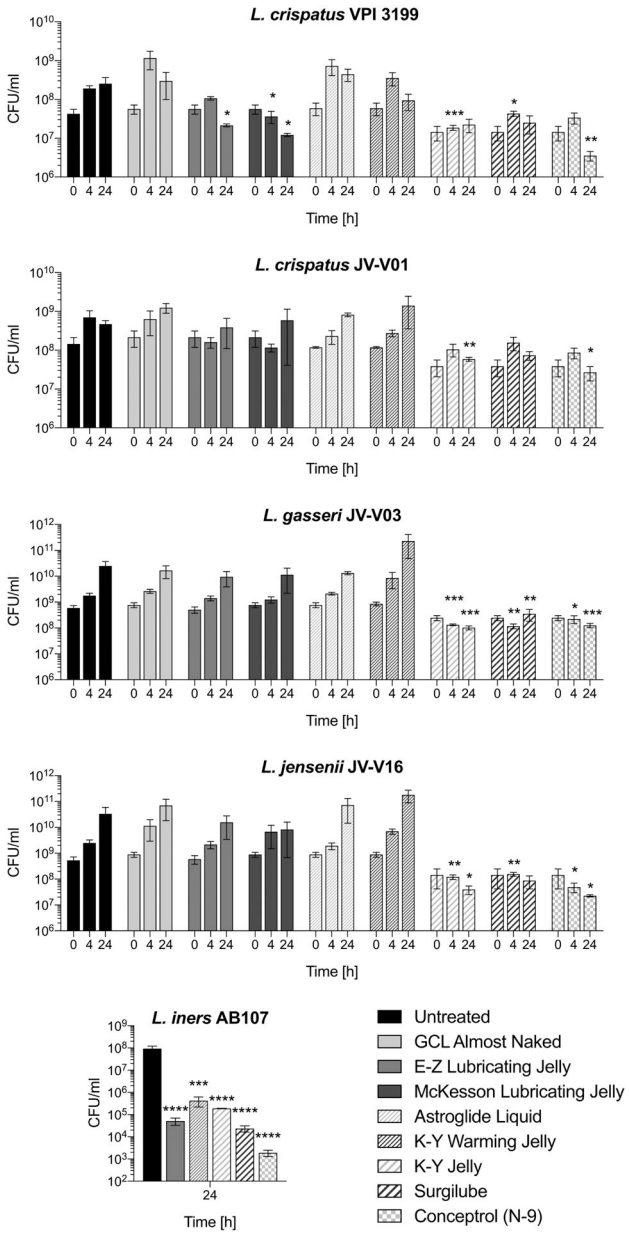

Figure 2. Select lubricants (K-Y™ Jelly, Surgilube® and Conceptrol®) exhibit bacteriostatic effect on vaginal *Lactobacillus* spp.

L. crispatus strain VPI 3199 (**A**), *L. crispatus* strain JV-V01 (**B**), *L. gasseri* strain JV-V03 (**C**), *L. jensenii* strain JV-V16 (**D**) and *L. iners* strain AB107 (**E**) were grown in MRS or supplemented TSB broth with 10% (v/v) lubricants at 37°C under 5% CO_2 or anaerobic conditions. Bacterial growth kinetics were assessed by measuring number of colony forming units (CFU) representing viable bacteria in the cultures in appropriate liquid media containing 10% solutions of lubricants at 4 and 24 h following the exposure to 10% (v/v) lubricants using standard plating assay. The broth without any lubricant was used as a positive control. Concentrations of viable bacteria in each culture were calculated as CFU/mL and compared to culture without lubricants at respective time points. Data are shown as means ± SE from three independent experiments. *P* values were calculated

using one-way ANOVA with Dunnett post-test (* $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$).

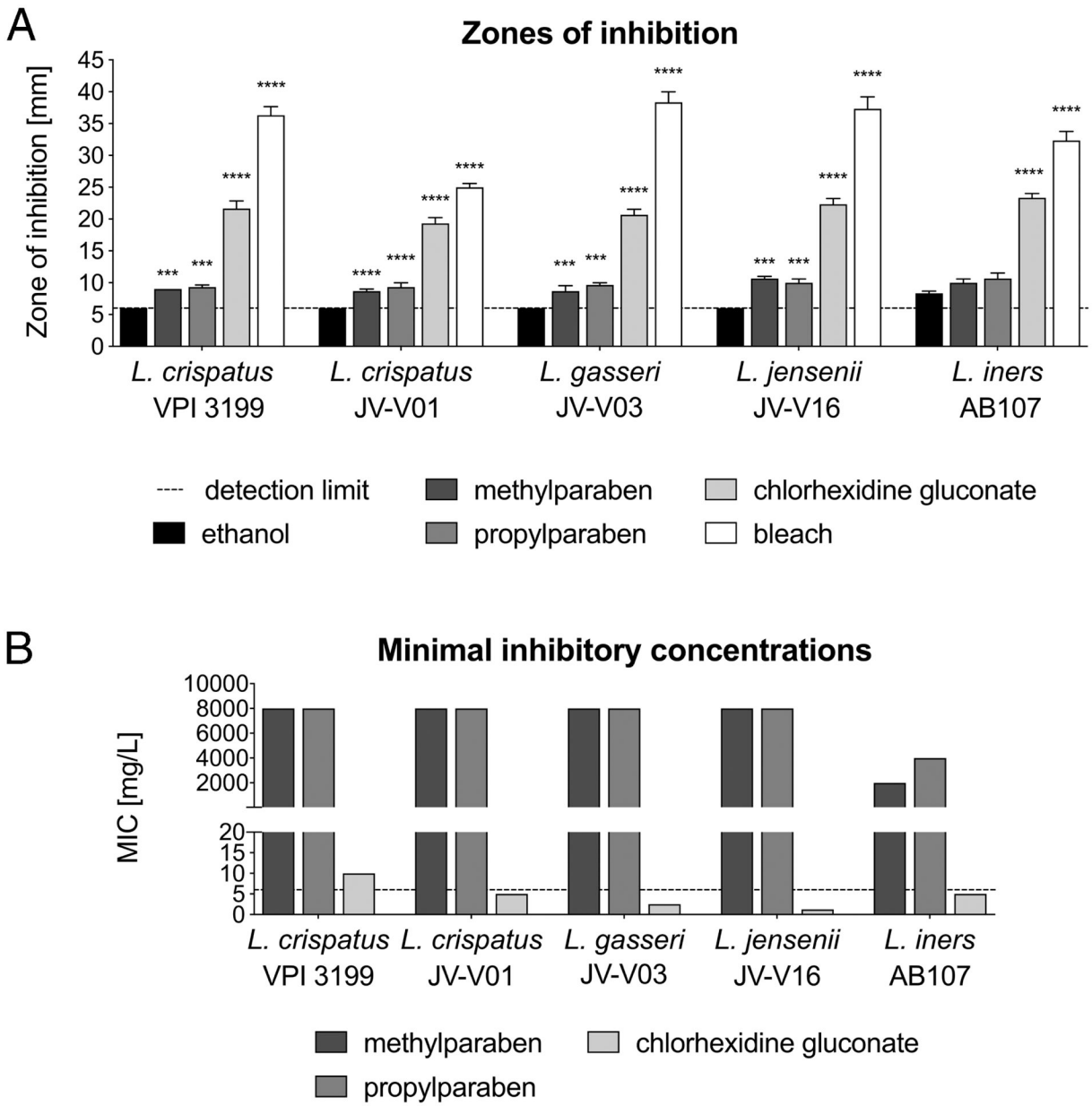

Figure 3. Chlorhexidine gluconate exhibit stronger antimicrobial properties than parabens across all tested vaginal *Lactobacillus* spp.

A. The impact of each excipient on the growth of *Lactobacillus* spp. was tested using disk diffusion assay. Bacteria were grown on appropriate agar plates with 6-mm disks impregnated with 20% solutions (w/w) of methylparaben, propylparaben and chlorhexidine gluconate (CHG). Ethanol (solvent for parabens) and bleach was used as negative and positive controls, respectively. Zones of inhibition were recorded 24 h post inoculation and compared to a negative control. Data are shown as means ± SE from three independent experiments. *P* values were calculated using one-way ANOVA with Bonferroni post-test (** $P<0.01$; *** $P<0.001$; **** $P<0.0001$). **B.** Minimal inhibitory concentrations (MIC) of methylparaben, propylparaben and CHG were determined using the broth microdilution

method. The MIC was defined as the lowest concentration of the excipient that inhibits the visible growth of the tested *Lactobacillus* spp.

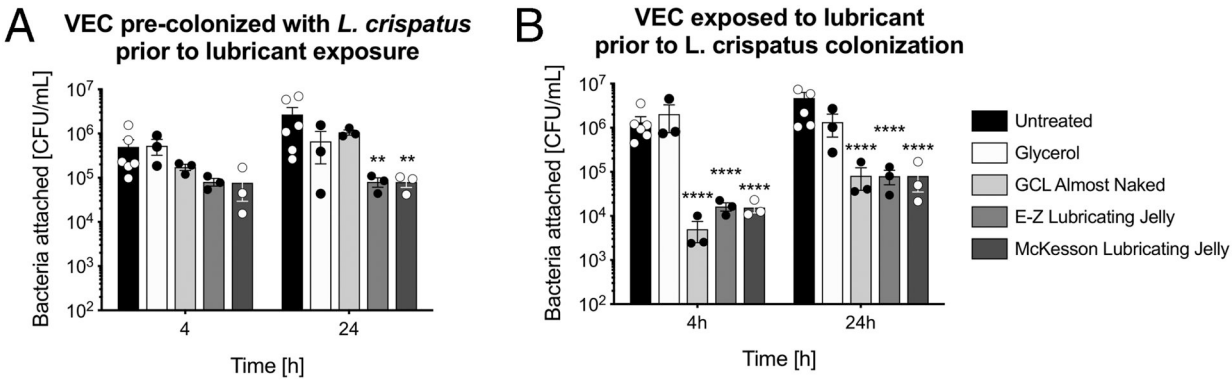

Figure 4. Lubricants reduce the colonization of vaginal epithelial cells (VEC) with *L. crispatus* particularly when VEC are colonized with bacteria following the lubricant exposure.

Three non-cytotoxic lubricants: Good Clean Love (GCL) Almost Naked, E-Z Lubricating Jelly, McKesson Lubricating Jelly) were tested to determine their impact on colonization of *in vitro* VEC model with *L. crispatus* strain JV-V01. **A.** VEC were pre-colonized with bacteria at the multiplicity of infection (MOI) 10 for 2 h prior to exposure to 10% (v/v) lubricants **B.** VEC were exposed to 10% (v/v) lubricants and immediately colonized with *L. crispatus* for 4 h or 24 h as described above. Colonization levels were reported as number of viable bacteria (CFU) attached to VEC per well. Data are shown as means ± SE from at least three independent experiments. *P* values were calculated using two-way ANOVA with Bonferroni post-test (** $P<0.01$; **** $P<0.0001$).

Table 1.
Formulations of tested clinical and personal lubricants.

Excipients exhibiting potential antimicrobial properties against vaginal *Lactobacillus* spp. are indicated in bold.

Lubricant	Osmolality [mOsm/kg]	Ingredients	Manufacturer
Clinical lubricants			
E-Z Lubricating Jelly	2243	Water, glycerin, carbomer, sodium hydroxide, PEG-150, **methylparaben**, **propylparaben**	Athena Medical Products
McKesson Lubricating Jelly	2125	Water, glycerin, sodium hydroxide, carbomer 140g, polyethylene glycol, **propylparaben**, **methylparaben**	McKesson Medical-Surgical Inc.
Surgilube® Surgical Lubricant	Not tested	Water, hydroxypropylmethylcellulose, propylene glycol, **chlorhexidine gluconate**	HR Pharmaceuticals Inc.
Personal lubricants			
Astroglide® Liquid	6100	Purified water, glycerin, propylene glycol, **polyquaternium 15**, **methylparaben**, **propylparaben**	Biofilm Inc.
Conceptrol®	1257	**Nonoxynol-9** (4%), lactic acid, **methylparaben**, povidone, propylene glycol, purified water, sodium carboxymethycellulose, sorbic acid, sorbitol solution	Caldwell Consumer Health LCC
Good Clean Love Almost Naked	270	Organic aloe barbadensis leaf juice, xanthan gum, agar, potassium sorbate, sodium benzoate, sodium lactate, lactic acid, natural flavors	Good Clean Love Inc.
K-Y™ Jelly	2500	Water, glycerin, hydroxyethylcellulose, **chlorhexidine gluconate**, gluconolactone, **methylparaben**, sodium hydroxide	Reckitt Benckiser Group plc
K-Y™ Warming Jelly	10300	Propylene glycol, PEG-8, hydroxypropylcellulose, tocopherol	Reckitt Benckiser Group plc