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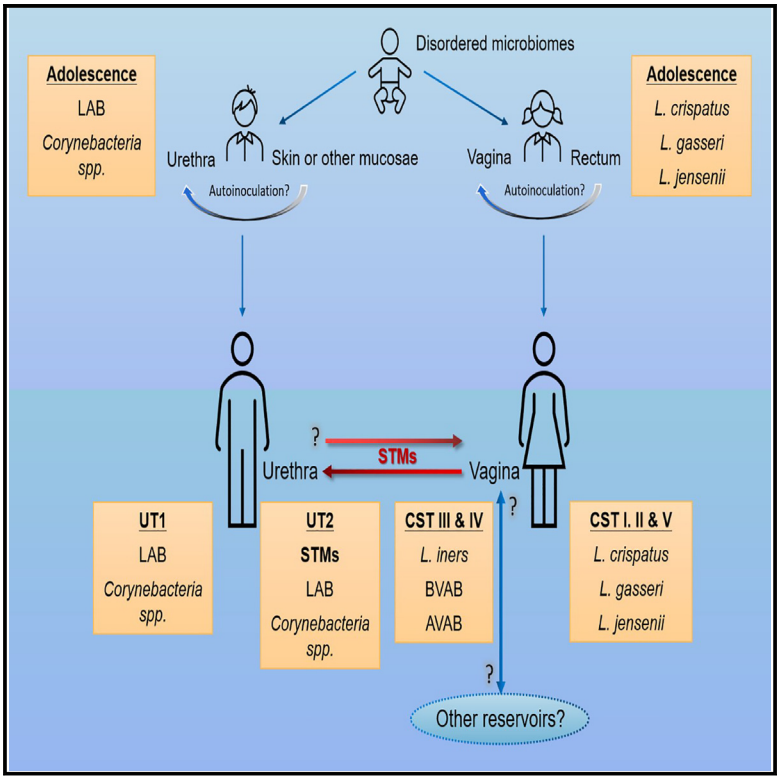
Sexual behavior shapes male genitourinary microbiome composition

Graphical abstract



Highlights

- The adult male urethra usually supports a characteristic core microbiome
- Bacteria associated with vaginal dysbiosis in women colonize some men
- Vaginal dysbiosis-associated bacteria are only detected in men who have vaginal sex
- Sexual behavior is an important determinant of microbiome composition

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In brief

Toh et al. characterize the urethral microbial communities in men who lack urethral symptoms and inflammation and discover that a few common bacteria are present in most men. However, several bacteria associated with reproductive tract disease in women only colonize men who have vaginal sex with women.

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Sexual behavior shapes male genitourinary microbiome composition

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SUMMARY

The origin, composition, and significance of the distal male urethral microbiome are unclear, but vaginal microbiome dysbiosis is linked to new sex partners and several urogynecological syndromes. We characterized 110 urethral specimens from men without urethral symptoms, infections, or inflammation using shotgun metagenomics. Most urethral specimens contain characteristic lactic acid bacteria and *Corynebacterium* spp. In contrast, several bacteria associated with vaginal dysbiosis were present only in specimens from men who reported vaginal intercourse. Sexual behavior, but not other evaluated behavioral, demographic, or clinical variables, strongly associated with inter-specimen variance in urethral microbiome composition. Thus, the male urethra supports a simple core microbiome that is established independent of sexual exposures but can be re-shaped by vaginal sex. Overall, the results suggest that urogenital microbiology and sexual behavior are inexorably intertwined, and show that the male urethra harbors female urogenital pathobionts.

INTRODUCTION

The male urinary and reproductive tracts merge at the post-prostatic urethra, and microorganisms that exit or enter the male urogenital tract traverse the penile urethra (PU). Similar to other mucosae, the PU is richly endowed with innate and adaptive immune cells that can detect and respond to microorganisms.[1] Nonetheless, the PU can be infected by and transmit a broad array of sexually transmitted bacterial, viral, and eukaryotic pathogens.[2] Communities of commensal microorganisms (microbiomes) associated with a healthy gastrointestinal tract and female reproductive tract (FRT) protect these organs against infection and promote health.[3,4] It is unclear if the healthy PU supports a characteristic microbiome, or microbiomes, that contribute to PU health or disease.

Several factors have impeded characterization of the PU microbiome. PU sampling is painful and is rarely indicated in healthy individuals; hence the PU microbiome has mostly been studied in men with sexually transmitted infections (STIs). Some PU microorganisms can be detected in non-invasive specimens, such as urine, but these approaches may oversample the upper urogenital tract and proximal urethra and undersample the PU epithelium.[5,6] The utility of PCR-based cultivation-independent microbial identification approaches, including 16S rRNA gene sequencing, has also been constrained by low microbial biomass in urogenital specimens, inability of these approaches to detect all the diverse types of microorganisms that have been documented in urogenital specimens (bacteria, viruses, fungi, protists, parasites), and signal to noise issues.[7,8]

There is increasing evidence that microorganisms do colonize the healthy PU. In a nongonococcal urethritis (NGU) case-control study in men, Bowie and colleagues cultivated streptococci, lactobacilli, and a broader variety of anaerobic bacteria from 91% of the urethral specimens from the controls.[9] Similar bacteria were detected in urine and urethral swabs of asymptomatic men using 16S rRNA gene sequencing in two other studies, which additionally showed that many of these corresponded to bacterial vaginosis associated bacteria (BVAB).[5,10] Other studies of urine,

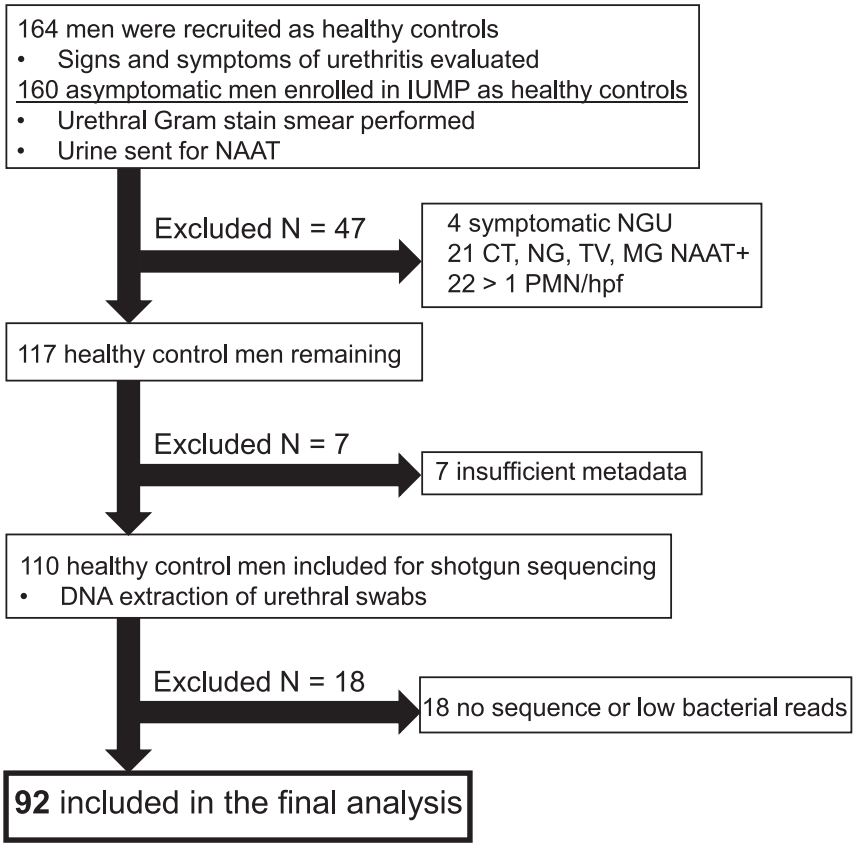

Figure 1. Study participant inclusion and exclusion flowchart

Participants were excluded from this analysis if they had urethral discharge, tested positive for *C. trachomatis*, *M. genitalium*, *N. gonorrhoeae*, *T. vaginalis*, or urethrotropic *Neisseria meningitidis* strain US_NmUC, exhibited >1 polymorphonuclear leukocytes per high-power field (PMN/HPF) on their urethral Gram stain smear, reported antibiotic use in the past month, had genitourinary tract symptoms, or genital skin conditions.

primarily from control men enrolled in studies of urogenital disease, have replicated these findings.[11–16] Although stability of the adult PU microbiome has not been evaluated, some PU bacteria that have been observed in adults were detected in consecutive monthly urine specimens collected from adolescents over a 3-month interval.[17] Conversely, some prevalent bacteria in adults were not detected in adolescents.[17]

Existing data suggest that the healthy PU microbiome is simple, stable, and may be linked to environmental and sexual exposures[17–19]; however, the taxonomic resolution of most studies has been low, so the identities of many PU microorganisms are unclear. The relationship between specific sexual behaviors and PU microbiome composition is also unknown.

Here, we use stringent inclusion criteria informed by clinical examination, STI testing, measurements of urethral inflammation, and behavioral surveys to identify healthy men with no signs or symptoms of urethral inflammation or disease to identify correlates of PU microbiome composition. PU specimens from 110 men and 24 vaginal specimens from a separate validation cohort were characterized using a shotgun metagenomic sequencing approach, allowing us to perform a detailed characterization of the PU microbiome. Roles of STI risk factors and sexual behaviors in PU microbiome composition were also evaluated. We show that the healthy PU supports a core microbiome that may be re-shaped by penile-vaginal (vaginal) sex. We also show that silent carriage of a wide array of FRT pathogens and pathobionts, but not protective *Lactobacillus* spp., is common in men who lack urethral disease.

RESULTS

Participant characteristics and enrollment strategy

We screened 164 volunteers who visited a public health clinic in Indianapolis for STI screening, to identify healthy adult *cis*-gender men without signs and symptoms of urethral inflammation, infection, or disease as part of the Idiopathic Urethritis Men's Project.[20–22] Fifty-four men were excluded for positive STI or urethral inflammation tests, antibiotic use in the past month, history of urogenital or systemic disease, other findings of urogenital abnormality including urogenital surgery, or incomplete survey results (Figure 1). The mean age of the remaining 110 men was 28.7 ± 10.7 years, 35% (38 of 110) were Black, 53% (58 of 110) were White, 13% (14 of 110) were other (Asian and more than one race), and 89% (98 of 110) identified as non-Hispanic or Latino. Seventy-five (68%) self-identified as heterosexual, 22 (20%) as homosexual, and 13 (12%) as bisexual/other, and most were sexually active in the prior year (108 of 110) (Tables S1 and S3).

PU can be colonized by FRT bacteria, but the microbiomes of the PU and FRT differ

The PU swabs were sequenced to an average depth of 31,303,028 ± 9,466,191 reads, and the sequences were annotated using MetaPhlAn3.[23] A total of 117 different bacterial and 26 viruses were detected (Table S4). Bacteria were detected in 92 specimens and these sequences accounted for 95.0% of the total microorganism sequences, on average per specimen (Figures 2A–2C). Viral sequences were less prevalent (41 of 92) (44.6%) and abundant (5.0%). *Streptococcus mitis*, a lactic acid bacterium (LAB) (bacterium in the order Lactobacillales that produce lactic acid) that has been cultured from male urogenital specimens previously,[24,25] accounted for 24.2% of the sequences. Many sequences corresponded to BVAB (e.g., *Gardnerella vaginalis*, *Atopobium vaginae* [recently renamed *Fannyhessea vaginae*[26]], *Prevotella amnii*) or aerobic vaginitis

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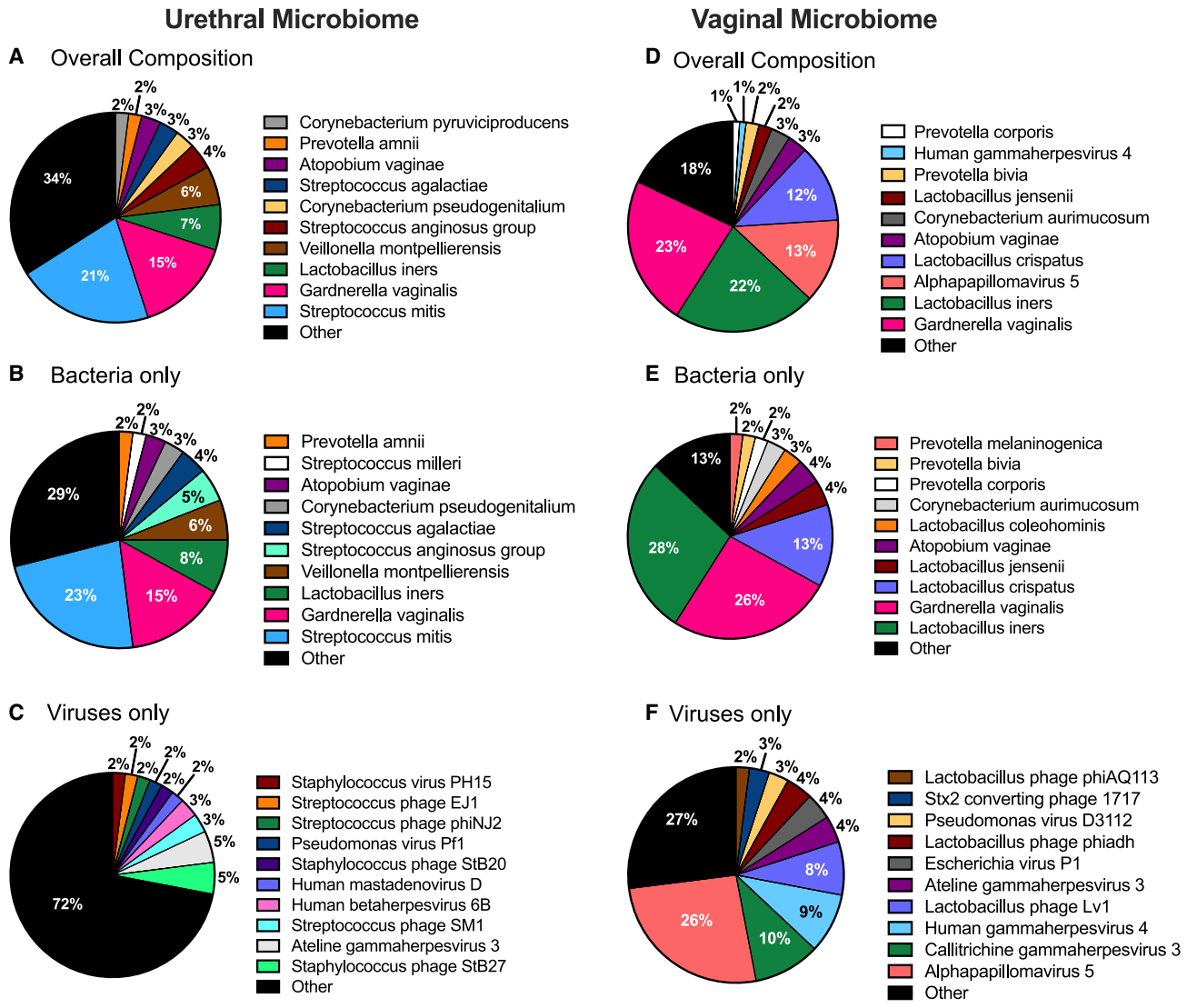

Figure 2. Pie charts depicting percent composition of the top 10 most abundant PU microorganisms based on relative abundance

"Other" includes all remaining taxa detected excluding the top 10 most abundant. Healthy controls: (A) overall microbial composition, (B) viruses only, (C) bacteria only; Vaginal specimens: (D) overall microbial composition, (E) viruses only, (F) bacteria only.

associated bacteria (AVAB) (*Streptococcus agalactiae* and *Streptococcus anginosis*).[27–29] Bacteriophages (phages) accounted for 62% of the viral sequences. *Streptococcus* phages were detected in 11 specimens, but no individual phages were prevalent. Sexually transmitted viruses that can silently colonize the PU such as Alphapapilloma viruses[30] and adenovirus[31] were found in two and one specimens, respectively. Systemic viruses that are commonly detected in male urogenital specimens, including cytomegalovirus[32] and Epstein-Barr virus,[33] were each found in two specimens. Human herpesvirus 6 was detected in six specimens.

Eighteen PU specimens yielded few or no microorganism sequences. This suggested that the urethral microbiome is sparse or absent in some men, or that our approach failed to detect or annotate sequences from microorganisms that were present. Separately, although sequences from FRT pathobionts were prevalent in the urethral specimens, sequences from several FRT health-associated *Lactobacillus* spp. were uncommon or absent. Therefore, we evaluated if our microbial annotation approach was too stringent using vaginal specimens collected in another study as positive controls. We obtained vaginal swabs from four women who enrolled in a prior study of incident bacterial vaginosis (iBV)[34] that were collected every other day before (−2 days), during, and up to 8 days before development of iBV (six specimens from each woman). These specimens were sequenced and annotated identically to the PU specimens. *Lactobacillus crispatus*, *Lactobacillus colehominis*, *Lactobacillus gasseri*, *Lactobacillus jensenii* and other less-common protective *Lactobacillus spp.* were abundant in the pre-BV specimens whereas a broad range of BVAB such as *G. vaginalis, A. vaginae,*

and *Prevotella bivia*, were detected in the iBV specimens, confirming that our approach could detect sequences from a wide range of FRT bacteria (Figures 2D–2F) (Table S4). *S. mitis* was not detected in the FRT specimens, consistent with this microorganism being an infrequent FRT colonizer.[35] Re-annotation of the PU sequences using a less stringent K-mer based approach (Kraken2)[36] (Table S5) yielded similar species-level identifications. This suggested that some PU specimens contain few or no microorganisms, and that our primary annotation approach captured most of the microbial diversity present. Subsequent analyses were performed using the species-level MetaPhlAn3 annotations because Kraken2 also identified many invariant taxa, possibly due to human contamination in reference microbial genome sequences.[37] Thus, the healthy male genital tract may harbor FRT pathobionts, but the microbiomes of the PU and FRT differ.

Two distinct microbiomes are associated with the PU

Clustering has been used to identify characteristic microbiome community state types or enterotypes in the vagina and gut,[38,39] respectively, and assess if specific organs support core microbiomes, defined by Shade and Handelsman as "the suite of members shared among microbial consortia from similar habitats."[40] Clustering performed with compositional datasets, like relative abundance, has limitations,[41] so the MetaPhlAn3 taxa counts were transformed from Simplex space into Euclidian space using two approaches, a default centered-log ratio (CLR) transformation and a custom additive log ratio (ALR) transformation that used human sequences as the invariant taxon.[42,43] To validate the ALR approach, the numbers of *G. vaginalis* genomes, measured by quantitative PCR, and the ratio of *G. vaginalis* genomes to human whole-genome sequences were compared. These were strongly positively correlated (Spearman correlation 0.82, $p = 1.2 \times 10^{-23}$) across several orders of magnitude (Figure S1), showing ALR abundance is a reasonable proxy for bacterial genome counts.[44] Clustering based on Euclidian distance, using ALR or CLR transformed data, sorted the specimens into clusters we called urethrotypes (UTs) (Figures 3A and S2A). Calinski-Harabasz (CH) and Silhouette index analyses determined that two clusters were the optimal number based on the maximum index values[45] using either the ALR or the CLR transformed data (Figures 3B, 3C, and S2B). Similar clusters were reproduced using bacterial and viral taxa or only the bacterial taxa (Figures 3A and S3A). Finally, similar clusters were also found when principal-component analysis separated the taxa using the ALR abundance of bacterial or all taxa (Figures 3Dand S3C). Thus, the clustering results were primarily driven by bacteria. Clusters from the ALR data were designated UT1 and UT2, and contained 66 and 26 specimens, respectively.

UT1 is dominated by simple communities of *Streptococcus* and *Corynebacterium* spp

One hundred total bacterial species were detected in the UT1 specimens, but the richness of the microbiomes in the individual specimens was low (Chao1: 6.13 ± 6.71; Ace: 6.12 ± 9.13) (Figure 4C). Only 16 of these bacteria were detected in more than 10% of UT1 specimens, while the other 84 bacteria only accounted for 19% of UT1 bacterial sequences (Table S4) (Figure 4A), indicating that UT1 harbors a simple microbiome. *S. mitis* was especially prevalent (54 of 66) and accounted for 35.5% of UT1 sequences.

S. mitis was the only taxon whose ALR abundance was significantly higher in UT1 than UT2 after application of Wilcoxon's signed rank test and a Benjamini-Hochberg (WBH) multiple test correction ($p = 0.0024$) (Table S6). Lower proportions of one or more of 15, primarily aerobic, *Corynebacterium* spp. were detected in 61% (40 of 66) of the specimens and accounted for 8.7% of UT1 bacterial sequences (Table S4). Other LAB including various viridans streptococci (*Streptococcus pseudopneumoniae*, *Streptococcus milleri*), *Lactobacillus iners,* and AVAB (*S. agalactiae* and *S. anginosis*) were detected in 92% (11 of 12) of the *S. mitis*-negative specimens, and 50% (27 of 54) of the *S. mitis*-positive specimens and accounted for 22.4% of UT1 sequences. All these *Streptococcus*,[24] and most of these *Corynebacterium* spp.[46] have been cultured from male urogenital specimens previously. Thus, like in adolescents,[17] core communities of LAB and *Corynebacterium* spp. colonize the PU in most adults.

UT2 specimens are dominated by BVAB

Eighty-three different bacteria were detected in the 26 UT2 specimens, 66 of which were also detected in UT1 specimens. The ALR abundance of 56 of these bacteria did not significantly differ between UT1 and UT2, although the prevalence and abundance of many of these was low (Table S6). Like in UT1, *Corynebacterium* and LAB spp. were prevalent (81% [52 of 66] and 88% [23 of 26], respectively) in UT2 specimens, suggesting that these organisms constitute a core PU microbiome (Table S4) (Figure 4B). In contrast, the richness of UT2 specimens (Chao1: 9.39 ± 4.26; Ace: 10.4 ± 5.48) was higher than UT1 specimens ($p = 0.00001$ for Chao1; $p = 0.00008$ for Ace, Wilcoxon's signed rank test) (Figure 4C). In addition, the ALR abundance of nine bacteria (*Aerococcus christensenii*, *G. vaginalis*, *A. vaginae*, *Veillonella montpellierensis*, *P. amnii*, *Dialister micraerophilus*, *Sneathia amnii* (recently renamed *Sneathia vaginalis*[47]), *Mageeibacillus indolicus*, and *L. iners*) was higher in UT2 than in UT1 specimens ($p < 0.05$ WBH) (Table S6) (Figure 5). All these bacteria were prevalent (range from 100% for *G. vaginalis* to 54% for *M. indolicus*) and collectively accounted for 85.9% of UT2 sequences (Table S4). All these bacteria are associated with BV, AV, or other non-optimal vaginal community state types,[27,38,48–50] and many can form inter-species biofilms with the keystone species *G. vaginalis*.[51]

Phenotypically diverse clinical isolates originally grouped into *G. vaginalis*[52] correspond to at least four named *Gardnerella* spp. and multiple additional unnamed genomospecies (GS).[53,54] Some GS are associated with specific BV phenotypes, and co-infection with multiple GS is associated with incident BV.[55] We applied a modification of the approach developed by Potter et al.,[54] to determine which GS were present in PU specimens. Sequences unique to all nine GS defined by Potter et al. were detected, and sequences from more than one GS (range 2–9, median 5) were detected in 93% of the *G. vaginalis*-positive specimens (Figure 4D) (Table S7). Sequences from GS03, associated with recurrent BV, were the most prevalent and abundant,

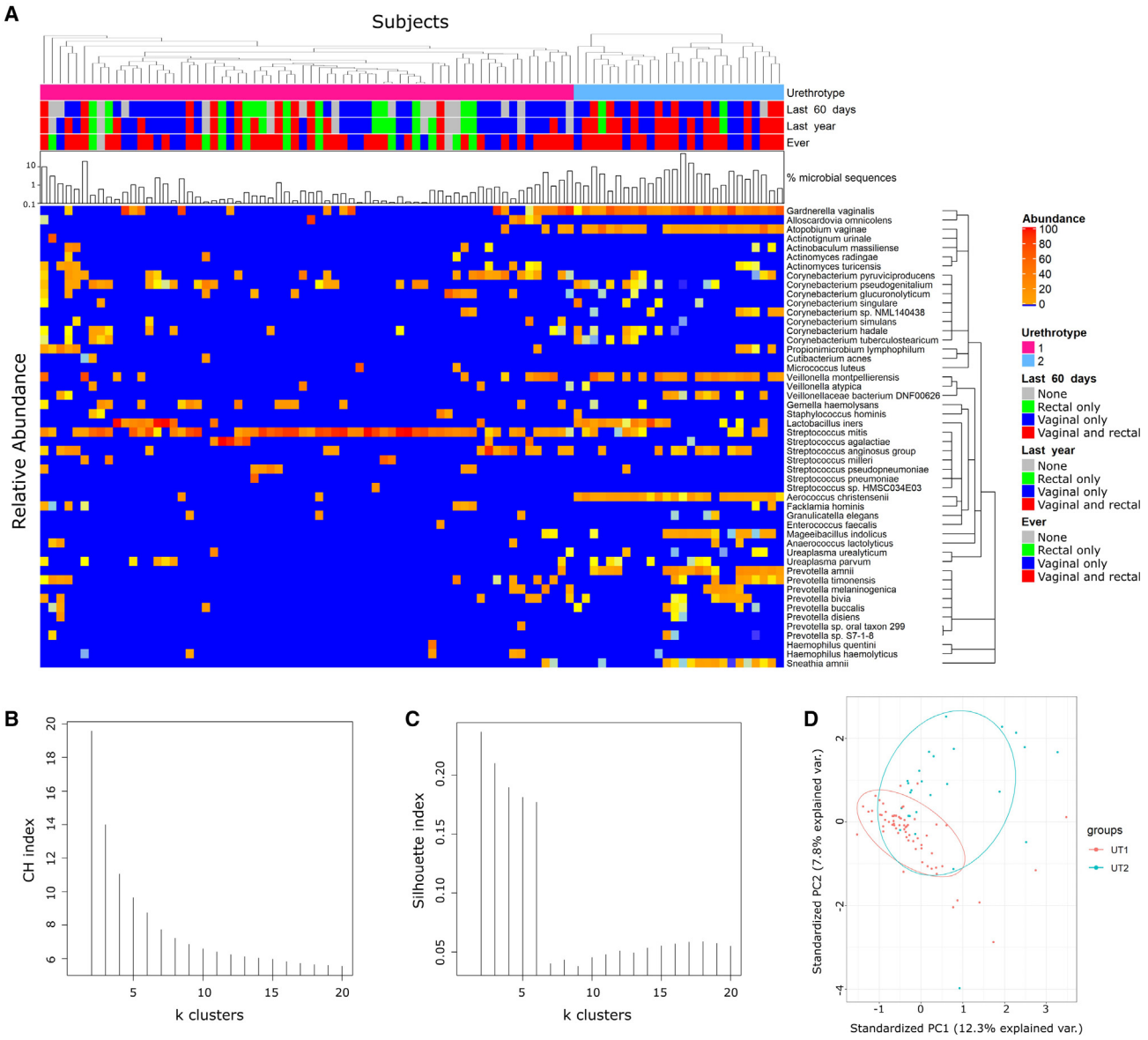

Figure 3. Clustering results based on Euclidian distance using ALR-transformed data reveals two urethrotype clusters

(A–D) (A) Heatmap of ALR-transformed proportions of the top 50 most abundant microbial taxa found in the penile urethral specimens of 92 men reveals two urethrotype clusters, UT1 and UT2. Metadata at the top of the heatmap include type of sexual activity (none, rectal only, vaginal only, vaginal and rectal sex) conducted at specific time intervals (last 60 days, last 1 year, lifetime) and urethrotypes (UT1 = pink, UT2 = blue). The bar graph depicts the absolute abundance of microbial sequences on a log scale. The colored bar indicates the relative abundance of a given species. As the color bar becomes redder, the relative abundance of the microorganism increases, (B) CH index analysis and (C) Silhouette analysis was used to determine the optimal number of UT clusters. (D) Relationships among communities visualized by principal-component analysis based on bacterial ALR abundance.

and sequences from GS04, associated with metronidazole treatment-refractory BV, were detected in several specimens.[55] Thus, phylogenetically distinct *G. vaginalis* strains can colonize the PU.

BVAB and core urethral bacteria may inhabit different PU niches

Given that the absolute number of bacteria in vaginal specimens from women with BV is higher compared with healthy women, and the reverse is true for women with AV,[56] the relationship between PU microbiome composition and ALR abundance was investigated. When all taxa were considered, their combined ALR abundance was significantly higher in UT2 compared with UT1 ($p = 3.8 \times 10^{-8}$, Wilcoxon's signed rank test). In contrast, the ALR abundance of core taxa was more similar ($p = 0.084$, Wilcoxon's signed rank test).

Competitive exclusion dictates that competition between sympatric species eventually leads to extinction of the less fit

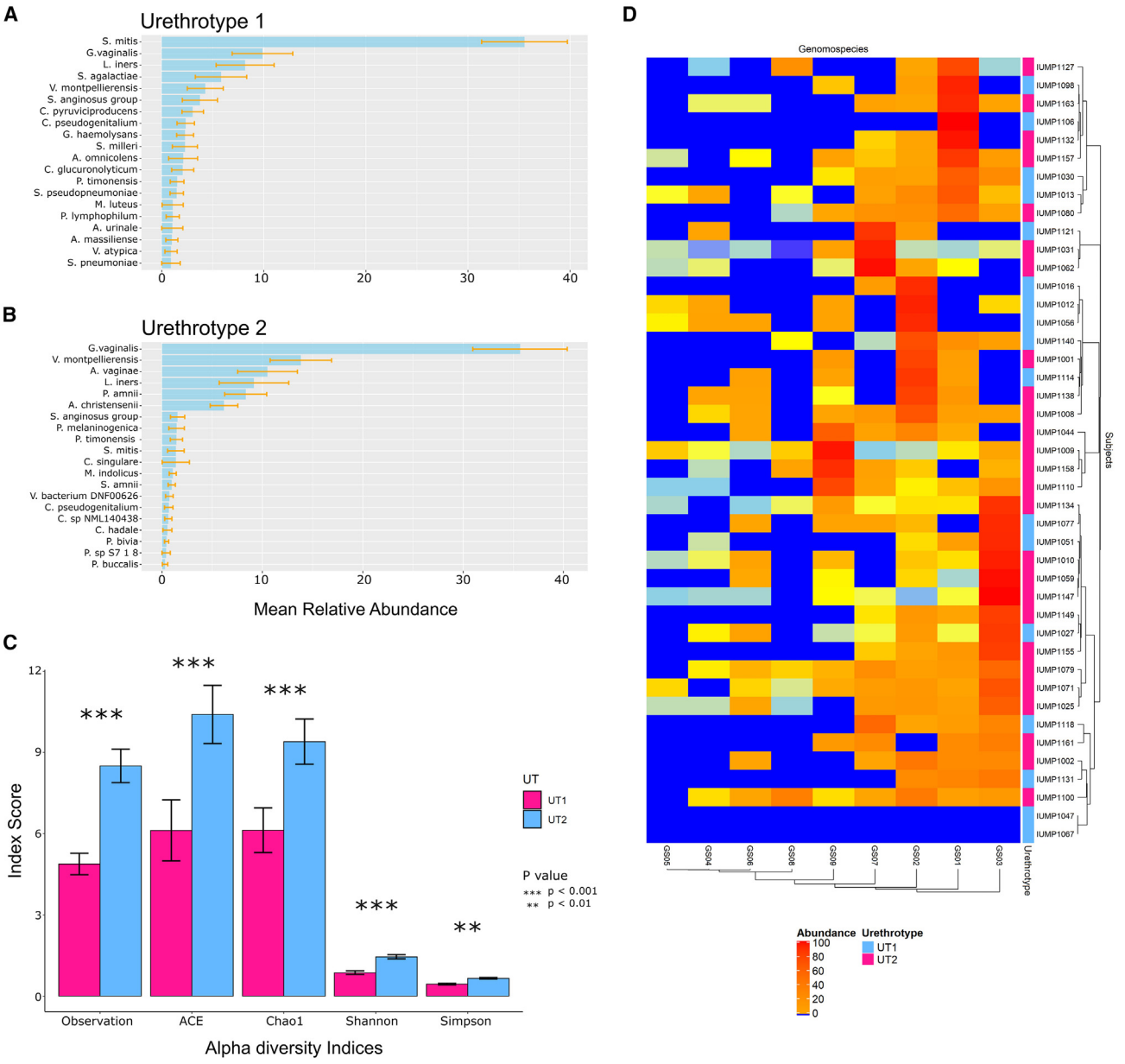

Figure 4. Species diversity (richness) of UT1 and UT2

(A) Alpha diversity between UT1 and UT2 measured by various indices. Significant p values are indicated (**p < 0.01, ***p < 0.001).

(B and C) (B) Top 20 most abundant bacterial species in UT1, (C) Top 20 most abundant bacterial species in UT2. Error bars indicate standard error.

(D) Heatmap with hierarchal clustering by Euclidean distance depicting the percentage of unique *Gardnerella* genomospecies-specific reads in participants in whom *Gardnerella* sequences were detected by MetaPhlAn3

species.[57] Two observations suggested that core bacteria and BVAB might inhabit different niches. First, many BVAB are obligate anaerobes, whereas most of the core bacteria that we detected are not.[58] Second, ALR abundance of core bacteria was similar in UT1 and UT2, suggesting that core organisms do not compete with BVAB. The taxa count data were analyzed using network analysis (SPIEC-EASI) (Figures 6A and 6B).[59] All the UT2 BVAB we identified were positively associated with, and many were connected to, one another by multiple edges in networks generated using either covariance selection (Glasso) or neighborhood selection (MB) approaches (Figures 6A and 6B) (Table S8). No edges were detected between *L. iners*, *S. mitis*, or other core LAB and other bacteria, indicating no strong ecological interactions among those taxa. In addition, gene ontology (GO) analysis determined that several genes and pathways that mediate anaerobic growth (e.g., fumarate reductase) and utilization of alternate urinary tract carbon and nitrogen sources (e.g., allantoin) were significantly enriched in the

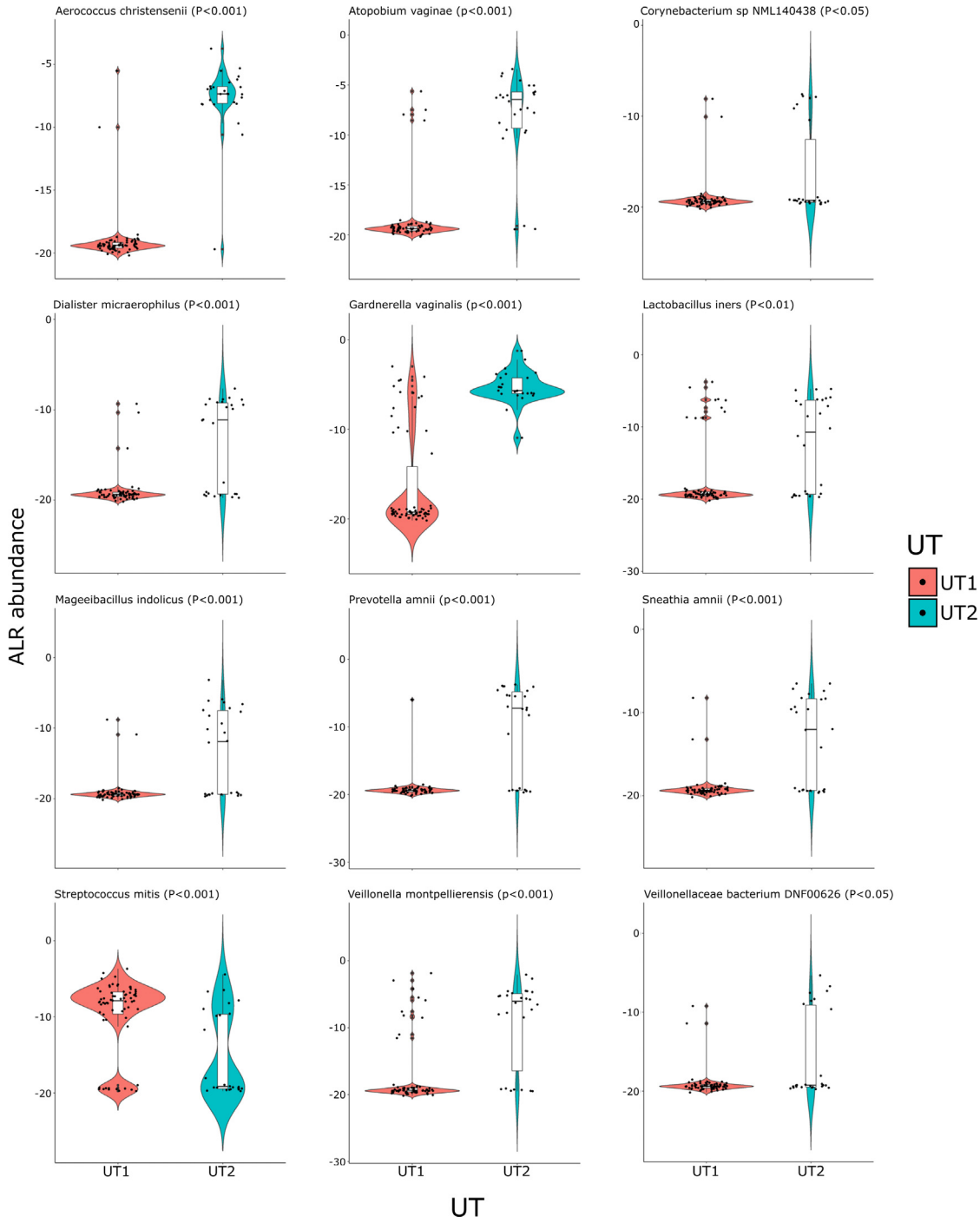

Figure 5. Violin plots showing the difference in ALR abundance of the 12 significant taxa between UT1 and UT2

The white bar represents the interquartile range, and the black bar represents the median value. Taxa with p value <0.05 were considered significantly different. The Wilcoxon Rank-Sum Test was used to generate the reported unadjusted p values. The violin plot outlines represent kernel probability density (the width of the shaded area represents the proportion of the data located there).

UT2 compared with the UT1 metagenome (Figures 6C–6E) (Table S8). Overall, these observations are consistent with the hypothesis that BVAB and core bacteria inhabit different niches in the urethra.

UT2, but not UT1, is associated with vaginal sex

Streptococcus spp. were among the most stable and abundant bacteria detected in the in urine of sexually inexperienced adolescents in one study,[17] whereas FRT bacteria were the most

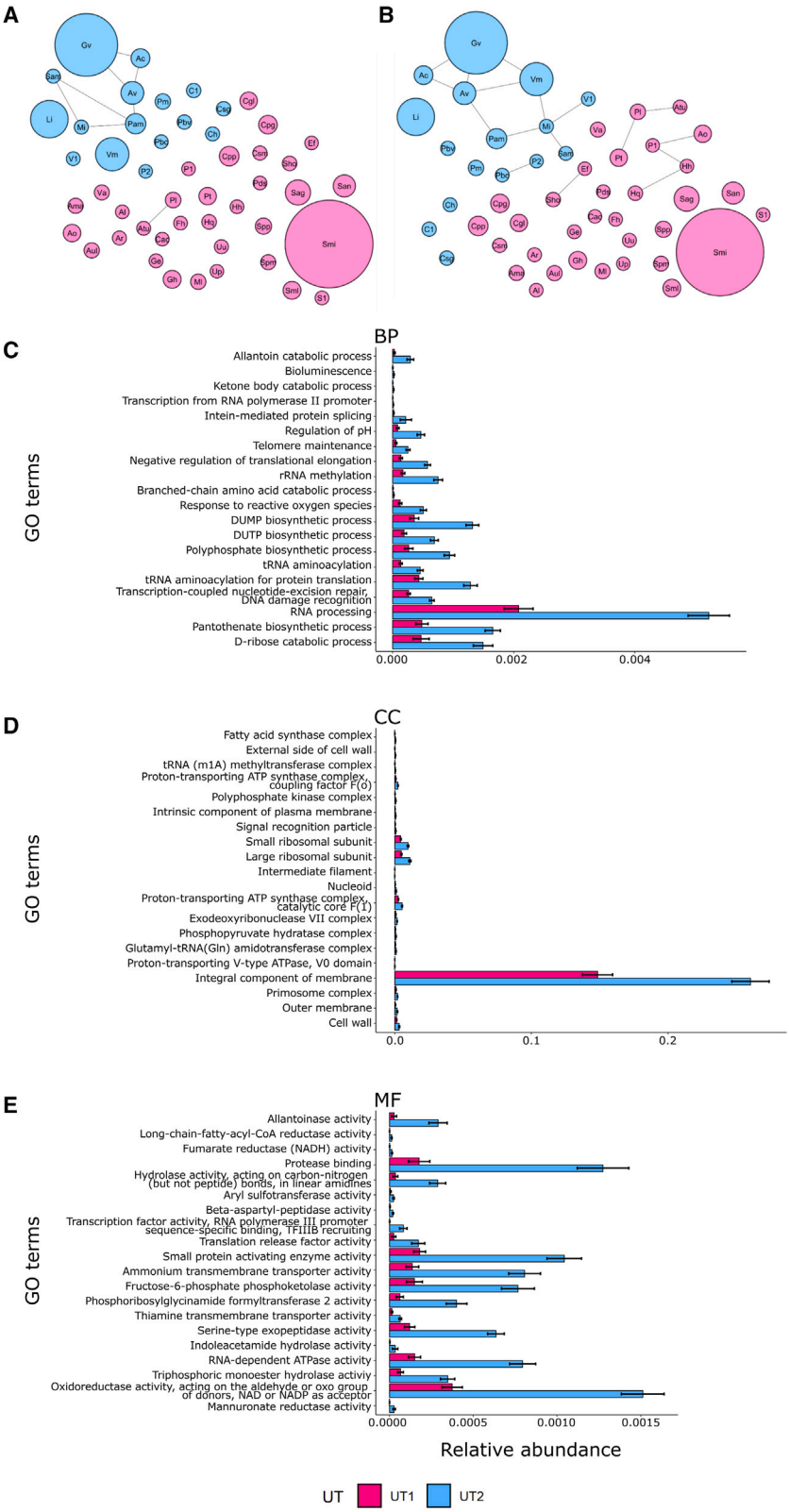

Figure 6. SPIEC-EASI network visualizations were generated by using two inference methods to construct a microbiome association network from all the bacterial operational taxonomic units (OTUs) (117)

Each node diameter is proportional to the mean of that OTU's relative abundance. Nodes are colored based on the urethrotype (UT1 = pink; UT2 = blue) in which the taxon is most abundant. Topology network using (A) graphical least absolute shrinkage and selection operator (Glasso), and (B) Meinshausen-Buhlmann's neighborhood selection (MB). Edges indicate the two nodes are connected. Taxa definitions: Ac = *Aerococcus christensenii*; Al = *Anaerococcus lactolyticus*; Ama = *Actinobaculum massiliense*; Ao = *Alloscardovia omnicolens*; Ar = *Actinomyces radingae*; Atu = *Actinomyces turicensis*; Aul = *Actinotignum urinale*; Av = *Atopobium vaginae*; Cac = *Cutibacterium acnes*; Cgl = *Corynebacterium glucuronolyticum*; Ch = *Corynebacterium hadale*; Cpg = *Corynebacterium pseudogenitalium*; Cpp = *Corynebacterium pyruviciproducens*; Csm = *Corynebacterium simulans*; Csg = *Corynebacterium singulare*; C1 = *Corynebacterium sp*. NML140438; Ef = *Enterococcus faecalis*; Fh = *Facklamia hominis*; Ge = *Gemella haemolysans*; Gv = *Gardnerella vaginalis*; Hh = *Haemophilus haemolyticus*; Hq = *Haemophilus quentini*; Li = *Lactobacillus iners*; Mi = *Mageeibacillus indolicus*; Ml = *Micrococcus luteus*; Pam = *Prevotella amnii*; Pbv = *Prevotella bivia*; Pbc = *Prevotella buccalis*; Pds = *Prevotella disiens*; Pl = *Propionimicrobium lymphophilum*; Pm = *Prevotella melaninogenica*; P1 = *Prevotella sp*. oral taxon 299; P2 = *Prevotella sp*. S7 18; Pt = *Prevotella timonensis*; Sag = *Streptococcus agalactiae*; Sam = *Sneathia amnii*; San = *Streptococcus anginosis* group; Sho = *Staphylococcus hominis*; Sml = *Streptococcus milleri*; Smi = *Streptococcus mitis*; Spm = *Streptococcus pneumoniae*; Spp = *Streptococcus pseudopneumoniae*; S1 = *Streptococcus sp*. HMSC034E03; Up = *Ureaplasma parvum*; Uu = *Ureaplasma urealyticum*; Va = *Veillonella atypica*; V1 = Veillonellaceae bacterium DNF00626; Vm = *Veillonella montpellierensis*.

(C–E) Relative abundance of top 20 significant gene ontology (GO) terms in UT1 and UT2 generated with HUMAnN 3.0 (all p values <0.001). GO terms were mapped from the gene families in the output, and the same GO terms of different taxa were combined. Wilcoxon signed rank test was used to identify the differentially abundant GO terms between UT1 and UT2, and the GO terms were ordered by p value. GO definitions: BP = Biological process; CC = Cellular component; MF = Molecular function.

prevalent bacteria detected in urogenital specimens in another study of adult male STI clinic attendees.[5,10]

Since the participants all completed detailed surveys, we tested if membership in UT1 or UT2 was associated with any demographic or STI risk factors, but failed to identify any significant associations (Table S9). However, use of self-reported sexual orientation to infer current and past patterns of sexual behavior has limitations[60] and we observed several instances where men who self-identified as heterosexual reported current or past same-sex behaviors as well as the reverse scenario (Table S3). Therefore, we tested if UT1 or UT2 associated with specific sexual behaviors in specific time intervals. UT2 was significantly associated with vaginal sex in the past 60 days (odds ratio [OR] = 6.01; 95% confidence interval [CI] 1.64–22.00), and in the past year (OR = 6.86; 95% CI 1.49–31.58), but not in an individual's lifetime (OR = 14.64; 95% CI 0.84–255.00) (Table S9). In contrast, insertive penile-anal (anal sex) and insertive penile-oral sex (oral sex) were not associated with UT1 or UT2 in these intervals (Table S9).

Specific bacteria are significantly associated with vaginal sex

Given that UT2 was associated with vaginal sex, we evaluated associations between specific bacteria, individual sexual behaviors, and combinations of sexual behaviors in three intervals (past 60 days, past year, ever) using participant survey data (Table S3). The odds of detecting several bacteria were elevated in men who reported vaginal sex (Table S9). For example, the odds of detecting *G. vaginalis* were higher in men who reported vaginal sex in the past 60 days (OR = 6.97; 95% CI 2.49–19.51), past year (OR = 11.79; 95% CI 3.21–43.30), or ever (OR = 35.5352; 95% CI 2.05–616.68) compared with the men who did not. Notably, in the 14 men who had never had vaginal sex, we did not detect *A. vaginae*, *P. amnii*, *L. iners*, *Streptococcus anginosus*, *M. indolicus*, *G. vaginalis*, *V. montpellierensis*, or *S. amnii*. Most of the associations we identified above remained significant when combinations of sexual behaviors were considered (Table S10). For example, the OR of detecting *G. vaginalis* in men who reported vaginal and rectal sex in the past year, compared with men who only reported rectal sex, was 12.09 (95% CI 2.31–63.42). Some bacteria were more prevalent in men who did not report vaginal sex. The OR of detecting *Corynebacterium glucuronolyticum* (OR = 0.27; 95% CI 0.08–0.91) was lower in men who reported vaginal sex in the past year compared with men who did not. A few bacteria were associated with rectal and oral sex, but these associations were comparatively weak and involved less prevalent bacteria (Table S10). All associations mentioned above remained significant when covariates including age, race, and urethral STI history were considered in multivariate analyses, and no associations were identified with non-sexual covariates (Table S11). Thus, colonization of the PU by many FRT bacteria may be contingent upon vaginal sex.

The effects of vaginal sex can be detected after extended intervals

To test if the associations with vaginal sex we observed were transient, we evaluated if any bacteria were more prevalent in men who had reported vaginal sex within 1 year, but not in the past 60 days (n = 6), compared with men who did not have vaginal sex in the past year (n = 26). Despite the small sample size, two FRT bacteria, *G. vaginalis* (OR = 7.67; 95% CI 1.04–56.77) and Veillonellaceae bacterium DNF00626 (OR = 29.44; 95% CI 1.20–719.88), were still associated with vaginal sex after 60 days, and several other BVAB were trending toward but did not reach significance (Tables S2 and S10). This indicated that bacteria remained associated with vaginal sex for at least 60 days after exposure. To assess if colonization with FRT bacteria persisted beyond a year, we tested if these organisms were enriched in men who reported vaginal sex in their lifetimes, but not the past year (n = 12), compared with men who never had vaginal sex (n = 14). FRT bacteria were detected in a few of the men who reported vaginal sex more than a year ago, but this comparison did not reach significance (Table S10).

Sexual behaviors significantly associate with variance in PU microbiome composition

Since specific bacteria were associated with vaginal sex, we investigated the contribution of specific sexual behaviors, relative to other variables we captured, on the variance in PU microbiome composition. Univariate PERMANOVA regression analysis was performed using the survey questions as independent variables. Except for sexual behavior, no other variables including age, race, and STI history were significant (data not shown). Next, sexual behaviors performed at different intervals (i.e., past 60 days, past year, ever, respectively) were treated as a polytomous variable (i.e., with the category of oral, rectal, vaginal sex, and their combinations). Polytomous sexual behavior in the past 60 days and year significantly contributed to the variance, and the effect of lifetime sexual behavior was trending toward significant ($p < 0.01$ within 60 days, $p < 0.02$ within 1 year, $p = 0.066$ ever), explaining 10.25%, 10.08%, and 4.74% of the variance of the PU microbiome composition, respectively (Table S11). Since sexual behavior was the key factor associated with PU composition, the effects of each individual sexual behavior were dissected using a multivariate PERMANOVA regression analysis where the behaviors were treated as separate independent variables (Table 1). Vaginal sex was the only independent variable associated significantly with variation in PU microbiome composition (p value <0.002 within 60 days, p value <0.001 within 1 year, and p value <0.005 ever) and explained 4.26%, 4.14%, and 3.37% of the variance in the past 60 days, past year, and ever, respectively.

DISCUSSION

PU microbiology is highly relevant to human health because the urethra can transmit STIs, and cryptic pathogens have been implicated in a variety of idiopathic urogenital syndromes.[11,61–64] Overall, our findings establish a baseline for studying this microbiome in male urogenital tract health and disease.

Consortia of LAB and *Corynebacterium* spp. similar to those we observed here have also been detected in adolescents[17] and adults,[65] so these may be resilient microbial communities. Alternately, these observations could reflect continuous seeding of the PU with microorganisms from other body surfaces without colonization.[17,18,66] In either case, the apparent lack of

Table 1. PERMANOVA regression analysis of effects of sexual behavior

	R^2	p value
A. Time of Behavior		
Past 60 days	0.1025	0.0060
Past 1 year	0.1008	0.0130
Lifetime	0.0474	0.0660
B. Time and Type of Behavior		
Oral 60 days	0.0096	0.5115
Vaginal 60 days	0.0426	0.0020
Rectal 60 days	0.0141	0.1588
Oral 1 year	0.0067	0.8432
Vaginal 1 year	0.0414	0.0010
Rectal 1 year	0.0182	0.0569
Vaginal lifetime	0.0337	0.0050
Rectal lifetime	0.0070	0.8821

A. Univariate effects at three time intervals (past 60 days, past year, lifetime). B. Univariate effects on all combinations of specific sexual behaviors (oral, vaginal, rectal) at three time intervals (past 60 days, past year, lifetime). R^2 depicts the proportion of variation in the data explained by the group being tested. P value indicates whether this result was a result of chance.

interactions between BVAB and core bacteria, similar loads of core bacteria in UT1 and UT2, numerous differences observed in the metagenomes of UT1 and UT2, and predicted oxygen requirements of BVAB and core bacteria all suggest that these groups of bacteria inhabit different urethral niches. We speculate that core bacteria colonize the urethral meatus where oxygen availability may be higher, whereas BVAB colonize the mucin-rich penile urethra.[67] Determining if the PU microbiota contributes to urethral health is a key area for future study. If PU core bacteria contribute to STI colonization resistance, like vaginal lactobacilli,[68] additional risks of the increasingly common use of broad-spectrum antimicrobials in STI prophylaxis need to be considered.[69] In contrast, if the PU microbiota is dispensable, broader application of prophylaxis may be warranted to eliminate male reservoirs of female urogenital pathogens.

Our observations suggest that PU colonization by FRT bacteria is contingent upon vaginal exposures and are consistent with prior reports that have documented suspected female to male transmission of undifferentiated *G. vaginalis* strains and BV biofilms.[70,71] Testing this and the reverse, if men can transmit these bacteria to women, directly will be difficult because this would ideally require sampling of sexual dyads before and after first partnered sexual activity, because it would be expected that existing dyads would already share readily transmissible microorganisms. Alternately, it might be possible to address these questions using a prospective dyad study design that incorporates interval antibiotic treatment of one or both partners or periods of voluntary abstinence. Nonetheless, incident BV is strongly associated with new male sex partners,[72] but attempts to prevent BV by treating male partners with antibiotics have been mostly unsuccessful.[73–78] Notably, none of these treatment trials assessed if all the BVAB we observed in men here were eliminated. Thus, improved understanding of how antibiotics impact the PU microbiota could inform future BV partner-treatment trials and provide insights into the role of sexual exposures in BV and other genitourinary syndromes.

Sexual behavior has been ignored in most human microbiome studies. Thus, our observation that sexual behavior explains more than 10% of variance in PU microbiota composition in specific intervals is even more striking when additional context is considered. Many participants in our study reported few, infrequent, or no vaginal exposures, and the prevalence of many FRT bacteria we identified is low in the overall adult female population. Much smaller effect sizes have been attributed to what are believed to be key drivers of the composition of other human microbiomes. For example, a recent study of more than 4,000 adults that considered hundreds of covariates was only able to account for 16% of gut microbiome variance.[79] However, the apparent powerful influence of vaginal sex on the PU microbiome may reflect that this site is less likely to be exposed to other sources of microorganisms than the skin and mucosal surfaces of the gut and lungs.

The associations between vaginal sex and BVAB that we observed may not be surprising in the context of what is known about the role of vaginal sex in the dispersal of sexually transmitted pathogens. The rarity of protective vaginal *Lactobacillus* spp. observed in our study specimens is consistent with some prior observations,[10,11,17] and may reflect that these bacteria persist in and seed the vagina from a gastrointestinal reservoir.[80,81] Our failure to detect microorganisms associated with oral and anal sex may not be due to differences in sample size; oral sex was more prevalent than vaginal sex in all intervals when partner gender was not considered (Table S3). We also failed to detect several known and putative sexually transmitted and urinary pathogens that have been observed in prior studies of men with urogenital disease or men in whom urethral inflammation was not evaluated.[11–16] Some of the differences might be explained by the more sensitive PCR approaches used in prior studies. Alternately, adaptations that permit specific BVAB to colonize the PU without eliciting inflammation may not be widely distributed, and we strictly excluded men with urethral/inflammation or disease. Natural history studies of the urethral microbiome in controlled settings seem warranted to differentiate these possibilities, considering their high public health significance.

We propose a model that integrates our findings with prior observations regarding the composition, development, and succession of urogenital microbiomes. Culture and metagenomic approaches have identified a broad array of bacterial taxa in pediatric vaginal and male urine specimens.[82–87] However, few common patterns have emerged across these studies, and there seems to be general agreement that pediatric urogenital microbiomes are disorganized and sparse. By adolescence, unknown behavioral and/or developmental changes in male individuals permit prolonged colonization of the distal PU by core microorganisms.[17] We hypothesize that vaginal sex might then promote colonization of the deeper PU by sexually transmitted microorganisms without replacement of the core PU microorganisms. In contrast, protective *Lactobacillus* spp., possibly from the rectum,[81] begin to stably colonize the vaginal mucosa,[88]

concomitant with an increase in glycogen levels proceeding menarche in adolescent females.[89] Sexually transmitted microorganisms may then be introduced by vaginal sex[71] or other types of sexual exposures.[90]

Limitations of the study

Our observations suggest that PU colonization by FRT bacteria is contingent upon vaginal exposures and are consistent with prior reports that documented suspected female to male transmission of undifferentiated *G. vaginalis* strains and BV biofilms.[70,71] Testing this and the reverse, if men can transmit these bacteria to women, could be difficult because this would ideally involve sampling of sexual dyads before and after first partnered sexual activity, as existing dyads might already share readily transmissible microorganisms. Alternately, it might be possible to address these questions using a prospective dyad study design that incorporates interval antibiotic treatment of one of the partners and/or periods of voluntary abstinence.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTERESTS

Y.X., X.G., T.A.B., B.V.D.P., N.G., L.J.F., J.D.F., and Q.D. have no conflicts of interest. S.J.J. has received honoraria and consulting fees from Hologic, Inc. E.T., J.A.W., and. D.E.N. retain the patent for the US_NmUC diagnostic assay used in this manuscript. C.A.M. is a consultant for Lupin Pharmaceuticals, BioFire Diagnostics, Cepheid, and PhagoMed. She has also received research funding support from Lupin Pharmaceuticals, Abbott Molecular, and Gilead as well as speaker honoraria from Abbott Molecular, Cepheid, Roche Diagnostics, and Becton Dickinson.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human-derived urethral swabs	This paper	
Human-derived vaginal swabs	Muzny et al. 2018[34]	
Chemicals, peptides, and recombinant proteins		
Lysozyme	Millipore Sigma	SAE0152
AMPure XP Reagent Beads	Beckman Coulter	A63881
Critical commercial assays		
DNeasy Blood & Tissue Kit	Qiagen	Cat. No. 69506
NexteraXT DNA Library Prep Kit	Illumina	FC-131-1096
NexteraXT Index Kit Set A	Illumina	FC-131-2001
Qubit 1X dsDNA HS Assay Kit	ThermoFisher	Q32854
Deposited data		
Raw metagenomic sequencing data of urethral swabs (human reads removed)	This paper	NCBI Bio Project Accession number: PRJNA785561
Raw metagenomic sequencing data of vaginal swabs (human reads removed)	This paper	NCBI Accession number: PRJNA707585
Sequences deposited	This paper	NCBI SRA Bio Sample accessions from SAMN23566502 to SAMN23566611
Oligonucleotides		
*Gardnerella vaginalis*_cpn60_Forward Primer- CGCATCTGCTAAGGATGTTG	Menard et al. 2008[91]	AF240579.3
*Gardnerella vaginalis*_cpn60_Reverse Primer- CAGCAATCTTTTCGCCAACT	Menard et al. 2008[91]	AF240579.3
*Gardnerella vaginalis*_cpn60_Probe- FAM-TGCAACTATTTCTGCAGCAGATCC-TAMRA	Menard et al. 2008[91]	AF240579.3
Recombinant DNA		
plasmid pGEMT-easy(cpn60)	This paper	
Mock community B, Even, Low Concentration v5.1L	BEI Resources, NIAID, NIH	HM782D
Software and algorithms		
Custom scripts for data analysis	This paper	https://github.com/qunfengdong/HealthyMaleUrethralMicrobiome
MetaPhlAn3	Beghini et al. 2021[23]	https://github.com/biobakery/biobakery/wiki/metaphlan3
Kraken2	Wood et al. 2019[36]	https://github.com/DerrickWood/kraken2
R	R Core Team 2022[92]	https://www.R-project.org/
R package "MASS"	Venables et al. 2002[93]	https://cran.r-project.org/web/packages/MASS/index.html
R package "microbiome"	Lahti, Shetty 2017[94]	https://www.bioconductor.org/packages/release/bioc/html/microbiome.html
R package "cluster"	Maechler et al. 2022[95]	https://cran.r-project.org/web/packages/cluster/index.html
R package "clusterSim"	Walesiak, Dudek 2020[96]	https://cran.r-project.org/web/packages/clusterSim/index.html
R package "circlize"	Gu et al. 2014[97]	https://cran.r-project.org/web/packages/circlize/index.html
R package "ComplexHeatmap"	Gu et al. 2016[98]; Gu 2022[99]	https://bioconductor.org/packages/release/bioc/html/ComplexHeatmap.html

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
R package "vegan"	https://github.com/vegandevs/vegan	https://cran.r-project.org/web/packages/vegan/index.html
R package "phyloseq"	McMurdie, Holmes 2013[100]	https://www.bioconductor.org/packages/release/bioc/html/phyloseq.html
R package "taxize"	Chamberlain, Szocs 2013[101]	https://cran.r-project.org/web/packages/taxize/index.html
R package "ggplot2"	Wickham 2016[102]	https://ggplot2.tidyverse.org
R package "ggbiplot"	https://github.com/vqv/ggbiplot	https://github.com/vqv/ggbiplot
R package "ggpubr"	https://rpkgs.datanovia.com/ggpubr/	https://rpkgs.datanovia.com/ggpubr/
Sparse InverseE Covariance Estimation for Ecological Association INference (SPIEC-EASI)	Kurtz et al. 2015[59]	https://github.com/zdk123/SpiecEasi
Cytoscape	Shannon et al. 2003[103]	https://cytoscape.org
Gardnerella genomospecies (GS) identification	Potter et al. 2019[54]	
HUMAnN 3.0	Beghini et al. 2021[23]	https://github.com/biobakery/humann
R package "epiR"		https://cran.r-project.org/web/packages/epiR/epiR.pdf
Microsoft Excel	Microsoft	https://www.microsoft.com/en-us/microsoft-365/excel
Prism	Graphpad	https://www.graphpad.com/scientific-software/prism/
Other		
HiSeq4000	Illumina	
NovaSeq6000	Illumina	

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, David E. Nelson (nelsonde@indiana.edu).

Materials availability

All primers, probe, and plasmid generated for this study are available from the lead contact with a completed Materials Transfer Agreement.

Data and code availability

Raw sequences from the PU specimens have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under Bio Project Accession number: PRJNA785561 (http://www.ncbi.nlm.nih.gov/bioproject/785561), and the vaginal specimen sequences are deposited under Accession number: PRJNA707585 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA707585/). All the sequences deposited in the NCBI SRA database are under the Bio Sample accessions from SAMN23566502 to SAMN23566611. All data supporting the findings of this study are included in the paper and supplemental information files.

Custom scripts are archived at GitHub (https://github.com/qunfengdong/HealthyMaleUrethralMicrobiome).

Any additional information required to reanalyze the data reported in this work paper is available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study population and cross-sectional design

This is an analysis of asymptomatic cis-gender men who enrolled as healthy controls in a case-control research study named the Idiopathic Urethritis Men's Project (IUMP), while undergoing STI screening, at the Bell Flower Clinic in Indianapolis, Indiana, between August 4th, 2016 and December 11th, 2019. Participants provided written informed consent for the collection of two distal urethral

swabs, followed by a first-catch urine specimen, and completed a detailed computer-assisted self-interviewing health and behavioral questionnaire (Methods S1, Table S1). A genital examination and a urethral Gram stain were performed to evaluate for urethral discharge, Gram-negative intracellular diplococci, and the number of polymorphonuclear leukocytes per high-power field (PMN/HPF). Participants were excluded if they tested positive for *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, or urethritis clade *Neisseria meningitidis* US_NmUC strains,[104] had urethral discharge, exhibited >1 PMN/HPF on their Gram stain smear, reported antibiotic use in the last month, or reported urethral symptoms. This study was approved by the Indiana University-Purdue University-Indianapolis (IUPUI) Institutional Review Board and the Marion County Public Health Department.

METHOD DETAILS

DNA extraction

DNA was extracted from urethral swabs using the DNeasy Blood and Tissue Kit (Qiagen, USA), and eluted using Tris-EDTA buffer. DNA was also extracted from molecular grade water spiked with a pure culture of *Thermus thermophilus* cells (an environmental hyperthermophile unlikely to be present in reagents or specimens) (reagent contamination control) or a mock bacterial community of known composition listed in the table below (HM782-D, BEI resources, NIAID, NIH) (Mock community B, Even, Low Concentration v5.1L) (extraction/annotation control). The mock bacterial community contains a pool of approximately 100 ng of the bacterial genomic DNA mixture (see table below) suspended in 25 μL TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH ∼ 7.4).

Organism	NCBI Reference Sequence
Acinetobacter baumannii, strain 5377	NC_009085
Actinomyces odontolyticus, strain 1A.21	NZ_AAYI02000000
Bacillus cereus, strain NRS 248	NC_003909
Bacteroides vulgatus, strain ATCC® 8482™	NC_009614
Clostridium beijerinckii, strain NCIMB 8052	NC_009617
Deinococcus radiodurans, strain R1 (smooth)	NC_001263, NC_001264
Enterococcus faecalis, strain OG1RF	NC_17316
Escherichia coli, strain K12, sub-strain MG1655	NC_000913
Helicobacter pylori, strain 26695	NC_000915
Lactobacillus gasseri, strain 63 AM	NC_008530
Listeria monocytogenes, strain EGDe	NC_003210
Neisseria meningitidis, strain MC58	NC_003112
Propionibacterium acnes, strain KPA171202	NC_006085
Pseudomonas aeruginosa, strain PAO1-LAC	NC_002516
Rhodobacter sphaeroides, strain ATH 2.4.1	NC_007493, NC_007494
Staphylococcus aureus, strain TCH1516	NC_010079
Staphylococcus epidermidis, FDA strain PCI 1200	NC_004461
Streptococcus agalactiae, strain 2603 V/R	NC_004116
Streptococcus mutans, strain UA159	NC_004350
Streptococcus pneumoniae, strain TIGR4	NC_003028

Vaginal swab DNA from a separate cohort of African American women enrolled in a prior incident bacterial vaginosis (iBV) study was also sequenced to test if our approaches could detect common FRT bacteria.[34]

Metagenomic shotgun sequencing

Dual-indexed sequencing libraries were prepared from 1 ng total swab or control DNA using the Nextera XT Library Preparation kit (Illumina Inc., USA), pooled, and pair end (2x150 b) sequenced on the Illumina HiSeq4000 or NovaSeq platforms at the Indiana University Center for Medical Genomics.

Data preprocessing

Most sequences from the 110 participants were human 27,092,065 ± 8,140,893 (86.6%), and 461,885 ± 1,411,457 (1.5%) were annotated to specific microorganisms. 18 participants with low or no bacteria reads (MetaPhlAn3 Bacteria/Human reads <= 0.01%) were subsequently removed from the downstream analyses. For the remaining 92 participants, the number of human and microbial sequences were 27,275,575 ± 8,461,033 (85.8%), and 551,858 ± 1,528,493 (1.7%) respectively.

Data analysis

MetaPhlAn3 taxa counts were subject to additive log transformation (ALR) using human sequences (the log of microbial counts plus 0.1 divided by human reads), and a default centered-log ratio (CLR) transformation by R package "microbiome". Urethrotype (UT) classification was performed with the partitioning around medoids clustering algorithm (pam by R package ‘‘cluster’’) based on Euclidean distances of both ALR (Figures 3 and S3) and CLR (Figure S2) transformed microbial taxon counts. Calinski-Harabasz (CH) and Silhouette index using the transformed ALR and CLR dataset were used to identify the optimal number of clusters,[105,45] which were designated as urethrotypes in this study (Figures 3, S2, and S3). In addition to pam, other R packages (clusterSim, circlize, ComplexHeatmap, vegan, phyloseq and taxize), and custom R scripts were used to generate the heatmaps for the ALR and CLR transformed data. Principal component analysis was performed and plotted by R using ALR transformed data.[41] Wilcoxon signed-rank test was used for detecting differential abundance in ALR transformed data and Fisher’s exact test was used to compare the prevalence of taxa between urethrotypes. R package vegan was used to calculate Chao I and Ace alpha diversity indices. Spearman correlations were calculated using *Gardnerella vaginalis* qPCR genome counts and ALR transformed sequence counts.

Taxonomic profiling analyses

Raw sequences were demultiplexed and annotated using MetaPhlAn3 and Kraken2 (Tables S2 and S3).[23,36] Human sequences were counted using Kraken2.[106] Only *T. thermophilus* sequences were detected in the reagent contamination control (Table S2). All microorganisms in the mock community were detected, confirming that our extraction approach could isolate genomic DNA from diverse bacteria, and our annotation approaches could detect these bacteria (Table S2).

Quantitative PCR

Gardnerella vaginalis-specific qPCR targeting the *cpn60* gene was performed using an Eppendorf Realplex4 cycler to quantify organism load, compared with a plasmid standard curve (pGEMT-easy(cpn60)), as previously described with modifications:[91] each reaction contained 0.25 nmol of probe, 0.5 nmol of gene-specific primers, 1 μl of extracted DNA, and 5 μl of 2XFastStart TaqMan Probe Master mix (Roche, USA) in a total of 10 μl. Results were expressed as copies of microorganism per sample.

Network visualization of positive associations

Raw taxa counts were further analyzed using a microbiome network analysis method, Sparse InversE Covariance Estimation for Ecological Association INference (SPIEC-EASI),[59] by applying either covariance selection (Glasso) or neighborhood selection (MB). The network was visualized using Cytoscape.[103]

Gardnerella genomospecies identification

PU sequences that matched nine *Gardnerella* genomospecies (GS) were identified using a modification of an approach that was developed to define these GS and identify GS-specific sequences (Potter et al., 2019). After human sequences were removed, *Gardnerella* sequences from the PU specimens were compared with the nine GS using NCBI BLAST+2.12.0. (Sayers et al., 2022). First, unique matches that mapped to only one GS were identified. Perfect matches that contained no mismatches or gaps were then identified from the unique matches. Those perfect matches were then blasted against the NCBI nt nucleotide database to check if they also matched perfectly to other non *Gardnerella* bacteria; if so, they were removed from the subsequent analysis. The numbers and proportions of the remaining GS-specific sequences for each subject were calculated.

Functional metabolic profiling

Raw PU sequences were also used to determine the abundance of microbial genes corresponding to specific metabolic pathways using HUMAnN 3.0 (Beghini et al., 2021). Gene Ontology (GO) terms were mapped from the corresponding genes in the HUMAnN output. The same GO terms from different taxa were then combined, and Wilcoxon signed rank test was used to identify differentially abundant GO terms between UT1 and UT2.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analysis was performed using R statistical programing, GraphPad Prism 9, and Microsoft Excel.

Chi-square tests were used to examine if there were associations between UTs and any of the individual items in the questionnaire, and p-values were adjusted by Benjamini-Hochberg correction.[107] Confusion matrices (with 0.5 added to each cell if at least 1 cell was 0) were then used to calculate odds ratios (ORs) with 95% confidence intervals (CI) between UTs and sexual behaviors (vaginal, rectal, and oral sex in the past 60 days, past year or lifetime) using R package ‘‘epiR’’.

Permutational multivariate analyses of variance (PERMANOVA) regressions were performed on the Euclidean distance matrix by R package vegan, derived from the ALR transformed taxa, to identify factors associated with variation in microbial composition.[108] Questionnaire covariates were individually tested in the univariate PERMANOVA regression, and p-values were adjusted by Benjamini-Hochberg correction.[107] Sexual behaviors at different intervals (past 60 days, past year, lifetime), were treated as a polytomous variable (i.e., multiple categories: none, vaginal-only, rectal-only, oral-only, oral-vaginal, oral-rectal, vaginal-rectal, and oral-rectal-vaginal) in the univariate PERMANOVA regression models. Finally, individual sexual behaviors were treated as separate independent variables in a multivariate PERMANOVA regression model.

Custom R scripts and confusion matrices (with 0.5 added to each cell if at least 1 cell was 0) for each taxon and sexual behavior (vaginal, rectal, oral, vaginal and rectal, vaginal and oral, rectal and oral, within the same time periods as above) were used to calculate ORs to test for association of individual taxa with specific sexual behaviors. The same taxa (response variable) and sexual behaviors (explanatory variables) were used in logistic regression models with covariates (age, race, STI diagnosis in the past 60 days and lifetime, history of self-reported chlamydia, gonorrhea, trichomoniasis, syphilis or NGU) to test if any of those covariates were significantly associated with the taxa. To investigate whether any taxa were associated with vaginal sex in certain time intervals, we also calculated ORs for 1) vaginal sex between the last 60 days and 1 year versus no vaginal sex in the last year, and 2) vaginal sex ever but not in the last year versus no vaginal sex ever.

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Supplemental information

Sexual behavior shapes male genitourinary microbiome composition

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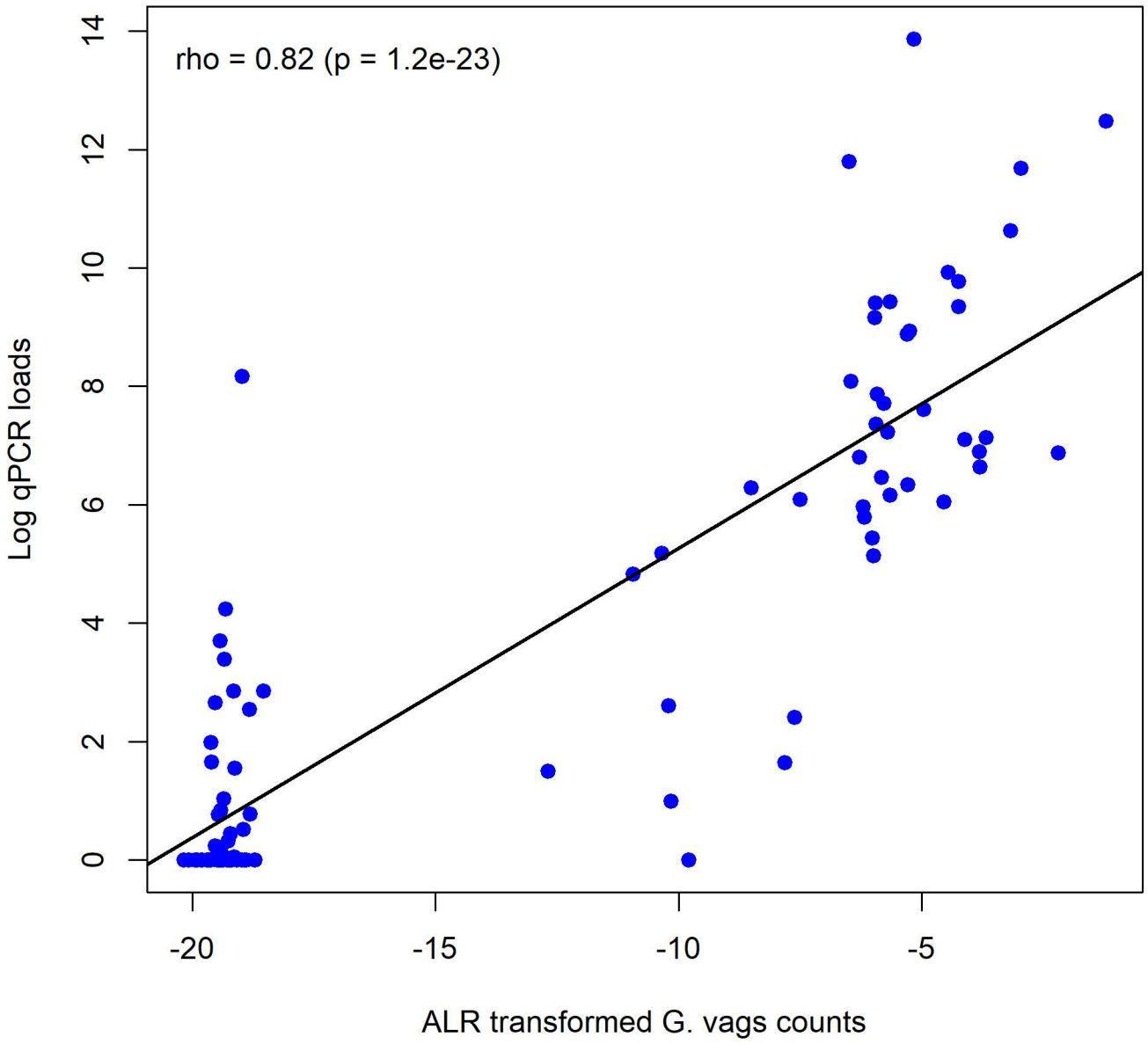
Supplemental Information.

Supplementary Table S1, related to Figure 1. IUMP Study Participant Characteristics (N = 110)

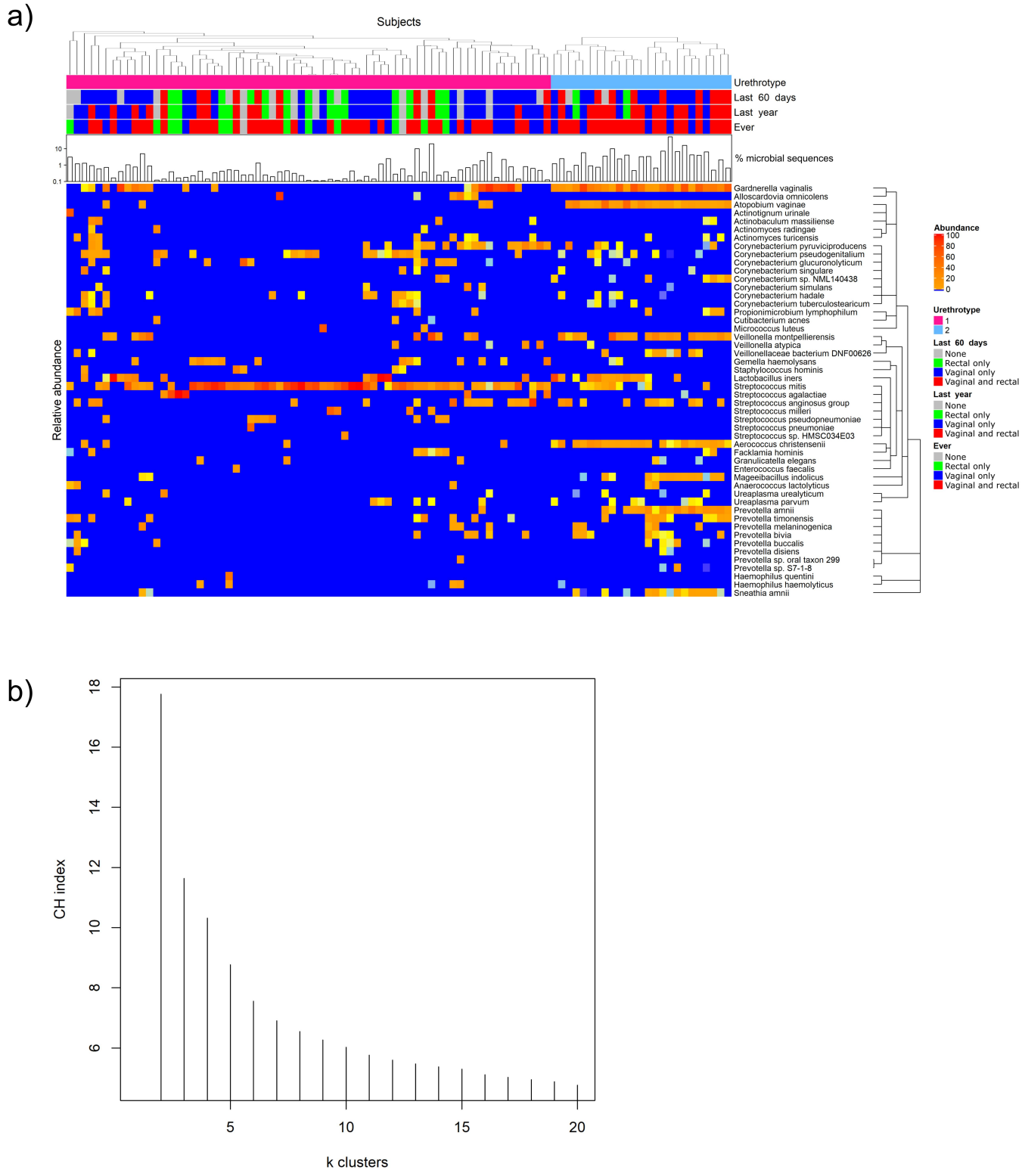
Characteristics	N (%)
Age, median (IQR)	28.7 (24.5–36.8)
Race	
Black/African American	38 (35%)
White	58 (53%)
Other	14 (13%)
Ethnicity	
Non-Hispanic	98 (89%)
Hispanic	12 (11%)
Self-reported sexual orientation	
Heterosexual	75 (68%)
MSM	22 (20%)
Other	13 (12%)
Prior self-reported history of STI	
Chlamydia (N = 106)	33 (31%)
Gonorrhea (N = 106)	26 (25%)
Trichomoniasis (N = 107)	5 (5%)
Herpes (N = 105)	5 (5%)
Syphilis (N = 105)	5 (5%)
NGU (N = 105)	11 (10%)
Genital warts (N = 107)	7 (7%)
Vaginal sex, most recent	
Never	15 (14%)
Within prior 60 days	71 (65%)
Within prior 1 year	8 (7%)
Lifetime/more than prior 1 year	15 (14%)
Received oral sex, most recent	
Never	1 (1%)
Within prior 60 days	90 (82%)
Within prior 1 year	9 (8%)
Lifetime/more than prior 1 year	10 (9%)
Insertive anal sex, most recent	
Never	29 (26%)
Within prior 60 days	41 (37%)
Within prior 1 year	13 (12%)
Lifetime/more than prior 1 year	27 (25%)
Reason for visit	
Diagnosed with STI	1 (1%)
Genital symptoms	3 (3%)
Worried about STI	21 (19%)
Partner diagnosed with STI	5 (5%)
General checkup/other	80 (73%)

Supplementary Table S2, related to Table 1 and STAR Methods: Temporal Odds Ratios of microorganisms that are significantly associated with vaginal sex at three-time intervals.

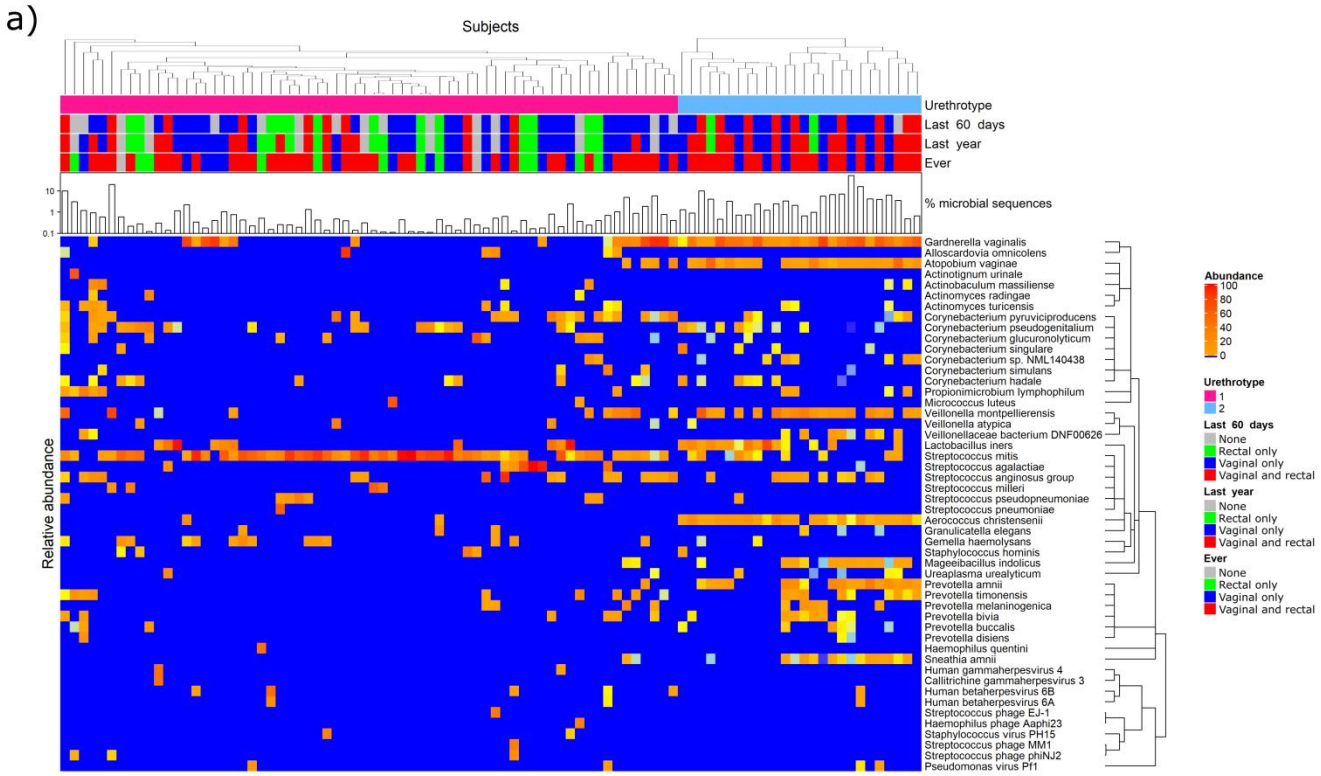
Taxa	Time	OR	CI2.5	CI97.5
Actinomyces radingae	Ever	0.0779	0.0065	0.9271
Aerococcus christensenii	1yr	4.381	1.1898	16.1309
Aerococcus christensenii	60d	4.3514	1.3508	14.017
Atopobium vaginae	1yr	4.381	1.1898	16.1309
Atopobium vaginae	60d	3.1263	1.052	9.2907
Corynebacterium glucuronolyticum	1yr	0.2714	0.0812	0.9069
Corynebacterium glucuronolyticum	60d	0.2727	0.0808	0.92
Cutibacterium acnes	Ever	0.0779	0.0065	0.9271
Gardnerella vaginalis	1yr	11.7949	3.2129	43.3004
Gardnerella vaginalis	60d	6.971	2.4912	19.5067
Gardnerella vaginalis	Ever	35.5352	2.0476	616.6845
Haemophilus parainfluenzae	Ever	0.0965	0.0145	0.6436
Haemophilus sp. HMSC71H05	Ever	0.0779	0.0065	0.9271
Lactobacillus iners	1yr	6.4186	1.3916	29.6059
Lactobacillus iners	60d	3.7692	1.1648	12.1968
Mageeibacillus indolicus	60d	10.3333	1.297	82.3244
Prevotella amnii	1yr	8.6735	1.0906	68.978
Prevotella amnii	60d	5.4545	1.1676	25.481
Staphylococcus hominis	Ever	0.0965	0.0145	0.6436
Streptococcus anginosus group	1yr	4.1008	1.1117	15.1272
Ureaplasma parvum	60d	20.2688	1.1671	352.0132
Veillonella montpellierensis	1yr	4.9833	1.3574	18.2944
Veillonella montpellierensis	60d	5	1.5571	16.0554

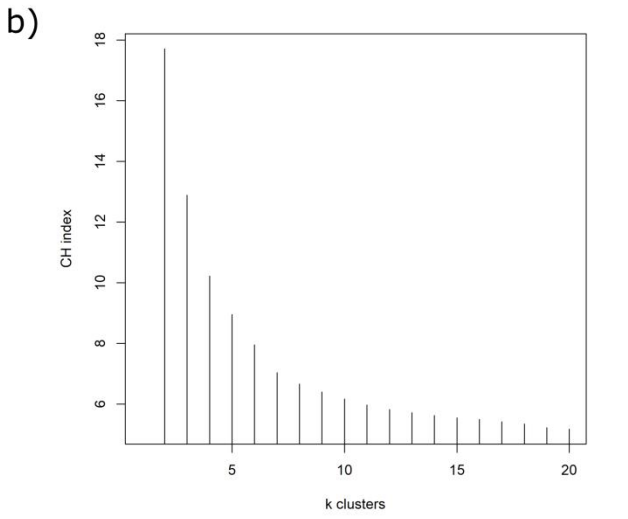


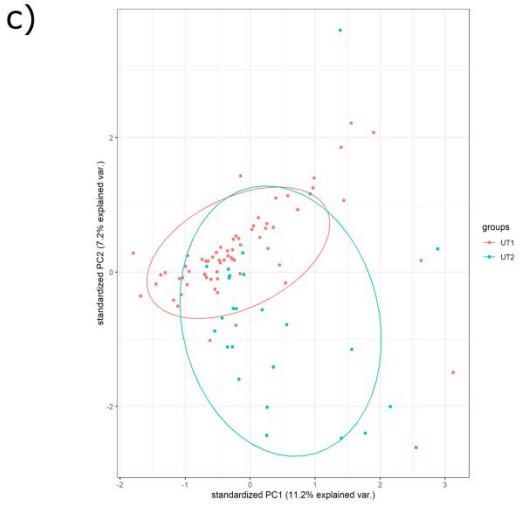
Supplementary Figure 1, Related to Figure 3 and STAR Methods: The Spearman's correlation coefficient of *Gardnerella vaginalis* QPCR genome counts and corresponding WGS reads illustrates strong positive correlations, validating the ALR transformation approach.



Supplementary Figure 2, Related to Figure 3 and STAR Methods: Clustering results based on Euclidian distance using CLR-transformed data reveals two urethrotype clusters. A) Heatmap of CLR-transformed proportions of the top 50 most abundant microbial taxa found in the PU specimens of 92 participants reveals two UT clusters UT1 and UT2. Metadata at the top of the heat map include type of sexual activity (none, rectal only, vaginal only, vaginal and rectal sex) in specific time intervals (last 60 days, last 1 year, lifetime) and urethrotypes (UT1 = pink, UT2 = blue). The bar graph depicts the absolute abundance of microbial sequences on a log scale. The intensity of the red scale bar correlates with the relative abundance of a given species. Darker red indicates higher relative abundance, B) CH index analysis was used to determine the optimal number of UT clusters.







Supplementary Figure 3, Related to Figure 3 and STAR Methods: Clustering results based on Euclidian distance using ALR-transformed data reveals two urethrotype clusters. A) Heatmap of ALR-transformed proportions of the top 50 most abundant microbial taxa found in PU specimens from 92 participants reveals two UT clusters UT1 and UT2. Metadata at the top of the heat map include type of sexual activity (none, rectal only, vaginal only, vaginal and rectal sex) in specific time intervals (last 60 days, last 1 year, lifetime) and urethrotypes (UT1 = pink, UT2 = blue). The bar graph depicts the absolute abundance of microbial sequences on a log scale. The intensity of the red scale bar correlates with the relative abundance of a given species. Darker red indicates higher relative abundance, B) CH index analysis was used to determine the optimal number of UT clusters, C) Relationships among communities visualized by principal component analysis based on bacterial ALR abundance.

Supplementary Methods S1, Related to Figure 1 and STAR Methods: Complete IUMP participant study survey.

Clinical History- Research Nurse

Record ID

[To AVOID DUPLICATES ALWAYS FIRST CHECK the Report 'Participant Identifiers And Time Point (Baseline/Followup)']

_____
(ALWAYS FIRST CHECK the Report 'Participant Identifiers And Time Point (Baseline/Followup)')

Visit

◯ Baseline
◯ Follow up
◯ Second Follow up

Data Form being filled by

◯
◯
◯
◯
◯
◯
◯

Please specify other not listed

_____

Is this participant a case or control?

◯ Case
◯ Control

Visit Date

_____
(MM/DD/YYYY)

1. Are you having eye symptoms?

◯ No
◯ Yes

2. If yes, what eye symptoms are you having?

☐ Erythema
☐ Pain, please describe:
☐ Blurry vision
☐ Increased tearing
☐ Discharge, please describe:
☐ Other, please describe:

2a.Please describe pain

_____

2b.Please describe discharge

_____

2c.Please specify other not listed

_____

3. Are you having throat symptoms?

◯ No
◯ Yes

4. If yes, what throat symptoms are you having?

☐ Sore throat
☐ Erythema
☐ Difficulties swallowing
☐ Swollen tonsils
☐ Exudate
☐ Other, please describe:

4a.Please specify other not listed

_____

5. Are you having any abdominal symptoms?

◯ No
◯ Yes

6. If yes, what abdominal symptoms are you having?

☐ Abdominal pain, describe (location and characteristics of the pain):
☐ Diarrhea
☐ Bloody stools
☐ Nausea
☐ Vomiting
☐ Other, please describe

6a.Please specify other not listed

_____

6b. Please describe location of abdominal pain

_____

6c. Please describe characteristics of the abdominal pain

_____

7. Are you having penile/urethral symptoms?

◯ No
◯ Yes

8. If yes, what penile/urethral symptoms are you having?

☐ Burning or tingling
☐ Itching
☐ Dysuria (pain when you pee)
☐ Erythema around meatus
☐ Discharge
☐ Lesions
☐ Other, please describe

8a.Please specify number of Lesions

◯ 1 - 5
◯ 6 - 10
◯ >10

8b.Please specify other not listed

_____

9. Are you having scrotal/testicular symptoms?

◯ No
◯ Yes

10. If yes, what scrotal/testicular symptoms are you having?

☐ Pain
☐ Lesions
☐ 'Bumps' or masses, describe (right or left or both sides, painful vs not painful):
☐ Swelling, describe (right or left or both sides):
☐ Other, please describe

10a.Please specify other not listed

_____

10b.Please describe side of 'Bumps' or masses

◯ Right side
◯ Left side
◯ Both right and left side

10c.Please describe pain of 'Bumps' or masses

◯ Painful
◯ Not painful

10d.Please indicate where swelling

◯ Right side
◯ Left side
◯ Both right and left side

11. Are you having anal/rectal/butt symptoms?

◯ No
◯ Yes

12. If yes, what anal/butt symptoms are you having?

☐ Discharge
☐ Bleeding
☐ Pain, describe (all the time, with defecation,etc):
☐ Other, please describe

12a.Please specify other not listed

_____

12b. Please describe the pain

◯ All the time
◯ With defecation
◯ Other

12b. Please describe the pain other not described above

_____

13. Are you having any problems with your skin?

◯ No
◯ Yes

14. If yes, what problems with your skin are you having?

☐ Rash, please describe (time frame ongoing):
☐ Dryness
☐ Pruritis
☐ Other, please describe

14a.Please specify other not listed

_____

14b.Please indicate start date of rash

_____
(MM/DD/YYYY)

14c.Did the rash stop or is it ongoing?

◯ Rash stopped
◯ Rash ongoing

14d.Please indicate stop date of rash

_____
(MM/DD/YYYY)

Clinical Examination - Research Nurse

Visit

◯ Baseline
◯ Follow up

Data Form being filled by

◯
◯
◯
◯
◯
◯
◯

Please specify other reason not listed

_____

Visit Date

_____
(MM/DD/YYYY)

Eyes:

☐ WNL
☐ Conjunctival injection (right, left, bilateral)
☐ Tearing (right, left, bilateral)
☐ Discharge (right, left, bilateral; color, quantity)
☐ Other findings (please describe):

Eyes:Conjunctival injection

◯ right
◯ left
◯ bilateral

Eyes:Tearing

◯ right
◯ left
◯ bilateral

Eyes:Discharge

◯ right
◯ left
◯ bilateral

Eyes: Discharge color

◯ Watery/clear
◯ Purulent
◯ Other

Please specify other not listed

_____

Eyes: Discharge quantity

◯ Small
◯ Moderate
◯ Copious

Eyes: Please describe other not listed

_____

Oropharynx:

☐ WNL
☐ Erythema
☐ Ulceration
☐ Exudates
☐ Lesions
☐ Other findings (please describe):

Please describe other oropharynx not listed

_____

Pubic hair:

☐ WNL
☐ Nits
☐ Other, please describe

Pubic hair WNL:

◯ Normal
◯ Shaved
◯ Waxed

Pubic hair Nits:

◯ No
◯ Yes

Pubic Hair: Please describe other not listed

_____

Penis:

☐ WNL
☐ Discharge
☐ Meatal erythema
☐ Lesions
☐ Other, please describe

Penis: WNL

◯ Circumcised
◯ Uncircumcised

Penis: Discharge

☐ None
☐ Minimal (with stripping only)
☐ Moderate
☐ Copious
☐ Clear
☐ Yellow/green
☐ White

Penis: Discharge

◯ None
◯ Minimal (with stripping only)
◯ Small
◯ Moderate
◯ Copious

Penis: Discharge Color

◯ Clear
◯ Yellow/green
◯ White

Penis: Meatal erythema

◯ No
◯ Yes

Penis: Lesions

☐ None
☐ Ulceration
☐ Genital warts
☐ Erythema
☐ Edema
☐ Other (describe):

Penis: Please specify other for lesions not described above

_____

Penis: Please specify other for not described above

_____

Penis: Location of lesions

☐ Peri-meatal
☐ Shaft
☐ Scrotum
☐ Suprapubic area
☐ Inguinal region
☐ N/A

Penis: Number of lesions

◯ 1 - 5
◯ 6 - 10
◯ >10

Scrotum and contents:

☐ WNL
☐ Epididymal tenderness
☐ Testicular tenderness
☐ Testicular mass
☐ Swelling
☐ Other, please describe

Scrotum and contents:Epididymal tenderness

◯ Right
◯ Left
◯ Bilateral
◯ None

Scrotum and contents:Testicular tenderness

◯ Right
◯ Left
◯ Bilateral
◯ None

Scrotum and contents:Testicular mass

◯ Right
◯ Left
◯ Bilateral
◯ None

Scrotum and contents:Swelling

◯ Right
◯ Left
◯ Bilateral
◯ None

Scrotum and contents: Please list other not listed above

External rectal exam:

☐ WNL
☐ Discharge, describe (color, quantity):
☐ Erythema
☐ Lesion

External rectal exam: Discharge Color

◯ watery/clear
◯ mucoid
◯ purulent

External rectal exam: Discharge Color

◯ small
◯ moderate
◯ copious

External rectal exam: Lesion

☐ None
☐ Ulceration (describe)
☐ Warts (describe)
☐ External hemorrhoid

External rectal exam: Number of Lesion

◯ 1 - 5
◯ 6 - 10
◯ >10

Skin:

☐ WNL
☐ Rash, describe:
☐ Erythema
☐ Other, please describe

Skin: Please describe other not listed

_____

Inguinal Lymph Nodes:

☐ WNL (not palpable)
☐ Nodes felt (describe size, tenderness)
☐ Other, please describe

Inguinal Lymph Nodes: describe size

_____

Inguinal Lymph Nodes: Tenderness

◯ No
◯ Yes

Inguinal Lymph Nodes: Please describe other not listed

_____

Bell Flower Urethral swab Gram Stain results

☐ None recorded
☐ < 2 WBCs, no GNID.
☐ 2-4 WBCs, no GNID.
☐ < 5 WBCs, no GNID.
☐ >5 WBCs, no GNID.
☐ >5 WBCs, positive for GNID.

Bell Flower Results

◯ Positive
◯ Negative
◯ Indeterminate

Study Samples Obtained:Remainder swab from initial urethral swab for Gram's stain

◯ No
◯ Yes

Study Samples Obtained:Urethral swab for Microbiome

◯ No
◯ Yes

Study Samples Obtained: First catch urine after swabs obtained

◯ No
◯ Yes

Study Samples Obtained: Saliva sample in collection kit (if subject consented)

◯ No
◯ Yes

Study Samples Obtained: rectal swab (if subject consented)

◯ No
◯ Yes

If yes rectal swab obtained, who obtained it?

◯ Participant obtained
◯ Clinician obtained

Treatment Provided

☐ Azithromycin 1000 mg orally directly observed in clinic
☐ Doxycycline 100 mg bid orally for 7 days
☐ Metronidazole 2 g orally directly observed in clinic
☐ None (controls)
☐ Other (specify):

Please specify other not listed

_____

Date and Time for Follow Up Appointment (cases only)

_____
(MM/DD/YYYY)

Enrollment Questionnaire Cases and Controls

Please complete the survey below.

Thank you!

---

1. Date of Visit

_____
(MM/DD/YYYY)

---

2. How old are you?

_____
(in years)

---

3. What is your birthdate?

_____
(MM/DD/YYYY)

---

4. What is your race?

◯ American Indian or Alaskan Native
◯ Asian
◯ Black or African American
◯ Native Hawaiian or other Pacific Islander
◯ White
◯ More than one race
◯ Other

4a. Please specify other

_____

---

5. This is about Hispanic ethnicity. Are you of Spanish, Hispanic, or Latino descent?

◯ No, I am not
◯ Yes, Mexican, Mexican-American Chicano
◯ Yes, Puerto Rican
◯ Yes, Cuban
◯ Yes, Central American
◯ Yes, South American
◯ Yes, Caribbean
◯ Yes, Other Spanish/Hispanic/Latino

6a. Which of the following best describes your current relationship status?

◯ Single and not dating
◯ Single and dating/hanging out with someone
◯ In a relationship but not living together
◯ Living together but not married
◯ Married and living together
◯ Married but not living together

6b. Is your current dating/relationship partner a:

◯ Man
◯ Woman

7. What is the highest level of education you completed (how much school did you complete)?

◯ No school or kindergarten
◯ 1st grade
◯ 2nd grade
◯ 3rd grade
◯ 4th grade
◯ 5th grade
◯ 6th grade
◯ 7th grade
◯ 8th grade
◯ 9th grade
◯ 10th grade
◯ 11th grade
◯ 12th grade/High school diploma/GED
◯ Vocational school (i.e. technical/secretarial/business)
◯ 1 year of college
◯ 2 years of college
◯ 3 years of college
◯ Graduated from college with a 4year degree
◯ At least some graduate work
◯ Completed a graduate degree

8. Are you currently a student?

◯ No
◯ Yes

9. Do you currently have a job?

◯ No
◯ Yes

10. What is the ZIP code of the area where you currently live?

_____

11. What is the main reason you came to the clinic today?

[Please select the one answer on the list below that comes closest to your main reason for coming]

◯ I am having genital symptoms (discharge from my penis, frequent or painful urination; burning/stinging/tingling/itching of the opening of my penis)
◯ I am worried that I might have a sexually transmitted infection
◯ I am a sexual partner of a person who has been diagnosed with a sexually transmitted infection
◯ I had a sexually transmitted infection and the doctor asked me to come back to be checked or treated
◯ I am here for a routine check to be tested for a sexually transmitted infection
◯ I need a general check-up-and physical exam
◯ Other [please describe]

11a. Please specify other

_____

12. Are there other reasons you came today?

	No	Yes
12a. I am having genital symptoms (discharge from my penis, frequent or painful urination; burning/stinging/tingling/itching of the opening of my penis	◯	◯
12b. I am worried that I might have a sexually transmitted infection	◯	◯
12c. I am a sexual partner of a person who has been diagnosed with a sexually transmitted infection	◯	◯
12d. I had a sexually transmitted infection and the doctor asked me to come back to be checked or treated	◯	◯
12e. I am here for a routine check to be tested for a sexually transmitted infection	◯	◯
12f. I need a general check-up and physical exam	◯	◯
12g. Other [please describe]	◯	◯

12g.Please specify other

_____

13. If you are having genital symptoms today, when did you first notice that something was wrong?

◯ Three days ago or less
◯ Four to seven days ago (more than 3 days but up to a week)
◯ Seven to fourteen days (1-2 weeks)
◯ More than fourteen days ago (2 weeks or more)
◯ Don't remember

14. Have you ever smoked cigarettes?

◯ No
◯ Yes

14a. How many years did you smoke cigarettes?

_____
(years)

14b. Do you currently smoke cigarettes?

◯ No
◯ Yes

14c. How many packs per day?

_____
(per day)

15. Have you ever consumed alcohol?

◯ No
◯ Yes

15a. Do you currently drink alcohol?

◯ No
◯ Yes

15b. On average, how often do you have drinks containing alcohol? One drink equals 1 bottle/glass of beer, 1 glass of wine, or 1 shot of liquor.

◯ Never
◯ Monthly or less
◯ 2-4 times a month
◯ 2-3 times a week
◯ 4 or more times a week
◯ Don't know
◯ Would prefer not to answer

15c. If you no longer drink alcohol, when did you quit drinking alcohol?

_____

16. Have you used any of the following substances in the past 30 days?

	No	Yes
16a. Marijuana	◯	◯
16b. Methamphetamine	◯	◯
16c. Cocaine	◯	◯
16d.Crack	◯	◯
16e. Heroin	◯	◯
16f. PCP	◯	◯
16g. Prescription pain medications	◯	◯
16h. Other	◯	◯

16g. Please specify other not listed above

_____

17. Whether you wanted to or not, how old were you when you engaged in sexual activity with another person for the first time?

_____
(in years)

17a. Any comments concerning question '17. Whether you wanted to or not, how old were you when you engaged in sexual activity with another person for the first time?'?

_____

18. How many sex partners have you had in your whole life?

(if unknown enter 999)

_____

19. How many sex partners have you had in the past 12 months?

(if unknown enter 999)

_____

20. How many sex partners have you had in the past 2 months (60 days)?

(if unknown enter 999)

_____

21. How many new sex partners have you had in the past 2 months (60 days)?

(if unknown enter 999)

_____

22. Have you ever been treated for a sexually transmitted disease (STD), which is also known as a venereal disease (VD)?

◯ No
◯ Yes
◯ Don't know

23. If you have been treated for a sexually transmitted disease or STD, was this within the last 2 months (60 days)?

◯ No
◯ Yes
◯ Don't know

Have you ever been told by a doctor or nurse that you had:

	No	Yes
24a. Chlamydia	◯	◯
24b.Gonorrhea	◯	◯
24c.Trichomonas	◯	◯
24d.Herpes	◯	◯
24e. Syphilis	◯	◯
24f. NGU (non-gonococcal urethritis)	◯	◯
24g. Genital warts	◯	◯

How recently have you engaged in the following sexual behaviors with a female partner?

	Done in past 60 days (two months)	Done in past year	Done during my lifetime (more than a year ago)	Never done this
25a) Masturbated with a woman	◯	◯	◯	◯
25b. I put my mouth on a woman's vagina, vulva, genitals ("giving oral sex")	◯	◯	◯	◯
25c. A woman put her mouth on my penis, genitals ("receiving oral sex")	◯	◯	◯	◯
25d. I used a condom while receiving oral sex from a woman	◯	◯	◯	◯
25e. I put my penis in a woman's vagina ("penile-vaginal sex")	◯	◯	◯	◯
25f. I used a condom during vaginal sex with a woman	◯	◯	◯	◯
25g. I put my penis in a woman's anus (butthole) ("anal sex")	◯	◯	◯	◯
25h. I used a condom during anal sex with a woman	◯	◯	◯	◯
25i. I put my mouth on a woman's anus/butthole? ("oral-anal sex," rimming)	◯	◯	◯	◯
25j. A woman put her mouth on my anus/butthole? ("oral-anal sex," rimming)	◯	◯	◯	◯
25k. Does your partner place her finger into or on your anus/butthole while engaging in sex?	◯	◯	◯	◯

25l.Do you or your partner place a sex toy (vibrator, butt plug, anal beads) into or on your anus/butthole while engaging in sex?	◯	◯	◯	◯

26. If you have had penile-vaginal sex, of the last 10 times that you had penile-vaginal sex, how many of those times did you use a condom?

◯ Every Time (10 out of 10 times)
◯ 9 out of 10 times
◯ 8 out of 10 times
◯ 7 out of 10 times
◯ 6 out of 10 times
◯ 5 out of 10 times
◯ 4 out of 10 times
◯ 3 out of 10 times
◯ 2 out of 10 times
◯ 1 out of 10 times
◯ Never (0 out of 10 times)

27. If you have had anal sex, of the last 10 times that you had anal sex, how many of those times did you use a condom?

◯ Every Time (10 out of 10 times)
◯ 9 out of 10 times
◯ 8 out of 10 times
◯ 7 out of 10 times
◯ 6 out of 10 times
◯ 5 out of 10 times
◯ 4 out of 10 times
◯ 3 out of 10 times
◯ 2 out of 10 times
◯ 1 out of 10 times
◯ Never (0 out of 10 times)

28. How recently have you engaged in the following sexual behaviors with a male partner?

	Done in past 60 days (two months)	Done in past year	Done during my lifetime (more than a year ago)	Never done this
28a. Masturbated with a man	◯	◯	◯	◯
28b. I put my mouth on a man's penis, genitals ("giving oral sex")	◯	◯	◯	◯
28c. A man put his mouth on my penis, genitals ("receiving oral sex")	◯	◯	◯	◯
28d. I used a condom during oral sex with a man	◯	◯	◯	◯
28e. I put my penis in a man's anus (butthole) ("insertive anal sex")	◯	◯	◯	◯

28f. I used a condom during insertive anal sex with a man	◯	◯	◯	◯
28g. A man put his penis in a my anus (butthole) ("receptive anal sex")	◯	◯	◯	◯
28h. I used a condom during receptive anal sex with a man	◯	◯	◯	◯
28i. I put my mouth on a man's anus/butthole? ("oral-anal sex," rimming)	◯	◯	◯	◯
28j. A man put his mouth on my anus/butthole? ("oral-anal sex," rimming)	◯	◯	◯	◯
28k. I used pre-exposure prophylaxis (PrEP) (routine medication to prevent HIV infection, taken daily)	◯	◯	◯	◯
28l. I used post-exposure prophylaxis (PEP) (temporary medication to prevent HIV infection, taken after I might have been exposed to HIV)	◯	◯	◯	◯
28m. Does your partner place his finger into or on your anus/butthole while engaging in sex?	◯	◯	◯	◯
28n. Do you or your partner place a sex toy (vibrator, butt plug, anal beads) into or on your anus/butthole while engaging in sex?	◯	◯	◯	◯

---

29. If you have insertive anal sex with a man, of the last 10 times that you put your penis in a man's anus/butthole, how many of those times did you use a condom?

◯ Every Time (10 out of 10 times)
◯ 9 out of 10 times
◯ 8 out of 10 times
◯ 7 out of 10 times
◯ 6 out of 10 times
◯ 5 out of 10 times
◯ 4 out of 10 times
◯ 3 out of 10 times
◯ 2 out of 10 times
◯ 1 out of 10 times
◯ Never (0 out of 10 times)

30. If you have receptive anal sex, of the last 10 times that a man put his penis in my anus/butthole, how many of those times did you use a condom?

◯ Every Time (10 out of 10 times)
◯ 9 out of 10 times
◯ 8 out of 10 times
◯ 7 out of 10 times
◯ 6 out of 10 times
◯ 5 out of 10 times
◯ 4 out of 10 times
◯ 3 out of 10 times
◯ 2 out of 10 times
◯ 1 out of 10 times
◯ Never (0 out of 10 times)

You are almost done. These are the last few questions.

31. Do you or your partner(s) use any products (i.e. lubricants, spermicides) when you have sex?

◯ No
◯ Yes

31a. What types of products do you use when you have sex (check all that apply)?

☐ None
☐ Saliva
☐ Lubricants (like KY jelly)
☐ Spermicide (not related to the condom)
☐ Oils
☐ Lotion
☐ Other, please list:

31a. Please specify other not listed

_____

32. Do you masturbate?

◯ No
◯ Yes

32a. How many times per week?

◯ None
◯ 1 time
◯ 2-3 times
◯ 4-6 times
◯ Every day
◯ 2 or more times a day

32b. Do you use lubrication when you masturbate?

◯ No
◯ Yes

32c. What do you use for lubrication when you masturbate? (check all that apply)

☐ Nothing
☐ Saliva
☐ Store bought lubrication (like KY jelly)
☐ Lotion
☐ Oil
☐ Other: please list:

32c. Please specify other

_____

32d. Do you use a masturbation sleeve or a "Fifi" when you masturbate?

◯ No
◯ Yes

32e. Do you use any other aids/toys when you masturbate (i.e. vibrator, anal beads)?

◯ No
◯ Yes

33. Which of the following commonly used terms best describes your sexual orientation?

◯ Straight/heterosexual (not gay)
◯ Gay or homosexual
◯ Bisexual
◯ Asexual (I am not sexually attracted to others)
◯ Other, please describe

33a. Please specify other

_____

Insight Test Results - Research Nurse

Date Form Filled out

◯
◯
◯
◯
◯
◯
◯

Please specify other not listed

_____

Date Form Filled out

_____
(MM/DD/YYYY)

1. Chlamydia tests (NAAT)

☐ None recorded
☐ Negative test(s), date(s):
☐ Positive test(s), date(s):

1a. Chlamydia tests (NAAT): Date of Negative Test

_____
(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

_____
(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

_____
(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

_____
(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

_____
(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

_____
(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

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(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

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(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

_____
(MM/DD/YYYY)

1a. Chlamydia tests (NAAT): Date of Negative Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

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(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

1b. Chlamydia tests (NAAT): Date of Positive Test

_____
(MM/DD/YYYY)

2. Gonorrhea tests (NAAT or culture)

☐ None recorded
☐ Negative test(s), date(s):
☐ Positive test(s), date(s):

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

_____
(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

_____
(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

_____
(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

_____
(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

(MM/DD/YYYY)

2a. Gonorrhea tests (NAAT or culture): Date of Negative Test

(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

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(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

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(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

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(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

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(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

____
(MM/DD/YYYY)

2b. Gonorrhea tests (NAAT or culture): Date of Positive Test

____
(MM/DD/YYYY)

3. Trichomonas tests (NAAT)

☐ None recorded
☐ Negative test(s), date(s):
☐ Positive test(s), date(s):

3a. Trichomonas tests (NAAT): Date of Negative Test

____
(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

____
(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

____
(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

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(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

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(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

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(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

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(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

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(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

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(MM/DD/YYYY)

3a. Trichomonas tests (NAAT): Date of Negative Test

____
(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

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(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

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(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

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(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

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(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

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(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

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(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

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(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

____
(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

____
(MM/DD/YYYY)

3b. Trichomonas tests (NAAT): Date of Positive Test

____
(MM/DD/YYYY)

4. Gram stain

☐ None recorded
☐ < 2 WBCs, no GNID. Date(s):
☐ 2-4 WBCs, no GNID. Date(s):
☐ < 5 WBCs, no GNID. Date(s):
☐ >5 WBCs, no GNID. Date(s):
☐ >5 WBCs, positive for GNID. Date(s):

4a. Gram stain: Date of < 5 WBCs, no GNID

____
(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

____
(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

____
(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

____
(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

____
(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

____
(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

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(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

____
(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

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(MM/DD/YYYY)

4a. Gram stain: Date of < 5 WBCs, no GNID

____
(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

____
(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

____
(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

____
(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

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(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

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(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

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(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

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(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

____
(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

____
(MM/DD/YYYY)

4b. Gram stain: Date of > 5 WBCs, no GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4c. Gram stain: Date of > 5 WBCs, positive for GNID

____
(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4d. Gram stain: Date of < 2 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

4e. Gram stain: Date of 2-4 WBCs, no GNID. Date(s):

____
(MM/DD/YYYY)

5. Prior diagnosis of NGU

◯ None recorded
◯ NGU diagnosis, date(s):

5a. Date of Prior diagnosis of NGU diagnosis

____
(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

____
(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

____
(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

____
(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

____
(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

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(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

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(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

____
(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

____
(MM/DD/YYYY)

5a. Date of Prior diagnosis of NGU diagnosis

____
(MM/DD/YYYY)

Specimen Lab

Barcode for U1a/Unspun Urine	_____ (U1a)
Sample of U1a/Unspun Urine	◯ Not Collected ◯ Not Run-Technical Error ◯ Other
Please specify other reason	_____
Barcode for U1ap/Unspun Urine	_____ (U1ap)
Sample of U1ap/Unspun Urine	◯ Not Collected ◯ Not Run-Technical Error ◯ Other
Please specify other reason	_____
Barcode for U1an/Unspun Urine	_____ (U1an)
Sample of U1an/Unspun Urine	◯ Not Collected ◯ Not Run-Technical Error ◯ Other
Please specify other reason	_____
Barcode for U1b/Unspun Urine	_____ (U1b)
Sample of U1b/Unspun Urine	◯ Not Collected ◯ Not Run-Technical Error ◯ Other
Please specify other reason	_____
Barcode for rs1/Rectal Swab	_____ (rs1)
Sample of rs1/Rectal Swab	◯ Not Collected ◯ Not Run-Technical Error ◯ Other
Please specify other reason	_____

Barcode for rs1a/Rectal Swab	_____ (rs1a)
Sample of rs1a/Rectal Swab	◯ Not Collected ◯ Not Run-Technical Error ◯ Other
Please specify other reason	_____
Date Form Filled Out	_____ (MM/DD/YYYY)
Name of Person filling out Form	◯ ◯ ◯
Please specify other	_____
Collection Date and Time	_____ (Date Time with Seconds (MMDDYYYY H:M:S))
Date Received in Laboratory	_____ (MMDDYYYY)
Aliquot Date and Time	_____ (Date Time with Seconds (MMDDYYYY H:M:S))
Urine Volume	_____ (ml)

Urine Test Results

Chlamydia trachomatis	◯ Positive ◯ Negative ◯ Indeterminate
Final Chlamydia trachomatis (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Bilogical Categorization of Chlamydia trachomatis (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Clinical Categorization of Chlamydia trachomatis (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate

Value	_____
Concentration	_____
Organism Load	_____
Comments	_____
Neisseria gonorrhoeae	◯ Positive ◯ Negative ◯ Indeterminate
Final Neisseria gonorrhoeae (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Biological Categorization of Neisseria gonorrhoeae (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Clinical Categorization of Neisseria gonorrhoeae (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Value	_____
Concentration	_____
Comments	_____
Trichomonas vaginalis	◯ Positive ◯ Negative ◯ Indeterminate
Final Trichomonas vaginalis (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Biological Categorization of Trichomonas vaginalis (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Clinical Categorization of Trichomonas vaginalis (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Value	_____

Concentration	_____
Comments	_____
Mycoplasma genitalium	◯ Positive ◯ Negative ◯ Indeterminate
Final Mycoplasma genitalium (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Biological Categorization of Mycoplasma genitalium (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Clinical Catogrization of Mycoplasma genitalium (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Value	_____
Concentration	_____
Comments	_____
Macrolide resistance testing result	◯ Positive ◯ Negative ◯ Indeterminate
Biological Categorization of Macrolide resistance testing result (if original result is indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Clinical Categorization of Macrolide resistance testing result (if original result is indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Macrolide resistance testing result date	_____ (MM/DD/YYYY)
Macrolide resistance testing result comments	_____
Quinolone resistance testing result	◯ Positive ◯ Negative ◯ Indeterminate

Biological Categorization of Quinolone resistance testing result (if original result is indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Clinical Categorization of Quinolone resistance testing result (if original result is indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Quinolone resistance testing result date	_____ (MM/DD/YYYY)
Quinolone resistance testing result comments	_____
Ureaplasma urealyticum	◯ Positive ◯ Negative ◯ Indeterminate
Final Ureaplasma urealyticum (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Biological Categorization of Ureaplasma urealyticum (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Clinical Categorization of Ureaplasma urealyticum (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Value	_____
Concentration	_____
Comments	_____

Urine Specimen Storage

Identifier of Abbott tube	_____
Date Frozen	_____ (MMDDYYYY)
Freezer Location	_____
Comments	_____

Identifier of Amplicor aliquot	_____
Date frozen	_____ (MMDDYYYY)
Freezer Location	_____
Comments	_____
Identifier of Neat Urine	_____
Date frozen	_____ (MM/DD/YYYY)
Freezer Location	_____
Comments	_____

Rectal Test Results

Chlamydia trachomatis	◯ Positive ◯ Negative ◯ Indeterminate
Final Chlamydia trachomatis (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Value	_____
Concentration	_____
Comments	_____
Mycoplasma genitalium	◯ Positive ◯ Negative ◯ Indeterminate
Final Mycoplasma genitalium (if originally indeterminate)	◯ Positive ◯ Negative ◯ Indeterminate
Value	_____

Concentration	
Comments	

Rectal Specimen Storage

Identifier of Abbott Tube	
Date frozen	(MM/DD/YYYY)
Freezer Location	
Comments	
Identifier of SPG aliquot	
Date frozen	(MM/DD/YYYY)
Freezer Location	
Comments	
Urethral Gram Stain	◯ < 5 ◯ >= 5 ◯ < 1 (PMN's/hpf)
Gram Negative Intracellular Diplococci (GNID) Present Urethral Gram Stain	◯ No ◯ Yes
Comments for Urethral Gram Stain	
Slide Identifier	
Slide Storage Box	
Any additional comments	

Cell Count

Barcode for SL1/Spent Urethral Swab

_____
(SL1)

Barcode for S1/Urethral Swab

_____
(S1)

Barcode for U1/Master Urine

_____
(U1)

Barcode for Sv/Saliva

_____
(Sv)

Barcode for U1c/UnSpun Urine

_____
(U1c)

Barcode for P1/Cell Pellet

_____
(P1)

Barcode for Sup/Master Supernatant

_____
(Sup)

Barcode for Sup1/Filtered Supernatant

_____
(Sup1)

Barcode for Sup2/Filtered Supernatant

_____
(Sup2)

Barcode for Sup3/Filtered Supernatant

_____
(Sup3)

Barcode for Sup4/Filtered Supernatant

_____
(Sup4)

Date Form Filled Out

_____
(MM/DD/YYYY)

Name of Person Filling Form

◯ Evelyn Toh
◯ Other

Please specify other name not listed

_____

Date received in lab

_____
(MM/DD/YYYY)

Urine Volume

_____

Value of Total Cell Count

_____

Urine Cell Count

Date Frozen

_____
(MM/DD/YYYY)

Freezer Location

_____

Comments

_____

Total Cell Count (Neat)

_____

Total Cell Count (Centrifuged)

_____

IUMP Target Baseline

Name of Person Filling out the Form	◯ ◯ ◯ ◯
Please specify other not listed	_____
Target Accruals Group	◯ IU ◯ Healthy ◯ CT only ◯ IUMP Other NGU Groups ◯ Enrolled as control, doesn't meet criteria ◯ Enrolled as case, doesn't meet criteria

If IUMP other NGU Groups, specify if

	Positive	Negative	Indeterminate	Not Done
MG	◯	◯	◯	◯
UU	◯	◯	◯	◯
TV	◯	◯	◯	◯
GC	◯	◯	◯	◯
NM	◯	◯	◯	◯
CT	◯	◯	◯	◯

Comments _____