Published in final edited form as:

Am J Reprod Immunol. 2015 December ; 74(6): 499–507. doi:10.1111/aji.12427.

Activity of Genital Tract Secretions and Synthetic Antimicrobial Peptides against Group B *Streptococcus*

Nidhi Agarwal¹, Niall Buckley², Natasha Nakra², Philip Gialanella³, Weirong Yuan⁴, and Jeny P. Gharthey⁵

¹Division of Neonatology, Department of Pediatrics, Children's Hospital At Montefiore, Albert Einstein College of Medicine, Bronx, NY, USA

²Department of Pediatrics, Albert Einstein College of Medicine, Bronx, NY, USA

³Department of Pathology, Division of Microbiology, Montefiore Medical Center, Bronx, NY, USA

⁴Institute of Human Virology, University of Maryland School of Medicine, Baltimore, MD, USA

⁵Department of Obstetrics & Gynecology and Women's Health, Albert Einstein College of Medicine, Bronx, NY, USA

Abstract

Problem—Genital tract secretions inhibit *Escherichia coli* (*E. coli*) through antimicrobial peptides (AMP) secreted by the host and vaginal microbiota. However, there are limited data against group B *Streptococcus* (GBS).

Method of Study—Group B *Streptococcus* were incubated with cervico-vaginal lavage (CVL) samples from healthy non-pregnant women ($n = 12$) or synthetic AMP and monitored for bacterial growth using a turbidimetric approach. *E. coli* inhibitory activity was determined by a colony-forming unit assay.

Results—None of the CVL samples inhibited GBS. The human neutrophil peptide-1 and human defensin 5 inhibited GBS growth by 80% at concentrations 20 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$, respectively, while human betadefensin 2 and LL-37 did not inhibit at highest concentration tested (100 $\mu\text{g/mL}$). In contrast, all AMP inhibited *E. coli*.

Conclusions—Antimicrobial peptides may protect against *E. coli* colonization but have more limited activity against GBS. Future studies will focus on augmenting host defense with specific AMP to prevent genitourinary infection with these pathogenic organisms.

Correspondence Jeny Gharthey, Department of Obstetrics & Gynecology, and Women's Health, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Block 307, Bronx, NY 10461, USA., jenyghartey@gmail.com.

Conflict of interest

'The authors report no conflict of interest'.

The findings of the study were presented at: (i) American Academy of Pediatrics National Conference & Exhibition, Orlando, FL, October 26–29, 2013. (ii) 83rd Perinatal & Developmental Medicine Symposium, Marco Island, FL, November 6–9, 2013.

Citation

Agarwal N, Buckley N, Nakra N, Gialanella P, Yuan W, Gharthey JP. Activity of genital tract secretions and synthetic antimicrobial peptides against group B *Streptococcus*. *Am J Reprod Immunol* 2015; 74: 499–507 doi:10.1111/aji.12427

Keywords

Antimicrobial peptides; cervico-vaginal lavage samples; defensins; *Escherichia coli*; female genital tract secretions; group B streptococcus

Introduction

The immune mechanisms in the female genital tract play an important role in preventing host infection both within and outside of pregnancy.^{1,2} Two bacterial pathogens, *Streptococcus agalactiae* [Group B *Streptococcus* (GBS)] and *Escherichia coli* (*E. coli*), have been associated with genital infection,^{3,4} urinary tract infection, preterm birth, and neonatal sepsis.^{5–7} GBS has also been associated with adverse perinatal outcomes including chorioamnionitis and endometritis.^{8,9} Efforts to reduce infection with these pathogens could have a significant impact on reproductive health outcomes in women.

The female genital tract maintains an array of defense mechanisms, which likely act in concert to prevent infection. These mechanisms include an intact epithelial barrier, acidic pH generated by H₂O₂ secreting vaginal lactobacilli, and soluble immune mediators (e.g., antimicrobial peptides, cytokines, and chemokines) that are secreted by epithelial and immune cells.¹⁰ Antimicrobial peptides (AMPs), such as defensins and cathelicidins, play an important role in the innate immune response against pathogens¹¹ and are small peptides secreted by immune and epithelial cells.¹² The family of defensins includes human neutrophil peptides 1–4 and human betadefensins 1, 2, and 3.¹³ Only one cathelicidin has been characterized in humans, LL-37, derived from the CAP18 protein.^{13,14} These AMPs contribute to innate host immunity by acting as ‘natural peptide antibiotics’ against potentially infectious bacteria, fungi, and viruses.^{12,15}

Genital tract secretions have consistently demonstrated *ex vivo* inhibitory activity against *E. coli*.^{16–18} This activity may reflect the cumulative interactions of host mucosal defenses including the AMPs and products of vaginal microbiota and may be critical during pregnancy to prevent pathogenic vaginal colonization leading to ascending infection, preterm birth, and neonatal sepsis.² In a recent crosssectional study, genital tract secretions from nearterm healthy pregnant women demonstrated high *E. coli* inhibitory activity that inversely correlated with *E. coli* vaginal colonization.¹⁶ Another study of cervico-vaginal lavage (CVL) samples from healthy non-pregnant women suggested that the *E. coli* inhibitory activity may be mediated, in part, by proteins secreted by lactobacilli.¹⁰ While host defense against *E. coli* may be attributed to the vaginal microbiota and soluble mediators including AMPs, there are limited data on the *ex vivo* activity of genital tract secretions against GBS. Moreover, few in vitro studies have shown that α -defensins [human neutrophil peptides 1–4 (HNP-1–4) and enteric human defensin 5 and 6 (HD5 and HD6)] have more potent activity against gram-positive bacteria [*Staphylococcus aureus* (*S. aureus*)], whereas all three human β -defensins (HBD-1–3) exhibited strong inhibitory activity against gram-negative bacteria (*E. coli*).^{19,20} To our knowledge, no prior study has examined the inhibitory activity of genital tract secretions against GBS in non-pregnant women. Furthermore, there are no data on the activity of AMPs and the vaginal microbiota

against GBS. Therefore, our objective was to evaluate the ability of genital tract secretions and representative synthetic AMPs (HNP-1, HD5, HBD-2, and LL37) to inhibit GBS growth. Additionally to assess the role of vaginal microbiota on GBS, we tested the ability of spent culture supernatants from major components of the vaginal microbiota to inhibit GBS growth, *in vitro*.

Materials and methods

Clinical Samples

A convenience sample of CVL collected from 12 healthy non-pregnant women who participated in a study examining the impact of semen/sex on soluble genital tract mucosal immunity was obtained. The parent study was approved by the Internal Review Board at the Albert Einstein College of Medicine. Exclusion criteria were as follows: age <18 years, evidence of active genitourinary infection based on history and symptoms, abnormal Pap smear result within the past year, antibiotic or steroid use within the past month, sexual intercourse within the last 72 hr, and history of human immunodeficiency virus infection. Demographic and clinical data were collected for each participant. We have previously shown that high inhibitory activity against *E. coli* is associated with combination of host proteins and *L. crispatus*-dominant microbiome,²¹ and therefore, we selected samples with high (>80%, $n = 8$) and low (<20%, $n = 4$) *E. coli* inhibitory activity to test the effect of the AMPs and vaginal microbiota on GBS growth. Non-pregnant females were selected to avoid the confounding effect of pregnancy on alteration of genital tract immunity and lower genital tract flora.²²

Cervico-vaginal lavage were previously collected by washing the cervico-vaginal area with 10 mL of sterile normal saline after insertion of a sterile speculum and allowing the fluid to pool in the posterior fornix prior to aspiration. The samples were transported on ice to the laboratory, centrifuged at 800 *g* for 10 min, and the supernatants divided into aliquots and stored at -80°C .

Synthetic AMPs and Spent Culture Supernatants

Synthetic AMPs were chemically produced, purified, and quantified at 280 nm using molar extinction coefficients in the laboratory of Dr. Wuyuan Lu at University of Maryland as previously described.¹⁹ α -Defensins (HNP-1, HD-5), β -defensin (HBD-2), and cathelicidin (LL-37)^{19,23} were assayed for antiGBS activity using a turbidimetric approach and for anti-*E. coli* activity using colony-forming unit assay (described in detail below). AMPs were dissolved in 0.01% acetic acid and stored at -20°C .

Spent culture supernatants (SCS) from *Lactobacillus crispatus* (*L. crispatus*) (ATCC 33197), *Lactobacillus iners* (*L. iners*) (DSM 13335, UPII 60B-BEI HM-131, and 143D-BEI HM-126), and Gardnerella vaginalis (*G. vaginalis*) (ATCC 49145, 14018) were prepared by centrifuging overnight cultures of bacteria at $2000 \times g$ for 15 min at room temperature and then filtering the supernatant to remove bacteria and particulate matter (0.22- μm syringe filter) in the laboratory of Dr. Adam Ratner at Columbia University as previously described.

²¹ SCS from each of these bacteria and their respective growth media were assayed for anti-GBS activity and *E. coli* inhibitory activity.

Anti-GBS Activity

Utilizing a turbidimetric approach, GBS (ATCC strain 27956) was grown overnight to stationary phase and then 30 μ L of bacteria were mixed with 30 μ L of CVL, synthetic AMPs, or bacterial SCS and added to the test wells on a 96-well plate in the presence of 60 μ L of Lim broth and 30 μ L of 0.01% acetic acid. The final concentrations of synthetic AMPs that were assayed were as follows: HD-5, HBD-2, and LL-37: 12.5 μ g– 100 μ g/mL; HNP-1: 2.5–20 μ g/mL. All experiments were performed in duplicate. The optical density (OD) reading of the bacteria was kept constant for all experiments (0.5–0.7 at OD_{620 nm}, corresponding to $\sim 5 \times 10^8$ cfu/mL)^{20,24} (Ultrospec 2100 pro, classic; GE Healthcare Life Sciences, Pittsburgh, PA, USA). A background well consisting of buffer only was subtracted from the test well to account for the turbidity of proteins other than GBS. The 96-well plate was incubated at 37°C for 6 hr (the peak of the logarithmic phase for GBS growth), and OD readings were taken at 1-hr intervals (Multimode Detector DTX 880 at 620 nm). Percent inhibition was calculated based on the OD readings in the test wells of CVL, synthetic AMPs, or bacterial SCS compared to OD readings in the negative control wells. The following served as negative controls: for CVL, a control buffer comprised of 20 mmol/L potassium phosphate, 60 mmol/L sodium chloride, 0.2 mg/mL albumin, pH 4.5; for AMPs, 0.01% acetic acid; and for bacterial SCS, the respective bacterial growth media. Penicillin/streptomycin (P/S) was included as a positive control (10,000 U/mL penicillin and 10,000 μ g/mL streptomycin; Gibco by Life Technologies, Carlsbad, CA, USA). After an unsuccessful attempt to replicate anti *E. coli* colony-forming assay, turbidimetric approach was adopted to run GBS assay. In additional experiments, the AMPs were also diluted in 154 mM NaCl to test whether the antimicrobial activity was salt sensitive as CVL samples were collected in normal saline.^{25,26} Finally, to evaluate whether GBS inhibition of genital tract secretions could be augmented with the addition of synthetic AMPs, two CVL samples were supplemented with HNP-1 (10 and 20 μ g/mL) and HD-5 (25 and 50 μ g/mL) and then assayed for GBS inhibitory activity.

E. coli Inhibitory Activity

E. coli (ATCC strain 4382627) was grown overnight to stationary phase, and then, 3 μ L of bacteria ($\sim 10^7$ cfu/mL) was mixed with 27 μ L of CVL, serial dilutions of synthetic AMPs, bacterial SCS, or control buffer and incubated at 37°C for 2 hr. The mixtures were then further diluted in control buffer to yield 800–1000 colonies on control plates and plated in duplicate on agar enriched with trypticase soy broth (TSB). Colonies were counted using ImageQuant TL v2005 after an overnight incubation at 37°C. All samples were tested in duplicate, and the percentage inhibition was determined relative to the colonies formed on the control plates. The AMPs were serially diluted in 10 mM NaPO₄ rather than acetic acid because in pilot studies we observed that *E. coli* (but not GBS) was inhibited by 0.01% acetic acid. The differences might be due to resultant pH effect (0.01% acetic acid = 0.01 mM acetic acid, pH 4.3; 10 mM sodium phosphate saline, pH 7.0).

Soluble Immune Mediators in Clinical Samples

Total protein concentration was measured by Micro BCA Protein Assay kit (Thermo Scientific, Rockford, IL, USA) in CVL diluted 1:4. The levels of HNP 1–3, HBD-2, and LL-37 were determined using commercial enzyme-linked immunosorbent assay kits with dilutions 1:200, 1:100, and 1:2, respectively (Alpha Diagnostic International, San Antonio, TX, USA and Hycult Biotech, Uden, The Netherlands). Concentrations that fell above the highest limit of detection were repeated at a higher dilution factor. The HNP ELISA does not differentiate between the closely related HNP-1, HNP-2, and HNP-3. There was no commercial ELISA kit available to measure HD5 levels at the time of experiments.

Statistical Analysis

For clinical and demographic data, standard descriptive statistics were calculated for the entire cohort. All experiments were performed in duplicate. The median percent inhibitory activity for GBS and *E. coli* were compared to their respective control with the Mann–Whitney U-test. Spearman rankorder correlation was calculated to assess correlations between mediators and bactericidal activity. A two-sided *P* value <0.05 was considered significant. All statistical tests were performed using STATA version 11.0 (College Station, TX, USA), R version 2.12.2, and GraphPad Prism version 5. (La Jolla, CA, USA).

Results

Female Genital Tract Secretions Lack Anti-GBS Activity

Cervico-vaginal lavage from 12 subjects, eight samples with high anti-*E. coli* activity (>80%) and four with low activity (<20%), were selected. Characteristics of the 12 subjects are summarized in Table I. To our surprise, none of the CVL samples demonstrated activity against GBS but a low level of enhancement (median percent inhibition –11.38, range: –42 to –1.98) was noted (Fig. 1). Notably, the median concentration of HNP1–3 was 0.117 µg/mL (range 0.007–1.06 µg/mL), which was significantly lower than the minimum concentration needed to inhibit GBS growth (Fig. 2). The median concentration of HBD-2 and LL-37 was 0.0015 µg/mL (range 0.0002–0.6671 µg/mL) and 0.00257 µg/mL (range 0.0001–0.0314 µg/mL), respectively. There was a significant correlation between LL-37 and *E. coli* inhibitory activity ($\rho = 0.6632$, $P = 0.02$). None of the mediators correlated with anti-GBS activity.

α -Defensins Exhibited Greater Anti-GBS Activity than β Defensins

Synthetic HNP-1 and HD-5 significantly inhibited GBS growth (as compared to negative control 0.01% acetic acid, $P < 0.05$) by 80% at concentrations of 20 and 50 lg/mL, respectively. In contrast, HBD-2 and LL-37 did not inhibit GBS growth even at highest concentrations tested 100 µg/mL (Fig. 2). All of the AMPs tested exhibited potent activity against *E. coli*; HBD-2 and LL-37 displayed significantly more *E. coli* inhibition than GBS inhibition, whereas HNP-1 had significantly more activity against GBS ($P < 0.001$, Fig. 3). Moreover, HNP-1 and HD-5 retained activity against GBS when diluted in normal saline (154 mM NaCl) (Fig. 4a), whereas the anti-*E. coli* activity of the defensins (but not LL-37) was significantly reduced (Fig. 4b).

Anti-GBS Activity is Restored with the Addition of Synthetic Defensins

To assess whether the absence of anti-GBS activity of CVL reflected insufficient concentrations of AMPs or an active inhibitor, synthetic HNP-1 and HD-5 were added to two CVL samples with >90% *E. coli* inhibition. The CVL modestly enhanced GBS growth (−42% and −17.5%, respectively), but supplementation with synthetic defensins resulted in a significant and dose-dependent additive increase in the inhibitory activity (Fig. 5).

Lactobacilli and *Gardnerella* SCS Inhibit *E. coli*, but not GBS

Spent culture supernatants from lactobacilli demonstrated only minimal inhibition (up to 30%) against GBS that was significantly different from its activity against *E. coli* as shown in Fig. 6. *L. crispatus* and *L. iners* 60B had the highest *E. coli* inhibitory activity (85 and 79%, respectively). *Gardnerella* also showed up to 30% inhibition to GBS, which did not significantly differ from *E. coli* inhibitory activity.

Discussion

There were marked differences in the antimicrobial activity of genital tract secretions, synthetic AMPs, and vaginal microbial SCS against GBS compared to *E. coli*. While α -defensins (HNP-1, HD-5) had *in vitro* activity against GBS, contrary to prior studies, we did not demonstrate anti-GBS activity of HBD-2 and LL-37.^{27,28} These *in vitro* findings translated into the absence of any inhibitory activity against GBS in clinical samples, but surprisingly, a modest increase in GBS growth was noted when the bacteria were mixed with CVL. We chose to restrict the CVL sample selection to a non-pregnant healthy female cohort with both high *E. coli* inhibitory activity (>80%) ($n = 8$) and low activity (<20%) ($n = 4$), as prior studies have suggested a modest correlation between *E. coli* inhibitory activity and the presence of AMPs (e.g. secretory leukocyte protease inhibitor, lactoferrin, lysozyme) and the vaginal microbiota^{10,16,17} and to avoid the potential confounding effect of pregnancy on the innate immunity within the genital tract.²²

Importantly, enhancement of inhibitory activity against GBS noted after supplementing representative CVL with HNP-1 or HD-5 suggested that either there were insufficient concentrations of inhibitory AMPs and/or an enhancer was present that could be overcome with the addition of synthetic AMPs. CVL likely reflects a 40–50-fold dilution of genital tract secretions based on studies using Normosol-R or lithium chloride as diluents.^{29,30} However, after correcting for the dilution effect, the concentrations of HNP-1 and HD-5 would still be insufficient to inhibit GBS, although the levels still contribute to *ex vivo* activity against *E. coli*. Noting that the CVL were collected in normal saline, this may have underestimated the activity of defensins in the genital tract, as the antimicrobial activity is salt sensitive (Fig. 3b). Studies with secretions collected by other methods such as swab or Softcup and/or diluted in other buffers such as sodium phosphate buffer might provide a more accurate assessment of the *E. coli* inhibitory activity of genital tract secretions.

Our data, consistent with prior studies, demonstrated that α -defensins (HNP-1, HD-5) had potent activity against GBS and *E. coli*²⁰; however, in contrast to prior studies, we found that HBD-2 and LL37 were selectively active against only *E. coli*.^{27,28} The disparate results

might have been due to differences in methodology.^{14,27,31} Differences in AMPs activity against specific bacteria may reflect bacterium-specific differences in the components of the cell wall and membrane of gram-positive compared to gram-negative organisms or enantiomeric structural differences of defensins.^{19,32} The common mechanism proposed for antimicrobial activity against bacteria is targeting of Lipid II, an essential precursor to cell wall synthesis.^{33,34} However, these data demonstrate the selective potent activity of α defensins against GBS but not against *E. coli*, and is consistent with other studies that have examined their activity against *S. aureus*.^{35–37}

Interestingly, we noted that LL-37 inhibited *E. coli* but not GBS, its activity against *E. coli* was resistant to salt, and its concentration significantly correlated with *E. coli* inhibitory activity of CVL. Studies have revealed that LL-37 has potent, direct or synergistic antimicrobial activity against different bacterial strains including *Klebsiella pneumoniae*,³⁸ *E. coli*, *S. aureus*,¹⁴ *Neisseria gonorrhoeae*,³⁹ and group A *Streptococcus*.⁴⁰ Taken together, LL-37 may play a major role in the prevention of colonization with pathogenic bacteria within the genital tract.

Also, significantly less activity of culture supernatants harvested from lactobacilli was seen against GBS compared to *E. coli* that might contribute toward the disparity in activity of the CVL samples. We previously found that the inhibitory activity against *E. coli* maps, at least in part, to proteins expressed by *L. crispatus* and/or *jensenii* and moreover, that women with high *E. coli* activity had a microbiome that was dominated by *L. crispatus*.²¹ Thus, the presence of a lactobacillus dominant microbiome may protect against *E. coli*, but not GBS through multiple mechanisms, including production of hydrogen peroxide and lactic acid,¹⁷ expression of inhibitory proteins including bacteriocins,⁴¹ and by competitive exclusion of other bacteria.⁴²

There are several limitations to this study including the small clinical sample size and reliance on dilute CVL collected in saline which may have impacted our results by interfering with AMPs that are salt sensitive. We did not collect data on the phase of menstrual cycle which would affect the concentrations of AMPs and possibly the activity of CVL against GBS.⁴³ We did not collect vaginal cultures to assess for *E. coli* or GBS colonization within the genital tract from our clinical cohort. The lack of this information precluded our ability to correlate the *E. coli* and GBS inhibition with colonization status.^{16,17} The use of two different methodologies in conducting the antibacterial assays may be a limitation. A colony-forming unit assay was used to assess *E. coli* inhibition and is the optimal method in quantifying bacterial growth. However, due to the opaque nature of growth media for GBS (blood agar) and the tendency for GBS colonies to aggregate together, it made counting individual colonies difficult and inaccurate. We therefore employed a turbidimetric approach but recognize the different methodologies may have impacted our results.

Our data suggest specificity in the antibacterial activities of AMPs and components of the vaginal microbiome against *E. coli* and GBS. To our knowledge, ours is the first study to examine the anti-GBS activity of female genital tract secretions. Future study on the impact of the vaginal microbiome and synthetic antimicrobial peptides on bacteria commonly

associated with preterm birth and neonatal infection could promote the identification of factors that might enhance host defense and improve outcomes for pregnant women and their newborns.

Acknowledgments

We would like to thank Dr. Betsy C. Herold for her mentorship and critical review of the manuscript and Dr. Adam J. Ratner for providing the bacterial spent culture supernatants. This work was funded by Einstein-Montefiore CTSA 1UL1RR025750, KL2RR025749 (J.G.).

References

1. Wira CR, Patel MV, Ghosh M, Mukura L, Fahey JV: Innate immunity in the human female reproductive tract: endocrine regulation of endogenous antimicrobial protection against HIV and other sexually transmitted infections. *Am J Reprod Immunol* 2011; 65:196–211. [PubMed: 21294805]
2. Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L: Innate and adaptive immunity in female genital tract: cellular responses and adaptive immunity in female genital tract: cellular responses and interactions. *Immunol Rev* 2005; 206:306–335. [PubMed: 16048557]
3. Foxman B, Gillespie B, Manning SD, Howard LJ, Tallman P, Zhang L, Marrs CF: Incidence and duration of group B Streptococcus by serotype among male and female college students living in a single dormitory. *Am J Epidemiol* 2006; 163:544–551. [PubMed: 16421237]
4. Donders GG, Vereecken A, Bosmans E, Dekeersmaecker A, Salembier G, Salembier G, Spitz B: Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. *BJOG* 2002; 109:34–43. [PubMed: 11845812]
5. Barcaite E, Bartusevicius A, Tameliene R, Maleckiene L, Vitkauskiene A, Nadisauskiene R: Group B streptococcus and Escherichia coli colonization in pregnant women and neonates in Lithuania. *Int J Gynaecol Obstet* 2012; 117:69–73. [PubMed: 22265188]
6. Kuhn P, Dheu C, Bolender C, Chognot D, Keller L, Demil H, Donato L, Langer B, Messer J, Astruc D: Incidence and distribution of pathogens in early-onset neonatal sepsis in the era of antenatal antibiotics. *Paediatr Perinat Epidemiol* 2010; 24:479–487. [PubMed: 20670228]
7. Stoll BJ, Hansen NI, Sanchez PJ, Faix RG, Poindexter BB, Van Meurs KP, Bizzarro MJ, Goldberg RN, Frantz ID, 3rd, Hale EC, Shankaran S, Kennedy K, Carlo WA, Watterberg KL, Bell EF, 23 Walsh MC, Schibler K, Laptook AR, Shane AL, Schrag SJ, Das A, Higgins RD; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network: Early onset neonatal sepsis: the burden of group B Streptococcal and *E. coli* disease continues. *Pediatrics* 2011; 127:817–826. [PubMed: 21518717]
8. Regan JA, Klebanoff MA, Nugent RP, Eschenbach DA, Blackwelder WC, Lou Y, Gibbs RS, Rettig PJ, Martin DH, Edelman R: Colonization with group B streptococci in pregnancy and adverse outcome. VIP Study Group. *Am J Obstet Gynecol* 1996; 174:1354–1360. [PubMed: 8623869]
9. Krohn MA, Hillier SL, Baker CJ: Maternal peripartum complications associated with vaginal group B streptococci colonization. *J Infect Dis* 1999; 179:1410–1415. [PubMed: 10228062]
10. Kalyoussef S, Nieves E, Dinerman E, Carpenter C, Shankar V, Oh J, Burd B, Angeletti RH, Buckheit KW, Fredricks DN, Madan RP, Keller MJ, Herold BC: Lactobacillus proteins are associated with the bactericidal activity against *E. coli* of female genital tract secretions. *PLoS ONE* 2012; 7:e49506. [PubMed: 23185346]
11. De Smet K, Contreras R: Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol Lett* 2005; 27:1337–1347. [PubMed: 16215847]
12. Pazgier M, Li X, Lu W, Lubkowski J: Human defensins: synthesis and structural properties. *Curr Pharm Des* 2007; 13:3096–3118. [PubMed: 17979752]
13. Wang G: Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. *J Biol Chem* 2008; 283:32637–32643. [PubMed: 18818205]

14. Travis SM, Anderson NN, Forsyth WR, Espiritu C, Conway BD, Greenberg EP, McCray PB, Jr, Lehrer RI, Welsh MJ, Tack BF: Bactericidal activity of mammalian cathelicidin-derived peptides. *Infect Immun* 2000; 68:2748–2755. [PubMed: 10768969]
15. Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI: Defensins. Natural peptide antibiotics of human neutrophils. *J Clin Invest* 1985; 76:1427–1435. [PubMed: 2997278]
16. Gharthey JP, Carpenter C, Gialanella P, Rising C, McAndrew TC, Mhatre M, Tugetman J, Einstein MH, Chazotte C, Herold BC: Association of bactericidal activity of genital tract secretions with *Escherichia coli* colonization in pregnancy. *Am J Obstet Gynecol* 2012; 207:297 e291–e298. [PubMed: 22867687]
17. Valore EV, Park CH, Igreti SL, Ganz T: Antimicrobial components of vaginal fluid. *Am J Obstet Gynecol* 2002; 187:561–568. [PubMed: 12237628]
18. Madan RP, Carpenter C, Fiedler T, Kalyoussef S, McAndrew TC, Viswanathan S, Kim M, Keller MJ, Fredricks DN, Herold BC: Altered biomarkers of mucosal immunity and reduced vaginal *Lactobacillus* concentrations in sexually active female adolescents. *PLoS ONE* 2012; 7:e40415. [PubMed: 22808157]
19. Wei G, de Leeuw E, Pazgier M, Yuan W, Zou G, Wang J, Ericksen B, Lu WY, Lehrer RI, Lu W: Through the looking glass, mechanistic insights from enantiomeric human defensins. *J Biol Chem* 2009; 284:29180–29192. [PubMed: 19640840]
20. Ericksen B, Wu Z, Lu W, Lehrer RI: Antibacterial activity and specificity of the six human {alpha}-defensins. *Antimicrob Agents Chemother* 2005; 49:269–275. [PubMed: 15616305]
21. Gharthey JP, Smith BC, Chen Z, Buckley N, Lo Y, Ratner AJ, Herold BC, Burk RD: *Lactobacillus crispatus* dominant vaginal microbiome is associated with inhibitory activity of female genital tract secretions against *Escherichia coli*. *PLoS ONE* 2014; 9:e96659. [PubMed: 24805362]
22. Anderson BL, Mendez-Figueroa H, Dahlke JD, Raker C, Hillier SL, Cu-Uvin S: Pregnancy-induced changes in immune protection of the genital tract: defining normal. *Am J Obstet Gynecol* 2013; 208:321 e321–e329. [PubMed: 23313311]
23. Wu Z, Ericksen B, Tucker K, Lubkowski J, Lu W: Synthesis and characterization of human alpha-defensins 4–6. *J Pept Res* 2004; 64:118–125. [PubMed: 15317502]
24. Dorschner RA, Lin KH, Murakami M, Gallo RL: Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: innate immunity during development of the adaptive response. *Pediatr Res* 2003; 53:566–572. [PubMed: 12612195]
25. Bals R, Wang X, Wu Z, Freeman T, Bafna V, Zasloff M, Wilson JM: Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. *J Clin Invest* 1998; 102:874–880. [PubMed: 9727055]
26. Yu Q, Lehrer RI, Tam JP: Engineered salt-insensitive alphadefensins with end-to-end circularized structures. *J Biol Chem* 2000; 275:3943–3949. [PubMed: 10660548]
27. Boldenow E, Jones S, Lieberman RW, Chames MC, Aronoff DM, Xi C, Loch-Caruso R: Antimicrobial peptide response to group B *Streptococcus* in human extraplacental membranes in culture. *Placenta* 2013; 34:480–485. [PubMed: 23562109]
28. Zaiou M, Nizet V, Gallo RL: Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence. *J Invest Dermatol* 2003; 120:810–816. [PubMed: 12713586]
29. Belec L, Meillet D, Levy M, Georges A, Tevi-Benissan C, Pillot J: Dilution assessment of cervicovaginal secretions obtained by vaginal washing for immunological assays. *Clin Diagn Lab Immunol* 1995; 2:57–61. [PubMed: 7719914]
30. Dezzutti CS, Hendrix CW, Marrazzo JM, Pan Z, Wang L, Louissaint N, Kalyoussef S, Torres NM, Hladik F, Parikh U, Mellors J, Hillier SL, Herold BC: Performance of swabs, lavage, and diluents to quantify biomarkers of female genital tract soluble mucosal mediators. *PLoS ONE* 2011; 6:e23136. [PubMed: 21858008]
31. Wang Y, Walter G, Herting E, Agerberth B, Johansson J: Antibacterial activities of the cathelicidins prophenin (residues 62 to 79) and LL-37 in the presence of a lung surfactant preparation. *Antimicrob Agents Chemother* 2004; 48:2097–2100. [PubMed: 15155206]
32. Scott MG, Hancock RE: Cationic antimicrobial peptides and their multifunctional role in the immune system. *Crit Rev Immunol* 2000; 20:407–431. [PubMed: 11145218]

33. Varney KM, Bonvin AM, Pazgier M, Malin J, Yu W, Ateh E, Oashi T, Lu W, Huang J, de Diepeveen- Buin M, Bryant J, Breukink E, Mackerell AD, Jr, De Leeuw EP: Turning defense into offense: defensin mimetics as novel antibiotics targeting lipid II. *PLoS Pathog* 2013; 9:e1003732. [PubMed: 24244161]
34. Schneider T, Kruse T, Wimmer R, Wiedemann I, Sass V, Pag U, Jansen A, Nielsen AK, Mygind PH, Raventos DS, Neve S, Ravn B, Bonvin AM, De Maria L, Andersen AS, Gammelgaard LK, Sahl HG, Kristensen HH: Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II. *Science* 2010; 328: 1168–1172. [PubMed: 20508130]
35. Giesbrecht P, Kersten T, Maidhof H, Wecke J: Staphylococcal cell wall: morphogenesis and fatal variations in the presence of penicillin. *Microbiol Mol Biol Rev* 1998; 62:1371–1414. [PubMed: 9841676]
36. Beveridge TJ, Graham LL: Surface layers of bacteria. *Microbiol Rev* 1991; 55:684–705. [PubMed: 1723487]
37. Jann K, Jann B: Polysaccharide antigens of *Escherichia coli*. *Rev Infect Dis* 1987; 9(Suppl 5):S517–S526. [PubMed: 2446369]
38. Smeianov V, Scott K, Reid G: Activity of cecropin P1 and FA-LL-37 against urogenital microflora. *Microbes Infect* 2000; 2:773–777. [PubMed: 10955957]
39. Bergman P, Johansson L, Asp V, Plant L, Gudmundsson GH, Jonsson AB, Agerberth B: *Neisseria gonorrhoeae* downregulates expression of the human antimicrobial peptide LL-37. *Cell Microbiol* 2005; 7:1009–1017. [PubMed: 15953032]
40. Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamas V, Piraino J, Huttner K, Gallo RL: Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature* 2001; 414:454–457. [PubMed: 11719807]
41. Reid G, Bruce AW, McGroarty JA, Cheng KJ, Costerton JW: Is there a role for lactobacilli in prevention of urogenital and intestinal infections? *Clin Microbiol Rev* 1990; 3:335–344. [PubMed: 2224835]
42. Carson HJ, Lapoint PG, Monif GR: Interrelationships within the bacterial flora of the female genital tract. *Infect Dis Obstet Gynecol* 1997; 5:303–309. [PubMed: 18476156]
43. Keane FE, Ison CA, Taylor-Robinson D: A longitudinal study of the vaginal flora over a menstrual cycle. *Int J STD AIDS* 1997; 8:489–494. [PubMed: 9259496]

Fig. 1.

Genital tract secretions lack anti- group B Streptococcus (GBS) activity: Twelve cervico-vaginal lavage samples were selected based on their level of inhibitory *E. coli* activity [$n = 8$ high ($>80\%$) activity and $n = 4$ low ($<20\%$) activity]. Anti-GBS activity was determined using a turbidimetric approach. None of the samples demonstrated activity against GBS.

Fig. 2.

HNP-1 and HD-5, but not HBD-2 and LL-37, inhibit group B Streptococcus (GBS) in a dose-dependent manner. Growth curve representing Group B Streptococcus in the presence of HNP-1, HD-5, HBD-2, LL-37, and controls (penicillin/streptomycin and 0.01% acetic acid). GBS was grown in Lim broth in the presence of antimicrobial peptide or control in a 1:1 ratio. The growth of GBS was monitored hourly by measuring the turbidity of the fluid. GBS growth in the presence of HBD-2 or LL-37 at 100 µg/mL was similar to the negative control (0.01% acetic acid). The asterisk (*) represents significant inhibition of GBS by HNP-1 and HD-5 at 20 and 50 µg/mL, respectively, as compared to the negative control (0.01% acetic acid).

Fig. 3.

Synthetic antimicrobial peptides (HNP-1, HD-5, HBD-2, and LL37) have potent activity against *E. coli*; only α -defensins (HNP-1 and HD-5) have anti-GBS activity. Bar graph depicting percent inhibition of *E. coli* (black bars) or GBS (white bars) of synthetic antimicrobial peptides at 50 $\mu\text{g/mL}$ (dissolved in 10 mM sodium phosphate for *E. coli* and 0.01% acetic acid for GBS). HBD-2 and LL-37 displayed significantly more *E. coli* inhibition than GBS (single asterisk represents $P < 0.05$; double asterisks represent $P < 0.01$, triple asterisks represent $P < 0.001$) and were found to be >95% inhibitory against *E. coli*. Also, HNP-1 and HD-5 showed 65 and 80% *E. coli* inhibition, respectively. Only HNP-1 and HD-5 demonstrated 84 and 78% GBS inhibition, respectively.

Fig. 4.

Salt sensitivity of antimicrobial peptides (AMPs) is likely bacterium-specific: Bar graph depicting percent inhibition of group B Streptococcus (GBS) (a) or *E. coli* (b) by synthetic AMPs at 25 µg/mL (a) or 50 µg/mL (b) dissolved in routine diluent (0.01% acetic acid for GBS experiments and 10 mM sodium phosphate for *E. coli* experiments) or 154 mM NaCl. Activity of HNP-1 and HD-5 against GBS is resistant to the effect of salt (a), whereas *E. coli* inhibitory activity of HNP-1, HD-5, and HBD-2 was significantly reduced in the presence of 154 mM NaCl (b) (single asterisk represents $P < 0.05$; double asterisks represent $P < 0.01$).

Fig. 5. Clinical samples gain anti- group B Streptococcus (GBS) activity with the supplementation of HNP-1 (a) and HD-5 (b): Anti-GBS activity was performed on two cervico-vaginal lavage (CVL) samples alone, on HNP-1 alone (a) or HD-5 alone (b), and on CVL samples + HNP-1 (a) or CVL samples + HD-5 (b). The samples displayed a significant and dosedependent additive increase in the inhibitory activity with the addition of HNP-1 (a) and HD-5 (b). The asterisks represent a significant increase (double asterisks represent $P < 0.01$, triple asterisks represent $P < 0.001$) in GBS inhibition for S1+ HNP-1 at 10 and 20 µg/mL as compared to S1 alone (a) and S1+ HD-5 at 25 µg/mL, S2+ HD-5 at 25 µg/mL, and S2+ HD-5 at 50 µg/mL (b) as compared to S1 or S2 alone.

Fig. 6.

Spent culture supernatants of *L. crispatus*, *L. iners*, and *G. vaginalis* have activity against *E. coli* but do not inhibit group B Streptococcus (GBS): Bar graph representing percent inhibition of *E. coli* (black bars) and GBS (white bars) of (SCS) of *L. crispatus*, 3 strains of *L. iners*, and two strains of *G. vaginalis*. Inhibitory *E. coli* activity was determined using a colony-forming unit assay. Anti-GBS activity was determined using a turbidimetric approach. Results are mean $\pm$ S.D. from two experiments conducted in duplicate. Only lactobacilli SCS demonstrated significantly greater inhibition against *E. coli* than GBS (double asterisks represent $P < 0.01$; triple asterisks represent $P < 0.001$).

Table I

Demographic and Clinical Characteristics of Healthy, Non-Pregnant Women

Characteristic	Total cohort (<i>n</i> = 12)
Mean age, years (S.D.)	32 (6.9)
Race, <i>n</i> (%)	
White	4 (33.3)
Black	1 (8.3)
Asian	2 (16.7)
Unknown	5 (41.7)
Current smoker, <i>n</i> (%)	2 (16.6)
History in past month	
Sexually transmitted infection	0
Urinary tract infection	0
History of genital wart, <i>n</i>	0
Contraception, <i>n</i> (%)	
Oral contraceptive pills	6 (50)
Intrauterine device	3 (25)
NuvaRing	1 (8.3)
None	2 (16.6)
History of tampon use, <i>n</i> (%)	7 (58.3)
History of chronic illness, diabetes, systemic lupus erythematosus, <i>n</i>	0
Lateral vaginal wall pH, mean (S.D.)	4.5 (0.3)
Current sexual partners, mean (S.D.)	1 (0)
Number sexual partners in past year, mean (S.D.)	1 (0)