GYNECOLOGY

Colonization of the upper genital tract by vaginal bacterial species in nonpregnant women

Caroline M. Mitchell, MD, MPH; Anoria Haick, MS; Evangelyn Nkwopara, BA; Rochelle Garcia, MD; Mara Rendi, MD, PhD; Kathy Agnew, BA; David N. Fredricks, MD; David Eschenbach, MD

OBJECTIVE: The objective of the study was to evaluate the upper genital tract (UGT) presence of vaginal bacterial species using sensitive molecular methods capable of detecting fastidious bacterial vaginosis (BV)—associated bacteria.

STUDY DESIGN: Vaginal swabs were collected prior to hysterectomy. The excised uterus was sterilely opened and swabs collected from the endometrium and upper endocervix. DNA was tested in 11 quantitative polymerase chain reaction (PCR) assays for 12 bacterial species: *Lactobacillus iners*, *L crispatus*, *L jensenii*, *Gardnerella vaginalis*, *Atopobium vaginae*, *Megasphaera* spp, *Prevotella* spp, *Leptotrichia/Sneathia*, BVAB1, BVAB2, BVAB3, and a broad-range16S ribosomal ribonucleic acid gene assay. Endometrial fluid was tested with Luminex and an enzyme-linked immunosorbent assay for cytokines and defensins and tissue for gene expression of defensins and cathelicidin.

RESULTS: We enrolled 58 women: mean aged 43 ± 7 years, mostly white (n = 46; 79%) and BV negative (n = 43; 74%). By species-specific quantitative PCR, 55 (95%) had UGT colonization with at least 1 species (n = 52) or were positive by 16S PCR (n = 3). The most common species were *L iners* (45% UGT, 61% vagina), *Prevotella* spp (33% UGT, 76% vagina) and *L crispatus* (33% UGT, 56% vagina). Median quantities of bacteria in the UGT were lower than vaginal levels by 2-4 $\log_{10}$ ribosomal ribonucleic acid gene copies per swab. There were no differences in the endometrial inflammatory markers between women with no bacteria, *Lactobacillus* only, or any BV-associated species in the UGT.

CONCLUSION: Our data suggest that the endometrial cavity is not sterile in most women undergoing hysterectomy and that the presence of low levels of bacteria in the uterus is not associated with significant inflammation.

Key words: endometritis, endometrium, intrauterine bacteria, reproductive tract microbiota, sterile, upper genital tract infection, uterine cavity

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Bacterial colonization of the uterus is associated with adverse reproductive health outcomes, including preterm delivery and chorioamnionitis,[1] pelvic inflammatory disease and endometritis,[2,3] and miscarriage.[4] Upper genital tract infection has been presumed to be due to the pathological ascent of vaginal bacteria in to the upper genital tract. The physical barrier of cervical mucous, its high concentrations of antimicrobial peptides and inflammatory cytokines,[5-9] and possibly immunoglobulins[10] or matrix-degrading enzymes[11] in the mucous plug is thought to provide a defense against bacterial ascent, and the uterine cavity of healthy women has long been considered sterile.

From the Departments of Obstetrics and Gynecology (Drs Mitchell and Eschenbach, Ms Haick, Ms Nkwopara, and Ms Agnew) and Pathology (Drs Garcia and Rendi), University of Washington School of Medicine, and Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center (Dr Fredricks), Seattle, WA.

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Dr Mitchell is now with the Vincent Center for Reproductive Biology, Massachusetts General Hospital, Boston, MA.

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Corresponding author: Caroline M. Mitchell, MD, MPH. caroline.mitchell@mgh.harvard.edu

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However, radioactively labeled albumin spheres placed in the vagina ascend into the uterus as early as 2 minutes after instillation,[12] suggesting that fluid and particles move between the vagina and uterus relatively freely. Studies of ostensibly healthy women report a variable rate of uterine bacterial colonization by culture, ranging from 0% to 82%.[13-22] This wide range is due in part to the differences in sample collection: studies using hysterectomy or transfundal sampling had lower rates (0-24%)[13-16,22] compared with those using transcervical sampling (33-82%).[17,18,21]

Many studies using molecular characterization of the microbiota have demonstrated the ubiquitous presence of bacteria throughout the body and their influence on health.[23,24] We hypothesized that bacterial colonization of the upper genital tract may be quite common and not pathological in many cases. We undertook this study to assess the prevalence and concentrations of bacteria in the upper genital tract (UGT) using sensitive molecular methods in sterilely sampled hysterectomy specimens. Additionally, we measured the endometrial immune response to determine whether intrauterine bacterial colonization was associated with epithelial inflammation, which could suggest an adverse effect of the bacteria.

Materials and Methods

Study cohort and sample collection

Women undergoing hysterectomy for noncancer indications were eligible. Exclusion criteria included presence of an intrauterine device, use of antibiotics, endometrial biopsy, intrauterine device removal or hysteroscopy in the past 30 days, or concern for cervical or endometrial neoplasia. Total laparoscopic or laparoscopically assisted vaginal hysterectomy specimens were collected only if the surgeon was able to complete the procedure using a noninvasive vaginal fornix delineator (Colpo-Probe; Cooper Surgical, Trumbull, CT) or a vaginal sponge stick rather than an intracervical manipulator.

The University of Washington Human Subjects Division approved the study. All subjects signed informed consent. All patients received standard preoperative antibiotic prophylaxis at least 30 minutes prior to surgery.

Prior to vaginal examinations or preparation, flocked swabs (Copan Diagnostics Inc, Murrieta, CA) were inserted 3-4 cm into the vagina for 5 seconds. One was smeared on a glass slide for Gram stain and Nugent scoring.[25] The uterus was removed, wrapped in a sterile towel, taken to pathology without fixation, and incised sagitally under sterile conditions, beginning at the fundus. Swabs were collected first from the endometrium and then from the upper endocervix by rolling the swab 2-3 times across the epithelium and frozen at −80°C.

In a subset of participants (n = 30, 52%), swabs were collected in the Port-A-Cul anaerobic system (Beckton, Dickinson and Co; Franklin Lakes, NJ) and cultured in standard fashion, including selective broth to allow growth of mycoplasma species and isolates identified by routine biochemical methods. Tissue sections were collected from the endometrium contralateral to the swab collection, cut into 1 × 1 cm blocks, placed in RNALater (Life Technologies, Grand Island, NY) at 4°C for 24 hours, and then placed at −80°C.

Bacterial polymerase chain reaction assays

Frozen swabs were thawed and 400 μL of phosphate-buffered saline added, mixed by a vortex shaker for 1 minute, then the swab removed, and the sample spun at 17,000 × *g* for 10 minutes (all at 4 degrees). The pellet underwent DNA extraction with the MoBio Bacteremia DNA isolation kit (MoBio, Carlsbad, CA), whereas the supernatant was aliquoted and frozen for Luminex analysis. DNA underwent taxon-directed 16S ribosomal ribonucleic acid (rRNA) gene TaqMan format quantitative polymerase chain reaction (qPCR) assays for the following bacterial species: *Lactobacillus crispatus*, *L jensenii*, *L iners*, *Gardnerella vaginalis*, *Atopobium vaginae*, *Megasphaera* genus, *Prevotella* genus, bacterial vaginosis–associated bacterium (BVAB)-1, BVAB2, BVAB3, and an assay detecting two closely related bacteria (*Leptotrichia* and *Sneathia*).[26,27]

For the *Prevotella* genus assay, the forward primer 384F (5′- GC CTG AAC CAG CCA AGT A-3′), reverse primer 513R (5′- GGA ATT AGC CGG TCC TTA TT-3′), and a taxon-specific probe (6FAM-GTG CAG GAI GAC GGC C-MGBNFQ) were used. The thermocycler program (ABI 7500 Thermocycler; Applied Biosystems, Foster City, CA) was 2 minutes at 50°C, 10 minutes at 95°C, and then 45 cycles of 15 seconds at 95°C, 39 seconds at 59°C, and 30 seconds at 72°C. UGT swabs were also tested using a broad-range 16S rRNA gene assay to assess for the presence of any bacteria. Limits of detection for the assays were as follows: *L crispatus* 75 gene copies/swab, *L jensenii* 125 gene copies/swab, all other species-specific assays 150 gene copies/swab, and broad-range 16S 6400 gene copies/swab.[28] Negative assays were assigned a value of half the lower limit of detection for that assay.

Measurement of cytokines, chemokines, and antimicrobial peptides

Supernatant from endometrial swabs was submitted for Luminex analysis (Luminex Corp, Austin, TX). Seven of the 14 analytes (interleukin [IL]-4, IL-10, IL-17, interferon-γ, interferon-α, tumor necrosis factor-α, macrophage inflammatory protein-1α) were undetectable in more than 95% of the samples and were not included in the final analysis. An enzyme-linked immunosorbent assay for human beta defensin (HBD)-2, HBD3 (Alpha Diagnostics International, San Antonio, TX) and human alpha defensins 1-3 (Hycult Biotech, Plymouth Meeting, PA) was performed. Homogenized endometrial tissue sections underwent ribonucleic acid (RNA) extraction using the RNEasy fibrous tissue kit (QIAGEN Inc, Valencia, CA). RNA was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Waltham, MA) and amplified using primers and probes from Applied Biosystems for HBD2, HBD3, cathelicidin, and IL-1β as well as the housekeeping gene β-actin.