GYNECOLOGY

Impact of contraceptive initiation on vaginal microbiota

Sharon L. Achilles, MD, PhD; Michele N. Austin, BS; Leslie A. Meyn, PhD; Felix Mhlanga, MD; Zvavahera M. Chirenje, MD; Sharon L. Hillier, PhD

BACKGROUND: Data evaluating the impact of contraceptives on the vaginal microbiome are limited and inconsistent.

OBJECTIVE: We hypothesized that women initiating copper intrauterine device use would have increased bacterial vaginosis and bacterial vaginosis-associated microbes with use compared to women initiating and using hormonal contraceptive methods.

STUDY DESIGN: Vaginal swabs (N = 1047 from 266 participants seeking contraception) for Nugent score determination of bacterial vaginosis and quantitative polymerase chain reaction analyses for assessment of specific microbiota were collected from asymptomatic, healthy women aged 18-35 years in Harare, Zimbabwe, who were confirmed to be free of nonstudy hormones by mass spectrometry at each visit. Contraception was initiated with an injectable (depot medroxyprogesterone acetate [n = 41], norethisterone enanthate [n = 44], or medroxyprogesterone acetate and ethinyl estradiol [n = 40]), implant (levonorgestrel [n = 45] or etonogestrel [n = 48]), or copper intrauterine device (n = 48) and repeat vaginal swabs were collected after 30, 90, and 180 days of continuous use. Self-reported condom use was similar across all arms at baseline. Quantitative polymerase chain reaction was used to detect *Lactobacillus crispatus*, *L jensenii*, *L gasseri/johnsonii* group, *L vaginalis*, *L iners*, *Gardnerella vaginalis*, *Atopobium vaginae*, and *Megasphaera*-like bacterium phylotype I from swabs. Modified Poisson regression and mixed effects linear models were used to compare marginal prevalence and mean difference in quantity (expressed as gene copies/swab) prior to and during contraceptive use.

RESULTS: Bacterial vaginosis prevalence increased in women initiating copper intrauterine devices from 27% at baseline, 35% at 30 days, 40% at 90 days, and 49% at 180 days ($P = .005$ compared to marginal prevalence at enrollment). Women initiating hormonal methods had no change in bacterial vaginosis prevalence over 180 days. The mean increase in Nugent score was 1.2 (95% confidence interval, 0.5−2.0; $P = .001$) in women using copper intrauterine devices. Although the frequency and density of beneficial lactobacilli did not change among intrauterine device users over 6 months, there was an increase in the log concentration of *G vaginalis* (4.7, 5.2, 5.8, 5.9; $P = .046$) and *A vaginae* (3.0, 3.8, 4.6, 5.1; $P = .002$) between baseline and 30, 90, and 180 days after initiation. Among other contraceptive groups, women using depot medroxyprogesterone acetate had decreased *L iners* (mean decrease log concentration = 0.8; 95% confidence interval, 0.3−1.5; $P = .004$) and there were no significant changes in beneficial *Lactobacillus* species over 180 days regardless of contraceptive method used.

CONCLUSION: Copper intrauterine device use may increase colonization by bacterial vaginosis−associated microbiota, resulting in increased prevalence of bacterial vaginosis. Use of most hormonal contraception does not alter vaginal microbiota.

Key words: bacterial vaginosis, hormonal contraception, intrauterine device, lactobacilli, vaginal microbiota

Introduction

Reproductive-aged women commonly use and frequently change contraceptive methods. Understanding the impact of contraceptive initiation and use on vaginal microbiota is important since perturbations often cause distressing symptoms and bacterial vaginosis (BV) has been associated with increased risk of sexually transmitted infections,[1-3] including HIV.[4-6] Systematic review of studies evaluating the risk of HIV acquisition and contraceptive use suggests that depot medroxyprogesterone acetate (DMPA) may increase the risk of HIV acquisition.[7] The possibility that contraceptive use may alter HIV susceptibility warrants further investigation of potential mechanisms, including understanding the impact on the vaginal microbiota.[8]

BV is associated with shifts in vaginal microbiota, characterized by a change in dominant bacterial species from *Lactobacillus*-predominant to a mixture of anaerobic species.[9-11] Women having a normal healthy pregnancy have lactobacilli as predominant members of the vaginal microbiome,[12] and nonpregnant women having *Lactobacillus*-dominant microbiota have reduced susceptibility to HIV and other sexually transmitted infections.[13,14] BV as assessed by Nugent criteria is common in reproductive-aged women, with an overall prevalence of 29% in healthy US women.[15] The impact of contraceptives on the vaginal microbiota and BV has been evaluated in several cross-sectional and longitudinal studies with inconsistent results. In these studies, women using oral contraceptives have generally been shown to have decreased risk of BV,[16] while women using intrauterine devices (IUDs) have had inconsistent associations with prevalent BV.[17,18] In cross-sectional studies that include evaluation of the vaginal microbiome, women using DMPA or oral contraceptives were reportedly less likely to be colonized by BV-associated microbiota, while women using levonorgestrel (LNG)-releasing intrauterine systems (IUS) had a trend toward more BV-associated microbiota.[19] There are fewer published longitudinal studies assessing the impact of contraceptives on vaginal microbiota, but those that have been published suggest that women using copper IUDs may have a modest increased risk of BV[20] and women using

Cite this article as: Achilles SL, Austin MN, Meyn LA, et al. Impact of contraceptive initiation on vaginal microbiota. Am J Obstet Gynecol 2018;volume:x.ex-x.ex.

0002-9378

https://doi.org/10.1016/j.ajog.2018.02.017

AJOG at a Glance

Why was this study conducted?
This study was conducted to evaluate the impact of contraceptive use on the vaginal microbiome of Zimbabwean women.

Key findings
Key findings include that hormonal contraceptive use did not alter vaginal microbiota over 6 months, while copper intrauterine device use was associated with increased bacterial vaginosis and associated microbiota, including *Gardnerella vaginalis* and *Atopobium vaginae*.

What does this add to what is known?
These data from a population of African women contribute to the body of evidence from the United States suggesting women using copper intrauterine devices are more likely to have changes in the vaginal microbiome including an increase in asymptomatic and symptomatic bacterial vaginosis.

the LNG-IUS had no increased risk of BV[21] and no changes in the microbiome consistent with BV.[22,23]

Our objective was to evaluate changes in prevalent BV and selected vaginal microbiota after initiation and use over 6 months of 6 contraceptive methods, including 3 hormonal injectables, 2 hormonal implants, and the copper IUD. We hypothesized that women initiating and using copper IUD would have increased BV and BV-associated microbiota compared to women initiating and using hormonal contraceptive methods.

Materials and Methods

Study population and sample collection

We performed a parallel longitudinal cohort study (ClinicalTrials.gov no: NCT02038335) of women initiating contraception with injectable (DMPA, norethisterone enanthate [Net-En], or medroxyprogesterone acetate and ethinyl estradiol [MPA/EE], implant (LNG or etonogestrel [ENG] subdermal implant), or intrauterine (copper T380A IUD [Cu-IUD] contraceptives. The primary objective was to assess the impact of initiation and continued use of contraceptives on HIV target cells in the lower genital tract at 1, 3, and 6 months of use and here we report on the secondary objective to assess the impact of contraceptive initiation and use on vaginal microbiota. The study was designed to assess changes compared to baseline with each woman serving as her own control; therefore, being free of exogenous steroid hormones at baseline and in a uniform phase of menses was central to the study design. Given the critical importance of the baseline values, laboratory confirmation by ultra-high-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) was performed to evaluate serum progesterone, LNG, ENG, norethindrone, and MPA concentrations, which covered the full spectrum of regionally available contraceptive progestins at the time this study was conducted. Baseline sampling was performed at the enrollment visit when all enrolled women were free of hormonal or intrauterine contraceptive use for the preceding 30 days and free of DMPA use for the preceding 10 months by self-report. All samples from participants found to have exogenous synthetic progestin blood levels contradictory to self-reported nonuse were retested to confirm biological results and to rule out contamination during sample processing. All retesting confirmed original results and the participants were disqualified from the study.

We calculated a sample size of 37 women in each group would be needed to have 80% power to detect a 1-log change in microbial densities, based on a paired samples *t* test and a common SD of the microbial density difference of 2.1 observed in a prior study.[24] To account for loss to follow-up, we planned a sample size of 40 women per contraceptive group.

The University of Pittsburgh Institutional Review Board and the Medical Research Council of Zimbabwe approved this study. All participants were enrolled at Spilhaus Family Planning Center in Harare, Zimbabwe, and signed informed consent before study participation.

The study population consisted of 451 women, age 18-34 years, seeking contraception in Harare, Zimbabwe. Eligible women were healthy, HIV negative, nonpregnant, and had regular menstrual cycles. Women were excluded if within 30 days of enrollment they: (1) used any hormonal or intrauterine contraceptive; (2) underwent any genital tract procedure (including biopsy); (3) were diagnosed with any urogenital tract infection; or (4) used any oral or vaginal antibiotics, oral or vaginal steroids, or any vaginal product or device except tampons and condoms (eg, spermicide, microbicide, douche, sex toys, and diaphragms). Women were also excluded if by self-report they used DMPA within 10 months of enrollment, were pregnant or breast-feeding within 60 days of enrollment, or had a new sexual partner within 90 days of enrollment. Exclusion criteria included having a contraindication, allergy, or intolerance to use of the contraceptive desired by the participant and having a prior hysterectomy or malignancy of the cervix or uterus.

Screening included urine pregnancy testing, 2 rapid HIV screening tests to rule out HIV infection, and collection of genital tract swabs for detection of *Neisseria gonorrhoeae*, *Chlamydia trachomatis* (ProbeTec; Becton Dickenson, Sparks, MD, or GeneXpert; Cepheid, Sunnyvale, CA), and *Trichomonas vaginalis* (OSOM; Sekisui Diagnostics, Lexington, MA).

Eligible participants presented for enrollment on a day when no vaginal bleeding was present and when they were in the follicular phase of menses (day 1-14) by self-reported last menstrual period. Participants were asked to refrain from any vaginal or anal intercourse for 48 hours prior to sample collection at