

The vaginal microbiota of transmasculine individuals on testosterone hormone therapy

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Background

Population

- **Transmasculine (tM)** is a term used to describe individuals who were assigned female at birth but who do not identify as female.
- tM individuals may receive gender-affirming medical care (GAMC) to help better align their body with their gender.
- **Testosterone hormone therapy (T-HRT)** is a common component of GAMC for tM individuals.¹
- Vaginectomy (surgical closure of the vagina) and other masculinizing genital surgeries are relatively rare.¹
- It is therefore important to understand the potential impacts of T-HRT on the vagina and gynecological/sexual health.

Vaginal Microbiota

- *Lactobacillus* spp. dominate the vaginal microbiota in the majority of reproductive age cisgender females (cF) and are highly beneficial in maintaining a protective environment against pathogens.²
- In the absence of *Lactobacillus* spp. dominance, vaginal microbiota are comprised of diverse strict and facultative anaerobic bacteria.² This state is sometimes referred to as **molecular bacterial vaginosis (mBV)** and is associated with increased inflammation, gynecological symptoms, and **risk of acquiring HIV** and other sexually transmitted infections.^{3,4}
- Estrogen supports lactobacilli colonization by promoting the production of glycogen by vaginal epithelial cells, as glycogen-derived polymers are a preferred nutrient source for lactobacilli.^{5,6}
- **Reductions in estrogen**, which are seen post-menopause but also result from T-HRT, are associated with reductions in *Lactobacillus* abundance and increases in gynecological symptoms including vaginal atrophy, dryness, and dyspareunia.^{5,7-8} However, local or systemic estrogen hormone therapy can restore *Lactobacillus* dominance in post-menopausal cF.⁸
- The single previous study of the microbiota in tM receiving T-HRT (n=28) found only three participants had *Lactobacillus*-dominated microbiota, two of which were using local estrogen.⁹

Objectives: 1) to characterize the vaginal microbiota of tM individuals receiving T-HRT, and
2) to compare with the vaginal microbiota of reproductive age and post-menopausal cF.

Methods

Study Procedures

- Participants were recruited through social media, community organizations, referrals from health care providers, and recontact of consenting Trans PULSE Canada participants.
- Participants were mailed a study kit (Figure 1) that included instructions, materials for vaginal swab sample self-collection, and pre-paid envelopes to return weekly samples.
- Once weekly over three weeks, participants self-collected vaginal swab samples and completed an online questionnaire detailing demographics, recent gynecological symptoms, and behaviours.

Microbiota Analyses

- Microbiota were characterized through amplification, sequencing, and bioinformatic analyses of the V3-V4 region of the 16S rRNA gene.
- Microbiota profiles from transmasculine participants were compared to those from reproductive age (n=100) and post-menopausal (n=100) cisgender females, previously published by our group.
- *Lactobacillus* spp. dominance was defined as relative abundance >50% and the proportion of *Lactobacillus*-dominated samples was compared across groups using chi-squared tests.

Sample

- Transmasculine individuals were eligible if they were at least 18 years of age, lived in Canada, had not undergone vaginectomy, and had been on testosterone hormone therapy for at least 1 year.
- Preliminary results from 157 samples provided by 63 of 91 enrolled tM participants are presented.



The analyses presented are part of a larger study, TransBiota, which aims to characterize the vaginal microenvironments of both transmasculine individuals on testosterone hormone therapy and transfeminine individuals who have undergone vaginoplasty.



Figure 1. TransBiota study kit.

Results

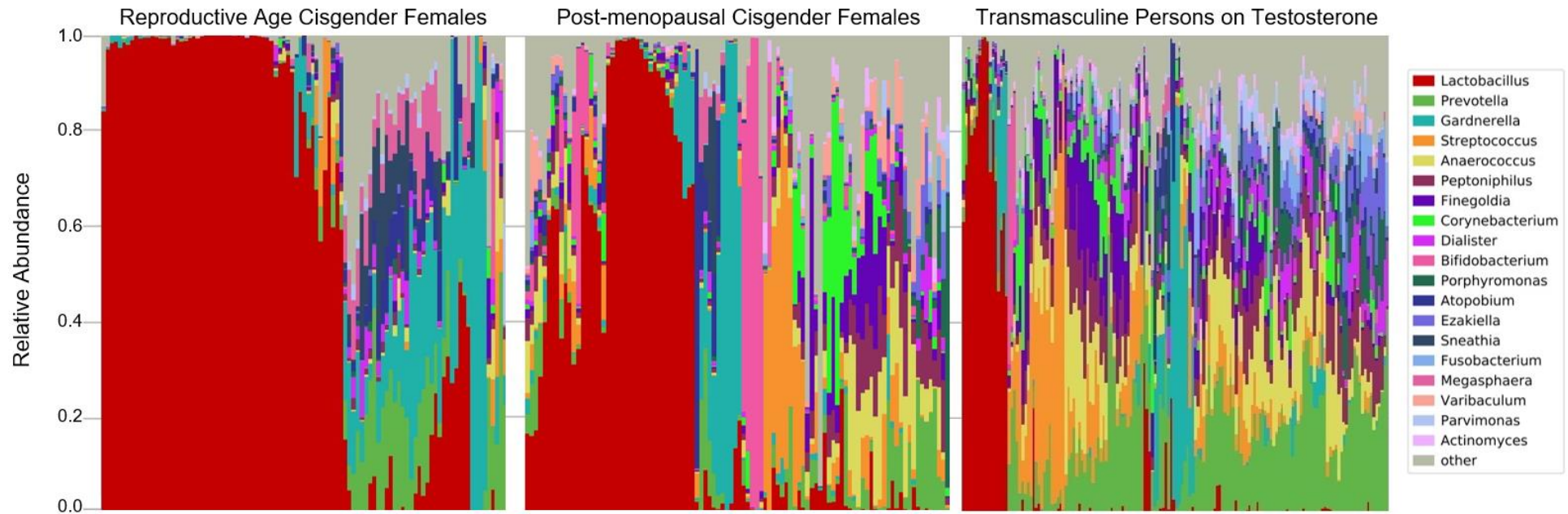


Figure 2. Relative abundance of the top 20 most abundant vaginal genera in reproductive age cF, post-menopausal cF, and tM on T-HRT.

Sample Characteristics

Participants had been on T-HRT for a median of 4.3 years (range 1.2 to 12.3 years). Nearly 1 in 3 tM participants (32%) reported experiencing vaginal dryness in the past 30 days. Itching/burning, pain, and bleeding were also commonly reported (24%, 22%, and 22%, respectively).

tM Microbiota

Lactobacillus spp. were detectable in 73% of tM samples but were dominant in less than 10%. Instead, the vaginal microbiota of tM were comprised of diverse communities of anaerobes, including species of *Prevotella*, *Streptococcus*, *Anaerococcus*, *Finegoldia*, and *Peptoniphilus*.

Comparison with Microbiota of Cisgender Females (cF)

The microbiota of tM were less likely to be dominated by *Lactobacillus* spp. than those of reproductive age (10% vs 59%, $p < 0.001$) and post-menopausal (10% vs. 29%, $p < 0.001$) cF. In the absence of *Lactobacillus* dominance, the microbiota profiles of tM were distinct from those of reproductive age cF but shared some resemblance with those of post-menopausal cF. Compared to reproductive age cF, species of *Sneathia*, *Megasphaera*, and *Atopobium* were scarcely observed and the prevalence of *Gardnerella* spp. was relatively low among tM. Compared to post-menopausal cF, *Bifidobacterium* spp. were less commonly observed among tM. Notably, the microbiota of tM appeared more likely to harbour species of *Ezakiella*, *Porphyromonas*, *Parvimonas*, and *Fusobacterium* than those of either reproductive age or post-menopausal cF.

Conclusion

- The microbiota of tM individuals receiving T-HRT were **unlikely to be dominated by *Lactobacillus* spp.**, but instead were comprised of **diverse communities of strict and facultative anaerobes**, including species of *Prevotella*, *Streptococcus*, *Anaerococcus*, *Finegoldia*, and *Peptinophilus*.
- The microbiota of tM shared some resemblance with those of *Lactobacillus*-deficient post-menopausal cF, but were distinguished by lower prevalence of *Bifidobacterium* and seemingly greater prevalence and abundance of *Ezakiella*, *Porphyromonas*, *Parvimonas*, and *Fusobacterium*. These distinctions suggest that **T-HRT may impact the vaginal microbiota beyond estrogen suppression**.
- Given the importance of the vaginal microbiota in HIV susceptibility and gynecological health, future work will investigate relationships between microbiota, inflammation, gynecological symptoms, and behavioural practices among tM on T-HRT.

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To learn more about the study, visit the TransBiota Facebook page: <https://www.facebook.com/TransBiota/>

