# Association between foreskin microbiota and local cytokines in men from Rakai, Uganda

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**Abstract: Background:** Male circumcision (MC) reduces the risk of HIV, and also reduces genital anaerobes. Foreskin inflammation induced by specific genital bacteria could elicit local immune responses, promoting inflammation and recruitment of HIV target cells. However, direct associations between penile microbiota and inflammatory markers have not been demonstrated. We assessed the penile microbiota and cytokine levels in 138 uncircumcised men from Rakai, Uganda.

**Methods**: Microbiota were characterized using DNA extracted from a coronal sulcus swab eluent by 16S rRNA gene-based qPCR and V3V4 pyrosequencing, obtaining a total of 371,653 sequences. Genital bacteria present at >0.05% of total sequences were included in the analysis. Using the total microbiota density measured by qPCR and taxa proportional abundance from sequencing, we calculated the taxa absolute abundance in 16S rRNA gene copies. Eluent levels of 14 cytokines were measured using the Meso Scale Discovery electrochemiluminescent detection platform, and correlated with the absolute abundance of genital bacteria using a quasi-Poisson regression model. Model comparisons were performed by chi-square test.

**Results:** Only IL-8 levels were sufficient to permit quantitative correlation (*Median* = 0.72 $\log_{10}$, *IQR* = 0.18-1.38 $\log_{10}$). Among penile bacteria significantly reduced by MC in prior studies, 11 were positively correlated with IL-8 levels in univariate analyses, including anaerobes: *Prevotella*, *Porphyromonas*, *Finegoldia*, *Peptoniphilus*, *Peptostreptococcus*, *Anaerococcus*, *Dialister*, *Mobiluncus*, *Actinomyces*, *Sutterella*, and Unclassified Clostridiales Family XI. In multivariate analysis *Prevotella* (Δ= +49.6% in IL-8 per 10-fold increase in *Prevotella spp*, *P*=0.048) and *Peptostreptococcus* (Δ= +34.0%, in IL-8 per 10-fold increase, *P*<0.001) abundances were associated with increased IL-8 levels, with a similar trend for *Staphylococcus* (Δ= +21.5%, *P*=0.062). In contrast *Corynebacterium*, which are increased post-MC, were associated with lower IL-8 levels (Δ= -22.8%, *P*=0.0058).

**Conclusions:** *Peptostreptococcus*, which is decreased by MC, was associated with significant increases in penile IL-8, while *Corynebacterium*, which increases post-MC, was associated with lower IL-8. These findings suggest that post-MC changes in microbiota may reduce inflammatory cytokines and potentially HIV susceptibility.

**Hypothesis:** Penile anaerobes reduced by male circumcision are associated with increased inflammatory cytokines the foreskin.

**Study Design & Methods.** Coronal sulcus swabs were collected from 155 HIV-negative, married men during the Rakai MC RCT trial. Fourteen pro-inflammatory cytokines and chemokines were assayed using the MSD multiplex platform. DNA was extracted from coronal sulcus swabs. Total penile bacterial density quantified using pan-bacterial real-time PCR targeting 16S rRNA gene. Proportional abundance of each penile bacterial taxon was evaluated by pyrosequencing analysis. Absolute abundances of each penile bacterial taxon was calculated as: (total penile bacterial density x proportional abundance of the taxon). IL-8 level and bacterial absolute of MC responders were log10-transformed. The absolute abundance data were converted into ten-fold categories and categories with greater than 10% of participants (i.e., more than 15 men) were visualized with IL-8 levels in boxplots. Univariate quasi-Poisson models were used to assess each taxon's association with IL-8 and significantly associated taxa were analyzed using a multivariate model.

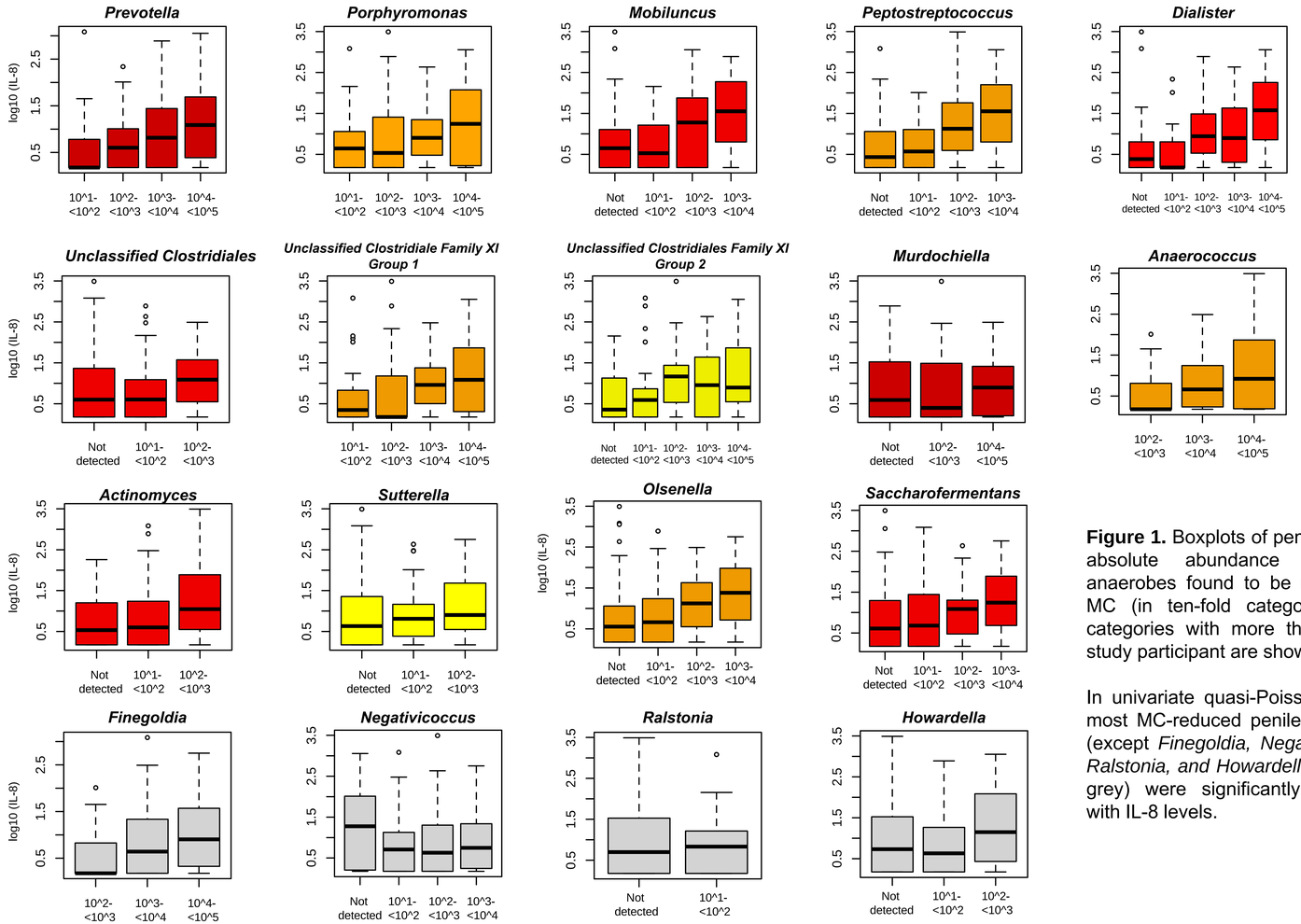



**Figure 1.** Boxplots of penile IL-8 and absolute abundance of penile anaerobes found to be reduced by MC (in ten-fold categories). Only categories with more than 10% of study participant are shown).

In univariate quasi-Poisson models, most MC-reduced penile anaerobes (except *Finegoldia, Negativococcus, Ralstonia, and Howardella*, shown in grey) were significantly correlated with IL-8 levels.

## Results

- ❑ MC significantly reduces the prevalence and absolute abundance of many penile anaerobes, including many taxa that have been previously associated with bacterial vaginosis (BV)
- ❑ In uncircumcised men, the penile anaerobes reduced by MC are significantly associated with elevated IL-8, a chemokine associated with increased density of HIV-target cells in foreskin tissue (ref: Poster #965 CROI 2014) (**Figure 1**)
- ❑ Multivariate analysis revealed that increased abundance of *Peptostreptococcus* spp. is significantly associated with elevated IL-8, while increased *Corynebacterium*—a bacterium that increases post-MC—is associated with significantly lower IL-8 levels.

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