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Colonization of the upper genital tract by vaginal bacterial species in non-pregnant women

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1 Title: Colonization of the upper genital tract by vaginal bacterial species in non-pregnant
2 women
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7

8 This study was conducted in Seattle, WA.
9

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13 Massachusetts General Hospital
14

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16

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19

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47 **Condensation:** Ninety-five percent of women undergoing hysterectomy had bacteria
48 detected in the upper genital tract using molecular diagnostic tests.
49 **Running title**: Intrauterine bacterial colonization
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70 **Objective:** Evaluate upper genital tract (UGT) presence of vaginal bacterial species
71 using sensitive molecular methods capable of detecting fastidious bacterial vaginosis
72 (BV)-associated bacteria.
73
74 **Study Design**: Vaginal swabs were collected prior to hysterectomy. The excised
75 uterus was sterilely opened and swabs collected from endometrium and upper
76 endocervix. DNA was tested in 11 quantitative PCR (qPCR) assays for 12 bacterial
77 species: *Lactobacillus iners, L. crispatus, L. jensenii, Gardnerella vaginalis*, *Atopobium*
78 *vaginae, Megasphaera spp., Prevotella spp., Leptotrichia/Sneathia*, BVAB1, BVAB2,
79 BVAB3 and a broad-range16S rRNA gene assay. Endometrial fluid was tested with
80 Luminex and ELISA for cytokines and defensins, and tissue for gene expression of
81 defensins and cathelicidin.
82
83 **Results**: We enrolled 58 women: mean age 43 $\pm$ 7 years, mostly white (n = 46; 79%)
84 and BV-negative (n = 43; 74%). By species-specific qPCR, 55 (95%) had UGT
85 colonization with at least one species (n = 52), or were positive by 16S PCR (n = 3).
86 The most common species were *L. iners* (45% UGT, 61% vagina), *Prevotella* spp. (33%
87 UGT, 76% vagina) and *L. crispatus* (33% UGT, 56% vagina). Median quantities of
88 bacteria in the UGT were lower than vaginal levels by 2-4 $\log_{10}$ rRNA gene copies/swab.
89 There were no differences in endometrial inflammatory markers between women with
90 no bacteria, *Lactobacillus* only or any BV-associated species in the UGT.
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92 **Conclusion:** Our data suggest that the endometrial cavity is not sterile in most women
93 undergoing hysterectomy, and that the presence of low levels of bacteria in the uterus is
94 not associated with significant inflammation.

95

96 **Key words:** Intrauterine bacteria; endometritis; upper genital tract infection;
97 reproductive tract microbiota; uterine cavity; endometrium; sterile

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115 **Introduction:**

116 Bacterial colonization of the uterus is associated with adverse reproductive health
117 outcomes, including preterm delivery and chorioamnionitis,[1] pelvic inflammatory disease
118 and endometritis[2,3] and miscarriage.[4] Upper genital tract infection has been presumed
119 to be due to pathologic ascent of vaginal bacteria in to the upper genital tract. The
120 physical barrier of cervical mucous, its high concentrations of antimicrobial peptides and
121 inflammatory cytokines,[5-9] and possibly immunoglobulins[10] or matrix degrading enzymes
122 [11] in the mucous plug are thought to provide a defense against bacterial ascent and the
123 uterine cavity of healthy women has long been considered sterile.
124
125 However, radioactively labeled albumin spheres placed in the vagina ascend into the
126 uterus as early as 2 minutes after instillation,[12] suggesting that fluid and particles move
127 between the vagina and uterus relatively freely. Studies of ostensibly healthy women
128 report a variable rate of uterine bacterial colonization by culture, ranging from 0-82%.[13-
129 22] This wide range is due in part to differences in sample collection: studies using
130 hysterectomy or transfundal sampling had lower rates (0-24%) [13-16,22] compared to
131 those using transcervical sampling (33-82%).[17,18,21]
132
133 Many studies using molecular characterization of the microbiota have demonstrated the
134 ubiquitous presence of bacteria throughout the body, and their influence on health. [23,24]
135 We hypothesized that bacterial colonization of the upper genital tract may be quite
136 common and not pathologic in many cases. We undertook this study to assess the
137 prevalence and concentrations of bacteria in the upper genital tract (UGT) using

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138 sensitive molecular methods in sterilely sampled hysterectomy specimens.
139 Additionally, we measured the endometrial immune response to determine whether
140 intrauterine bacterial colonization was associated with epithelial inflammation, which
141 could suggest an adverse effect of the bacteria.
142
143 **Materials & Methods:**
144 *Study cohort and sample collection:* Women undergoing hysterectomy for non-cancer
145 indications were eligible. Exclusion criteria included presence of an IUD, use of
146 antibiotics, endometrial biopsy, IUD removal or hysteroscopy in the past 30 days, or
147 concern for cervical or endometrial neoplasia. Total laparoscopic or laparoscopically-
148 assisted vaginal hysterectomy specimens were only collected if the surgeon was able to
149 complete the procedure using a non-invasive vaginal fornix delineator (Colpo-Probe,
150 Cooper Surgical, Trumbull, CT) or a vaginal sponge stick rather than an intracervical
151 manipulator. The University of Washington Human Subjects Division approved the
152 study. All subjects signed informed consent. All patients received standard pre-
153 operative antibiotic prophylaxis at least 30 minutes prior to surgery.
154
155 Prior to vaginal exams or prep, flocked swabs (Copan Diagnostics Inc., Murrieta, CA)
156 were inserted 3-4cm into the vagina for 5 seconds. One was smeared on a glass slide
157 for Gram stain and Nugent scoring.[25] The uterus was removed, wrapped in a sterile
158 towel, taken to pathology without fixation and incised sagitally under sterile conditions,
159 beginning at the fundus. Swabs were collected first from the endometrium and then
160 from the upper endocervix by rolling the swab 2-3 times across the epithelium and

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161 frozen at -80ºC. In a subset of participants (n = 30, 52%) swabs were collected in the
162 Port-A-Cul anaerobic system (Beckton, Dickinson and Company, Franklin Lakes, NJ),
163 cultured in standard fashion, including selective broth to allow growth of mycoplasma
164 species and isolates identified by routine biochemical methods. Tissue sections were
165 collected from the endometrium contralateral to the swab collection, cut into 1 x 1 cm
166 blocks, placed in RNALater at 4ºC for 24 hours, then placed at -80ºC.
167
168 *Bacterial PCR assays*: Frozen swabs were thawed and 400 uL of PBS added, mixed by
169 vortex shaker for 1 minute, then the swab removed and the sample spun at 17,000 x g
170 for ten minutes (all at 4 degrees). The pellet underwent DNA extraction with the MoBio
171 Bacteremia DNA Isolation Kit (MoBio, Carlsbad, CA), while the supernatant was
172 aliquoted and frozen for Luminex analysis. DNA underwent taxon-directed 16S rRNA
173 gene TaqMan format qPCR assays for the following bacterial species: *Lactobacillus*
174 *crispatus, L. jensenii, L. iners, Gardnerella vaginalis, Atopobium vaginae, Megasphaera*
175 genus*, Prevotella* genus, Bacterial Vaginosis Associated Bacterium 1 (BVAB1), BVAB2,
176 BVAB3 and an assay detecting two closely related bacteria (*Leptotrichia* and
177 *Sneathia*).[26 27] For the *Prevotella* genus assay, the forward primer 384F (5' - GC CTG
178 AAC CAG CCA AGT A – 3'), reverse primer 513R (5' - GGA ATT AGC CGG TCC TTA
179 TT - 3') and a taxon-specific probe (6FAM - GTG CAG GAI GAC GGC C – MGBNFQ)
180 were used. The thermocycler (ABI 7500 Thermocycler, Applied Biosystems, Foster
181 City, CA) program was 2 minutes 50°C, 10 minutes 95°C, and then 45 cycles of 15
182 seconds 95°C, 39 seconds 59°C and 30 seconds 72°C. UGT swabs were also tested
183 using a broad-range 16S rRNA gene assay to assess for the presence of any bacteria.

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184 Limits of detection for the assays were as follows: *L. crispatus* 75 gene copies/swab, *L.*
185 *jensenii* 125 gene copies/ swab, all other species-specific assays 150 gene
186 copies/swab, and broad-range 16S 6,400 gene copies/swab. [28] Negative assays were
187 assigned a value of half the lower limit of detection for that assay.
188
189 *Measurement of cytokines, chemokines and antimicrobial peptides:* Supernatant from
190 endometrial swabs was submitted for Luminex (Luminex Corporation, Austin, TX)
191 analysis. Seven of the 14 analytes (IL4, IL10, IL17, IFN-γ, IFNα, TNFα, MIP1α) were
192 undetectable in over 95% of samples and were not included in the final analysis. ELISA
193 for human beta defensin 2 (HBD2), HBD3 (Alpha Diagnostics International, San
194 Antonio, TX) and human alpha defensins 1-3 (HNP 1-3; Hycult Biotech, Plymouth
195 Meeting, PA) was performed. Homogenized endometrial tissue sections underwent
196 RNA extraction using the RNEasy Fibrous Tissue Kit (Qiagen Inc., Valencia, CA). RNA
197 was reverse transcribed using iScript cDNA synthesis kit (Bio-Rad Laboratories,
198 Waltham, MA) and amplified using primers and probes from Applied Biosystems (Foster
199 City, California) for HBD2, HBD3, cathelicidin (CAMP) and IL1β, as well as the
200 housekeeping gene β-actin.
201
202 *Statistical analysis:* All analysis was performed using Stata v.10. Prevalences were
203 compared between groups using the chi square test. Quantities of bacteria and
204 concentrations of cytokines were not normally distributed, so were compared across
205 groups using Wilcoxon rank-sum or Kruskall Wallis tests.
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207 **Results:**

208 *Cohort:* We enrolled 58 women with mean age of 43 $\pm$ 7 years. Participants were

209 primarily white (n = 46; 79%), with a small proportion of African American (n = 6; 10%)

210 and Hispanic (n = 4; 7%) women (2 declined to answer the question). All underwent

211 hysterectomy for benign disease: primarily bleeding (n = 20; 34%), fibroids (n = 15;

212 26%), pain (n = 17; 29%). Most had a normal Nugent score (n = 43; 74%), while 6

213 (10%) had bacterial vaginosis, 7 (12%) had an intermediate score and 2 (3%) could not

214 be scored. Most (37; 64%) were on no hormonal medications, 5 (9%) were taking oral

215 contraceptives, 13 (22%) were using Lupron, and 2 (3%) were using a different

216 hormonal medication (testosterone, hormone replacement therapy). Eight women

217 (14%) reported being menopausal. Only 37/50 (74%) pre-menopausal women provided

218 information about last menstrual period (LMP). The median number of days since LMP

219 was 28 (IQR 12, 64), and of the 24 women reporting < 40 days since their LMP only 8

220 (33%) were in the first 14 days of their cycle. Most women had never douched (n = 32;

221 55%) or douched more than 1 week prior to surgery (n = 10; 17%), with a minority who

222 had douched within the past week (n = 2; 3%) and 14 (24%) who did not answer the

223 question. Nineteen women (33%) reported sexual intercourse in the week prior to

224 surgery.

225

226 *UGT colonization:* By species-specific qPCR, 55 (95%) of women had UGT colonization

227 (i.e. in the endometrium or upper endocervix) with at least one of the assayed species

228 (n = 52), or were positive by broad range 16S PCR (n = 3). The most commonly

229 detected species in the vagina were *Prevotella* spp. (76%) *L. iners* (61%), and *L.*

230 *crispatus* (56%). These were also the most commonly detected species in the UGT: *L.*
231 *iners* (45%), *Prevotella* spp. (33%) and *L. crispatus* (33%)(Figure 1a). *G. vaginalis, A.*
232 *vaginae* and *L. jensenii,* were detected in the vagina in over 40% of women, but were
233 detected less frequently in the UGT (in 19%, 10% and 20%, of women). Mean
234 quantities of bacteria detected in the UGT were lower than levels in the vagina by 2-4
235 $\log_{10}$ rRNA gene copies (Figure 1b). When detected in the vagina, *A. vaginae* was the
236 least likely species to also be detected in the UGT, while BVAB1 and *L. iners* were the
237 most likely (Figure 1c). The mean vaginal quantity of *L. crispatus* and *G. vaginalis* was
238 significantly higher in women who had UGT colonization with those species: 7.7 ± 1 vs.
239 5.5 ± 2.5 gene copies/swab for *L. crispatus* ($p = 0.006$) and 7.8 ± 1.2 vs. 4.9 ± 1.5 gene
240 copies/swab for *G. vaginalis* ($p < 0.001$). There were no significant differences in
241 vaginal quantity between women with and without UGT colonization with other bacteria
242 (data not shown). The median number of species detected in the UGT was 2 (IQR 1,3;
243 range 0-8), while the median number of species detected in the vagina was 3 (IQR 2,5;
244 range 0-9). There was no correlation between number of species detected in the
245 vagina and the UGT (correlation coefficient 0.21, $p = 0.12$). Of note, in several cases an
246 organism present in the UGT was not present in the vagina (Figure 2).
247
248 As almost all women had at least one species detected in the UGT we were unable to
249 evaluate risk factors for colonization in general. We divided women into those with no
250 bacteria detected in the UGT (n = 3), *Lactobacillus* species only (n = 18; 31%), any non-
251 *Lactobacillus* species (n = 34; 59%), 16S positive only (n = 3). The only demographic
252 difference between these groups was race: UGT colonization with a non-*Lactobacillus*

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253 species was more common in African American women (5/6; 83%) and Hispanic
254 women (3/4; 75%) than white women (25/46; 54%)(p = 0.01). Rates of BV were slightly
255 different between these groups: 17% for African American, 0% for Hispanic and 11% for
256 white women. There was a trend to increasing UGT colonization by non-*Lactobacillus*
257 species with increasing Nugent score: with Nugent score 0-3 the rate was 51% (22/43),
258 score of 4-6 71% (5/7) and score 7-10 83% (5/6) (p = 0.24). However, the six women
259 with the highest levels of non-*Lactobacillus* species detected in the UGT all had a
260 Nugent score < 7. Age, menopausal status, treatment with GnRH agonist, gravidity,
261 parity, douching or sex in the past week were not significantly different between the
262 groups. (data not shown)
263
264 Of the subset of 30 women who also had cultures performed of upper genital tract
265 swabs, 28 (93%) had bacteria detected by qPCR, and 26/30 (87%) had bacteria
266 detected by culture. Both women with negative qPCR results were also negative by
267 culture. The most commonly cultured organisms were *Diphtheroids* (n = 15; 50%),
268 followed by anaerobic gram-positive cocci (12; 40%), *Proprionibacterium* spp. (n = 9;
269 30%) and *Lactobacillus* species (n = 8 species from 5 women; 17%) (Supplementary
270 Table 1).
271
272 *Immune response:* Soluble markers of inflammation were measured from endometrial
273 swabs by Luminex, antimicrobial peptides by ELISA, and gene expression for
274 defensins, cathelicidin and IL1β from tissue RNA and results compared between women
275 with no bacteria, only *Lactobacillus* species or any non-*Lactobacillus* species detected

276 in the upper genital tract. (Figure 3) There were no significant differences in the median
277 values of these markers between groups. However, the lowest quantities of beta-
278 defensin proteins seemed to be samples from women with non-*Lactobacillus* species in
279 the UGT. The one woman with high (> 100,000 gene copies/swab) of *L. iners* in the
280 UGT had relatively high levels of several inflammatory markers – but the lowest levels
281 of gene expression for the beta defensins, cathelicidin and IL1β. When compared
282 between women who had surgery for fibroids, bleeding, pain or other reasons, the only
283 analyte that was significantly different between the groups was IL6: median 6 pg/mL
284 (Interquartile Range (IQR) 1, 28) in women having surgery for fibroids, 21.9 pg/mL (IQR
285 9,154) in women having surgery for bleeding, 32 pg/mL (IQR 14, 323) in women having
286 surgery for pain, and 1 (IQR 1, 7.8). There was no difference in the distribution of
287 women with only *Lactobacillus spp.* in the UGT versus non-*Lactobacillus* species
288 between the surgical indications. (data not shown)
289
290 **Comment**
291 We detected UGT bacteria by PCR in 95% of women undergoing hysterectomy for
292 benign gynecologic conditions. These results confirm the growing consenus that the
293 endometrial cavity is not sterile. However, the quantity of bacteria present in the uterus
294 and high endocervix was significantly lower than that in the vagina, suggesting that
295 either the cervix serves as a partial filter to ascent, or that the endometrial immune
296 response clears bacteria that do ascend, or a combination of both. We found a much
297 higher prevalence of UGT colonization, but less correlation between vaginal and UGT
298 samples than we anticipated. In women with vaginal colonization by a given species,

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299 rates of UGT colonization varied widely, suggesting differences in microbial ability to
300 evade cervical immunity, or greater permissiveness to some species. Many groups
301 have shown that the vaginal microbial community is dynamic.[27,29] Our results suggest
302 that microbes may remain in the UGT after they disappear from the vagina and/or have
303 better growth in the UGT than the vagina.
304
305 Studies using a similar strategy of incising a hysterectomy specimen to collect samples,
306 but using culture to identify bacterial colonization, report rates of intrauterine bacterial
307 colonization ranging from 0% among 10 women from Finland[22] to 31% in a cohort of
308 100 women from England.[16] Studies using transcervical sampling report higher rates of
309 intrauterine bacterial colonization, ranging from 33%[18] to 60%,[17] but the degree of
310 cervical or vaginal contamination of the endometrial specimen is unknown.[30] Our qPCR
311 results from surgically obtained samples suggest an even higher rate of low-level
312 bacterial presence in the upper genital tract than culture-based studies using
313 transcervical sampling. Many of the bacteria identified by qPCR in this study, such as
314 BVAB1-3 and *Leptotrichia/Sneathia*, are fastidious and difficult to culture, which may
315 account for the differences between our data and previous reports. The bacteria we
316 identified by culture from a subset of women include several taxa that have been
317 identified in vaginal communities but were not targeted by our PCR assays:
318 *Corynebacteria (Diptheroids), Proprionibacteria, Ureaplasma*, coagulase-negative
319 *Staphylococcus*, as well as several anaerobic colonies that could represent any number
320 of other common vaginal species. All women in this study received pre-operative
321 antibiotics intravenously, which likely affected our culture results.

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322

323 Surprisingly, we saw few differences in endometrial immune markers between women
324 with and without upper genital tract colonization by BV-associated microbes. This could
325 be due to trauma-induced cytokine release at the time of surgical removal of the tissue,
326 but our median values are similar to reported median values from endometrial aspirates
327 in women with intact uteri undergoing in-vitro fertilization procedures[31] suggesting this is
328 not the case. Alternatively, cytokines may be impacted by hormonal status, the
329 underlying pathology leading to hysterectomy, or by viral or fungal pathogens not
330 measured in our study. Our data suggest that a low quantity of upper genital tract
331 bacterial colonization by common vaginal species does not induce a strong
332 inflammatory stimulus in most cases.

333

334 This is an exploratory analysis with a small sample size, which limits our ability to detect
335 small associations or to perform well-powered subgroup analyses to look at factors
336 associated with UGT colonization for different species. However, it is the first study
337 using molecular methods to assess upper genital tract colonization in non-pregnant
338 women. Our analysis is cross-sectional, which limits our ability to make conclusions
339 about causation or direction of associations. However, opportunities to sterilely collect
340 endometrial samples with minimal risk of contamination from the lower genital tract are
341 becoming scarce, and preclude longitudinal sample collection from the UGT. Changing
342 patterns of surgery mean that fewer hysterectomies are being performed, and many are
343 now performed use minimally invasive techniques where an intracervical manipulation
344 device is used. Intracervical instrumentation could introduce an uncontrolled amount of

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345 endometrial contamination, so these cases were excluded. Another limitation of our
346 study is the use of selective qPCRs, which do not capture the entire microbiota.
347 However, we did not have sufficient concentrations of bacterial DNA to perform broad
348 range bacterial PCR with pyrosequencing. We did obtain additional information using
349 bacterial culture in some cases, but prophylactic antibiotics potentially impact the
350 sensitivity of cultures, and culture is not able to identify fastidious bacterial species.
351 Finally, since these samples were collected from surgical specimens, all participants
352 had pathology and thus the study population may not reflect the conditions present in
353 normal, healthy women.
354
355 Recent advances in our understanding of the human microbiome reveals the important
356 role that microbes play in many facets of human health.[32] The microbiome plays an
357 important role in the immunologic homeostasis of the gut, encouraging proper
358 development of mucosal immunity and preventing excessive inflammation (reviewed in
359 [33]). T-regulatory cells in the gut mucosa maintain a tolerogenic environment and appear
360 to be selected by interactions with commensal gut microbiota.[34] In the uterus, T-
361 regulatory cells are important for implantation of the embryo and early placental
362 development.[35,36] In one study, the presence of hydrogen peroxide producing
363 *Lactobacillus* species on the tip of the embryo transfer catheter for in-vitro fertilization
364 increased the chance of live birth compared to women who did not have these bacteria
365 detected by culture.[37] This finding raises the possibility that the presence of intrauterine
366 commensal bacteria may have a similar role in the selection of uterine T-regulatory cells
367 as commensal gut microbiota do for colonic T-regulatory cells. While many other

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368 factors contribute to a successful pregnancy, it is worth noting that germ-free mice
369 raised in sterile conditions have lower rates of reproductive success after embryo
370 transfer than conventional animals,[38] suggesting a potential role for intrauterine bacteria
371 in successful pregnancy. This potentially critical issue is largely unexplored.
372
373 It is clear that intrauterine bacterial colonization is not always benign, nor positive. In
374 patients undergoing in vitro fertilization cycles, the presence of *Streptococcus viridans*
375 on the embryo transfer catheter tip was associated with decreased chance of live birth
376 compared to women without *S. viridans* detected.[37] Women with preterm birth are more
377 likely to have intra-uterine placental infection than women who deliver at term.
378 (reviewed in [39]) Women with significant inflammatory sequelae in the upper genital tract
379 with pelvic inflammatory disease often have anaerobic Gram-negative rods and mixed
380 communities of bacteria in the uterus and fallopian tubes.[3,40] Pathologic effects of
381 intrauterine bacteria may occur only with particularly virulent strains or species, only
382 with high concentrations of bacteria, or only in the presence of a mixed bacterial
383 community at the endometrial surface.
384
385 In summary, these data indicate that the endometrial cavity is not sterile in most women
386 undergoing hysterectomy for benign indications. Additionally, detection of bacteria in
387 the upper genital tract is not associated with a significant inflammatory immune
388 response. While bacterial concentrations in the endometrium are much lower than that
389 in the vagina, a low-level bacterial presence in the uterus appears common and not
390 pathologic.

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392 developed the *Prevotella* genus assay in Dr. Fredricks' laboratory at the Fred
393 Hutchinson Cancer Research Institute, as well as the surgeons in the Gynecology
394 Division at the University of Washington who facilitated collection of specimens.

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Figure Legends

Figure 1: Distribution of upper genital tract bacterial colonization. A. Proportion of participants with detection of bacteria by species-specific qPCR in the vagina alone, both vagina and upper genital tract (UGT) and UGT alone. B. Comparison of mean quantity of bacteria in the vagina and UGT of women with either vaginal or upper genital tract detection of that species. * denotes comparisons that are significantly different ($p < 0.05$) by t-test. C. Proportion of women with vaginal detection of a given species by qPCR who also had UGT detection of that species.

Figure 2: Comparison of vaginal and upper genital tract detection of bacteria. Detection and quantity of each species in the vagina and UGT for each participant, organized by Nugent score. Each bacterium is represented by a row, and the quantity is represented by a gradient of color, with darker colors representing higher quantities. The color gradients represent grouping of 1-100, 101-10,000, 10,000 – 1,000,000 and > 1,000,000 16S rRNA gene copies/swab. A white space means that the bacterium was not detected in that sample.

Figure 3: Endometrial immune markers. Comparison of markers of the immune response in the upper genital tract between women with no bacteria detected by PCR in the upper genital tract, only *Lactobacillus* species detected, or any non-*Lactobacillus* species detected. Numbers in boxes are multiple of the median, calculated by taking the median value for the whole cohort and dividing the individual group value by that

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number. There were no significant differences between these three groups. Values highlighted in red are higher than the group median, and those in blue are lower than the group median.

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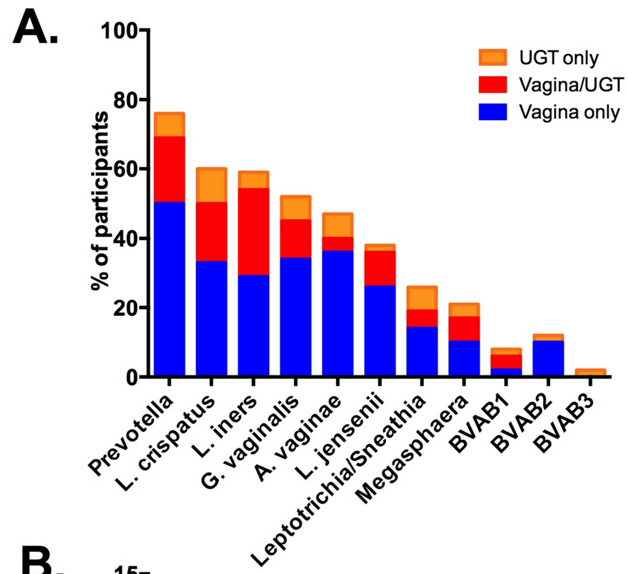

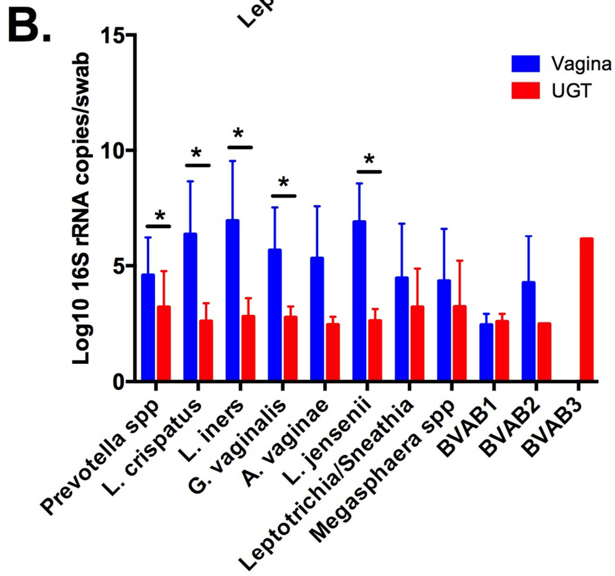

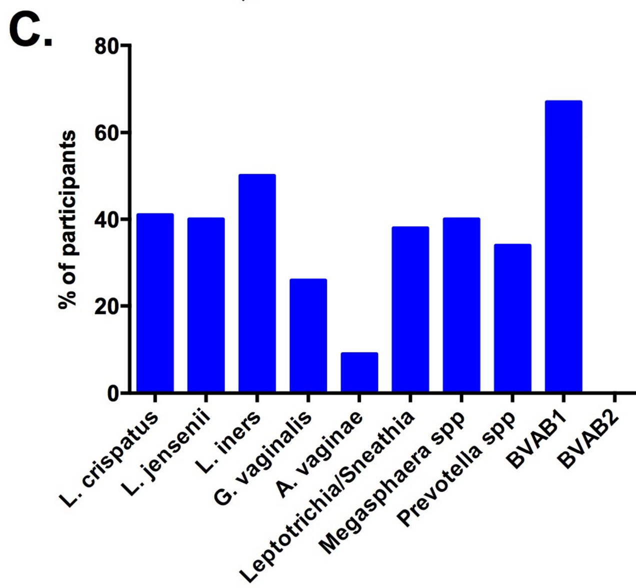

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Supplemental Table 1: Comparison of qPCR and culture results of women who had both assays completed. Unless otherwise noted, all bacteria detected by culture were present at low levels (< 10^2 cfu/swab)

	UGT culture	UGT qPCR
1015	*Diphtheroids*	*L. iners* *Prevotella spp*
1016	--	*L. crispatus* *L. iners* *16S*
1017	Coagulase negative staphylococcus Anaerobic GPC	*L. iners* *Prevotella spp.* *Leptotrichia/Sneathia*
1018	Viridans streptococcus (2 x 10^2), *Lactobacillus spp* Anaerobic GPC	*L. iners* 16S
1026	Coagulase negative staphylococcus *Diphtheroids* *Propionibacterium spp* Anaerobic GNR Anaerobic GPC	*L. crispatus* *Prevotella spp.* *16S*
1028	*Diphtheroids*	*L. iners* *Leptotrichia/Sneathia* 16S
1032	*Propionibacterium spp* Coagulase negative staphylococcus	*L. iners*
1033	*Propionibacterium acnes*	*Prevotella spp* 16S
1034	--	--
1035	Coagulase negative staphylococcus	*L. iners*
1037	--	*L. crispatus* *Prevotella spp* 16S

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1038	*G. vaginalis* (10^5) *Lactobacillus* (2 spp, each 10^3) Anaerobic GPC (10^2)	*G. vaginalis* *Prevotella spp*
1044	Coagulase negative staphylococcus	*L. crispatus* *Leptotrichia/Sneathia* *Prevotella spp.* 16S
1046	Anaerobic GNR	*L. jensenii* *16S*
1049	*Proprionibacterium acnes*	*A.vaginae* *Leptotrichia/Sneathia* *Prevotella spp* BVAB1 16S
1050	*Diphtheroids,* *Propionibacterium acnes*	*L. iners,* *Leptotrichia/Sneathia* *Prevotella spp.*
1053	*Lactobacillus spp* #1(9 x 10^2), *Lactobacillus spp* #2 (10^3), Coagulase negative staphylococcus *Diphtheroids,* *Lactobacillus* spp #3 Anaerobic GPC	*L. jensenii* *L. iners* *G. vaginalis* 16S
1054	--	--
1055	*Lactobacillus spp* (2 x 10^2) Anaerobic GPR (2spp)	*Prevotella*
1056	*Diphtheroids* Aerobic GPC	*L. crispatus,* *Leptotrichia/Sneathia*
1057	*Diphtheroids* (2 types)	*Leptotrichia/Sneathia*
1060	Aerobic GPC *E. coli* Coagulase negative staphylococcus *Diphtheroids*	*L. iners* *Prevotella spp* 16S

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	Anaerobic GPC Anaerobic GNR Anaerobic GNC Anaerobic GPR	
1061	Proteus spp (presumptive mirabilis) Diptheroids	*L. jensenii* *L. iners*
1062	*Diphtheroids* *Propionibacterium spp* Anaerobic GPR	*L. crispatus* *L. jensenii* *L. iners* *G, vaginalis* BVAB2
1064	*Diphtheroids* Coagulase negative staphylococcus Anaerobic GPC (3 isolates)	*G. vaginalis,* *Megasphaera spp* 16S
1066	Coagulase negative staphylococcus (2 isolates) Anaerobic GNR, Anaerobic GPC	*L. crispatus*
1067	Anaerobic GPR *Lactobacillus spp* *Diphtheroids*	*L. crispatus*
1071	Viridans streptococci, *Diphtheroids* Anaerobic GPC Anaerobic GPR	*Prevotella spp.* 16S
1073	*Diphtheroids* *Propionibacterium spp (2 types),* *Propionibacterium acnes* Anaerobic GPC	*G. vaginalis,* *A. vaginae,* *Megasphaera spp* *Leptotrichia/Sneathia* *Prevotella spp* 16S
1075	*Ureaplasma urealyticum*	16S

GPC = Gram positive cocci; GNC = Gram negative cocci; GNR = Gram negative rods; GPR = Gram positive rods

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