Original Article
Safely unsterile: understanding the anatomical and immunologic niches of placental, urinary and vaginal microbiomes

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Abstract: This paper details the anatomic and immunologic environment in the placenta, urinary tract and vagina that allows for the establishment of a commensal or symbiotic bacterial population in each of those areas. It describes the placental microbiome and hypothesizes implications for the initiation of labor. It details how the host bladder cells and behavior form the foundation of the urinary microbiome and attempts to explain the observed variations in a normal population. It concludes with a review of the vaginal microbiome and discussion of probiotic use. Comparison of these three microbiomes illustrates that neither microbial sterility nor constancy have been observed in healthy populations and highlights the ubiquitous role that microorganisms play in normal human physiology. The paper summarizes the local anatomic and immunologic factors that contribute to the establishment and maintenance of the microbiome, and considers what processes may alter its function. Recalibrating dysfunctional microbiota remains challenging but is an ambitious and important goal for clinical practice and future experimental design.

Keywords: Vaginal microbiome, placental microbiome, urinary microbiome, asymptomatic bacteriuria, vaginosis

Introduction

Humans co-evolved with a multitude of bacteria, viruses and fungi that colonized and occasionally invaded their body surfaces and spaces. It is now widely accepted that these potential pathogens are kept at bay by a sophisticated innate and acquired immune system that recognizes and neutralizes pathogens and signals for needed repairs [1]. Bacteria cultured from surfaces of healthy hosts that have regular contact with the environment are not usually associated with a disease state because of a healthy host immune system, physical barriers to invasion and clearance mechanisms.

As our understanding of the gaps and failures in the immunologic defense system increase, more and more antimicrobials have been developed with the goal of treating disease by killing the organisms hypothesized to be the cause. However, intentional bacterial eradication in many cavities of the human body has proven unhealthy as normal organ function may be inadvertently interrupted when populations of flora, which help maintain physiologic homeostasis, are decimated by broad spectrum antibiotic use. An example of this disruption is the reduction in Vitamin K, needed for clotting, calcium retention in bone and other functions, following broad spectrum antibiotic use which decreases the vitamin K synthesizing flora usually present in the gut. Exogenous sources of vitamin K must be used to compensate [2, 3].

The concept of a normal flora composing a microbiome, a population of microbes that exist on and in the human body in symbiotic, commensal or pathogenic relationships, was described in 1988 [4] and more broadly accepted in the two decades following its recognition [http://microbe.net/2015/04/08/what-does-the-term-microbiome-mean-and-where-did-it-come-from-a-bit-of-a-surprise/] as technologic advances allowed researchers to identify non-pathologic and non-dominant bacterial populations

more easily. The labor involved in usingculture based techniques to investigate the multitude of organisms in a microbiome limited its exploration; it was too difficult to obtain a sufficient sample, uncontaminated by sampling methods, grow many organisms, which may be fastidious or difficult to isolate, and identify known bacteria. Non-culture based molecular methods, which extract DNA from a swab or fluid sample, use polymerase chain reaction (PCR) techniques to amplify and sequence bacterial 16SrRNA and attempt to match it with known bacterial rRNA sequences, allow for easier identification of bacteria but do not mitigate the issues with sampling. With the use of culture and non-culture based testing, over a dozen distinct microbiomes have been discovered in anatomic spaces of healthy individuals, including in some spaces that had traditionally been considered sterile using conventional culture techniques.

These distinct microbiomes are not only influenced by non-infectious disruptions in human health, but the absence of a robust heterogeneous bacterial population in certain non-sterile spaces can rapidly become pathologic. The differences between populations of bacteria identified in the gut microbiome in healthy individuals are now thought to be a result of the interactions between diet, genetic make-up and antibiotic exposure [5]. In turn, gut microbiota alter nutrient metabolism in obese or underweight states, [6] or influence the level of chronic inflammation in bowel disease [7]. After broad-spectrum antibiotic use, the substrates and space originally used by a mixture of organisms may be used for the overgrowth of a single bacterial or fungal population. The overgrowth commonly precipitates an immune response as occurs in oral or vulvo-vaginal candidiasis [8] or invasive disease like *Clostridium difficile* associated colitis [9].

For the clinician who prescribes antibiotics, the presence and genomic identity of the microbiomes in any body space are less directly applicable to practice than an understanding of the relationship that exists between bacterial and fungal elements in the microbiome and its anatomic niche. Then assessment of the microbiome can be combined with discrete microbial manipulation in disease states. Yet the functional relationship is not completely defined in many of the descriptive studies outlining and categorizing either the normal or altered microbiome.

This paper aims to investigate the relationship between the identified microbiota of placental, urethral and vaginal spaces, three spaces with very different anatomic relationships to normal bacterial flora in the perineum (**Table 1**). It will explore the type of relationship the microbiome has with its anatomic space by using the understanding of the normal function and operation of the placenta, bladder and vagina, the depth of immunologic response in those spaces, and characteristics of the bacteria identified in the microbiome in healthy subjects. We speculate on a physiologic role for the placental microbiome, explain the role of cellular niches and local host defenses in the bladder and urethra in shaping the urinary microbiome, and briefly review the role of the vaginal microbiome in health and disease.

Placental microbiome

Normal placental function and immunologic environment

The human placenta forms in the fertilized egg, supports and protects the developing fetus during the course of a healthy pregnancy. Around the sixth day after egg fertilization, the expanding blastocyte moves from the fallopian tubes to the uterus where, initially, its growth is supported by uterine secretions. After the blastocyte implants, the connection it makes with the uterine wall eventually matures into decidua basalis from maternally derived tissue on the basal plate of the placenta and the choric villi from the fetus on the chorionic plate side of the placenta [10].

The largest cellular interface between the blood of the developing fetus and the blood of the mother is the fused multinucleated trophoblasts, called syncytiotrophoblast, in the placenta's chorionic villi. Trophoblasts independent of the syncytiotrophoblast invade the uterine wall to form the spiral arteries which connect the myometrium and the maternal blood supply. In the first trimester, natural killer cells, macrophages and regulatory T-cells enter the decidua and accumulate around extravillus trophoblasts [11]. The syncytiotrophoblast allows the essential exchange of nutrients, gases, and waste between the maternal and fetal blood streams. It also protects the fetus from exposure to foreign genetic material, maternal immune system interrogation and sensitization, and transmission of pathogens [12].

Table 1. Normal and abnormal flora of the placenta, bladder and vagina

Location			Flora detected in absence of dysfunction (Microbiome)	Flora associated with dysfunction, and/or Prior Infection (resolved)	References
Placenta			Oral Flora (Lactobacilli, Bifidobacterium, Proteobacteria, Bacteroidetes and Fusobacteria) Bifidobacterium and Lactobacterium	*Ureaplasma urealyticum*, *Esherichia coli*, Enterococcus species, Group B Streptotoccus, *Listeria monocytogenes* and mixed gram negative and gram positive aerobes	[15, 17]
Urine					
	Urethra Male				
		Foreskin intact	Lactobacilli, Corynebacterium, Pseudomonal genera, anaerobes		[45]
		No Foreskin	Lactobacillli, Corynebacterium		[45]
	Urethra Female		Lactobacilli		[46, 50]
	Bladder		Intracellular enterobacteriacea (following resolution of prior infection)	Gram negative enteric organisms; streptococci	[48]
Vagina					
	Pregnancy		Dominance of Lactobacilli-*L. iners* and *L. crispatus*; stable throughout pregnancy		[60]
	Non-pregnancy		Five community state types: Four dominated by *Lactobacillus: L iners* or *L crispatus* most common; *L gasseri* or *L. jenesnii* less common; Fifth with fewer lactic acid producing bacteria; Prevotellas sp, *Gardnerellavaginalis* and more strict anaerobes. Variation over time	Bacterial dysbiosis associated with fewer lactic acid producing organisms; Gardnerella sp, Prevotella sp, *Atopobium vaginae*, Candida sp associated with HIV, human papilloma virus and *Trichomonas vaginalis*	[55, 60, 61]

Both the activation and modulation of the maternal immune system are essential for the progression of pregnancy and completion of a successful delivery. The development of a strong connection between the uterus and placenta is promoted by a pro-inflammatory state driving rapid cell recruitment and tissue growth in the first trimester [11]. The immune cells accumulating around the extravillous trophoblasts are demonstrably essential to the progression of the pregnancy, but Mor and Cardenas [11] hypothesized that the function of these immune cells must be carefully modulated to allow for tolerance of the connection between mother and placenta. In the second trimester, the inflammatory state is suspended so the maternal immune system will permit the connection through the placenta. This allows an unrestricted supply of the substances essential for the maturation of fetal organs, creates a protected space for cell activity that does not occur at any other time in human development. However, the extravillous trophoblasts in the decidua and the syncytiotrophoblast in the chorionic villi may also be a site of potential microbial invasion or colonization [12]. Gene expression changes in the transcriptional profiles of chorioamniotic membranes of women in normal labor are similar to the genes expressed with local inflammation, [13] as a second pro-inflammatory state in the third trimester spurs uterine contraction, delivery of a neonate and disconnection of the placenta from the uterus [11].

Describing placental microbiota

The presence of bacteria in the amniotic fluid of a pregnancy has long been associated with miscarriage, preterm labor and fetal infection, however this is not exactly the case for the presence of bacteria in the placenta. In a study by Pettker, 183 women undergoing trans-abdominal amniocentesis either for preterm labor and suspected infection or for fetal lung maturity (used as the control population) also had swab, tissue of the placenta, and tissue of the amnion-chorion taken immediately after delivery [14]. Bacteria including *Ureaplasmaurealyticum*, *Esherichia coli*, Enterococcus species, Group B Streptotoccus and mixed gram negative and gram positive aerobes, grew from the placenta in women in preterm labor, but over 50% of women's placental samples from their control group also grew bacteria. Then in a study of samples of placental tissue of full term

pregnancies from both vaginal and cesarean section births, the DNA of common vaginal flora Bifidobacterium and Lactobacterium was found in placental tissue, though those bacterial strains were not cultivated in the lab [15].

Before the concept of a placental microbiome, it was unclear why these oral or intestinal commensal bacteria would be found in placental tissue from healthy pregnancies with such high prevalence. Efforts to prevent contamination of samples from the vaginal tract had been made and other colonizers of the vagina were cultivated, mostly at low concentrations of <1,000 colony forming units (CFU). In another cross sectional study aimed at identifying intracellular and therefore uncultivable bacteriain placental tissues published in 2013 [18], 27% of 195 placentas harvested less than 12 hours after delivery had evidence of intracellular bacteria of many morphologies and cell wall characteristics in basal plate samples from both term and preterm placentas. Researchers only noted a significant increase in the incidence of this intracellular bacteria in very premature spontaneous births; Group B Streptococcus carriage, or clinical chorioamniotis diagnosis were not linked to increased bacterial detection [16].

In 2014, an investigation of the placenta's microbiota using PCR-sequencing based, rather than cultivation based techniques suggested that the bacteria found in the placenta told a partial microbiotic history of the pregnancy [17]. Researchers took samples from 320 women with a variety of pregnancy histories and determined the taxonomic classification of the placental microbiome and then compared it to other body niches. The placental microbiome, with taxonomic classifications matching phyla of commensal bacteria with low pathogenic potential including Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria, was far more similar to oral microbes from non-pregnant subjects than to the microbesin the vagina, gut, or nares. Bacterial contamination was not a plausible explanation of the results, though it is worth noting the authors did not attempt to culture these bacteria so there is no evidence that organisms were alive or replicating at the time of delivery. The content of the microbiome was altered in those subjects with remote history of antenatal infection, such as a urinary tract infection in the first trimester of pregnancy, and in those subjects with preterm

birth. However, the microbiome was not altered in those with Group B Streptococcus colonization, maternal obesity or by mode of delivery and it was unclear if the microbiota in cases of antenatal infection were altered by the infection or by the exposure to antibiotics to treat the infection.

The critiques of the construction of a placental microbiome independent of culture based techniques are that sequence based techniques may amplify fragmented or dead bacteria, have a higher risk of sample contamination given the increased sensitivity, and maternal blood infusing the placental tissue may contain nonviable bacteria present transiently during the process of labor and birth [18]. However, studying the way that PCR and culture based techniques have been used in conjunction to identify placental bacteria suggests that the PCR based techniques are unlikely to amplify bacteria from maternal blood or bacteria fragments in samples. In one study, approximately half of 1365 samples of placental tissues of neonates delivered between 23 and 27 weeks gestation had positive bacterial cultures [19]. Due to freezing of the tissues prior to PCR analysis, none of the samples randomly selected for PCR analysis had bacteria identified. In the process of investigating why and how this occurred, researchers discovered that it was difficult to amplify bacterial DNA from placental tissue and speculated the tissue itself degraded the DNA. More vaginal deliveries than deliveries by cesarean section had bacteria identified, which could suggest vaginal contamination, except the biopsy was taken from a different tissue plane that did not have direct contact with the birth canal and did not have high rates of lactobacillus identified (as would be expected for tissue contaminated by vaginal secretions).

Role of placental microbiota

The presence of bacteria in the placenta may be incidental. Transient bacteremia with oral flora occurs in individuals without periodontal disease, following the typical activities of teeth brushing or flossing. Such bacteria are rapidly cleared by innate host defenses. Human oral bacteria can spread hematogenously into a murine placenta and it may do the same throughout a normal pregnancy because of the unique immune regulation that occurs in pregnancy, coupled with massive blood flow through an organ that also has immense filtering capac-

ity [20]. The presence of periodontal disease is associated with pre-term birth, [21] but the placental bacteria were identified in women without periodontal disease who carried their gestations to term in the Aagaard study [17].

A certain density of bacteria in the placenta may play a role in stimulating the second pro-inflammatory state of the immune system in pregnancy and lead to cervical ripening and uterine contractions as part of the interaction between stimulation and suppression of the mother's immune response at the maternal-fetal interface [22]. When biopsy specimens were obtained from placentas from neonates up to 28 weeks gestation, samples from neonates of lower gestational age had a higher rate of bacterial identification by culture [23]. When tissue was examined by pathologists, this bacterial colonization was associated with the presence of neutrophils in the fetal stem vessels or vessels of the umbilical cord. It is possible that the bacteria populating the placental microbiome may only become pathogenic when they replicate too quickly in the protected space or, as is possibly the case with periodontal disease, when the placenta was exposed to frequent or concentrated bacterial loads throughout pregnancy. Even if the placental bacterial load reaches a critical density too soon, it may elicit an immune response and set off an inflammatory cascade which eventually leads to birth [10].

The theory of placental bacteria as part of the normal initiation of laboris conjectural on our part; studies to date have focused on identifying bacterial pathogens that prematurely initiate labor, rather than suggesting that the presence of bacteria is part of a continuum. The dominant species of microbes in the microbiome of both mother and neonate are different in term and preterm labor. The differences may be a cause of the pre-term labor or a reflection of labor initiation in a different stage of pregnancy as the maternal microbiota in the vagina, gut and mouth also change during pregnancy [24]. While it may be hypothesized that the changes result in a more-metabolically active bacterial population similar to how changes in the gut microbiota are noted in bacteria concentrations in lean and obese subjects, it is not clear whether the behavior or hormonal state of the subject or their personal microbiota changes first or how long these alterations persist.

Urinary microbiome

Gender differences in usual urinary tract function and dysfunction

The anatomy of the upper urinary tract, kidneys and bladder is nearly identical in both genders and the traditional standpoint is that urine is produced sterile from the kidneys and should remain so throughout the urinary tract unless an infection is present. As with the assumption of placental sterility, this concept of urine sterility does not account for the presence of a stable population of bacteria in a healthy and normally functioning bladder. Moreover, as with the placental microbiome, the presence of a urinary microbiome in a healthy functioning bladder has more ramifications than would an incidental colonization, easily removed with antibiotic treatment. Unlike the placental microbiome, which exists only for the duration of the pregnancy, the urinary microbiome spans a wide range of physiologic and pathologic conditions in the urinary tract over an individual's lifetime and has more variability than the placental microbiome.

The anatomic differences between the urinary tracts of adult men and women are a source of diversity in the microbiologic populations of the bladder and likely influence the rates of symptomatic urinary tract infection. The female urinary tract has a shorter urethra with only a brief distance to the bladder and has a wealth of both lower gastrointestinal tract and vaginal flora exposure. Accidental contamination of urine with this surrounding flora may occur when samples are collected by spontaneous voiding. The male urinary tract has a longer urethra, less diverse potential bacterial exposures and lower risk of accidental contamination of samples.

It has been accepted that not all bacteria in urine equate to an infection in a patient who has no symptoms of a urinary tract infection, but the precise definition differs between men and women. Asymptomatic bacteriuria in women is defined as the same strain of bacteria with quantitative counts of at least 100,000 colony forming units per milliliter of urine from at least two consecutive clean-catch urine samples without clinical symptoms of a urinary tract infection (dysuria, urinary frequency). In men, asymptomatic bacteriuria is defined as bacteria growing from only one clean-catch

urine sample without symptoms. If the urine sample is taken by catheterization from either gender without symptoms, only up to 100 colony forming units per milliliter of urine may grow for it to be deemed asymptomatic bacteriuria. Except in pregnant women, it is not felt necessary to treat asymptomatic bacteriuria as an infection [25]. Rates of asymptomatic bacteriuria change as men and women age. In women, it may occur from 3% to greater than 18% in post-menopausal women over 60 years old [26]. Asymptomatic bacteriuria is very uncommon in young men. In men over 80, the rate is still less than 10% [27].

In either gender, substantial host urinary tract defenses must be breached in order for bacteria to cause a urinary tract infection. High salt content and low pH make urine a poor media for bacterial growth [28]. Bacteria may ascend from the external mucosal surfaces to the bladder via the urethra or a colonized urinary catheter and replicate without causing an infection during a period of urinary stasis. Regular micturition eliminates bacteria which are not adherent to the bladder walls; bladder epithelial cells called facet cells form an impermeable barrier which protect the transitional and basal cells in deeper cell layers in the bladder. The uroepithelium responds to bacterial invasion by releasing inflammatory cytokines, recruiting host immune cells and subsequently shedding the infected uroepithelial cells. These processes cause the clinical symptoms of urinary frequency, dysuria or hematuria and microscopic changes seen in urinalysis in lower urinary tract infection. Infections occur with greater frequency in women than in men and sexual intercourse and the use of spermicides or a new sexual partner increases the risk of a urinary tract infection in women [29]. Infrequent urinary infections are observed in young men with normal anatomy.

Bacterial persistence in the urinary tract is the foundation of the microbiome

The adaptive responses of bacteria to immune system defenses in the bladder allow for the creation of deep quiescent intracellular bacterial colonies which may persist even after a symptomatic infection has been treated. Many different enterobacteriacea are capable of invading the facet cells via uroplakins on the cell surface [30]. Most successful uropathogenic strains of *E. coli* replicate in the epithelial cells' cytoplasm but leave the intracellular space

before the cell can be shed [28]. Some *E. coli* strains augment the cell signaling and cytokine production after the cell has been infected which accelerates the shedding of superficial cells and results in the exposure of transitional and basal cell layers of the bladder [30]. With increased facet cell shedding, there is increased exposure of deeper cell layers to potentially pathogenic bacteria before the transitional cells can differentiate into new, mature facet cells. In this way, bacteria form a cellular reservoir below the facet cells, partially circumventing the host immune system and antibiotics.

This cellular reservoir may be a source of recurrent bacteriuria. In a 2 month cycle, superficial bladder epithelial cells are shed, exposing mature new facet cells to urine [31]. Thirty years ago, cultures and scanning electronic microscopy analysis of bladder biopsies from women with recurrent urinary tract infections had identified bacteria within bladder tissue that were not cultured in their urine [32]. In a mouse model of recurrent bladder infections constructed by Mysorekar and Hultgren, quiescent intracellular reservoirs were identified in superficial facet cells 2 weeks after *E. coli* initial infection despite sterile urine without inflammatory cells [33]. Mice bladders were then treated with protamine sulfate, which causes exfoliation of the superficial bladder cells similar to the inflammatory shedding during an infection. When transitional cells differentiated to reform superficial facet cells, around half of the mice developed bacteriuria with identical levels and cell changes to an acute infection with the *E. coli* bacteria with which the bladder had originally been inoculated. This might explain findings from follow up of human urinary tract infections; sampling of urine from women with recurrent cystitis found that one third of the recurrent episodes were caused by the index episode strain [34]. With the right stimulus for facet cell shedding, quiescent intracellular reservoirs in the deeper transitional cells might release bacteria which could lead to a symptomatic urinary tract infection.

Not every bacteriuria with a pathogenic organism leads to symptoms or pathologic evidence of a urinary tract inflammation, and bacteriuria, even in the presence of increased bladder cell turn-over, may not always result in infection. Consider interstitial cystitis/painful bladder syndrome (IC/PBS), which is a disease characterized by pain with a full bladder, relieved by voiding, with a sterile urine culture at the time of diagnosis. Its histological appearance, hypothesized to be due to longstanding inflammation or intrinsic dysfunction of the associated cells, is thinning or ulcerations of the epithelial cell wall in the bladder [35]. The initiation for the cascade and vulnerability to development of the disease is not well known, but studies suggest that the inflammatory cascade results in increased cell apoptosis in IC/PBS [36]. In the setting of increased cell turn over, bacteriuria would be expected, and in fact, in a study following urine cultures of 100 patients diagnosed with IC/PBS collected at active visits over 2 years, 31% had urine cultures with bacteria traditionally considered pathogenic [37]. Subjects with positive cultures reported no difference in several symptomatic measures, including bladder pain, frequency and urgency. Furthermore, oral antibiotic administration, even for presumed unmeasured or uncultivable bacterial infections precipitating the inflammatory cascade, has not been successful in IC/PBS treatment [38]. When a continually intravesical system allows for lidocaine, which may work as a local anti-inflammatory agent, [39] to be instilled into the bladder for days without further mechanical disruption, the ulcerations, bladder wall thinning and bladder pain characteristic of IC/PBC may resolve and not return for months after the device is removed [40]. As another example, when urine was collected by catheterization from 182 women planning treatment for urgency urinary incontinence (median ages approximately 55-60) and 16S ribosomal RNA gene sequencing was used to identify presence or absence of bacterial DNA in their urine, a little more than half of the samples contained bacterial DNA [41]. Moreover, those womens' urinary urgency episodes were more likely to respond to the tested anticholinergic medication and those women with bacterial DNA in their urine were less likely to experience urinary tract infections after anticholinergic treatment was introduced. So the pursuit of urine sterility is not the solution in IC/PBS or urgency urinary incontinence, as asymptomatic bacteriuria may not resolve with antibiotics nor always evolve into an infection.

Influences of cell type, environment, and voiding pattern on the urinary microbiome

The characteristics of the mucosal cells in the bladder coupled with emptying patterns may predetermine which bacteria are present in urinary microbiota. For example, following a radi-

cal cystectomy, a neobladder can be surgically constructed from an intestinal segment that has been resected and diverted, complete with its own blood supply. This neobladder does not have the same immune response and protection as the native bladder which may influence the patterns of colonization that subsequently occur. Post-urinary diversion procedure, mucus and urine are first colonized by yeast while on post-surgical antibiotic regiments, then by gram-positive cocci (Streptococcus and Staphylococcus species found on the skin surface) and gram negative enteric bacteria (*E. coli*, Proteus species, and Klebsiella species) [42]. Non-continent diversions, with constant drainage of urine through a stoma in the abdominal wall, had colonization of mixed gram negative enteric bacteria and gram positive skin flora. Orthotopic diversions, where the neobladder was connected to the subject's intact urinary sphincter, had urine colonized by mostly *E. coli* and other gram-negative enterobacteriacae. The concentration of bacterial colonization correlated strongly with the amount of residual urine in the neobladder, and in a separate study, patients with neurogenic bladder had different bacterial populations if they could spontaneously void [43]. Ileal neobladders (made from small bowel) were more frequently colonized by anaerobic bacteria than colonic neobladders (made from large bowel) suggesting that different bowel epithelium promoted different populations of bacterial colonization, even with optimal neobladder emptying [44].

The similarities and differences between the male urinary microbiome and the female urinary microbiome reflect the environment around the urethra of each gender. Researchers have hypothesized the Lactobacillus colonization in men and women is present from maternal transmission at birth and able to persist in the urogenital area of both genders due to shared embryonic origins of the urogenital tissue cells [45]. Lactobacillus species, well known colonizers of the female vaginal tract, are present by 16S rRNA sequencing in healthy women's urine even when urine is obtained by transurethral catheterization or sterile suprapubic sampling [46, 47]. Lactobacilli can even be cultured with the appropriate media suggesting that live Lactobacillus species are nearly always part of a healthy woman's urinary microbiota [48].

In men, the urinary microbiome may not be so dominated by a single bacterial genera and may transiently change with sexual activity and circumcision status. In a study of 19 first-catch urine samples in men, researchers found that the urogenital microbiome identified by PCR sequencing of 16S rRNA sequences reflected known bacterial colonizers of skin, colon and vagina including Lactobacillus and Corynebacterium species, usually found on the skin [49]. The coronal sulcus and urethral bacterial microbiota in circumcised and uncircumcised men were distinguished by increasing proportions of Pseudomonal genera and anaerobic bacteria in uncircumcised men, and more abundant Lactobacillus species in the urine versus the coronal sulcus. In a second study, serial urine samples over 3 months taken from healthy men aged 14-17 with and without history of sexual activity identified a few bacteria genera only transiently present in males with sexual experience, while Lactobacillus was consistently found in their urine, even without reported sexual activity [45].

In the native and normally functioning urinary tract, the urinary microbiota changes with type and pattern of voiding. In a cross-sectional study of the urine samples from in men and women with and without spinal cord injury associated neurogenic bladder, 16S rDNA sequencing was performed on urine samples obtained from 53 participants, 27 of which had neurogenic bladder due to spinal cord injury [43]. Healthy female controls and female neurogenic bladder patients who could spontaneously void were distinguishable from other patients by dominant populations of lactobacillus, a normal colonizer of the vaginal tract, in their urinary microbiota. These urinary bacterial populations were decreased in women who needed intermittent catheterizations or an indwelling catheter. Healthy male patients had populations of Corynebacterium that were also decreased in men with neurogenic bladder and men requiring intermittent or continuous catheterization [43].

While the identification of urinary microbiota in healthy individuals suggests its presence alone is not pathologic, it is not yet clear if the contents of the urinary microbiome influence infectious risk or bladder dysfunction or if they only a reflection of past urinary tract infections or

colonization. When a urinary microbiota survey was obtained in women who suffer from urgency urinary incontinence without infection, researchers found that though the urinary microbiota was less diverse than microbiomes of the human skin, gut or mouth, the urinary microbiota contained a greater number of bacterial species in women with urge incontinence than in women with normal bladder control [46]. It was also less likely to contain Lactobacillus than normal women. It has been hypothesized that lactobacillus may help acidify the urine and provide a less fertile environment for more virulent bacteria as a decreased lactobacilli colony counts in stool and urine have been observed in infants with urinary tract infections, [43] though the observed decrease may have been due to the presence of a urinary tract infection itself [50]. However, in adults it has been demonstrated that voiding patterns, gender, history of antibiotic usage and presence of in dwelling catheters all also change the risk of a urinary tract infection and likelihood of Lactobacillus isolation. So changes in organisms found in urinary microbiota reflect bladder cell origin and previous bacterial exposure, subject voiding patterns, gender and circumcision status, and presence of indwelling catheters without yet reliably predicting the risk of urinary tract disease.

Vaginal microbiome

"Normal" vaginal flora and function

The vagina is the muscular canal which extends from the vulva to the uterus located between the anus and the urethra. Its surface is a stratified squamous epithelium whose squamous outermost layer is in a state of constant exfoliation. Intermediate and basal cell layers are filled with glyocogen with estrogen stimulus and thinner without. There are regulated changes in hormonal stimuli and pH that occur with menstruation cycle to make the vagina and its secretions support the delicate process of human reproduction. The discomfort of vaginal bacterial disruption will likely affect every woman with exposure to antibiotics through her life time.

After the gut microbiome, the symbiotic role of lactobacilli in the vaginais perhaps the most well established and well accepted. In 1894, Albert Döderlein, a German obstetrician and gynecologist, first identified the 'Döderleinbacillus,' now called lactobacilli, in vaginal secretions. Lactobacilli remain the dominant organisms of a healthy vaginal microbiota; however, improvements in culture technique and methods of bacterial identification increased our understanding of their behavior and that of smaller subgroups of anerobic bacteria that were subsequently identified. Decades ago, researchers found that presumed stable population of bacteria in the healthy vagina underwent a number of fluctuations in both quantity and relative proportions of sub-populations through out a woman's entire life time, changing from pre-puberty, to maturity with variations during menstruation, pregnancy and in the post-partum period, to menopause [51]. Alterations invaginal flora were also observed in disease states such as vaginosis and vulvovaginal candidiasis, and after gynecologic surgery, antibiotic use and immunosuppression [51].

The bacteria in the vaginal microbiome are believed to have an active role in preventing urogenital disease. Vaginal acidity is maintained by the bacterial production of lactic acid and hydrogen peroxide by Lactobacillis species, and a low pH inhibits the growth of many other bacteria including *Gardnerella vaginalis* and *Candida albicans*, both associated with disease states in overgrowth [52, 53]. Lactobacilli are also thought to produce other inhibitory proteinaceous molecules, generally called bacteriocins or bacteriocin-like compounds, which help to maintain their dominance in the vaginal microbiota [54]. Disruptions in the vaginal flora are associated with increased risk of urinary tract infections and contraction of sexually transmitted infections including chlamydia, trichomonas, and HIV [55].

Physiologic and genetic variations in the vaginal microbiome

Hundreds of species of Lactobacilli and anaerobic subpopulations in the vaginal microbiome have been identified and despite its relative proximity to other skin surfaces, vaginal microbiota in all stages of reproduction is distinct from the microbiota of the nose, nasopharynx, mouth, gut or skin [17]. Analysis of the vaginal microbiome in healthy woman of varying races and ethnicities has revealed further variations of microbiota than anticipated. Ravel identified

five distinct "communities" of vaginal microbiota containing from 21 to 135 known bacterial taxa when they characterized the vaginal microbiota of 396 asymptomatic sexually active women of four self-reported ethnic groups (white, black, Asian and Hispanic) via phylogenetic analysis of 16SrRNA gene sequences [55]. Four of these 5 states were dominated by Lactobacillis species while one more heterogeneous group had higher proportions of anaerobic bacteria including Prevotella and Gardnerella species. Though all communities contained bacteria in genera known to produce lactic acid, the more heterogeneous group had the highest median pH (5.3±0.6) while the community dominated by *L. crispatus* had the lowest median pH (4.0±0.6). When ethnic variations in the microbiome were examined, lactobacillus dominated groups were found in 80-90% of self-identified white and Asian women but only in around 60% of the black and Hispanic women, where the more heterogeneous community group was more prevalent and the median vaginal pH was higher. Similar findings of differences in homogeneity of the vaginal microbiota have been demonstrated in studies in Chinese, Japanese and self-identified white and black women, suggesting that the definition of healthy and normal vaginal may have been skewed by the ethnicity of women included in the earlier studies [55]. It is also worth noting that American populations of women self-identifying as black, African American, or Hispanic reflect a more genetically heterogeneous group than researchers assumed [56].

The differences in the vaginal microbiota decrease during normal pregnancy, when the vaginal microbiome has low diversity and high stability. Studies published by Hillier noted that pregnant women could be categorized into different groups based on the predominance of lactobacilli in their vaginal flora [57]. The absence of lactobacilli did initially seem to be associated with pathology such as preterm labor, bacterial vaginosis and intrauterine growth restriction, [58, 59] though more recent research found no significant difference between the vaginal microbiota of pregnant women who carried to term and those who did not [60]. Walter-Antonio [61] compared samples of vaginal swabs taken from a small group of pregnant Caucasian women four times (one during each trimester and one prior to labor) to data collected by Romero [60] who sampled from pregnant and non-pregnant predominantly Af-

rican-American women. Both groups found that bacterial species diversity in the vaginal microbiome significantly decreased during pregnancy so that Lactobacillus species dominated all vaginal microbiota types. Inter-subject variability between African American women also appeared lower in pregnancy than non-pregnancy [61]. Though the meta-analysis was limited by fact that the differences in the 16S rRNA regions amplified by the primers used in the two studies and sequencing platforms made a number of direct comparisons impossible, it suggests that lactobacillus dominates the vaginal microbiota in uncomplicated pregnancy. Monthly fluctuations in hormones or alterations in cervical or vaginal secretions associated with men struation may drive vaginal microbiologic diversity and their absence during pregnancy may contribute to the relative stability of the microbiome. The protective aspects of a stable vaginal microbiome during pregnancy are still being investigated.

Years of study of the vaginal microbiota have suggested that some demographic and behavioral characteristics may influence the contents of the microbiome [62]. The vaginal microbiome does not appear to vary substantially in adolescents, reproductive age or post-menopausal woman, though the quantity and diversity of the microbiota may be reduced in post-menopausal women. The influence of sexual behavior, while presumed to have some influence on the microbiota as the levels of *Gardneralla vaginalis* are higher after sexual debut, lacks detailed surveillance data needed for comprehensive study. Studies on the effect of the menstrual cycle suggest that increased estradiol, both during menstruation and pregnancy, promotes lactobacilli growth but the passage of menses disrupt the usual flora, decreasing lactobacilli concentration and introducing streptococci or other gram positive cocci, and some organisms associated with bacterial vaginosis (BV). In a study done in post-menopausal women with vaginal atrophy and dryness, increased vaginal dryness was associated with decreased lactobacilli dominance and a large amount of detectable species diversity [63]. It remains unclear if this decreased lactobacilli dominance is due to the down-regulation of the genes associated with vaginal epithelial cell proliferation and decreased in epithelial cell glycogen levels after menopause or part of the cause of vaginal dryness as some studies have not shown significant differences

Vaginal microbiota dysfunction

Most attention has been devoted to the altered communities of microorganisms in the vagina associated with a variety of conditions including postpartum endometritis, neonatal infections, miscarriage, pre-term birth, pelvic inflammatory disease and increased HIV acquisition and transmission. Those associated with BV will be very briefly reviewed here. Bacterial vaginosis is now known to be imbalance of the vaginal microbiota associated with a range of diverse facultative anaerobic bacteria where no single organism predominates. There is an inverse association between hydrogen peroxide production by various lactobacilli and the diagnosis of BV, and while *G. vaginalis* and *Atopobium vaginae* are the common organisms by prevalence and abundance, neither are invariably present in BV. In recurrent BV, no single organism is identified as the cause, though >90% of a biofilm identified on vaginal epithelial cells of women with BV was composed of *A. vaginae* and *G. vaginalis* and this biofilm may have interfered with treatment. Other potential explanations for recurrent BV include inadequate treatment with residual organisms after treatment (i.e. relapse), reinfection from sexual partners or disruption of normal flora from other exogenous factors.

The end goal of the focus on the vaginal microbiota dysfunction is the development of a probiotic which could re-establish a healthy and protective community of vaginal flora after inevitable interruption with antibiotic treatment or other conditions, but current treatments remains limited by our understanding of the natural variation of the vaginal microbiome in healthy women and ability to influence vaginal microbiota in a disease state. Bisanz investigated the effect of three days of vaginal administration of two strains of lactobacilli in postmenopausal women [64]. Women had no significant improvement in Nugent score (which is believed to predict the risk of development of BV) or change in vaginal pH. There was an increase in total lactobacillus abundance, either of the study strains or of indigenous lactobacillus, but the application of any vaginal supplement (with study strains or placebo) seemed to increase levels of Staphylococcus. For now, the safety and efficacy of attempts to influence the vaginal microbiota with the instillation of specific strains of lactobacilli remains uncertain.

Swapping microbiomes

Each of the three microbiomes of the urogenital tract described in this paper have the potential to be altered by external pressures-from alteration of their growth environment, the health of the individual host or from exogenous chemicals and enzymes. Though antibiotics are generally thought to have a powerful effect, the effect is clearly not always beneficial. For example, breast milk has its own microbiome helps to populate the neonatal gut. One of the breast microbiome's dysfunctional states causes infectious mastitis during lactation [64]. A trial using two strains of lactobacilli isolated from breast milk as an oral probiotic to treat infectious mastitis found that women in the probiotic group had more improvement and lower recurrence rates than women who were treated with antibiotics [65]. The study also found that there was an increase in the populations of *Staphylococcus epidermidis* and *Staphylococcus aureus* in breast milk of the antibiotic treated group when compared to the probiotic groups. This finding suggests that antibiotic use might select populations of more pathogenic bacteria.

In reviewing the studies of the placental microbiome, there have been few attempts to influence the placental microbiome with agents other than antibiotics. In contrast, researchers have attempted to manipulate the urinary microbiome by establishing a colonization of non-pathogenic, non-virulent E. coli in men and women who had recurrent urinary tract infection due to incomplete bladder emptying [66]. The strain of E. coli used in the study lacks virulence factors and is sensitive to common antibiotics. The study protocol involved attempted sterilization of the urine, a short infection-free interval and then emptying of bladder using a catheter followed by instillation of a solution of *E. coli* 83972. Researchers found it took approximately 5 months longer for study subjects to develop urinary tract infections after successful colonization with E. coli 83972 than subjects who had placebo procedures or unsuccessful colonization.

The evidence on influencing the vaginal microbiome with probiotics is more robust, but the

effect has not been clearly beneficial as some studies found no significant difference between placebo and the vaginal instillation of lactobacillus species [67]. It may be that the differences from probiotic administration are too subtle for studies that do not use 16S rRNA sequencing for assessment to detect [68]. It may also be that it takes more than just a single installation of a few strains of lactobacillus or an antibiotic to alter the population of a complex and established microbiome that has been developing over an individual's lifetime. When studies have looked at the risk of bacterial vaginosis in women who have sex with women, there was a high rate of bacterial vaginosis in both members of monogamous couples. Bacterial vaginosis was also more common in women who had a higher number of lifetime female sex partners, did not clean insertive sex toys between partners and reported oral-anal sex [69]. This suggests that there are many ways and ongoing practices that might introduce disruptive microbiota into the vaginal microbiome.

Conclusion

The microbiota of every surface of the human body are influenced by the physiologic functions, the exposures to other bacteria, available substrates and the cell type and immune function both generally of the host and specific to the microbiota's anatomical niche. It is clear after investigating the available literature that the understanding of the complex microbiotic ecosystems in the urogenital tract is still nascent and there are many questions left to be investigated including the role of antibiotics and probiotics, even in situations where a change in the make-up of the microbiome is thought to be the cause of the clinical symptoms. Many of the studies correlate certain bacterial species or levels of bacterial diversity with healthy states, but are unable to identify which activities or strains of bacteria might most strongly promote those healthy states. The ability to fine tune dysfunctional microbiota remains limited, but one of the ultimate goals in the study of the human microbiome should be the development of new physiologic methods of replenishing a microbiota depleted by antibiotic exposure.

Disclosure of conflict of interest

None.

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