**Title:** The role of the penile microbiome in explaining differential mucosal inflammatory cytokines between men who have sex with men and men who have sex with women

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**Background:** Men who have sex with men (MSM) are disproportionately affected by HIV and STIs as compared to men who have sex with women (MSWo). This is hypothesized to be due in part to different burdens of mucosal inflammation, but has not been directly evaluated. We characterized the penile microbiome of MSM and MSWo, to evaluated whether penile microbial composition was associated with mucosal inflammation.

**Methods:** In this cross-sectional study, we enrolled 43 HIV negative MSM and 43 HIV negative MSWo in Kisumu, Kenya. Subjects were matched on age and circumcision status. The penile microbiome was assessed via a shallow meatal swab, with 16s rRNA amplicon sequencing of the V3-V4 regions. Urinary cytokine concentrations were measured using Luminex LabMAP multiplex system. After filtering and normalization, Random Forest (RF) with 5-fold cross validation was used to identify genus level taxa differing between MSM and MSWo. Used as feature selection, taxa from RF were regressed on cytokine outcomes, with multiple testing correction.

**Results:** Men were median age 24 and 77% circumcised. There were substantial differences in educational attainment, employment status, alcohol consumption, drug use, condom use, and number of sexual partners, with MSM in general having lower SES and greater behavioral risks. Microbiome composition differed substantially between MSM and MSWo: based on 10 taxa, RF discriminated between MSM and MSWo with 89% accuracy. Most important (i.e., highest Gini coefficient) discriminating taxa were *Lactobacillus, Anaerococcus,* and *Staphylococcus*. In crude analysis, cytokines TNF-α, IL-1β, IL-10, and IP-10 were statistically significantly elevated among MSWo as compared to MSM, while IL-8 did not differ. Microbiome composition did not account for the difference in TNF-α between MSM and MSWo, while the difference in IL-1β and IP-10 became non-significant once accounting for microbiome composition. Expected value of cytokines increased mostly in response to *Lactobacillus*, *Corynebacterium* and *Peptoniphilus*.

**Conclusions:** To our knowledge, this is the first direct comparison between MSM and MSWo of penile microbiome and urinary cytokines. Differences in penile microbial community composition may be important factors associated with markers of potential inflammation. Future studies should examine whether microbiome and mucosal inflammation differences between MSM and MSWo affect the efficacy of interventions for HIV and STIs.