ORIGINAL ARTICLE: REPRODUCTIVE SCIENCE

Investigation of the microbiota of the reproductive tract in women undergoing a total hysterectomy and bilateral salpingo-oopherectomy

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Objective: To conduct a pilot study to investigate the possible presence of bacteria throughout the female reproductive tract and to make a preliminary assessment of whether there are differences in the composition of the microbial communities between these body sites and/or between patients.
Design: Prospective pilot study followed by 16S amplification and high-throughput sequencing.
Setting: Tertiary care military hospital.
Patient(s): A total of 10 women underwent a total hysterectomy with bilateral salpingo-oopherectomy; tissue samples were collected from the vagina, resected cervix, uterus, fallopian tubes, and ovaries.
Intervention(s): None.
Main Outcome Measure(s): Microbial composition of samples within patients and between body sites.
Result(s): The microbial composition of each sample was characterized by amplification and sequencing of the V1-V3 region of the 16S rRNA gene. Bacteria were identified in 95% of the samples; the remaining 5% of samples showed no evidence of bacterial 16S rRNA. The microbial communities present at each anatomical location were highly related across the samples and across the patients. The Firmicutes phylum was highly abundant as was the *Lactobacillus* genus.
Conclusion(s): This study is the first global evaluation of the distribution of bacteria throughout the female reproductive tract in its entirety. Bacteria were detected by 16S sequencing from anatomical sites including the fallopian tubes and ovaries. The microbial profiles were closely related regardless of which body site or patient the samples originated from. The results of this trial will serve as the basis for future work correlating the colonization of the female reproductive tract with both obstetric and gynecologic conditions. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Reproductive tract microbiota, upper genital tract colonization, ovarian microbiota, salpingitis, fallopian tube microbiota

Microbiota characterization is used to better understand the effect of normal flora on various disease states (1–5), and advances in high-throughput sequencing have allowed for unprecedented analysis of the microbiota present at various body sites. Despite extensive characterization of much of the human body, the microbiota of the female upper reproductive tract, including the uterus, fallopian tubes, and ovaries, has not been thoroughly investigated. This is in contrast to the cervix and vaginal microbiotas, which have been characterized and shown to have different microbial communities that fluctuate over time (6–12). Furthermore, the vaginal microbiota has been shown to be dynamic in its composition during IVF ET (13) and pregnancy (14). Thus, it is possible that similar fluctuations in the microbial communities exist elsewhere in the female upper reproductive tract. This is particularly true given that the vaginal microbiome may serve as a source for seeding more distant sites. A recent study showed that in some cases, bacteria detected from postsurgical endometrial specimens were similar to the patient's vaginal flora; however, it should be noted that

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in several subjects the bacteria noted in the endometrium were not detected in the vaginal samples (15). Thus, further work is needed to define the extent to which the vaginal microbiome serves as the source of microbial spread to the upper reproductive tract.

Characterization of the microbiota of the upper reproductive tract is important to understand whether there are any factors that may be associated with various upper reproductive tract diseases. Practice changes, such as opportunistic salpingectomy (16) as a means to reduce ovarian cancer, may ultimately be related to the microorganisms that are present within the fallopian tubes, which may induce changes in the epithelia. Similarly, understanding the structure of these microbial communities may create opportunities for alternative treatments for patients who would normally undergo salpingectomy for hydrosalpinx as a means to improve pregnancy rates with IVF (17–19). The presence of microorganisms in the ovaries may also play a role in follicle development, polycystic ovarian syndrome, the climacteric experiences of women, premature ovarian failure, and so on. Exploratory studies of these upper reproductive tract sites may be key to understanding many of these gynecologic and obstetric conditions.

To investigate the possible presence of bacteria throughout the female reproductive tract in its entirety and to make a preliminary assessment of whether there are differences in the composition of the microbial communities between these body sites and/or between patients, we proposed a pilot study to examine the composition of the microbial communities throughout the vagina, cervix, uterus, fallopian tubes, and ovaries within and between individuals. High-throughput sequencing techniques are able to identify microorganisms that are in low abundance and/or uncultivable. Because previous microbiota studies have shown that 16S RNA gene libraries can be used to reproducibly determine bacterial presence and phylogeny (20, 21), we chose to isolate total DNA from the various body sites. These samples were then used to amplify by polymerase chain reaction (PCR) the V1-V3 region of the bacterial 16S rRNA gene, which was then subjected to high-throughput sequencing. Specimens from 10 women undergoing total hysterectomy and bilateral salpingo-oopherectomy were sampled. Our hypothesis was that we would observe the presence of bacteria throughout the female reproductive tract (i.e., vagina, endocervix, endometrium, fallopian tubes, and ovaries). This pilot study was undertaken with the understanding that if this hypothesis was proven correct, further studies would need to be conducted to determine whether bacterial prevalence is associated with reproductive health and potentially pathologic conditions.

MATERIALS AND METHODS

This was a prospective pilot study of women undergoing a total hysterectomy and bilateral salpingo-oopherectomy from September to November 2013 at Walter Reed National Military Medical Center (WRNMMC). This study was approved by the Institutional Review Board of WRNMMC (IRB no. 387884-2). A total of 10 women were recruited from the gynecology and gynecologic oncology divisions at our institution and were enrolled in the study. Informed consent was obtained from each woman. Participants with any of the following criteria were excluded: <18 years of age, positive HIV status or other immunosuppressive condition, pregnancy, evidence of active opportunistic infections or immune deficiencies leading to active opportunistic infections, documentation of pelvic inflammatory disease within the preceding 6 months, antibiotic treatment within 30 days, and diagnosed sexually transmitted infections including known treatment of chlamydia in the preceding 6 months. Consistent with current practice guidelines, all patients were treated with antibiotics within 30 minutes before skin incision. Patients received cefazolin unless they were penicillin allergic, in which case they received levofloxacin and metronidazole (patient no. 4). The average time from incision to surgical specimen removal was 294 minutes (range, 88–431 minutes).

From each patient, samples were taken from the vagina, cervix, endometrium, fallopian tube, myometrium, and ovary. All samples were collected after administration of antibiotics as described above. Additional samples were collected from any gross pathology (i.e., hydrosalpinx, fibroid, cyst, endometrial implants, and suspected malignancy). Vaginal samples were collected with dry, sterile, cotton-tipped swabs under anesthesia before the vaginal preparation for surgery in the operating room; samples were immediately frozen at –80°C. For sampling, a sterile speculum was inserted, and then swabs were inserted 3–4 cm into the vagina and swabbed as follows for approximately 5 seconds: swabs were applied along the vaginal wall and then withdrawn while applying to the posterior wall of the vagina with removal of the speculum to the hymenal ring. The cervix, uterus, fallopian tubes, and ovaries were then removed en bloc under sterile conditions, wrapped in a sterile towel, and taken to pathology without fixation.

Fimbriae, endometrial implants, and other external areas of interest were sampled before both chlorhexidine wipe of the surgical specimen and sagittal incision under sterile conditions beginning at the fundus. Sagittal incision of the ovaries and tubes was also performed. Cervical specimens were collected by rolling the cotton-tipped swab 3–4 times across the upper endocervical epithelium; samples were frozen at –80°C. Tubal specimens were collected from the ampullary region of the transected fallopian tubes. Swabs were then collected from the endometrium, ovaries, and other areas of internal interest and frozen at –80°C. The samples were transferred to Research and Testing Laboratory, Inc., of Lubbock, TX, where genomic DNA was isolated from patient tissue and the bacterial 16S ribosomal RNA V1-V3 genomic DNA regions were amplified and then sequenced using 454 sequencing technology.

Samples were prepared for 454 pyrosequencing following standard techniques. Briefly, the forward primer was constructed with (5′-3′) the Roche A linker (CCATCTCATC CCTGCGTGTCTCCGACTCAG), an 8–10 bp barcode, and the GAGTTTGATCNTGGCTCAG 28F primer. The reverse primer was constructed with (5′-3′) the Roche B linker