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Microbiota and Pelvic Inflammatory Disease

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Abstract

Female genital tract microbiota play a crucial role in maintaining health. Disequilibrium of the microbiota has been associated with increased risk of pelvic infections. In recent years, culture-independent molecular techniques have expanded understanding of the composition of genital microbiota and the dynamic nature of the microbiota. There is evidence that upper genital tract may not be sterile and may harbor microflora in the physiologic state. The isolation of bacterial vaginosis-associated organisms in women with genital infections establishes a link between pelvic infections and abnormal vaginal flora. With the understanding of the composition of the microbiota in healthy and diseased states, the next logical step is to identify the function of the newly identified microbes. This knowledge will further expand our understanding of the causation of pelvic infections, which may lead to more effective prevention and treatment strategies.

Keywords

vaginal microbiota; bacterial vaginosis; pelvic inflammatory disease

It has long been recognized that humans coexist with complex bacterial communities, but the scope of their relationship and the role of microbiota in health and disease have only recently been recognized. The exploration of the genital tract microbiota began over 150 years ago.[1] Most of our knowledge about the composition of microbiota in female genital tract was based on cultivation-dependent methods.[2–5] In recent years, cultivation-independent molecular-based methods have broadened our understanding of the composition of normal and abnormal genital microflora, which complements our existing knowledge from cultivation-based techniques. Recently, 16s rRNA sequencing has led to the understanding that the vaginal microbiota includes one or two lactobacilli from a range of

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four main species (*Lactobacillus iners, L. crispatus, L. gasseri, L. jensenii*).[6–8] Furthermore, the vaginal microbiota undergoes shifts in their representative species and virulence over time, which is affected by several factors.[9–11] The newer techniques of isolation demonstrate far greater diversity of microbiota associated with bacterial vaginosis (BV) than previously understood by cultivation-dependent techniques.[12–14] Technological advances of microbial isolation have revealed a wide variety of previously uncultured bacteria and have aroused curiosity to research the role of the newly discovered microorganisms in health and disease.

Microbiota Diversity of the Female Genital Tract

The vaginal microbiota is dominated by *Lactobacillus* species; however, an appreciable proportion of asymptomatic healthy women have microbiota lacking significant numbers of *Lactobacillus* spp. and harboring diverse array of facultative bacteria and anaerobes.[8,15–17]

Ravel et al[15] evaluated vaginal microflora and vaginal pH in 396 asymptomatic sexually active women representing four ethnic groups (white, black, Hispanic, and Asian) by pyrosequencing of 16S rRNA genes. *Lactobacillus* species dominated four groups (*L. iners*, *L. crispatus*, *L. gasseri*, and *L. jensenii*), but one group accounting for 27% of women (termed as group IV community state type) lacked significant lactobacilli and was heterogeneous with higher proportion of strictly anaerobic bacteria including *Prevotella*, *Dialister*, *Atopobium*, *Gardnerella*, *Megasphaera*, *Peptoniphilus*, *Sneathia*, *Eggerthella*, *Aerococcus*, *Finegoldia*, and *Mobiluncus*. This latter group was over represented in black and Hispanic women. Data from Ravel et al[15] suggest that one-fourth of asymptomatic women lack lactobacillus-dominated microbiota and almost half have a pH > 4.5. These data suggest that vaginal bacterial communities lacking lactobacilli are common and species composition of vaginal microbiota might be genetically determined.

Dynamics of the Genital Tract Microbiota

Understanding the dynamics of the female genital microbiota may provide insight into the factors influencing the susceptibility toward a diseased state. The existing studies on vaginal microbiology either have used a cross-sectional design with sampling at a single point of time or feature long intervals between sampling and therefore offer little information of short-term variations of microbiota. However, frequent fluctuation in the microbiota composition has been reported in various studies using molecular tools, microscopy, and culture-based methods.[9–11,18]

Srinivasan et al[19] studied 22 women assessed with daily quantitative PCR assays and reported that microbiota of human vagina was highly dynamic and that *Lactobacillus* sp. levels changed over course of a month.[19] Using Gram stain analysis, Brotman et al[10] demonstrated rapid fluctuation of the microbial communities and indicated association of physical disturbances like lubricant use or rectal sex with BV. The authors also reported that women had rapid fluctuation of vaginal microbiota sometimes leading to short episode of BV with spontaneous remission.[10] Moreover, Aagaard et al[20] recently showed using 16S rRNA gene sequencing that pregnancy state is associated with significant reduction in overall diversity and richness of vaginal microbiota. Recently, a 16-week longitudinal study

of women, sampled twice weekly, demonstrated that some bacterial communities changed markedly over a short period, whereas others were relatively stable, including communities lacking *Lactobacillus*. Menses were identified as the most negative factor affecting microbiota stability.[21] These findings are supported by numerous studies that have demonstrated that menses, hormonal fluctuation, sexual behaviors, hygiene practices, new sexual partner, and vaginal microbiota composition contribute to the fluctuation of bacterial communities.[11,22–24] Longitudinal studies with frequent sampling and detailed behavior analysis are required to advance understanding of the dynamics of vaginal microbiota and the events leading to BV.

Is the Upper Genital Tract Sterile?

Despite the microbial colonization of the cervico-vaginal epithelium, the upper genital tract was considered sterile,[25] and recovery of bacteria from the upper tract was associated with a diseased state.[26] Several culture-based investigations have challenged this notion and demonstrated the presence of bacteria in the endometrium of healthy asymptomatic women.[27–29] However, these findings were questioned and contamination from the lower genital tract was documented as a potential explanation.[30] Later studies overcame this limitation by studying the endometrial cultures from specimens of asymptomatic hysterectomy patients. These later studies have demonstrated that 25 to 30% of patients harbored one or more bacteria in the endometrium including *Lactobacillus* spp., *Mycoplasma hominis*, *Gardnerella vaginalis*, and *Enterobacter* spp.[31,32] Recently, Steel et al[33] reported detection of bacteria in both preterm and term cesarean deliveries in absence of labor using in situ hybridization of bacterial RNA in fetal membrane. The authors suggested that the presence of intrauterine bacteria are common and may not cause inflammatory response and preterm labor.[33]

Based on currently available data, there is evidence that upper genital tract may harbor microflora in asymptomatic state, but their role in health and disease is not yet fully understood.

Disturbances of the Genital Tract Microbiota in Disease States

BV is a clinical syndrome characterized by disequilibrium in the vaginal microbiota with decline in the number of lactobacilli.[34–36] BV has been identified as an independent risk factor for the acquisition of sexually transmitted infections (STIs),[37–40] human immunodeficiency virus (HIV),[38,41–45] pelvic inflammatory disease (PID),[46] and reproductive and obstetric sequelae.[47–51]

Cultivation-based methods identified BV as a shift from the relative abundance of lactobacillus to microbial diversity including anaerobic bacteria such as *Gardnerella*, *Prevotella*, *Mobiluncus*, *Ureaplasma*, and *Mycoplasma*. As the majority (> 99%) of bacterial species resist cultivation,[52] recent molecular studies have broadened our understanding of the microbial flora associated with BV.[12,53] Using cloning and sequencing methods, Fredricks et al[12] confirmed the association of *Atopobiumvaginae* with BV and for the first time described three organisms strongly associated with BV with 16S r RNA gene sequences designated as BV-associated bacteria (BVAB) 1, 2, and 3.

Traditionally known *G. vaginalis* and *A. vaginae*, both have high sensitivity and specificity in predicting BV.[54] Newer identified fastidious anaerobic BVAB including *clostridia* like BVAB 1, 2, and 3, *Leptotrichia* and *Sneathia* genera, and uncultivated *Megasphaera*-like phylotype have been associated with BV and its known risks.[8,12–14,16,55,56] Fethers et al[57] demonstrated that majority of the BV candidate organisms were absent in sexually unexposed women and were associated with increasing sexual exposure. Similarly, Ness at al[46] identified two discrete clusters of genital microbes among 1,140 women: BV-associated microflora; *Gardnerella*, *Mycoplasma*, and anaerobic rods and non–BV-associated microflora; *Enterococcus* species; and *Escherichia coli*. The authors demonstrated that BV-associated microbes and a new sexual partner were most strongly associated with PID, and identified that BV-related microbiota greatly increased the risk of acquiring PID.[46] Molecular techniques have demonstrated that BV is characterized by microbial diversity that includes many novel bacterial species previously undetected by conventional cultivation techniques.

Vaginal Microbiota and Pelvic Inflammatory Disease

Numerous studies using Nugent score and cultures have demonstrated that BV-associated vaginal microbiota predisposes to acquiring PID[46] and STI.[37–39] The largest longitudinal cohort study followed up 3,620 women quarterly for 1 year using microscopic evaluation and culture methods of endocervical swab and vaginal smears and found that intermediate and high Nugent scores were associated with a 1.5- to 2-fold increased risk for trichomonal, gonococcal, and/or chlamydia infection.[58] The hazard ratio was 1.5- to 2-fold, suggesting that women with high Gram stain scores were at increased risk of STIs.[58] This study is consistent with other studies associating vaginal microbiota with PID risk. A report by Cherpes et al[37] on 670 women in Pittsburg area found that a high Nugent score was associated with twofold increased risk for herpes simplex virus type 2 (HSV-2) seroconversion.[37] In the “GYN Infections Follow-through (GIFT) study” in the United States involving 1,140 women, clusters of BV-associated vaginal microbiota identified by culture methods were associated with development of PID.[46] Allsworth and Peipert[59] reported that the highest abnormal Gram stain (Nugent) scores had the greatest risk of STIs among 535 women. Korn et al[60] reported that in a group of nonpregnant women who presented to a STD clinic with vaginal symptoms, BV was associated with an increased rate of both plasma cell endometritis and endometrial colonization with BVAB. Collectively, these observations establish that BV microbiota, as reflected by the Nugent score, is a risk factor for pelvic infections.

BV has also been linked to various pregnancy complications, including preterm labor and delivery, preterm premature rupture of membranes (PPROM), chorioamnionitis, and postpartum endometritis.[61] The most consistent association was between BV and preterm delivery.[61] The suggested hypothesis was that BV promotes the ascension of the bacteria from the lower to the upper genital tract or that BV may be an indication of upper genital microbial tract colonization.[62–64] Thus, vaginal microbiota consistent with BV is a recognized risk factor for upper genital tract infections in both gynecologic and obstetric patients.

Bacterial Vaginosis as a Risk Factor for Trichomonas

Trichomonas vaginalis represents a critical health issue with > 85% asymptomatic cases[65] and its association with increased susceptibility to and transmission of HIV infection.[66,67] The hazard ratios for incident trichomonal infection demonstrated a dose–response trend with increasing Nugent scores.[58] Brotman et al[68] evaluated the association of vaginal microbiota determined by molecular analysis and *T. vaginalis* in 394 women and found a disproportionate burden of *T. vaginalis* in women whose vaginal microbiota composed of higher proportions of the genera *Mycoplasma*, *Parvimonas*, and *Sneathia*.[68] A cross-sectional study by Hillier et al[69] in 7,918 pregnant women reported that trichomonal infection was more strongly associated with intermediate Gram stain smears. Collectively, studies using diverse techniques (microscopy, culture, and molecular methods) indicate that lack of lactobacilli in vaginal microbiota of women with BV is an independent risk factor for acquisition of pelvic infections.

Vaginal Microbiota and Human Immunodeficiency Virus

Microbial imbalance as seen in BV has also been identified as a risk factor for acquisition of HIV. The leading explanation for this association is the lack of H_2O_2 production by lactobacilli, but other mechanisms may also be contributory. The alkaline environment activates the CD4 lymphocytes, which then become target cells for HIV.[70] TNF- and IL-1β are found in high levels in cervical secretions from women with BV and these cytokines can upregulate HIV replication in the vagina.[71] BV-associated microflora (*Peptostreptococcus asaccharolyticus* and *Prevotella bivia*) has been shown to stimulate HIV expression in monocytoid cells and T cells.[72]

Associations between BVAB and HIV have been demonstrated in several observational studies. One of the first reports was from Thailand,[41] showing correlation between BV and HIV seropositivity among female commercial sex workers. Prospective studies have confirmed these cross-sectional research findings. A study of pregnant women in Malawi[45] found that clinical BV was associated with an increased risk of HIV-1 seroconversion during pregnancy and delivery. Another prospective study in Kenya[38] followed up commercial sex workers and demonstrated that abnormal vaginal flora was significantly associated with acquisition of HIV and gonorrhea. Sewankambo et al[73] found that women with Nugent scores of 9 or 10 had the highest rates of acquisition of HIV-1 infections.

Many studies have reported that BV-associated microbiota is linked to increased HIV shedding. Coleman et al[42] showed that women with diminished *lactobacillus* have a greater endocervical HIV-1 RNA load than women with normal *lactobacillus* levels.[42] In another study, *G. vaginalis* count, *M. hominis* count, Nugent score, and the presence of lower genital tract infection were associated with increased HIV RNA levels in the cervicovaginal lavage evaluation by PCR.[74] Recently, Spear et al[75] using 16srRNA gene sequencing demonstrated a trend ($p = 0.07$) toward higher microbial diversity in the vaginal secretions of HIV + BV+ subjects versus HIV-BV + women. BV has been identified as an important risk factor in acquisition of HIV and current research efforts focus on studying control of BV as an intervention in decreasing incidence of HIV infection.

Diversity of Organisms Isolated from Upper Genital Tract Infections

Traditionally, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* have been linked with PID, but recent data suggest that PID is polymicrobial.[60,76] During 1980s, six studies[77–82] using laparoscopically identified cases of PID in 387 women, isolated anaerobes and facultative microorganisms, *C. trachomatis*, and *N. gonorrhoeae* in 61, 31, and 27% from the upper genital tract, respectively. Using culture-based techniques, Soper et al[83] reported that *N. gonorrhoeae* was recovered from the fallopian tubes or cul-de-sac in 38% patients, *C. trachomatis* from only a single patient, and anaerobic and facultative organisms were isolated from the endometrium and cul-de-sac in 16 (31.4%) and 12 (14.3%), respectively, from laparoscopic-confirmed PID cases. Jossens et al[84] described the microorganisms cultured from 580 PID patients admitted to the San Francisco General Hospital. In that study, the most common organism isolated was *N. gonorrhoeae* from 55.8% patients; *C. trachomatis* from 22.2% patients, and anaerobes and/or facultative bacteria alone were recovered from 30% of the patients.[84] Anaerobes and facultative bacteria were also isolated from 50% of the patients from whom *Chlamydia* and *Neisseria* were recovered; thus, anaerobes and facultative bacteria were present in the upper genital tract of nearly two-thirds of the PID patients.[84]

Only a few molecular studies and, to date, no longitudinal molecular studies have investigated the interaction between vaginal microbiota and upper genital tract infections. The newer culture-independent techniques using the pathogen-specific PCR and serological tests have associated extremely fastidious organism with endometritis,[85] PID,[86] and tubal factor infertility[87] (Table 1). In Kenya, Hebb et al[88] used broad-range 16S rDNA PCR to identify novel uncultivable bacteria associated with cases of salpingitis. The authors identified bacteria in fallopian tube samples in 11 (24%) of the 45 case patients with laparoscopically confirmed acute salpingitis and 0 of 44 controls with no evidence of infection.[88] Bacterial phylotypes most closely related to *Leptotrichia* spp. were detected as the only type in one specimen but were mixed with other bacterial phylotypes in two specimens.[88] Novel bacterial phylotypes and microbiotas associated with BV including *A. vaginae* were identified in three specimens.[88] *N. gonorrhoeae* and *Streptococcus pyogenes* were identified in two and one specimens, respectively.[88] The study detected several BVAB using the rDNA PCR including *Prevotella* spp. and *Peptostreptococcus* spp. from salpingitis indicating the association of BV and BVAB in upper genital tract infections.[88]

Like PID, tuboovarian abscesses (TOAs) are also considered polymicrobial infections. The STD organisms, *Gonorrhoeae* and *Chlamydia*, are infrequently isolated from TOAs. Most of the TOAs follow pelvic infections, but many cases of TOAs have been demonstrated without microbial evidence of known STD organisms. The initial step in an abscess formation is damage and necrosis of the tubal epithelium by a pathogen, which establishes a favorable environment for anaerobic invasion and growth.[89] Initial reports of role of anaerobes in TOA were demonstrated by Altemeier in the early 1940s with the isolation of anaerobes from 92% of TOA specimen which were previously labeled as no growth.[90] Cohen et al[91] studied 11 women with presumptive TOA who failed antibiotic therapy and required surgical drainage in Kenya. Nine (82%) specimens were culture positive and aerobes were present in all nine specimens. Seven of the nine cultures (78%) were

polymicrobial out of which five specimens contained both anaerobes and aerobes. Anaerobic gram-negative bacilli (*Prevotella* sp., *Porphyromonas* sp. and *Bacteroides* sp., and *E. coli*) and *Streptococcus* sp. (*S. viridans* and *S. agalactiae*) were the most common microorganisms isolated. Interestingly, *N. gonorrhoeae* and *C. trachomatis* were not isolated by culture or PCR in any specimen.[91]

Landers and Sweet[92] evaluated 232 patients with TOAs out of which 70% had unilateral TOA and 66 of 232 patients with TOAs were intrauterine device (IUD) users. The most common organisms isolated from TOAs were *E. Coli*, *Bacteroides fragilis*, *Bacteroides* species, *Peptostreptococcus*, *Peptococcus*, and aerobic streptococcus.[92,93] TOAs have been seen in 20 to 54% of IUD users[89] and in most of these abscesses, *Actinomyces israelii*, a gram-positive anaerobe, has been identified.[94] Unusual microorganisms isolated from TOAs have been reported including *Pasteurella multocida*,[95] *Pseudomonas aeruginosa*,[96] Mycobacterium tuberculosis in a patient with HIV,[97] Salmonella,[98] and *Streptococcus pneumonia* in premenarchal child.[99] To our knowledge, the only published report of isolation of the microorganisms using the newer molecular techniques in TOA was a case report in 2003,where *A. vaginae* was isolated by 16S rDNA sequencing from a TOA subsequent to transvaginal oocyte recovery.[100] TOAs have been demonstrated to be a mixture of anaerobic, aerobic, and facultative bacteria with surprising absence of *Gonorrhoeae* and *Chlamydia*. The current literature clearly demonstrates that many bacteria colonizing the upper genital tract represent novel species and even novel genera with the availability of newer pyrosequencing methods.[88] The isolation of BVAB from patients with salpingitis and now from TOAs emphasizes the need for further longitudinal molecular investigations of the link between disturbed microflora to the causation of upper genital tract infections, which may lead to new treatment and prevention strategies.

Conclusion

Vaginal microbiota disequilibrium in the BV state has long been recognized as a risk factor for acquisition of pelvic infections, but newer molecular techniques isolating BVAB from the upper genital tract in pelvic infections suggest a direct microbiological link between pathological vaginal microflora and upper genital tract infections. The key to the puzzle will be to answer two questions. First, is there a physiological upper genital tract microbiota? Second, are the same factors that predispose the normal vaginal microbiota to BV state, associated with the imbalance of the upper tract microbiota and pelvic infections? Future efforts should aim to characterize the microbiota of the upper genital tract in health and disease using molecular techniques. Further studies are required to explore whether treating BV can result in decreasing upper genital tract infections and subsequent reproduction sequelae. We envision the future will permit recognition of factors that predispose women to a state of microbiota imbalance and hence increased pelvic infections. Ultimately, this understanding may lead to development of new intervention strategies aimed at shifting the vaginal microbiome into, and maintaining, more protective states.

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Table 1

Organisms recovered from pelvic infections

Organisms identified with cultures/microscopy[77–84]
Chlamydia trachomatis
Neisseria gonorrhoeae
Nongonococcal, non-chlamydia microbes
Anaerobes[83,84,91,92]
Prevotella species
Prevotella bivia
Prevotella disiens
Bacteroides species
Peptostreptococcus asaccharolyticus
Peptostreptococcus anaerobius
Facultative bacteria[83,84,91,92]
Gardnerella vaginalis
Escherichia coli
Group B streptococcus
α-Hemolytic streptococcus
Coagulase-negative staphylococcus
Novel phylotypes identified with molecular techniques[88,100]
Atopobium vaginae
Acinetobacter sp.
Dialister sp.
Fusobacterium gonidiaformans
Gemella sp.
Leptotrichia sp.
Mogibacterium sp.
Porphyromonas sp.
Propionibacterium acnes
Sphingomonas sp.
Veillonella sp.