Characterizing Post-Coital Vaginal and Penile Microbial Transfer and Persistence

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Disclaimers

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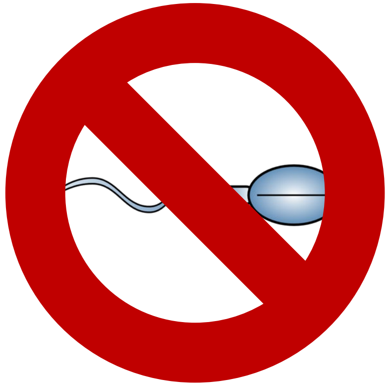
The opinions, findings, conclusions, and/or recommendations expressed in this presentation are those of the authors.

Sexual Assault Evidence Processing

Serology → Differential Extraction → Quantification → STR Amplification → Capillary Electrophoresis → STR Analysis



No spermatozoa?
No male DNA?

OSAC RESEARCH NEEDS ASSESSMENT FORM

Title of research need: 1. Applications of the microbiome in DNA transfer and human identification.

U.S. Department of Justice
Office of Justice Programs
National Institute of Justice

Forensic Science Technology Working Group Operational Requirements
April 2021

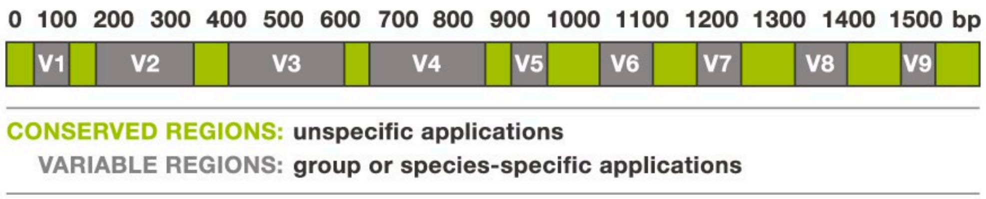
The ability to differentiate, physically separate, and selectively analyze DNA and/or cells from multiple donors or multiple tissue/cell types contributing to mixtures, with minimal or no sample loss	Methods by which to identify areas on a swab with DNA type of interest (e.g., semen) to determine how much is needed for testing prior to beginning extraction
Better understanding of advanced approaches to removing steps from typical DNA processing workflows (e.g., extraction, quantitation, amplification)	The ability to associate cell type and/or fluid with DNA profile, primarily for mixture DNA profiles

Human Microbiome

- Human microbiota: 10-100 trillion symbiotic microorganisms found in each human body
- Inter- and intravariability found within and between body sites
- Vaginal microbiome: well-characterized, clinical relevance
- Penile microbiome: fewer studies

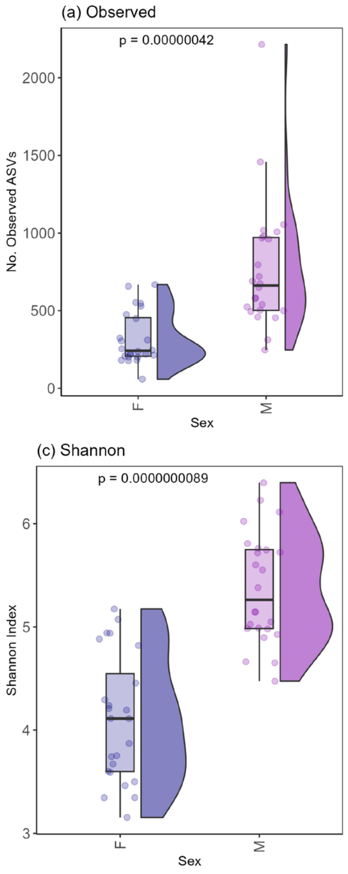
Experimental Design

- Target 16S rRNA gene hypervariable regions V3 and V4
- Samples from donor couples (N =4, 6 coital events):
  - Pre-coital penile and vaginal swabs
  - Post-coital penile and vaginal swabs (no showering)
  - Post-coital penile and vaginal swabs (**with post-coital showering**)
- Illumina MiSeq FGx
- DADA2 (SILVA v132 and NCBI BLAST)
- R (4.1.1) and RStudio (v1.4)

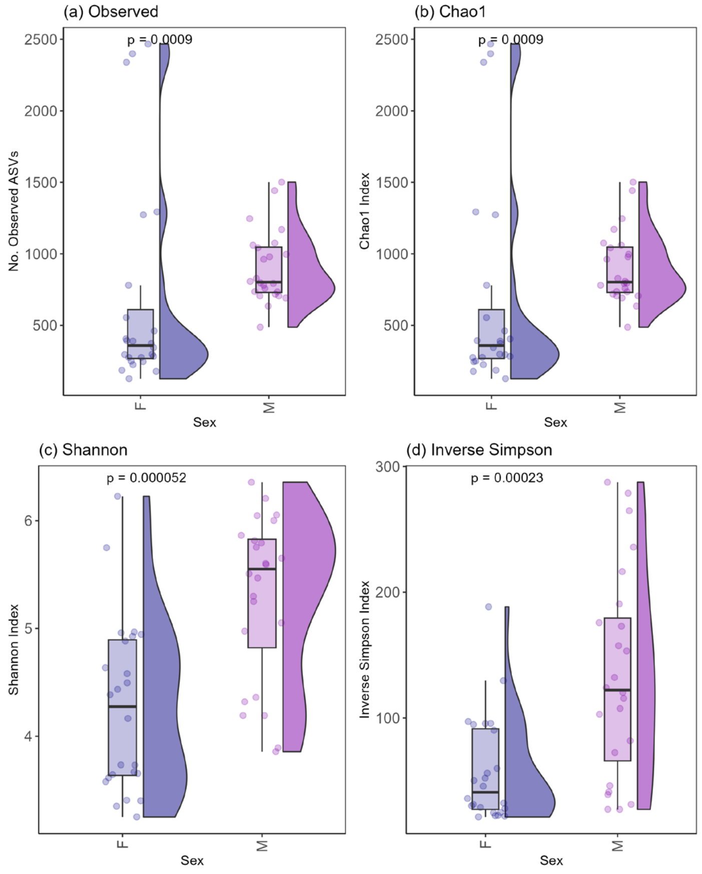


Alpha Diversity by Activity

PRE-COITAL

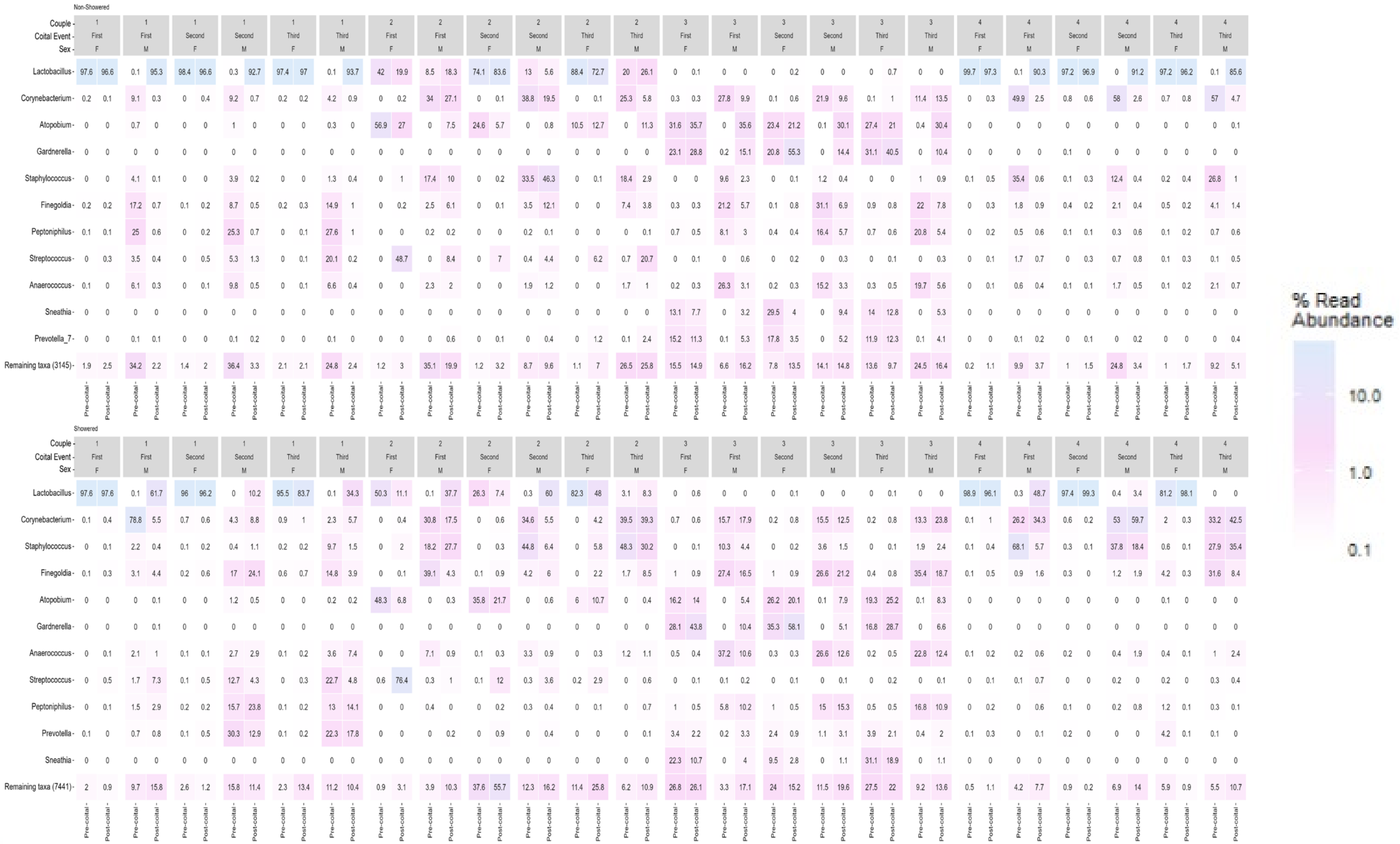


POST-COITAL



- Pre-coital: male samples more diverse.
- Post-coital: female samples more diverse.

Taxonomy



Non-Showered

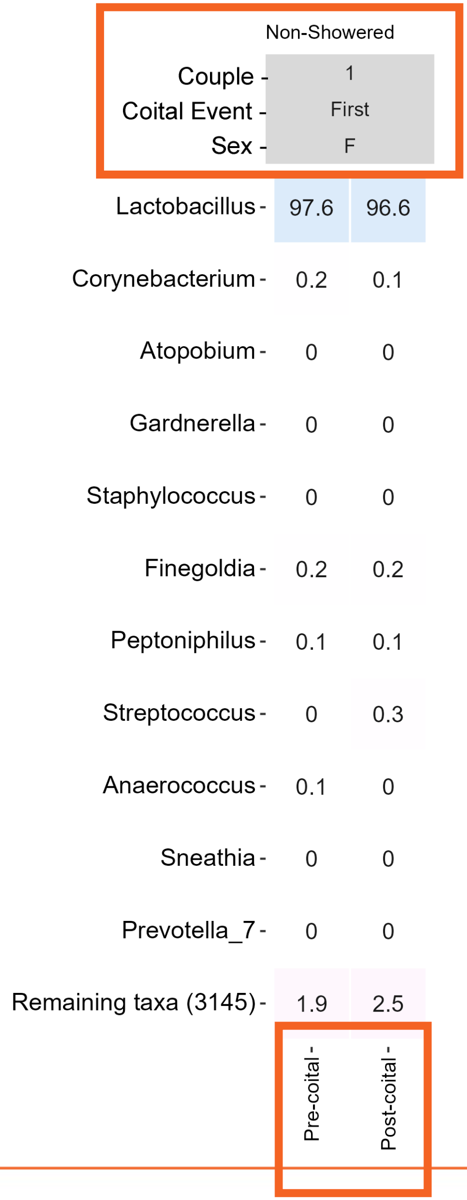
Couple	1		1		1		1		1		1		2		2		2		2		2		2	
Coital Event	First		First		Second		Second		Third		Third		First		First		Second		Second		Third		Third	
Sex	F		M		F		M		F		M		F		M		F		M		F		M	
Lactobacillus	97.6	96.6	0.1	95.3	98.4	96.6	0.3	92.7	97.4	97	0.1	93.7	42	19.9	8.5	18.3	74.1	83.6	13	5.6	88.4	72.7	20	26.1
Corynebacterium	0.2	0.1	9.1	0.3	0	0.4	9.2	0.7	0.2	0.2	4.2	0.9	0	0.2	34	27.1	0	0.1	38.8	19.5	0	0.1	25.3	5.8
Atopobium	0	0	0.7	0	0	0	1	0	0	0	0.3	0	56.9	27	0	7.5	24.6	5.7	0	0.8	10.5	12.7	0	11.3
Gardnerella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staphylococcus	0	0	4.1	0.1	0	0	3.9	0.2	0	0	1.3	0.4	0	1	17.4	10	0	0.2	33.5	46.3	0	0.1	18.4	2.9
Finegoldia	0.2	0.2	17.2	0.7	0.1	0.2	8.7	0.5	0.2	0.3	14.9	1	0	0.2	2.5	6.1	0	0.1	3.5	12.1	0	0	7.4	3.8
Peptoniphilus	0.1	0.1	25	0.6	0	0.2	25.3	0.7	0	0.1	27.6	1	0	0	0.2	0.2	0	0	0.2	0.1	0	0	0	0.1
Streptococcus	0	0.3	3.5	0.4	0	0.5	5.3	1.3	0	0.1	20.1	0.2	0	48.7	0	8.4	0	7	0.4	4.4	0	6.2	0.7	20.7
Anaerococcus	0.1	0	6.1	0.3	0	0.1	9.8	0.5	0	0.1	6.6	0.4	0	0	2.3	2	0	0	1.9	1.2	0	0	1.7	1
Sneathia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prevotella_7	0	0	0.1	0.1	0	0	0.1	0.2	0	0	0.1	0	0	0	0	0.6	0	0.1	0	0.4	0	1.2	0.1	2.4
Remaining taxa (3145)	1.9	2.5	34.2	2.2	1.4	2	36.4	3.3	2.1	2.1	24.8	2.4	1.2	3	35.1	19.9	1.2	3.2	8.7	9.6	1.1	7	26.5	25.8
	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital

Couple	3		3		3		3		3		3		4		4		4		4		4		4	
Coital Event	First		First		Second		Second		Third		Third		First		First		Second		Second		Third		Third	
Sex	F		M		F		M		F		M		F		M		F		M		F		M	
Lactobacillus	0	0.1	0	0	0	0.2	0	0	0	0.7	0	0	99.7	97.3	0.1	90.3	97.2	96.9	0	91.2	97.2	96.2	0.1	85.6
Corynebacterium	0.3	0.3	27.8	9.9	0.1	0.6	21.9	9.6	0.1	1	11.4	13.5	0	0.3	49.9	2.5	0.8	0.6	58	2.6	0.7	0.8	57	4.7
Atopobium	31.6	35.7	0	35.6	23.4	21.2	0.1	30.1	27.4	21	0.4	30.4	0	0	0	0	0	0	0	0	0	0	0	0.1
Gardnerella	23.1	28.8	0.2	15.1	20.8	55.3	0	14.4	31.1	40.5	0	10.4	0	0	0	0	0.1	0	0	0	0	0	0	0
Staphylococcus	0	0	9.6	2.3	0	0.1	1.2	0.4	0	0	1	0.9	0.1	0.5	35.4	0.6	0.1	0.3	12.4	0.4	0.2	0.4	26.8	1
Finegoldia	0.3	0.3	21.2	5.7	0.1	0.8	31.1	6.9	0.9	0.8	22	7.8	0	0.3	1.8	0.9	0.4	0.2	2.1	0.4	0.5	0.2	4.1	1.4
Peptoniphilus	0.7	0.5	8.1	3	0.4	0.4	16.4	5.7	0.7	0.6	20.8	5.4	0	0.2	0.5	0.6	0.1	0.1	0.3	0.6	0.1	0.2	0.7	0.6
Streptococcus	0	0.1	0	0.6	0	0.2	0	0.3	0	0.1	0	0.3	0	0.1	1.7	0.7	0	0.3	0.7	0.8	0.1	0.3	0.1	0.5
Anaerococcus	0.2	0.3	26.3	3.1	0.2	0.3	15.2	3.3	0.3	0.5	19.7	5.6	0	0.1	0.6	0.4	0.1	0.1	1.7	0.5	0.1	0.2	2.1	0.7
Sneathia	13.1	7.7	0	3.2	29.5	4	0	9.4	14	12.8	0	5.3	0	0	0	0	0	0	0	0	0	0	0	0
Prevotella_7	15.2	11.3	0.1	5.3	17.8	3.5	0	5.2	11.9	12.3	0.1	4.1	0	0	0.1	0.2	0	0.1	0	0.2	0	0	0	0.4
Remaining taxa (3145)	15.5	14.9	6.6	16.2	7.8	13.5	14.1	14.8	13.6	9.7	24.5	16.4	0.2	1.1	9.9	3.7	1	1.5	24.8	3.4	1	1.7	9.2	5.1
	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital

Showered

Couple	1		1		1		1		1		1		2		2		2		2		2		2	
Coital Event	First		First		Second		Second		Third		Third		First		First		Second		Second		Third		Third	
Sex	F		M		F		M		F		M		F		M		F		M		F		M	
Lactobacillus	97.6	97.6	0.1	61.7	96	96.2	0	10.2	95.5	83.7	0.1	34.3	50.3	11.1	0.1	37.7	26.3	7.4	0.3	60	82.3	48	3.1	8.3
Corynebacterium	0.1	0.4	78.8	5.5	0.7	0.6	4.3	8.8	0.9	1	2.3	5.7	0	0.4	30.8	17.5	0	0.6	34.6	5.5	0	4.2	39.5	39.3
Staphylococcus	0	0.1	2.2	0.4	0.1	0.2	0.4	1.1	0.2	0.2	9.7	1.5	0	2	18.2	27.7	0	0.3	44.8	6.4	0	5.8	48.3	30.2
Finegoldia	0.1	0.3	3.1	4.4	0.2	0.6	17	24.1	0.6	0.7	14.8	3.9	0	0.1	39.1	4.3	0.1	0.9	4.2	6	0	2.2	1.7	8.5
Atopobium	0	0	0	0.1	0	0	1.2	0.5	0	0	0.2	0.2	48.3	6.8	0	0.3	35.8	21.7	0	0.6	6	10.7	0	0.4
Gardnerella	0	0	0	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anaerococcus	0	0.1	2.1	1	0.1	0.1	2.7	2.9	0.1	0.2	3.6	7.4	0	0	7.1	0.9	0.1	0.3	3.3	0.9	0	0.3	1.2	1.1
Streptococcus	0	0.5	1.7	7.3	0.1	0.5	12.7	4.3	0	0.3	22.7	4.8	0.6	76.4	0.3	1	0.1	12	0.3	3.6	0.2	2.9	0	0.6
Peptoniphilus	0	0.1	1.5	2.9	0.2	0.2	15.7	23.8	0.1	0.2	13	14.1	0	0	0.4	0	0	0.2	0.3	0.4	0	0.1	0	0.7
Prevotella	0.1	0	0.7	0.8	0.1	0.5	30.3	12.9	0.1	0.2	22.3	17.8	0	0	0	0.2	0	0.9	0	0.4	0	0	0	0.1
Sneathia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Remaining taxa (7441)	2	0.9	9.7	15.8	2.6	1.2	15.8	11.4	2.3	13.4	11.2	10.4	0.9	3.1	3.9	10.3	37.6	55.7	12.3	16.2	11.4	25.8	6.2	10.9
	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital

Couple	3		3		3		3		3		3		4		4		4		4		4		4	
Coital Event	First		First		Second		Second		Third		Third		First		First		Second		Second		Third		Third	
Sex	F		M		F		M		F		M		F		M		F		M		F		M	
Lactobacillus	0	0.6	0	0	0	0.1	0	0	0	0.1	0	0	98.9	96.1	0.3	48.7	97.4	99.3	0.4	3.4	81.2	98.1	0	0
Corynebacterium	0.7	0.6	15.7	17.9	0.2	0.8	15.5	12.5	0.2	0.8	13.3	23.8	0.1	1	26.2	34.3	0.6	0.2	53	59.7	2	0.3	33.2	42.5
Staphylococcus	0	0.1	10.3	4.4	0	0.2	3.6	1.5	0	0.1	1.9	2.4	0.1	0.4	68.1	5.7	0.3	0.1	37.8	18.4	0.6	0.1	27.9	35.4
Finegoldia	1	0.9	27.4	16.5	1	0.9	26.6	21.2	0.4	0.8	35.4	18.7	0.1	0.5	0.9	1.6	0.3	0	1.2	1.9	4.2	0.3	31.6	8.4
Atopobium	16.2	14	0	5.4	26.2	20.1	0.1	7.9	19.3	25.2	0.1	8.3	0	0	0	0	0	0	0	0	0.1	0	0	0
Gardnerella	28.1	43.8	0	10.4	35.3	58.1	0	5.1	16.8	28.7	0	6.6	0	0	0	0	0	0	0	0	0	0	0	0
Anaerococcus	0.5	0.4	37.2	10.6	0.3	0.3	26.6	12.6	0.2	0.5	22.8	12.4	0.1	0.2	0.2	0.6	0.2	0	0.4	1.9	0.4	0.1	1	2.4
Streptococcus	0	0.1	0.1	0.2	0	0.1	0	0.1	0	0.2	0	0.1	0	0.1	0.1	0.7	0	0	0.2	0	0.2	0	0.3	0.4
Peptoniphilus	1	0.5	5.8	10.2	1	0.5	15	15.3	0.5	0.5	16.8	10.9	0	0.2	0	0.6	0.1	0	0.2	0.8	1.2	0.1	0.3	0.1
Prevotella	3.4	2.2	0.2	3.3	2.4	0.9	1.1	3.1	3.9	2.1	0.4	2	0.1	0.3	0	0.1	0.2	0	0	0	4.2	0.1	0.1	0
Sneathia	22.3	10.7	0	4	9.5	2.8	0	1.1	31.1	18.9	0	1.1	0	0	0	0	0	0	0	0	0	0	0	0
Remaining taxa (7441)	26.8	26.1	3.3	17.1	24	15.2	11.5	19.6	27.5	22	9.2	13.6	0.5	1.1	4.2	7.7	0.9	0.2	6.9	14	5.9	0.9	5.5	10.7
	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital	Pre-coital	Post-coital

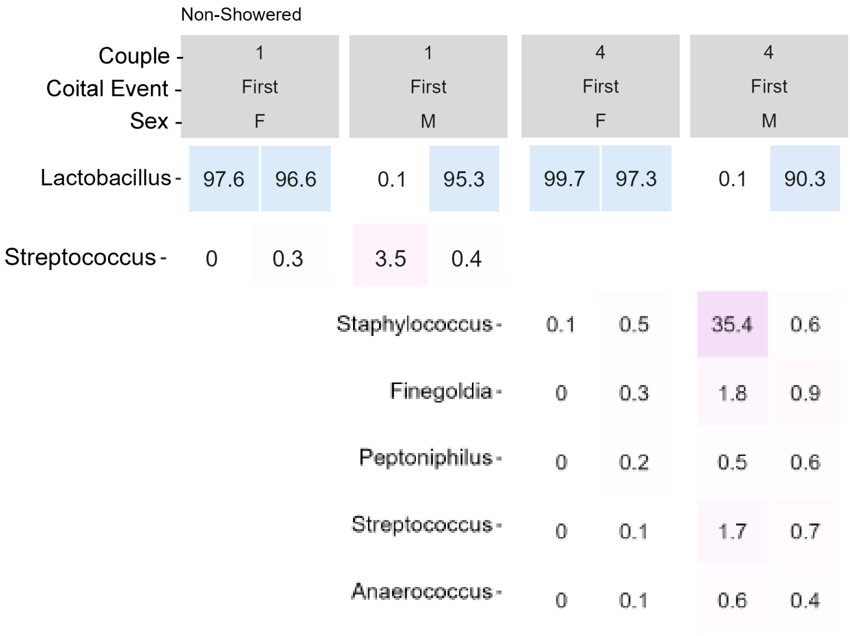


Non-Showered

Couple	1	
Coital Event	First	
Sex	F	
Lactobacillus	97.6	96.6
Corynebacterium	0.2	0.1
Atopobium	0	0
Gardnerella	0	0
Staphylococcus	0	0
Finegoldia	0.2	0.2
Peptoniphilus	0.1	0.1
Streptococcus	0	0.3
Anaerococcus	0.1	0
Sneathia	0	0
Prevotella_7	0	0
Remaining taxa (3145)	1.9	2.5
	Pre-coital	Post-coital

Couples 1 & 4

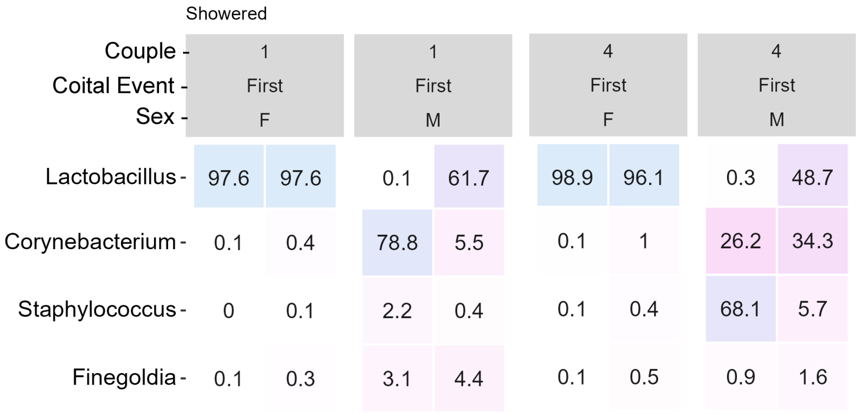
Non-showered



Non-Showered

Couple	1		1		4		4	
Coital Event	First		First		First		First	
Sex	F		M		F		M	
Lactobacillus	97.6	96.6	0.1	95.3	99.7	97.3	0.1	90.3
Streptococcus	0	0.3	3.5	0.4				
Staphylococcus					0.1	0.5	35.4	0.6
Finegoldia					0	0.3	1.8	0.9
Peptoniphilus					0	0.2	0.5	0.6
Streptococcus					0	0.1	1.7	0.7
Anaerococcus					0	0.1	0.6	0.4

Showered



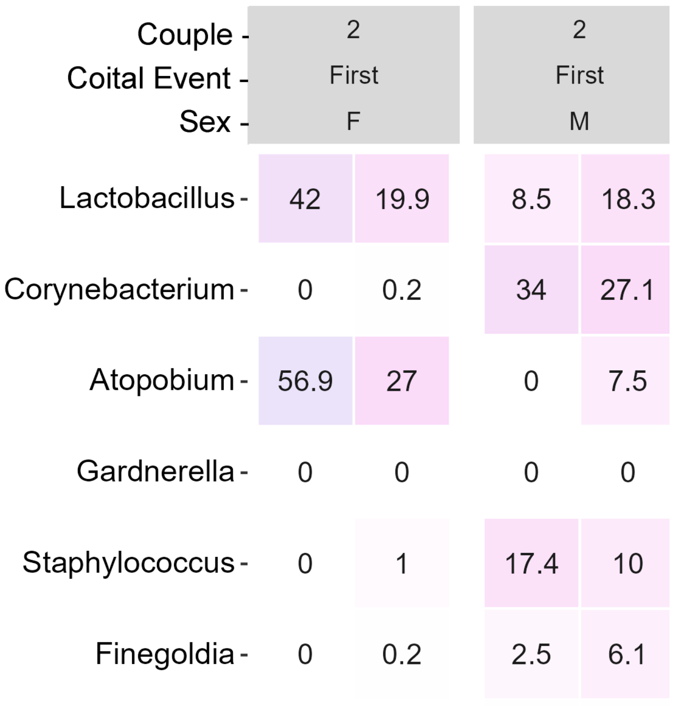
Showered

Couple	1		1		4		4	
Coital Event	First		First		First		First	
Sex	F		M		F		M	
Lactobacillus	97.6	97.6	0.1	61.7	98.9	96.1	0.3	48.7
Corynebacterium	0.1	0.4	78.8	5.5	0.1	1	26.2	34.3
Staphylococcus	0	0.1	2.2	0.4	0.1	0.4	68.1	5.7
Finegoldia	0.1	0.3	3.1	4.4	0.1	0.5	0.9	1.6

- Transfer of *Lactobacillus* dominant vaginal microbiomes to penile microbiomes persists through showering.
- Transfer of penile microbial genera persists through showering.

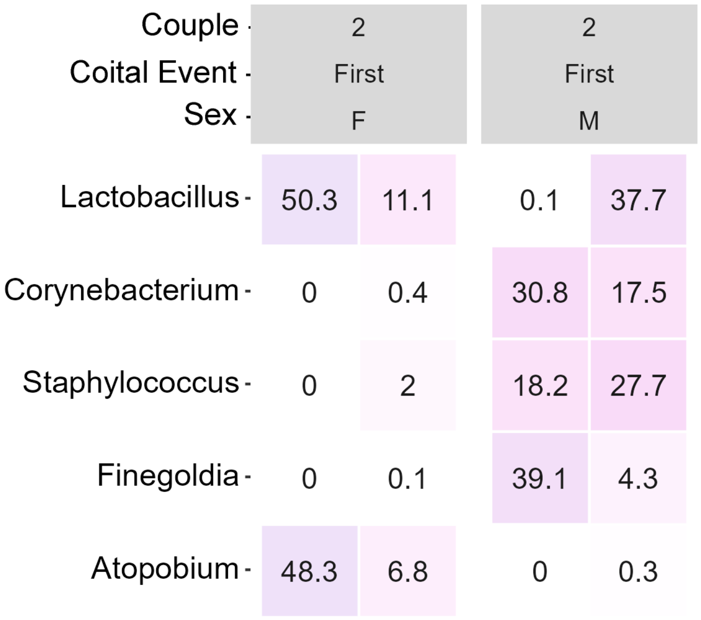
Couple 2

Non-showered



Couple	2		2	
Coital Event	First		First	
Sex	F		M	
Lactobacillus	42	19.9	8.5	18.3
Corynebacterium	0	0.2	34	27.1
Atopobium	56.9	27	0	7.5
Gardnerella	0	0	0	0
Staphylococcus	0	1	17.4	10
Finegoldia	0	0.2	2.5	6.1

Showered

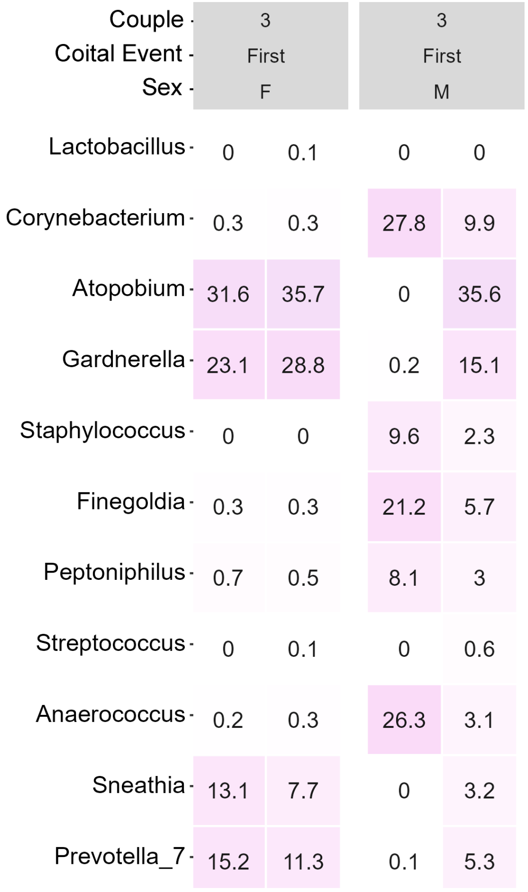


Couple	2		2	
Coital Event	First		First	
Sex	F		M	
Lactobacillus	50.3	11.1	0.1	37.7
Corynebacterium	0	0.4	30.8	17.5
Staphylococcus	0	2	18.2	27.7
Finegoldia	0	0.1	39.1	4.3
Atopobium	48.3	6.8	0	0.3

- Transfer of *Lactobacillus* and *Atopobium* dominant vaginal microbiomes to penile microbiomes persists through showering.
- Transfer of penile microbial genera persists through showering.

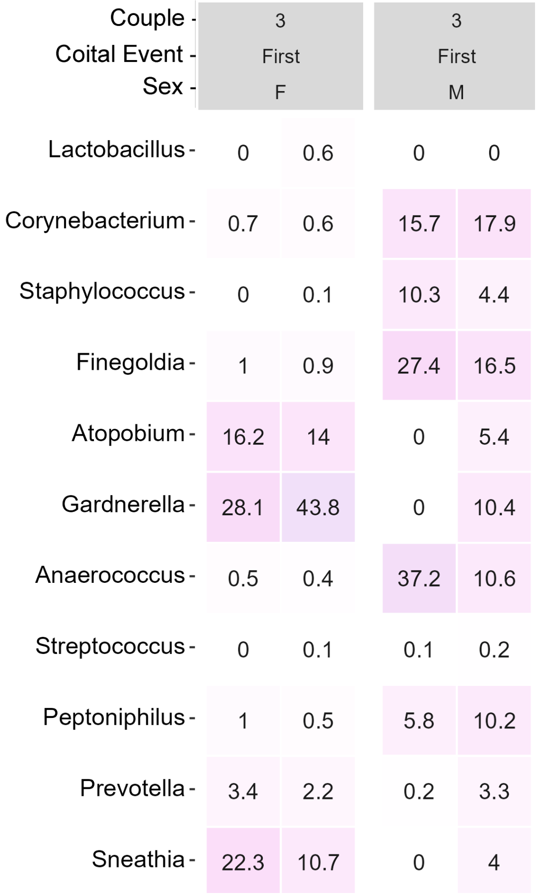
Couple 3

Non-showered



Couple	3	3	3	3
Coital Event	First	First	First	First
Sex	F	F	M	M
Lactobacillus	0	0.1	0	0
Corynebacterium	0.3	0.3	27.8	9.9
Atopobium	31.6	35.7	0	35.6
Gardnerella	23.1	28.8	0.2	15.1
Staphylococcus	0	0	9.6	2.3
Finegoldia	0.3	0.3	21.2	5.7
Peptoniphilus	0.7	0.5	8.1	3
Streptococcus	0	0.1	0	0.6
Anaerococcus	0.2	0.3	26.3	3.1
Sneathia	13.1	7.7	0	3.2
Prevotella_7	15.2	11.3	0.1	5.3

Showered



Couple	3	3	3	3
Coital Event	First	First	First	First
Sex	F	F	M	M
Lactobacillus	0	0.6	0	0
Corynebacterium	0.7	0.6	15.7	17.9
Staphylococcus	0	0.1	10.3	4.4
Finegoldia	1	0.9	27.4	16.5
Atopobium	16.2	14	0	5.4
Gardnerella	28.1	43.8	0	10.4
Anaerococcus	0.5	0.4	37.2	10.6
Streptococcus	0	0.1	0.1	0.2
Peptoniphilus	1	0.5	5.8	10.2
Prevotella	3.4	2.2	0.2	3.3
Sneathia	22.3	10.7	0	4

Transfer from *Atopobium*, *Gardnerella*, *Sneathia*, and *Prevotella* dominant vaginal microbiomes persists through showering.

Conclusions

1. Post-coital increase in vaginal diversity and simultaneous loss of penile diversity.
2. Transfer observed both in vaginal and penile genera.
3. Variable levels of post-showering persistence observed across donor couples.

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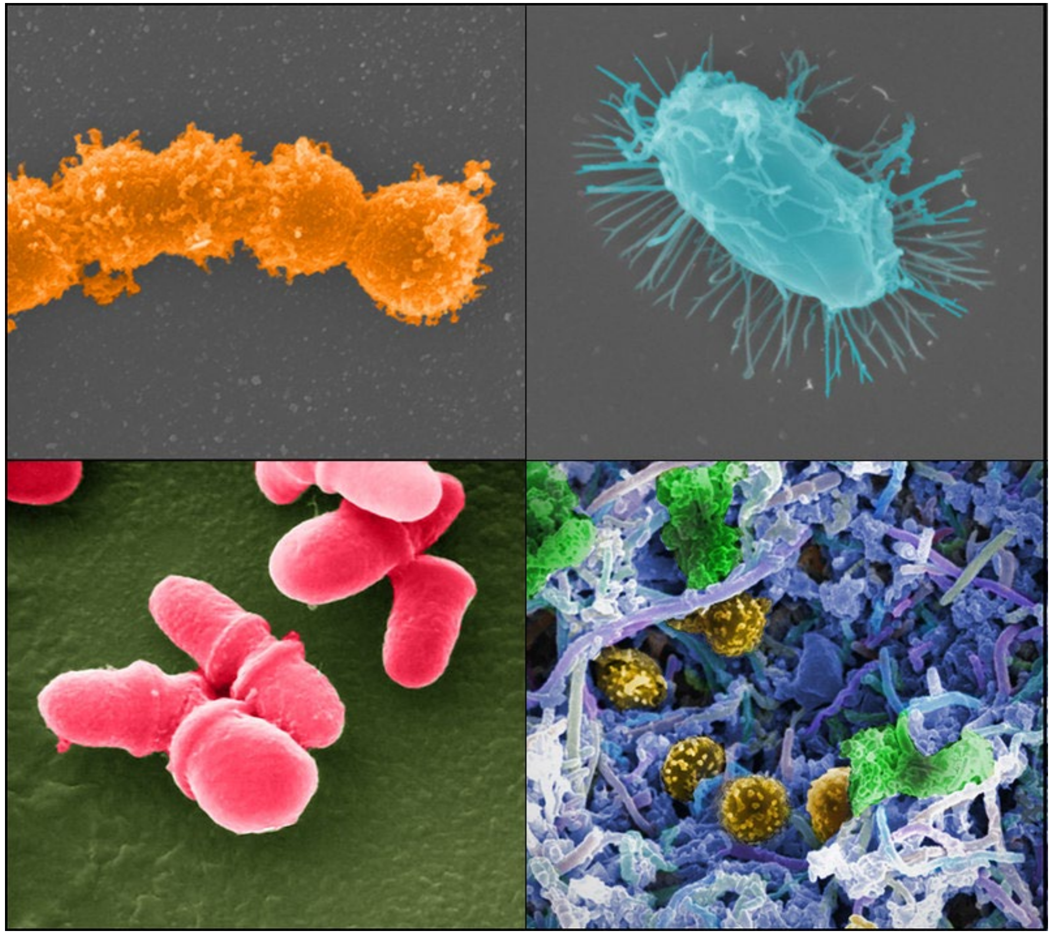

Thank you!

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