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# ♦ Protocols for "The female urinary microbiota in relation to the reproductive tract microbiota." V.3

Chen Chen<sup>1</sup>, Lilan Hao<sup>1</sup>, Weixia Wei<sup>2</sup>, Fei Li<sup>1</sup>, Liju Song<sup>1</sup>, Xiaowei Zhang<sup>1</sup>, Juanjuan Dai<sup>2</sup>, Zhuye Jie<sup>1</sup>, Jiandong Li<sup>1</sup>, Xiaolei Song<sup>1</sup>, Zirong Wang<sup>1</sup>, Zhe Zhang<sup>1</sup>, Liping Zeng<sup>2</sup>, Hui Du<sup>2</sup>, Huiru Tang<sup>2</sup>, Tao Zhang<sup>1</sup>, Huanming Yang<sup>1</sup>, Jian Wang<sup>1</sup>, Susanne Brix<sup>3</sup>, Karsten Kristiansen<sup>1</sup>, Xun Xu<sup>1</sup>, Ruifang Wu<sup>2</sup>, Huijue Jia<sup>1</sup>

<sup>1</sup>BGI-Shenzhen, Shenzhen 518083, China.; <sup>2</sup>Peking University Shenzhen Hospital, Shenzhen 518036, China.;

<sup>3</sup>Department of Biotechnology and Biomedicine, Technical University of Denmark, Soltofts Plads, Building 221, 2800 Kgs. Lyngby, Denmark.

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#### ABSTRACT

Lilan Hao

Human urine is traditionally considered to be sterile, and whether the urine harbours distinct microbial communities has been a matter of debate. Potential links between female urine and reproductive tract microbial communities is currently not clear. Here, we collected urine samples from 147 Chinese women of reproductive age and explored the nature of colonization by 16S rRNA gene amplicon sequencing, quantitative real-time PCR, and live bacteria culture. To demonstrate the utility of this approach, the intra-individual Spearman's correlation was used to explore the relationship between urine and multiple sites of the reproductive tract. PERMANOVA was also performed to explore potential correlations between the lifestyle and various clinical factors and urinary bacterial communities. Our data demonstrated distinct bacterial communities in urine, indicative of a non-sterile environment. *Streptococcus*-dominated, *Lactobacillus*-dominated, and diverse type were the three most common urinary bacterial community types in the cohort. Detailed comparison of the urinary microbiota with multiple sites of the female reproductive tract microbiota demonstrated that the urinary microbiota were more similar to the microbiota in the cervix and uterine cavity than to those of the vagina in the same women. Our data demonstrate the potential connectivity among microbiota in the female urogenital system and provide insight and resources for exploring diseases of the urethra and genital tract.

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Human urine is traditionally considered to be sterile, and whether the urine harbours distinct microbial communities has been a matter of debate. Potential links between female urine and reproductive tract microbial communities is currently not clear. Here, we collected urine samples from 147 Chinese women of reproductive age and explored the nature of colonization by 16S rRNA gene amplicon sequencing, quantitative real-time PCR, and live bacteria culture. To demonstrate the utility of this approach, the intra-individual Spearman's correlation was used to explore the relationship between urine and multiple sites of the reproductive tract. PERMANOVA was also performed to explore potential correlations between the lifestyle and various clinical factors and urinary bacterial communities. Our data demonstrated distinct bacterial communities in urine, indicative of a non-sterile environment. *Streptococcus*-dominated, *Lactobacillus*-dominated, and diverse type were the three most common urinary bacterial community types in the cohort. Detailed comparison of the urinary microbiota with multiple sites of the female reproductive tract microbiota demonstrated that the urinary microbiota were more similar to the microbiota in the cervix and uterine cavity than to those of the vagina in the same women. Our data demonstrate the potential connectivity among microbiota in the female urogenital system and provide insight and resources for exploring diseases of the urethra and genital tract.

#### **FILES**

	DNA extraction for human microbe samples.  Version 1 by Lilan Hao
	A Bioinformatics Analysis workflow for 16S rRNA Amplicon Sequencing data  Version 1 by Lilan Hao
	Quantitative real-time PCR for the four Lactobacillus species  Version 1 by Lilan Hao