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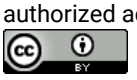

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Genomic relatedness and clinical significance of *Streptococcus mitis* strains isolated from the urogenital tract of sexual partners

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Abstract

Research into the lower urinary tract (LUT) microbiota has primarily focused on its relationship to LUT symptoms (LUTS), taking snapshots of these communities in individuals with and without LUTS. While certain bacterial taxa have been associated with LUTS, or the lack thereof, the temporal dynamics of this community were largely unknown. Recently, we conducted a longitudinal study and found that vaginal intercourse resulted in a shift in species richness and diversity within the LUT microbiota. This is particularly relevant as frequent vaginal intercourse is a major risk factor for urinary tract infection (UTI) in premenopausal women (Aydin *et al. Int Urogynecol J* 2015;26:795–804). To further investigate the relationship between vaginal intercourse and LUT microbiota, here we present the results of a 3 week study in which daily urogenital specimens were collected from a female participant and her male sexual partner. Consistent with our previous findings, the LUT microbiota changed after vaginal intercourse, most notably a high abundance of *Streptococcus mitis* was observed post-coitus. We isolated and sequenced *S. mitis* from both sexual partners finding that: (i) the *S. mitis* isolates from the female partner's urogenital tract were genomically similar throughout the duration of the study, and (ii) they were related to one isolate from the male partner's oral cavity collected at the end of the study, suggesting transmission between the two individuals. We hypothesize that blooms in *S. mitis* after vaginal intercourse may play a role in coitus-related UTI. We found that a *S. mitis* isolate, in contrast to a *Lactobacillus jensenii* isolate displaced after vaginal intercourse, cannot inhibit the growth of uropathogenic *Escherichia coli*. Thus, this bloom in *S. mitis* may provide a window of opportunity for a uropathogen to colonize the LUT.

DATA SUMMARY

DNA sequences have been deposited in the SRA and Assembly databases at the National Center for Biotechnology Information (NCBI) with the BioProject accession number PRJNA316969.

INTRODUCTION

The discovery of resident microbiota in the bladders (i.e. urobiome) of women [1–5] led researchers to investigate its role and relatedness to lower urinary tract (LUT) health and disease. While prior reports of studies sought to determine the relationship between the urobiome and LUT symptoms (LUTS), few studies have assessed the urinary microbiota

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Keywords: lower urinary tract; metaculturomics; microbiome; sex; *Streptococcus mitis*; urinary microbiota.

Abbreviations: ANI, average nucleotide identity; EQUC, expanded quantitative urine culture; JSD, Jensen–Shannon divergence; LUT, lower urinary tract; LUTS, lower urinary tract symptoms; MSU, midstream voided urine; NIH, National Institutes of Health; ProFUM, Probiotics and the Female Urinary Microbiome; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

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Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. Four supplementary tables and three supplementary figures are available with the online version of this article.

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longitudinally, and only one has sought to measure the impact of daily personal factors as a primary objective [6].

In our prior study [6], we assessed the LUT microbiota of asymptomatic, premenopausal women using midstream voided urine (MSU) specimens to investigate relationships between LUT microbiota dynamics and participant-reported personal factors. We found that measures of microbial dynamics related to specific factors, particularly menstruation and vaginal intercourse. Further investigation of these trends revealed differences in LUT microbiota composition and diversity within and across participants. These data, in combination with previous studies showing relationships between LUT microbiota and LUTS [2, 5, 7–20], suggest that personal factors relating to the genitourinary system may be an important consideration in the aetiology, prevention and/or treatment of LUTS.

Vaginal intercourse is a major risk factor for urinary tract infection (UTI) in women [21–24]. However, with some exceptions, it has been shown that UTI-causing pathogens (e.g. *Escherichia coli*) are not introduced during intercourse. Instead, researchers have found other, mainly Gram-positive, non-pathogenic organisms in female urine specimens following vaginal intercourse, including *Corynebacterium*, *Staphylococcus* and *Streptococcus* [23]. These findings are consistent with data published by our group [6], where we found increased abundance of *Streptococcus* species in LUT specimens following participant-reported vaginal intercourse.

We chose to investigate the relationship between vaginal intercourse and altered LUT microbiota by enrolling a participant from our prior study in a follow-up 3 week trial, in which daily urogenital specimens were collected from the female participant and her male sexual partner. Isolates of *Streptococcus mitis*, which were found in high abundance following vaginal intercourse, were isolated and whole-genome sequencing was performed to assess the genomic relatedness of *S. mitis* isolates within and between the sexual partners. Bacterial isolates obtained from the female participant during our prior study also were examined for their *in vitro* characteristics relating to persistence in the urogenital tract and effect on UTI-causing pathogens. We found that all the *S. mitis* isolates from the female partner's urogenital tract were very closely related and that they were related to one isolate from the male partner's oral cavity. A member of this *S. mitis* lineage did not inhibit the growth of a strain of uropathogenic *E. coli* (UPEC), in contrast to a *Lactobacillus jensenii* isolate from the female partner's LUT. Based on these data, we present a hypothesis for the development of coitus-related UTI.

Impact Statement

Prior research discovered that the species richness and diversity within the microbiota of the lower urinary tract (LUT) in women can change as a result of vaginal intercourse. This is particularly relevant as frequent vaginal intercourse is a major risk factor for urinary tract infection (UTI) in premenopausal women. We conducted a 3 week study in which daily urogenital specimens were collected from a female participant and her male sexual partner. After vaginal intercourse, *Streptococcus mitis* bloomed, temporarily replacing *Lactobacillus jensenii* as the predominant bacterial species. Genome sequences of multiple *S. mitis* isolates from each partner revealed that: (i) the isolates from the female partner's urogenital tract were genomically similar throughout the duration of the study, and (ii) these isolates were related to one isolate from the male partner's oral cavity collected at the end of the study. This suggests transmission between the two individuals. We hypothesize that blooms of *S. mitis* after vaginal intercourse may play a role in coitus-related UTI. We found that one *S. mitis* isolate, in contrast to one *L. jensenii* isolate, cannot inhibit the growth of uropathogenic *Escherichia coli*. Thus, this *S. mitis* bloom may provide a window of opportunity for a uropathogen to colonize the LUT.

METHODS

Clinical study enrollment

Following Institutional Review Board (IRB) approval and consent, one female participant and her male sexual partner were enrolled in a clinical survey study to investigate the effects of sexual intercourse on the microbiota of the female LUT. The female participant was previously enrolled as participant ProFUM05 in a registered clinical trial (NCT03250208) as part of the Probiotics and the Female Urinary Microbiome (ProFUM) study [6, 25, 26]. Study eligibility criteria as well as temporal changes and the effects of probiotics and personal factors on the LUT microbiota have been published [6, 25].

Both participants completed an inclusion/exclusion questionnaire and met the following criteria: sexual relationship with the other participant; age 18 years or older; not currently taking antibiotics; not planning a holiday for more than 7 days during the time of specimen collection (i.e. 3 weeks); can read English and sign a consent form detailing the requirements and voluntary nature of the study. Additionally, the female participant met the following criteria: not pregnant, lactating nor planning pregnancy within 6 months; without use of an indwelling catheter. The following demographics were collected on each participant: age, race/ethnicity, height/weight, blood pressure, prior urogynaecological/urological surgery, sexual activity (frequency, type, partners), condom use, dietary preferences, alcohol consumption, number of bowel movements in an average week, use of cigarettes, frequency of bathing, current medications, and history of UTI and kidney stones. The male participant was asked about circumcision status. The female participant was asked the following additional questions: vaginal parity, birth control method, typical length of menstrual cycles, use of menstrual hygiene products, and history of urinary

Table 1. Participant demographics

Feature	PARTNER01 (female)	PARTNER02 (male)
General		
Age (years)	25	27
Race/ethnicity	White	White
BMI	23.5	22.5
Clinical history		
Urological/ urogynaecological surgery	No	No
UTI	No	No
Kidney stones	No	No
Urinary incontinence	No	–
Faecal incontinence	No	–
Hygiene		
No. of bowel movements per week	10	10
No. of showers/baths per week	10	10
Social history		
Special diet	No	No
Alcohol use	Yes	Yes
Tobacco use	No	No
Sexual history		
Condom use	No	No
How often sexually active	Weekly	Weekly
Sexual partners	One male partner	One female partner
Female questions		
Vaginal parity	0	–
Birth control method	Mirena IUD	–
Length of menstrual cycles	None	–
Male questions		
Circumcised	–	Yes

BMI, Body mass index; IUD, intrauterine device.

incontinence and faecal incontinence. These data are shown in Table 1. Participants were each compensated $200 (£144; £1=$1.39) for their time.

The participants were given sufficient supplies (described below) and instructed on how to self-collect, label and deliver daily specimens to the research team. Participants were assigned unique study identifiers by the research nurse, and were instructed to attach labels with their study ID and date of collection to each specimen prior to delivery to a locked drop-box in an accessible room at Loyola University Medical Center. Participants were instructed on proper specimen collection through use of a standardized video detailing proper collection prepared by the clinical team. The female participant was instructed to collect an MSU specimen, a peri-urethral swab, a vaginal swab and an oral/buccal swab. The male participant collected an MSU specimen, a meatal swab, a penile swab and an oral/buccal swab. MSU specimens were collected by voiding into a toilet to discard the initial void (i.e. approximately the first 10 ml urine). The remaining specimen was then collected into a sterile collection cup. A portion of each urine sample was placed in a sterile manner into a BD Vacutainer Plus C and S preservative tube for culturing. Specimens that were collected more than 72 h prior to receipt were excluded from analyses. All swab specimens were collected using a BD ESwab liquid Amies collection and transport system. The flocked swabs allow for optimal elution of the specimen into the medium. Specimens that were collected more than 48 h prior to receipt were excluded from analyses. Peri-urethral (female) and meatal (male) swabs were collected by swabbing approximately 1 cm around the urethral opening. Vaginal swabs were collected by inserting the swab approximately 2 cm into the vaginal opening and rotating. Penile swabs were collected by swabbing the shaft of the penis. Oral/buccal swabs were collected by swabbing the inner cheeks of the mouth.

Specimen collection

The clinical design for this study is described in Fig. 1. The female participant (PARTNER01) self-collected MSU specimens, peri-urethral swabs and vaginal swabs each morning for 21 consecutive days. The male participant (PARTNER02) self-collected MSU specimens, meatal swabs and penile swabs before and after sexual intercourse (i.e. within 30 min). Both participants self-collected an oral/buccal swab once per week. The participants were instructed to engage in sexual intercourse once per week (i.e. during days 1–7, 8–14 and 15–21). Responses to the lifestyle questionnaire (described below) showed compliance.

Participants completed a non-validated personal questionnaire that they turned in daily with their specimens. The questionnaire included the following: alcohol consumption, bathing, swimming, number of bowel movements, medications used, illness, diet (presented as broad food categories) and sexual activity. The questionnaire included the following questions regarding sexual activity: type of sexual activity (oral, vaginal, anal, manual), products used (condom and lubricant), brand of products used (if applicable), time of day (morning, afternoon, evening), and habits immediately following the sexual activity (i.e. within 30 min) (bathing and urination). The female participant's questionnaire also included questions regarding menstruation and menstrual hygiene product use.

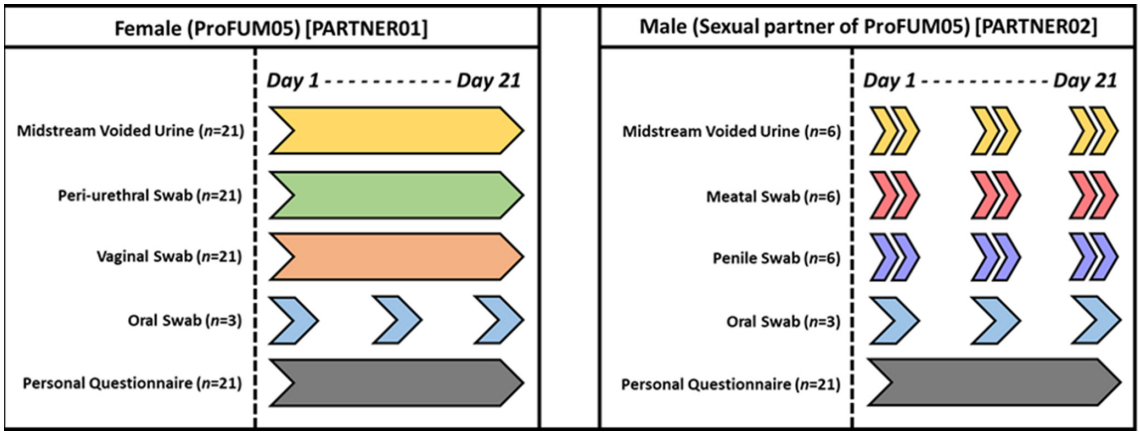

Fig. 1. Clinical study design. The female participant (PARTNER01) self-collected MSU and peri-urethral and vaginal swab specimens daily for 3 weeks (days 1–21). The male participant (PARTNER02) self-collected MSU and meatal and penile swab specimens before and after (within 30 min of) sexual intercourse for 3 weeks. Additionally, both participants self-collected an oral/buccal swab once per week for 3 weeks. The participants were instructed to engage in sexual intercourse once per week for the duration of the study. Daily personal questionnaires were completed for all 21 days by both participants.

Laboratory analysis of specimens

A modified version of the expanded quantitative urine culture (EQUC) protocol was used to identify the microbiota of the specimens [1, 6, 27]. For the MSU specimens, 0.01 ml (female specimens) or 0.1 ml (male specimens) urine was spread quantitatively onto diverse media types [BAP, CNA agar and CDC anaerobe 5% BAP (ABAP)] and incubated in appropriate environments at appropriate temperatures (5% CO_2 at 35 °C for 48 h or anaerobic conditions at 35 °C for 48 h). CNA agar contains approximately 10 mg colistin l^{-1} and 15 mg nalidixic acid l^{-1} for the growth inhibition of Gram-negative organisms. ABAP contains additional growth factors required by common obligate anaerobes including haemin (5 mg l^{-1}), L-cystine (400 mg l^{-1}) and vitamin K1 (10 mg l^{-1}). Each morphologically distinct colony type was counted and isolated on a different plate of the same medium to prepare a pure culture for identification by MALDI-TOF MS, using the Bruker MALDI Biotyper research (RUO) system. All swab specimens underwent the same protocol using 0.01 ml of the liquid elution medium after the swab was vortexed in the collection tube for 10 s. The anaerobic condition was not used for the oral/buccal swabs.

Genomic DNA was extracted from pellets using a phenol/chloroform method. Briefly, pellets were resuspended in 0.5 ml DNA extraction buffer; 50 µl lysozyme (20 mg ml^{-1}) and 30 µl mutanolysin (5 KU) were then added, followed by a 1 h incubation at 37 °C. Next, 80 µl of 10% SDS and 20 µl proteinase K (>600 mAU) were added, followed by a 2 h incubation at 55 °C. Then, 210 µl 6M NaCl and 700 µl phenol/chloroform were added for density separation. Following room temperature incubation and constant rotation for 1 h, the samples were centrifuged at 13500 r.p.m. for 10 min. The aqueous phase was aliquoted to a new tube and an equal volume of isopropanol was added, followed by an additional 10 min centrifugation. The supernatants were decanted and washed with 600 µl of 70% ethanol. The DNA was pelleted, the supernatants were decanted, and the tubes were left at room temperature to dry before the DNA was resuspended in 50 µl nuclease-free water and stored at −20 °C.

Genome sequencing and bioinformatics analysis

A total of 54 isolates of *S. mitis* from specimens from both participants were collected and 39 were selected for whole-genome sequencing (Table 2). Those selected for sequencing included isolates from voided urine specimens, peri-urethral swabs, vaginal swabs, penile swabs and oral swabs. Genome sequencing details can be found in Mores *et al.* (2020) [26]. Briefly, the Illumina Nextera kit was used for whole-genome library preparation. Each isolate was sequenced using the Illumina MiSeq system producing paired-end 2×250 bp reads. Quality control and de-multiplexing of sequence data was done with onboard MiSeq Control software and MiSeq Reporter v3.1. Raw reads were trimmed using Sickle v1.33 (https://github.com/najoshi/sickle) and assembled using SPAdes v3.13.0 [28] with the 'only-assembler' option for $k = 55, 77, 99$ and 127. Genome coverage was calculated using BBMap v38.47 (https://sourceforge.net/projects/bbmap/). The National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) v4.8 [29] was used to annotate the genome sequences. Unless previously noted, default parameters were used for each software tool.

Average nucleotide identity (ANI) values were calculated on the whole-genome level using the software FastANI [30]. Pairwise calculations were performed to obtain ANI values between all the genomes. Hierarchical clusters were generated in R studio (v1.3.959) with ANI data. ANI comparisons and Tetra Correlation Searches to publicly available genomes were conducted using JSpeciesWS [31].

Table 2. List of bacterial isolates used

Isolate no.	Species	Participant ID	Specimen type	Collection day	Genome assembly no.
ProFUM study isolates (Price *et al.* 2020) [6]					
ProFUM05_SM34	*S. mitis*	ProFUM05	MSU	34	–
ProFUM05_LJ35	*L. jensenii*	ProFUM05	MSU	35	–
***S. mitis* isolates**					
SM01	*S. mitis*	PARTNER01	MSU	1	–
SM02	*S. mitis*	PARTNER01	MSU	1	–
SM03	*S. mitis*	PARTNER01	MSU	1	–
SM04	*S. mitis*	PARTNER01	MSU	2	WIJS00000000
SM05	*S. mitis*	PARTNER01	MSU	2	WIKE00000000
SM06	*S. mitis*	PARTNER01	MSU	2	WIJR00000000
SM07	*S. mitis*	PARTNER01	PU	2	WIKD00000000
SM08	*S. mitis*	PARTNER01	PU	2	WIJQ00000000
SM09	*S. mitis*	PARTNER01	OS	2	WIKC00000000
SM10	*S. mitis*	PARTNER01	OS	2	WIJP00000000
SM11	*S. mitis*	PARTNER01	OS	2	WIJO00000000
SM12	*S. mitis*	PARTNER02	OS	1 (WK1-B)	WIKB00000000
SM13	*S. mitis*	PARTNER02	OS	1 (WK1-B)	WIJN00000000
SM14	*S. mitis*	PARTNER02	OS	1 (WK1-B)	WIJM00000000
SM15	*S. mitis*	PARTNER02	PS	1 (WK1-A)	WIJL00000000
SM16	*S. mitis*	PARTNER02	PS	1 (WK1-A)	WIJK00000000
SM17	*S. mitis*	PARTNER01	MSU	3	WIJA00000000
SM18	*S. mitis*	PARTNER01	MSU	3	WIIZ00000000
SM19	*S. mitis*	PARTNER01	MSU	3	WIIY00000000
SM20	*S. mitis*	PARTNER01	VS	3	WIKA00000000
SM21	*S. mitis*	PARTNER01	MSU	4	–
SM22	*S. mitis*	PARTNER01	MSU	5	–
SM25	*S. mitis*	PARTNER01	MSU	7	–
SM26	*S. mitis*	PARTNER01	OS	8	WIJZ00000000
SM27	*S. mitis*	PARTNER01	MSU	9	WIJY00000000
SM28	*S. mitis*	PARTNER01	MSU	9	WIJJ00000000
SM29	*S. mitis*	PARTNER01	MSU	9	WIJI00000000
SM30	*S. mitis*	PARTNER01	PU	9	WIJX00000000
SM31	*S. mitis*	PARTNER01	PU	9	–
SM32	*S. mitis*	PARTNER01	PU	9	–
SM33	*S. mitis*	PARTNER02	OS	8 (WK2-B)	WIJW00000000
SM34	*S. mitis*	PARTNER02	OS	8 (WK2-B)	WIJH00000000
SM35	*S. mitis*	PARTNER01	MSU	10	WIIX00000000
SM36	*S. mitis*	PARTNER01	MSU	10	WIIW00000000

Continued

Table 2. Continued

Isolate no.	Species	Participant ID	Specimen type	Collection day	Genome assembly no.
SM37	*S. mitis*	PARTNER01	MSU	10	WIIV00000000
SM38	*S. mitis*	PARTNER01	MSU	15	–
SM39	*S. mitis*	PARTNER01	OS	15	WIJV00000000
SM40	*S. mitis*	PARTNER01	OS	15	WIJG00000000
SM41	*S. mitis*	PARTNER01	OS	15	WIJF00000000
SM42	*S. mitis*	PARTNER01	MSU	16	WIIU00000000
SM43	*S. mitis*	PARTNER01	MSU	16	WIIT00000000
SM44	*S. mitis*	PARTNER01	MSU	16	WIIS00000000
SM45	*S. mitis*	PARTNER01	MSU	17	WIJU00000000
SM46	*S. mitis*	PARTNER01	MSU	17	WIJE00000000
SM47	*S. mitis*	PARTNER01	MSU	17	WIJD00000000
SM48	*S. mitis*	PARTNER02	OS	16 (WK3-B)	WIJT00000000
SM49	*S. mitis*	PARTNER02	OS	16 (WK3-B)	WIJC00000000
SM50	*S. mitis*	PARTNER02	OS	16 (WK3-B)	WIJB00000000
SM51	*S. mitis*	PARTNER01	MSU	18	–
SM52	*S. mitis*	PARTNER01	MSU	18	–
SM53	*S. mitis*	PARTNER01	MSU	18	–
SM54	*S. mitis*	PARTNER01	MSU	19	–
SM55	*S. mitis*	PARTNER01	MSU	19	–
SM56	*S. mitis*	PARTNER01	MSU	19	–

LJ, *L. jensenii*; SM, *S. mitis*; OS, oral swab; PS, penile swab; PU, peri-urethral swab; VS, vaginal swab; WK, week; -B. before intercourse; -A, after intercourse.

The USEARCH algorithm (v11.0.667) was used to identify high identity proteins with the 'cluster_fast' option for id=0.8 to both strands, generating clusters for each homologue protein. Each cluster was manually inspected. A presence and absence matrix was generated using Python where each column was a genome and each row a homologue, in order to identify genes that were present or absent from each genome sequenced. The homologues for each cluster were aligned using MAFFT (v6.240) [32] and imported to Geneious Prime v2019.2 for manual inspection and identification of point mutations. A mutation matrix was manually generated, and hierarchical clustering was performed in R studio (v1.3.959).

Assembled genomes were compared using Mash v2.2 [33]. Given the expected genomic similarity of the strains isolated, we used 1 million *k*-mers (for *k*=21) to conduct genome comparisons. All pairwise comparisons were conducted resulting in a similarity matrix based on *k*-mer usage similarity. Hierarchical clustering was performed in R studio (v1.3.959).

The sequence of the *spxB* gene was examined from the *S. mitis* genomes produced in this study. The gene sequences were extracted from the genome annotations and aligned using MAFFT (v6.240) [32]. Polymorphisms were identified using Geneious Prime v2019.2.

In vitro experiments

For *in vitro* studies, strains of *L. jensenii* (strain ProFUM05_LJ35) and *S. mitis* (strain ProFUM05_SM34) isolated from MSU specimens obtained from the female participant during our previous study [6] were used; these isolates had been frozen at −80 °C (Table 2). The *L. jensenii* isolates were grown in De Man, Rogosa and Sharpe (MRS) broth or brain heart infusion (BHI) broth with 10% FBS and incubated under 5% CO_2 conditions at 37 °C for 48 h. The *S. mitis* isolates were grown in BHI broth with 10% FBS and incubated under 5% CO_2 conditions at 37 °C for 48 h. Clinical UPEC strains isolated from patients with cystitis (NU14 [34] and UTI89 [35]) were used, as well as a *fimH* deletion mutant strain of NU14 (NU14-1), which shows poor *in vitro* adherence to surfaces [36]. The UPEC isolates were grown in tryptic soy broth (TSB) and incubated aerobically at 37 °C for 24 h.

To test the abilities of urinary bacterial isolates to influence UPEC growth, strains ProFUM05_LJ35 and ProFUM05_SM34 were grown in MRS broth and cell-free supernatants collected using a 0.2 micron filter. UPEC strains NU14 and UTI89 were grown as described above, diluted to an optical density (OD_{600}) of 0.1 in fresh TSB. Supernatants from the bacterial isolates or media controls were added to the UPEC cells in a 1:1 volume ratio. Periodically, growth was measured by OD_{600} and cells plated for assessment of viable c.f.u. numbers.

To test the ability of the urinary bacterial isolates to produce H_2O_2, strains of *L. jensenii* (strain ProFUM05_LJ35 and ProFUM_LJ70) and *S. mitis* (strain ProFUM05_SM34, PARTNER_SM6, PARTNER_SM8, PARTNER_SM10, PARTNER_SM33, PARTNER_SM48 and PARTNER_SM50) were grown in BHI broth with 10% FBS and incubated under 5% CO_2 conditions at 37 °C for 48 h, and cell-free supernatants collected using a 0.2 micron filter. Using an Amplex red hydrogen peroxide/peroxidase assay kit (ThermoFisher), H_2O_2 levels were measured by reading the absorbance at 560 nm.

Biofilm assays were performed. UPEC strains were diluted and 100 µl aliquots were placed into a 96-well microtiter plate. At various timepoints (0, 0.5, 1, 2, 4 and 6 h), an equal volume of urinary bacterial supernatants was added. Media controls were included as negative controls. Plates were incubated aerobically for 24 h total. UPEC biofilm formation was assessed using a standard crystal violet assay. Wells were decanted and washed with PBS three times. A 200 µl aliquot of crystal violet stain was added to each well and left for 10 min. The crystal violet stain was then decanted, and the plates were left to dry for 30 min before adding 200 µl of 70% ethanol to each well. OD_{600} measurements were taken. NU14-1 was used as a negative control.

Urothelial cell adherence assays were performed. A non-malignant urothelial cell line (HURO23A) [37] was used to assess the ability of bacterial strains to adhere to bladder tissue *in vitro*. Urothelial cells were grown in Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM/F-12) with 5% FBS under 5% CO_2 conditions at 37 °C. Cells were enumerated using a haemocytometer and approximately 10^5 cells (ml tissue culture medium)$^{-1}$ (i.e. DMEM/F-12 with 5% FBS) were seeded into 24-well microtiter plates in triplicate. Cells were incubated overnight to promote adherence. Bacterial cells were either resuspended in tissue culture medium and added in a 1:1 volume ratio to the urothelial cells, or the bacterial cells were first pre-treated with supernatants of other strains for 1 h in a 1:1 volume ratio prior to adding the bacterial cells to the urothelial cells. Between 10^7 and 10^8 c.f.u. ml^{-1} of bacterial cells were used. Co-cultures of bacterial and urothelial cells were incubated in 5% CO_2 at 37 °C for 3 h. Empty wells (i.e. no urothelial cells) were used as negative controls to determine the level of bacterial adherence to the plastic surface of the 24-well microtiter plates. After 3 h, the wells were washed with PBS three times and 500 µl of 0.1% Triton X was added to each well. The contents of each well were then serially diluted and plated on BAP medium and incubated under 5% CO_2 conditions at 37 °C for 24–48 h. The c.f.u. values were enumerated and mean values obtained from the negative controls were subtracted from the experimental conditions (i.e. wells with urothelial cells).

Statistical analyses

Statistical analyses of microbiota data were performed using SAS software version 9.4. Microbiota stability measures were calculated using Jensen–Shannon divergence (JSD) as described elsewhere [38]. First, we calculated a representative microbiota distribution for each participant, averaging the abundance data for the microbiota across all collection days. JSD values were then calculated between each day's microbiota and the average microbiota for each participant. These values were then applied to metadata, such as questionnaire results, to determine whether a statistical relationship exists between microbiota stability and personal factors. Wilcoxon rank sum tests and Mann–Whitney U tests compared mean or median JSD values with participant-reported personal factors.

RESULTS

Participant microbiota characteristics

Both participants completed all personal questionnaires. Responses showed compliance by engaging in sexual activity once per week. Questions regarding sexual activity were consistent between the participants for each occasion (i.e. no discrepancies were noted). Vaginal intercourse was reported for the evenings of days 1, 8 and 16. Condom or lubricant use were not reported. Receptive oral intercourse was reported only on day 8.

All specimens were collected; some were excluded from analyses because the specimen was received beyond the acceptable hold time. Fig. 2 shows the relative abundance of the microbiota as assessed by modified EQUC over time for PARTNER01 (female). As reported in our prior study [6], *Lactobacillus* and *Gardnerella* alternately predominated in the female's LUT microbiota. Quantitatively, however, JSD values revealed a disruption in the stability of the MSU microbiota following vaginal intercourse that approached statistical significance (P=0.109) (Table S1, available with the online version of this article). Qualitative trends in the microbiota were consistent with previous findings, showing an association between participant-reported vaginal intercourse and subsequent detection of *Streptococcus* species [6]. This trend applied to the MSU (Fig. 2a) and vaginal (Fig. 2c) microbiota, but not the peri-urethral microbiota (Fig. 2b). The species *S. mitis* was found in high abundance in the MSU microbiota following vaginal intercourse, but was only found once in the vaginal microbiota. *S. mitis* also was detected, but in very low abundance, in the peri-urethral microbiota on days 2 and 9, both of which were after participant-reported vaginal intercourse (Fig. 2b). *S. mitis* also was detected in the oral swab on all three days (Fig. 2d). In PARTNER02 (male), *S. mitis* was not detected in the MSU specimens nor genital swabs collected prior to vaginal intercourse. *S mitis* was detected in low abundance in the penile swab collected after intercourse during week 1, and in high abundance in all three oral swabs (Fig. S1).

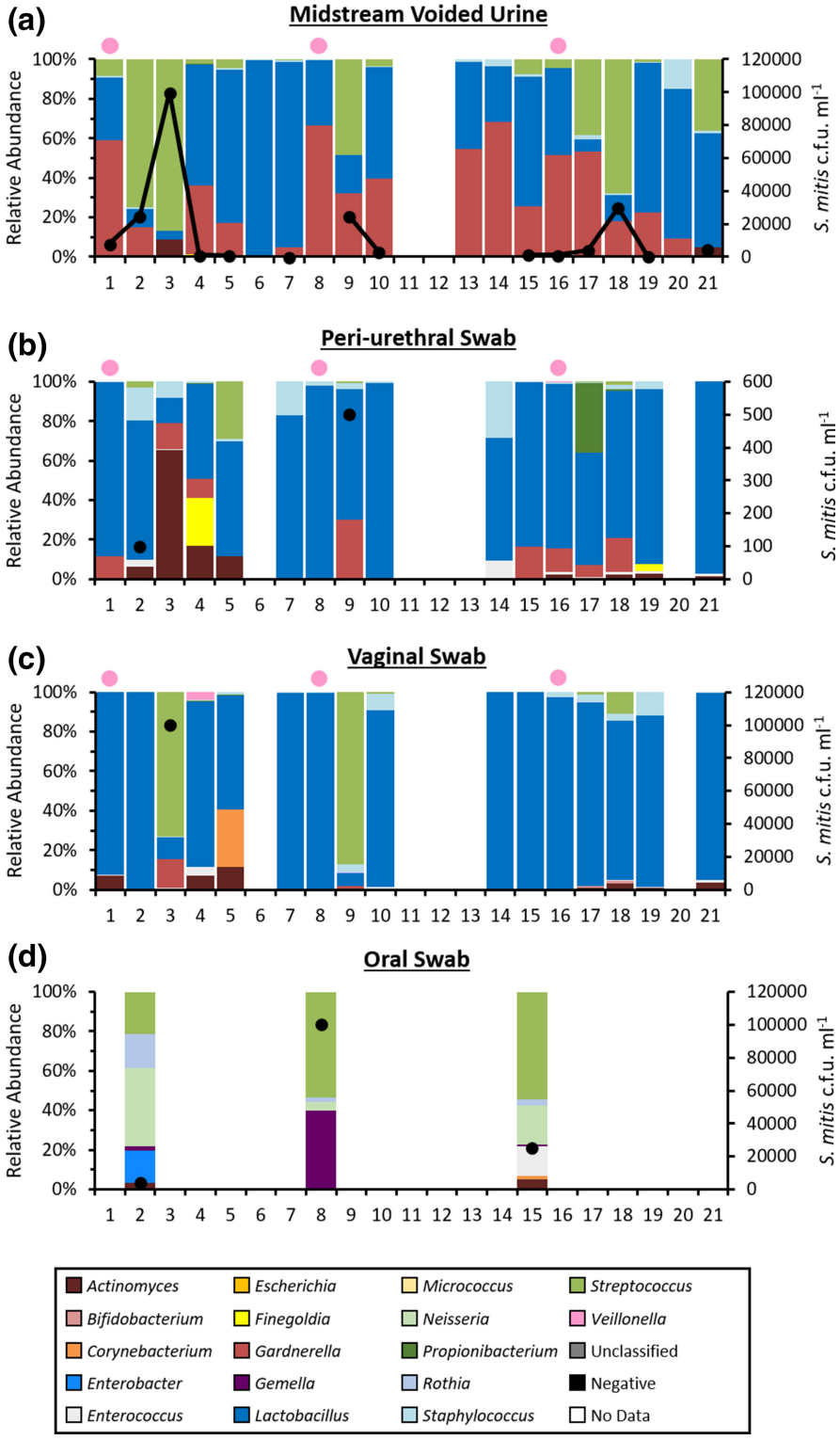

Fig. 2. *S. mitis* abundance and microbiota profiles of specimens from the female participant (PARTNER01). Microbiota profiles are shown as stacked bar graphs depicting the relative abundance (left *y*-axes) of various genera over time and the abundance of *S. mitis* (right *y*-axes) in chronological order (*x*-axes) from various specimens. Black dots indicate *S. mitis* levels. Bar positions that appear 'white' (blank) refer to days where no specimen was collected, received or stored. A key containing the most common genera is found underneath the charts. Data were generated using modified EQUC. Vaginal intercourse was reported on the evenings of days 1, 8, and 16 (pink circles). Oral intercourse was reported on day 8. (a), (b), (c) and (d) show data for MSU, peri-urethral vaginal, and oral microbiota, respectively.

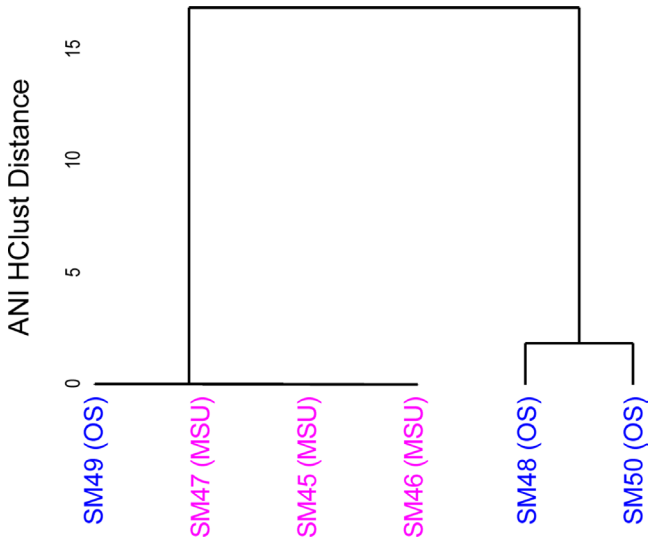

Fig. 3. Dendrogram based on ANI of *S. mitis* isolates. The genomes of six isolates of *S. mitis* isolated on day 17 were compared using ANI. Three isolates were from the MSU specimen of PARTNER01 (female; pink) and three isolates were from the oral swab (OS) specimen of PARTNER02 (male; blue).

Genomic relatedness of *S. mitis* isolates within and between participants

A total of 54 *S. mitis* isolates from various specimens of both participants were collected (Table 2). Multiple isolates collected from the same specimen on the same day were included. Pure bacterial cultures were grown, genomic DNA was extracted, and 39 isolates were selected for whole-genome sequencing and analysis here: 29 from PARTNER01 and 10 from PARTNER02. Genomes for each isolate were assembled and annotated.

The genomic relatedness of *S. mitis* isolates from day 17 (i.e. the day after vaginal intercourse was reported) from MSU specimens of PARTNER01 and oral swab specimens of PARTNER02 was measured. Hierarchical clustering based on ANI between the six isolates is shown in Fig. 3. The genomes from three MSU isolates from PARTNER01 (pink) were found to be highly similar to one another, suggesting a clonal population. In contrast, genomes from three oral isolates from PARTNER02 (blue) did not cluster, suggesting that they represent three genomically distinct populations of oral *S. mitis*. One of these isolates, SM49, had a high ANI score (99.99%) relative to the genomes of the MSU isolates from PARTNER01. These data show that some isolates of *S. mitis* found in the oral cavity of PARTNER02 are clonally related to isolates found in the LUT of PARTNER01.

We expanded our analysis to include all 39 genome sequences and calculated similarity using the ANI metric (Table S2). ANI values ranged from 84.07% (SM16 and SM07 isolated from the penile swab of PARTNER02 on day 1 and the peri-urethral swab of PARTNER01 on day 2, respectively) to 100% (SM40 and SM41, both isolated from the oral swab of PARTNER01 on day 15). While the mean ANI value amongst these 39 genomes was 95.16%, SM16 had an ANI value <85% with the other 38 genomes. SM16 showed similarly low ANI values (~82%) when compared to publicly available *S. mitis* genomes. This prompted us to conduct a Tetra Correlations Search through the JSpecies web server against their available genome database. No *z*-score values above the species threshold were found. Although hits to *S. mitis* strain 29/42 (GenBank Assembly accession no. ATAB00000000) were 'in range' (>0.989, with a *z*-score=0.991), several *Streptococcus infantis* strains had greater *z*-score values, the greatest being *S. infantis* strain DD18 (*z*-score=0.997).

Given the high level of similarity observed, we next compared genomes using a *k*-mer approach. This method is more sensitive to mutations such that even closely related strains can be differentiated. Twenty-nine genomes were compared using *k*-mer content. These genomes were selected to include all of the PARTNER02 genomes (n=10) and high-quality genomes from PARTNER01 that had an ANI value ≥99% to SM49 (n=19). The PARTNER01 genomes were from isolates of MSU specimens, peri-urethral swabs and vaginal swabs collected on days 2, 3, 9, 10, 16 and 17. The *k*-mer contents of these 19 genomes are 99.67% similar (Fig. 4a). The PARTNER02 genome SM49 shares 99.31% of the same genome sequence content with these PARTNER01 genomes. While SM49 shows similarity to the female participant's strains, it is distinctly different (mean *k*-mer similarity=16.24%) from other strains isolated from PARTNER02's oral swabs (Fig. 4a). As the *k*-mer analysis reveals, genomically distinct populations – SM48, SM49 and SM50 – were isolated from PARTNER02's oral swab on day 17 post-coitus, further confirming the observations from our ANI analysis (Fig. 3).

Both ANI and *k*-mer analyses suggest that many of the female isolates and SM49 are nearly identical. To ascertain if this is in fact true, we determined the core genome of the 19 PARTNER01 genomes included in our *k*-mer analysis and the PARTNER02 SM49 genome. The core genome for these strains includes 1828 genes. We next identified mutations within the core genome as a means of distinguishing between isolates. In total, 1788 of the 1828 genes (97.81%) were predicted to encode identical amino acid sequences in all 20 genomes signifying very little variation in these conserved gene sequences. We manually identified mutations in the 40 gene sequences in the core genome with variation (Table S3) and created a matrix of amino acid mutations. On the basis of these mutations, the genomes fell into three clusters. Two clusters contain genomes from PARTNER01's MSU specimens, vaginal swabs and/or peri-urethral swabs. Each cluster includes genomes from different sample sites collected throughout the study (Fig. 4b). The genome of the male isolate SM49 and one female urogenital isolate were less closely related based on these 40 genes.

In vitro activity against UPEC

Isolates of *S. mitis* (seen in high levels in the LUT microbiota of the female participant following vaginal intercourse)

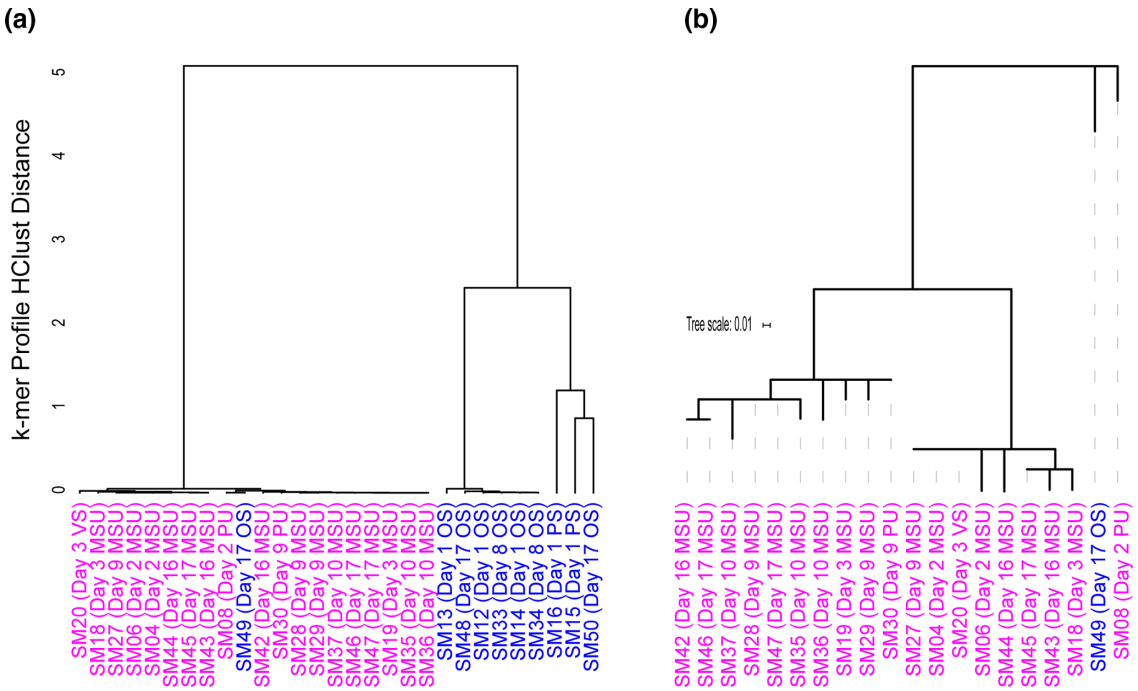

Fig. 4. Dendrograms based on *k*-mer and point mutation analysis of *S. mitis* genomes. Isolates from different specimen of PARTNER01 (female) are shown in pink and the isolates of PARTNER02 (male) are presented in blue. (a) Explores the relatedness of the 29 isolates, 19 from PARTNER01 and 10 from PARTNER02, using *k*-mer analysis. (b) Shows the dendrogram based on point mutations between the genomes in the left clade of (a). Samples include MSU, peri-urethral swab (PU), oral swab (OS), vaginal swab (VS) and penile swab (PS). The tree scale indicates the number of substitutions per site.

and *L. jensenii* (seen in high levels on other days) were isolated from PARTNER01 during our previous study [5]. A representative isolate of each species was used to study the clinical significance of these trends *in vitro*. *S. mitis* strain ProFUM05_SM34 was isolated in high abundance from an MSU specimen the day after vaginal intercourse was reported (day 35). *L. jensenii* strain ProFUM05_LJ35 was isolated in high abundance from an MSU specimen 2 days after vaginal intercourse was reported (day 36).

Vaginal intercourse is a major risk factor for UTI in women [21–24], but the mechanism underlying this correlation is unclear. We theorized that the altered LUT microbiota may play a role. More specifically, we hypothesized that *S. mitis* isolates are less inhibitory against the common UTI-causing pathogen, UPEC, than *L. jensenii* isolates, which represent the normal LUT flora for the female participant. Thus, we added supernatants from ProFUM05_SM34 or ProFUM05_LJ35 to cultures of two UPEC strains, NU14 and UTI89, and monitored their growth. In the presence of the ProFUM05_LJ35 supernatants, both UPEC strains showed no additional growth, as measured by OD_{600} (Fig. 5a, b) and c.f.u. (Fig. 5c, d), suggesting that the *L. jensenii* supernatants are bacteriostatic. In the presence of the ProFUM05_SM34 supernatants, however, both UPEC strains showed comparable growth to the BHI medium control (Fig. 5). The bacteriostatic effect of the ProFUM05_LJ35 supernatants was dose-dependent (Fig. S2). These data show that the supernatants of *L. jensenii* are inhibitory towards UPEC growth, while supernatants of *S. mitis* are not.

Others have reported that *Lactobacillus* species inhibit *E. coli* growth in part through their production of hydrogen peroxide (H_2O_2) [39, 40]. To determine whether this compound contributes to the bacteriostatic effect, we assessed UPEC growth in the presence of the ProFUM05_LJ35 supernatant with or without the addition of catalase. Fig. 6 shows that this addition reversed the inhibitory effect of the supernatant seen in Fig. 5. These data suggest that H_2O_2 production by ProFUM05_LJ35 is involved in the bacteriostatic effects against UPEC. To test this hypothesis, we measured the H_2O_2 production of ProFUM05_SM34 and ProFUM05_LJ35, as well as another *L. jensenii* isolate from our prior study, ProFUM05_LJ70. We also measured H_2O_2 production of six *S. mitis* isolates sequenced in this study. The *L. jensenii* strains produced >10× more H_2O_2 than any of the *S. mitis* strains (Table 3). All 39 *S. mitis* genomes included *spxB*, the gene encoding pyruvate oxidase, the enzyme that generates H_2O_2. Sequence analysis of the *spxB* coding region within the 39 *S. mitis* genomes identified numerous nonsynonymous mutations (Table S4).

We next asked whether the urinary bacterial isolates affect the ability of the UPEC strain NU14 to form a biofilm *in vitro*. NU14Δ*fimH*, an isogenic strain deleted for the *fimH*

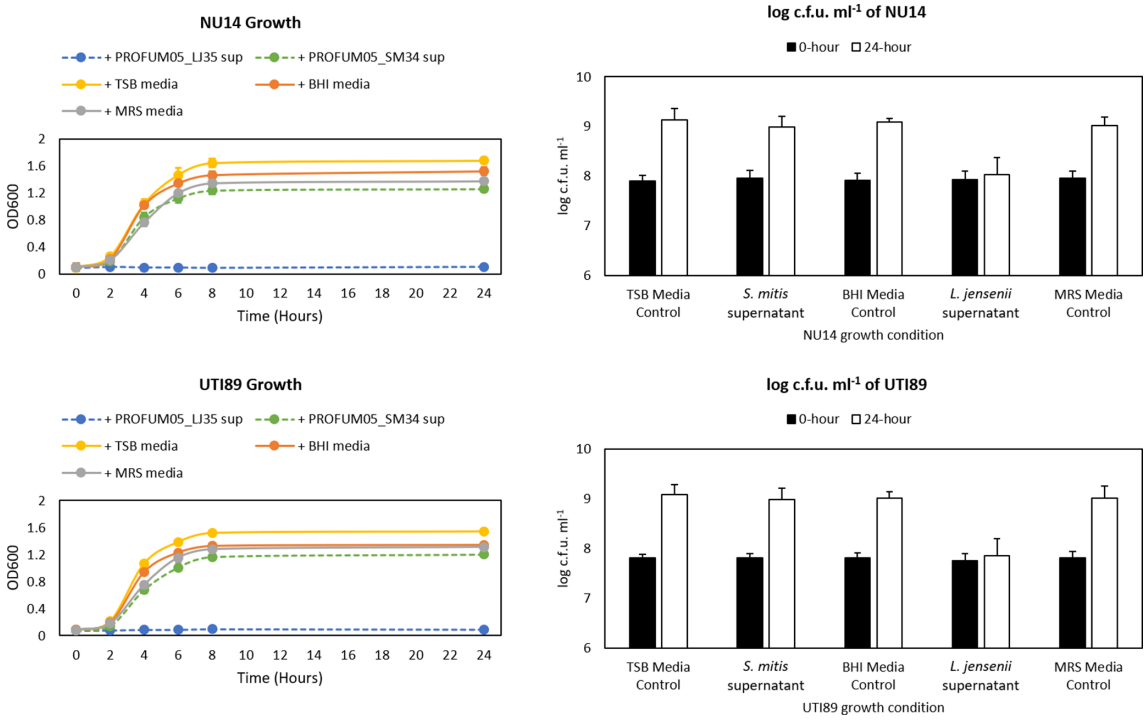

Fig. 5. Effect of LUT bacterial supernatants on UPEC growth. UPEC (i.e. NU14 or UTI89) cells were grown overnight in TSB. Cells were diluted to an OD_{600} of 0.1. Supernatants from bacterial isolates or media controls were added to UPEC cells in a 1:1 volume ratio. Two strains of UPEC were used: NU14 (a, c) and UTI89 (b, d). OD_{600} measurements of UPEC growth were taken at 0, 2, 4, 6, 8 and 24 h (a, b). UPEC c.f.u. values were measured at 0 h (black bars) and 24 h (white bars) (c, d). Figures show data using one *L. jensenii* (ProFUM05_LJ35) and one *S. mitis* (ProFUM05_SM34) isolate. Experiments were performed in triplicate.

gene, was used as a negative control. Fig. S3(a) shows the OD_{600} of the WT NU14 over the mutant. In most conditions, the NU14 strain showed twice as much biomass as the mutant; the exception was in the presence of the ProFUM05_LJ35 supernatant. The ProFUM05_SM34 supernatant did not show the same effect. Furthermore, the raw NU14Δ*fimH* OD_{600} data for the ProFUM05_LJ35 condition were lower than other conditions (Fig. S3b). These data show that the *L. jensenii* supernatants likely do not specifically affect NU14 biofilm formation, but rather NU14 growth in general, because they affect NU14 irrespective of *fimH* status.

Bacterial adherence to urothelial cells *in vitro*

The high abundance of *S. mitis* is transient in the LUT microbiota of the female participant. This may suggest that these bacteria are ill-adapted to the LUT environment compared to *L. jensenii*. We hypothesized that ill-adapted bacteria would adhere poorly to urothelial cells *in vitro*. ProFUM05_LJ35 and ProFUM05_SM34 were grown, washed, resuspended in DMEM/F-12 and 1 ml of each resuspension added to 10^4 urothelial cells. Fig. 7 shows that ProFUM05_LJ35, but not ProFUM05_SM34, could adhere to the urothelial monolayer.

DISCUSSION

In this study, we investigated the relationship between vaginal intercourse and the LUT microbiota. We enrolled a female participant from a prior study [6] and her male sexual partner. As observed in the prior study, the female participant's LUT microbiota alternated every few days between *Lactobacillus* and *Gardnerella* predominance (Fig. 2a). The cause of this pattern remains unknown. However, following vaginal intercourse, *Lactobacillus* and *Gardnerella* abundance diminished and *S. mitis* abundance increased in the female partner's MSU specimen, but not her other urogenital samples. This observation recapitulates our prior study of eight young women who had no evidence of LUTS. Following vaginal intercourse, *Streptococcus* species abundance tended to increase [6].

We do not know why *S. mitis* abundance increased as *Lactobacillus* and *Gardnerella* abundance decreased. One possibility could relate to the male ejaculate. *Streptococcus* is common in the semen microbiota [41]. Furthermore, unprotected sex is negatively associated with the prevalence of *Lactobacillus* species [42], which might be related to the increase in vaginal pH due to the alkalinity of seminal fluid [43]. Given that this couple did not use a condom during vaginal intercourse these are reasonable explanations; indeed, the pH of the MSU sample increased following vaginal intercourse [44]. However, in our prior study

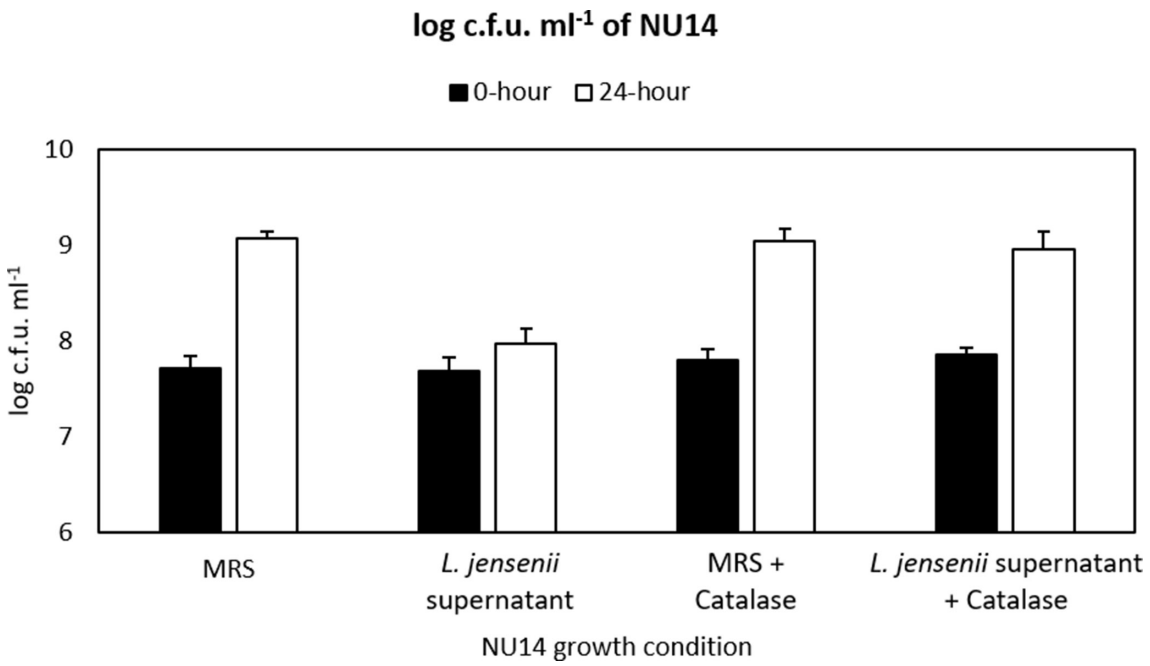

Fig. 6. Effect of addition of catalase on inhibition of UPEC growth by *L. jensenii* supernatants. UPEC (NU14) cells were grown overnight in TSB. Cells were diluted to an OD_{600} of 0.1. Supernatants from one *L. jensenii* isolate (ProFUM05_LJ35) were added in a 1:1 volume ratio with the NU14. Catalase (0.5 mg ml^{-1}) was added to select cultures. MRS broth was used as a medium control for the ProFUM05_LJ35 supernatant. Bars depict NU14 growth (log c.f.u. ml^{-1}) on BAP at 0 h (black bars) and 24 h (white bars) after incubation. The experiment was performed in triplicate.

[6], we observed the same trends even when condom use was reported; therefore, we consider it is unlikely that the observed phenomenon is due to the male's ejaculate.

Since *S. mitis* was also found in high abundance in the oral swabs of both partners, another possible explanation for the increased abundance of *S. mitis* is receptive oral sex. However, receptive oral intercourse was reported on only one of the three instances of vaginal intercourse (i.e. day 8), whereas *S. mitis* abundance increased in the MSU specimens following all three instances of vaginal intercourse (i.e. days 2, 9 and 17). Thus, the elevated abundance of *S. mitis* cannot be related to receptive oral intercourse. Instead, it is likely a bloom of a population resident to the female's urogenital tract. That the female's *S. mitis* population is clonal and persists within her LUT supports this hypothesis. All her sequenced isolates were closely related; there were very few polymorphisms. Interestingly, mutations did not accumulate over time; mutations identified in the day 1 isolate's genome were not present in the genomes of isolates obtained on subsequent days. These results suggest that mutations are explored within this clonal population.

Table 3. H_2O_2 production by *L. jensenii* and *S. mitis* isolates

For each strain, biological duplicates were performed, each with technical triplicates. BHI medium control: mean=0.02, SD <0.01.

Study	Strain	Mean H_2O_2 (µM)	Standard deviation
ProFUM	LJ70	1.67	0.46
	LJ35	1.54	0.17
	SM34	0.07	0.02
Partner	SM06	0.07	0.01
	SM08	0.08	0.01
	SM10	0.09	0.02
	SM33	0.14	0.04
	SM48	0.06	0.02
	SM50	0.10	0.03

Our genome analysis also identified one isolate that may represent a species other than *S. mitis*. SM16, from the penile swab of PARTNER02 on day 1, has an ANI value <85% with the other 38 *S. mitis* genomes examined here. ANI has emerged as a method for defining species now that whole-genome sequencing is routine; typically, a threshold of ≥95% ANI is used for determining a species [30, 45]. While prior studies have shown that this threshold is not valid for the *mitis* group of *Streptococcus* [46, 47], and have highlighted issues with taxonomic assignment in *S. mitis* and other streptococcal species [48], the ANI value for *S. mitis* SM16 and other *S. mitis* genomes is substantially lower than the range measured for this species [47]. This suggests that SM16 may not be an *S. mitis* strain, but rather a member of another *Streptococcus* species.

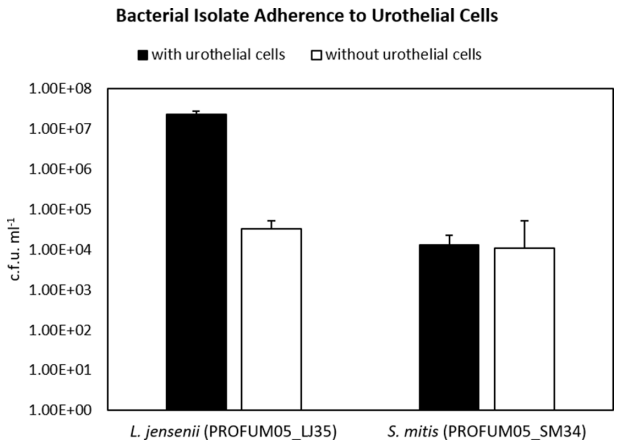

Fig. 7. Bacterial isolate adherence to urothelial cells. Bacterial isolates of *S. mitis* (ProFUM05_SM34) and *L. jensenii* (ProFUM05_LJ35) were grown for 48 h in BHI or MRS broth, respectively. OD_{600} measures were approximately 0.8. This corresponded to approximately 5×10^7 c.f.u. ml^{-1} of ProFUM05_SM34 and 2×10^8 c.f.u. ml^{-1} of ProFUM05_LJ35. Cells were pelleted, washed and resuspended in DMEM/F-12. A 1 ml sample of the bacterial cultures was added to microtiter plates containing urothelial cell monolayers (approximately 10^4 cells per well) and incubated in 5% CO_2 for 3 h. The figure depicts the number of cells recovered from Triton X-treated urothelial cell cultures following the incubation. White bars indicate bacteria binding to empty wells (i.e. without urothelial cells); black bars indicate binding to wells containing urothelial cell monolayers. The experiment was performed in triplicate.

The genome of one isolate (SM49) obtained from the male's oral swab prior to vaginal intercourse was highly similar to the genomes of his female partner's urogenital isolates. This is evidence of microbial transmission between the partners; however, we do not know the direction of the transfer or when transmission occurred. While our investigation of these two individuals suggests that microbial transmission during vaginal intercourse is possible, a larger cohort is needed to ascertain the frequency of such events.

To better understand the clinical significance of our observations, we examined *in vitro* characteristics of a *S. mitis* isolate (ProFUM05_SM34) obtained from the female participant during our prior study and an isolate of *L. jensenii* (ProFUM05_LJ35), the predominant species of her LUT microbiota [6]. We found that cell-free supernatants of the *L. jensenii* isolate were bacteriostatic towards both UPEC strains tested, but supernatants of the *S. mitis* isolate were not. Unsurprisingly, this bacteriostatic behaviour depended upon production of hydrogen peroxide by the *L. jensenii* isolate, as addition of catalase to the supernatant prevented bacteriostasis. In contrast to the *L. jensenii* strains tested, the *S. mitis* strain ProFUM05_SM34 and *S. mitis* strains SM06, SM08, SM10, SM33, SM48 and SM50 did not produce significant levels of H_2O_2. Sequence analysis of the *spxB* coding region within the 39 *S. mitis* genomes identified numerous nonsynonymous mutations that may contribute to the observed phenotype. Finally, we found that *L. jensenii* adheres better to urothelial cells than does *S. mitis.* This could be why the *S. mitis* bloom does not persist within the female participant's LUT microbiota, whereas the *L. jensenii* population recovers.

These data also provide a novel explanation for the association between vaginal intercourse and increased UTI risk. Based upon the data presented here and in our previous report [6], we conclude that vaginal sex can cause a transient decrease in the abundance of a protective bacterium (e.g. *L. jensenii*) and a simultaneous increase in the abundance of a non-protective bacterium (e.g. *S. mitis*). This would represent a window of opportunity for a uropathogen (e.g. UPEC), either introduced by her sexual partner or present as a minor component of her urogenital microbiota, to bloom, causing symptoms. If so, then it may be possible to reduce risk in women prone to coitus-related UTI by enhancing their protective female LUT microbiota. Future studies should test this hypothesis.

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Author contributions
A. J. W. and L. B. obtained funding. T. K. P., B. W., L. B., E. R. M., C. P. and A. J. W. designed the study. T. K. P. and B. W. recruited participants. T. K. P. processed the specimens. T. H. prepared specimens for DNA sequencing. R. L. sequenced the specimens. C. R. M., T. K. P., C. P. and A. J. W. analysed the data. C. R. M., T. K. P., C. P. and A. J. W. wrote the manuscript. C. R. M., T. K. P., B. W., T. H., R. L., L. B., E. R. M., C. P. and A. J. W. reviewed and edited the manuscript.

Conflicts of interest
E. R. M. discloses research support from NIH and Astellas Scientific and Medical Affairs, membership on the Advisory Boards of Boston Scientific, Ferring, UroCure, UpToDate Royalties and Butler Snow/Ethicon Legal Review. L. B. discloses research funding from NIH and editorial stipends from Female Pelvic Medicine and Reconstructive Surgery, UpToDate and JAMA. C. P. discloses research support from NSF. A. J. W. discloses research support from NIH, the DOD, Astellas Scientific and Medical Affairs, and the Kimberly Clark Corporation, and membership on the Advisory Board of Urobiome Therapeutics. The remaining authors (C. R. M., T. K. P., B. W., T. H., R. L.)
declare no conflicts of interest.

References

1. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ *et al*. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol* 2014;52:871–876.
2. Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S *et al*. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J Transl Med* 2012;10:174.
3. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K *et al*. Evidence of uncultivated bacteria in the adult female bladder. *J Clin Microbiol* 2012;50:1376–1383.
4. Karstens L, Asquith M, Davin S, Stauffer P, Fair D *et al*. Does the urinary microbiome play a role in urgency urinary incontinence and its severity? *Front Cell Infect Microbiol* 2016;6:78.
5. Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K *et al*. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. *mBio* 2014;5:e01283-14.

6. Price TK, Wolff B, Halverson T, Limeira R, Brubaker L *et al*. Temporal dynamics of the adult female lower urinary tract microbiota. *mBio* 2020;11:e00475-20
7. Siddiqui H, Lagesen K, Nederbragt AJ, Jeansson SL, Jakobsen KS. Alterations of microbiota in urine from women with interstitial cystitis. *BMC Microbiol* 2012;12:205.
8. Thomas-White KJ, Hilt EE, Fok C, Pearce MM, Mueller ER *et al*. Incontinence medication response relates to the female urinary microbiota. *Int Urogynecol J* 2016;27:723–733.
9. Abernethy MG, Rosenfeld A, White JR, Mueller MG, Lewicky-Gaupp C *et al*. Urinary microbiome and cytokine levels in women with interstitial cystitis. *Obstet Gynecol* 2017;129:500–506.
10. Thomas-White KJ, Kliethermes S, Rickey L, Lukacz ES, Richter HE *et al*. Evaluation of the urinary microbiota of women with uncomplicated stress urinary incontinence. *Am J Obstet Gynecol* 2017;216:55.e1–55.e16.
11. Komesu YM, Richter HE, Carper B, Dinwiddie DL, Lukacz ES. The urinary microbiome in women with mixed urinary incontinence compared to similarly aged controls. *Int Urogynecol J* 2018;29:1785–1795.
12. Chen Z, Phan M, Bates LJ, Peters KM, Mukerjee C *et al*. The urinary microbiome in patients with refractory urge incontinence and recurrent urinary tract infection. *Int Urogynecol J* 2018;29:1775–1782.
13. Nickel JC, Stephens A, Landis JR, Mullins C, van Bokhoven A *et al*. Assessment of the lower urinary tract microbiota during symptom flare in women with urologic chronic pelvic pain syndrome: a MAPP network study. *J Urol* 2016;195:356–362.
14. Willner D, Low S, Steen JA, George N, Nimmo GR *et al*. Single clinical isolates from acute uncomplicated urinary tract infections are representative of dominant in situ populations. *mBio* 2014;5:e01064-13.
15. Dune TJ, Price TK, Hilt EE, Thomas-White KJ, Kliethermes S *et al*. Urinary symptoms and their associations with urinary tract infections in urogynecologic patients. *Obstet Gynecol* 2017;130:718–725.
16. Price TK, Hilt EE, Dune TJ, Mueller ER, Wolfe AJ *et al*. Urine trouble: should we think differently about UTI? *Int Urogynecol J* 2018;29:205–210.
17. Curtiss N, Balachandran A, Krska L, Peppiatt-Wildman C, Wildman S *et al*. A case controlled study examining the bladder microbiome in women with overactive bladder (OAB) and healthy controls. *Eur J Obstet Gynecol Reprod Biol* 2017;214:31–35.
18. Groah SL, Pérez-Losada M, Caldovic L, Ljungberg IH, Sprague BM *et al*. Redefining healthy urine: a cross-sectional exploratory metagenomic study of people with and without bladder dysfunction. *J Urol* 2016;196:579–587.
19. Bresler L, Price TK, Hilt EE, Joyce C, Fitzgerald CM *et al*. Female lower urinary tract microbiota do not associate with IC/PBS symptoms: a case-controlled study. *Int Urogynecol J* 2019;30:1835–1842.
20. Jacobs KM, Price TK, Thomas-White K, Halverson T, Davies A. Cultivable bacteria in urine of women with interstitial cystitis: (not) what we expected. *Female Pelvic Med Reconstr Surg*.
21. Moore EE, Hawes SE, Scholes D, Boyko EJ, Hughes JP *et al*. Sexual intercourse and risk of symptomatic urinary tract infection in postmenopausal women. *J Gen Intern Med* 2008;23:595–599.
22. Nicolle LE, Harding GK, Preiksaitis J, Ronald AR. The association of urinary tract infection with sexual intercourse. *J Infect Dis* 1982;146:579–583.
23. Buckley RM, McGuckin M, MacGregor RR. Urine bacterial counts after sexual intercourse. *N Engl J Med* 1978;298:321–324.
24. Foxman B, Somsel P, Tallman P, Gillespie B, Raz R *et al*. Urinary tract infection among women aged 40 to 65: behavioral and sexual risk factors. *J Clin Epidemiol* 2001;54:710–718.
25. Wolff BJ, Price TK, Joyce CJ, Wolfe AJ, Mueller ER. Oral probiotics and the female urinary microbiome: a double-blinded randomized placebo-controlled trial. *Int Urol Nephrol* 2019;51:2149–2159.
26. Mores CR, Price TK, Brassil B, Putonti C, Wolfe AJ. Complete genome sequences of *Streptococcus mitis* strains isolated from the oral cavity and urogenital tract of a woman and her male sexual partner. *Microbiol Resour Announc* 2020;9:e01379-19
27. Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S *et al*. The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. *J Clin Microbiol* 2016;54:1216–1222.
28. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
29. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP *et al*. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res* 2016;44:6614–6624.
30. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 2018;9:5114–5119.
31. Richter M, Rossello-Mora R, Oliver Glockner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
32. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 2013;30:772–780.
33. Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH *et al*. Mash: fast genome and metagenome distance estimation using MinHash. *Genome Biol* 2016;17:132.
34. Langermann S, Palaszynski S, Barnhart M, Auguste G, Pinkner JS *et al*. Prevention of mucosal *Escherichia coli* infection by FimH-adhesin-based systemic vaccination. *Science* 1997;276:607–611.
35. Chen SL, Hung CS, Xu J, Reigstad CS, Magrini V *et al*. Identification of genes subject to positive selection in uropathogenic strains of *Escherichia coli*: a comparative genomics approach. *Proc Natl Acad Sci USA* 2006;103:5977–5982.
36. Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC *et al*. Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* 1998;282:1494–1497.
37. Le PT, Pearce MM, Zhang S, Campbell EM, Fok CS *et al*. IL22 regulates human urothelial cell sensory and innate functions through modulation of the acetylcholine response, immunoregulatory cytokines and antimicrobial peptides: assessment of an *in vitro* model. *PLoS One* 2014;9:e111375.
38. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW *et al*. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2014;2:4.
39. Martin R, Suarez JE. Biosynthesis and degradation of H_2O_2 by vaginal lactobacilli. *Appl Environ Microbiol* 2010;76:400–405.
40. Hertzberger R, Arents J, Dekker HL, Pridmore RD, Gysler C *et al*. H_2O_2 production in species of the *Lactobacillus acidophilus* group: a central role for a novel NADH-dependent flavin reductase. *Appl Environ Microbiol* 2014;80:2229–2239.
41. Sacks D, Baxter B, Campbell BCV, Carpenter JS, Cognard CMulti-society consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke. *Int J Stroke* 2018;13:612–632.
42. Lewis FM, Bernstein KT, Aral SO. Vaginal microbiome and its relationship to behavior, sexual health, and sexually transmitted diseases. *Obstet Gynecol* 2017;129:643–654.
43. Cao X-W, Lin K, Li C-Y, Yuan C-W. [A review of WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition)]. *Zhonghua Nan Ke Xue* 2011;17:1059–1063.
44. Price TK. Assessing the temporal dynamics of the lower urinary tract microbiota and the effects of lifestyle. PhD Thesis, Loyola University, Chicago, USA; 2019.
45. Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci USA* 2005;102:2567–2572.

46. Kilian M, Poulsen K, Blomqvist T, Håvarstein LS, Bek-Thomsen M *et al*. Evolution of *Streptococcus pneumoniae* and its close commensal relatives. *PLoS One* 2008;3:e2683.

47. Jensen A, Scholz CFP, Kilian M. Re-evaluation of the taxonomy of the Mitis group of the genus *Streptococcus* based on whole genome phylogenetic analyses, and proposed reclassification of *Streptococcus dentisani* as *Streptococcus oralis* subsp. *dentisani* comb. nov., *Streptococcus tigurinus* as *Streptococcus oralis* subsp. *tigurinus* comb. nov., and *Streptococcus oligofermentans* as a later synonym of *Streptococcus cristatus*. *Int J Syst Evol Microbiol* 2016;66:4803–4820.

48. Velsko IM, Perez MS, Richards VP. Resolving phylogenetic relationships for *Streptococcus mitis* and *Streptococcus oralis* through core- and pan-genome analyses. *Genome Biol Evol* 2019;11:1077–1087.

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