vaginal microbiota and immune markers were associated with higher concentrations of VLY in reproductive-age women.

Methods Forty women self-collected mid-vaginal swabs in a cross-sectional study. Microbial communities were characterised by 16S rRNA gene amplicon sequence analysis. VLY (ng/ml) was detected by ELISA and normalised by a cube root transformation. Absolute bacterial abundance of *G. vaginalis* (log_{10} transformed) was estimated by multiplying its relative abundance by the sample total bacterial burden estimated by qPCR. Pro-inflammatory immune markers were quantified by Luminex and categorised above and below the median. Multivariate linear regression models evaluated factors associated with VLY abundance and controlled for confounders, including smoking and history of vaginal douching.

Results Vaginal microbiota clustered into 3 community state types (CSTs); 2 dominated by *Lactobacillus* spp. (*Lactibacillus iners* (CST-III) or *L. crispatus* (CST-I)), and one lacking *Lactobacillus* spp. and characterised by BV-associated bacteria including *G. vaginalis* and *Atopobium vaginae* (CST-IV). In the multivariate analysis, CST-IV, *G. vaginalis* bacterial load, a high Nugent Gram stain score, and a more basic vaginal pH (all $p<0.03$) were positively associated with increasing concentrations of VLY. TNF-α, TGF-β and IL-8 were inversely associated with VLY but only TNF-α remained significant in multivariate analysis ($p=0.01$).

Conclusion This study confirms that vaginal microbiota lacking lactobacilli, as well as other clinical indicators of BV, were associated with higher concentrations of VLY *in vivo*. Inflammatory markers were inversely associated with VLY. Because VLY may alter the vaginal microbiota and local inflammation, the role of VLY in BV warrants further evaluation.

O06.5 DEVELOPMENT OF A HUMAN URETHRAL EQUIVALENT TO STUDY *CHLAMYDIA TRACHOMATIS* INVASION

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Introduction *Chlamydia trachomatis* (Ct) genovars D-K cause non-invasive urogenital infections, which often remain asymptomatic. Little is known about the invasion of the epithelial layer and the subsequent effects of Ct on the epithelium in humans. The objective of this study was to develop a human urethral 3D *in vitro* equivalent to gain a better insight into the invasiveness of Ct in host tissue.

Methods Human urethral equivalents were constructed by seeding primary urethral keratinocytes and fibroblasts on top of and into a collagen matrix. Urethral cells were isolated from urethral clinical specimens of transgender patients undergoing gender surgery at VUMC. Urethral equivalents were incubated with a Ct genovar D strain, by placing a Ct impregnated nylon gauze on top of each model. Standard Ct cell culture, existing of HeLa cells grown on coverslips, were used as a control to assess growth of Ct strains used for infections of the urethral equivalents. Ct invasion was assessed after 2, 4 and 6 days of incubation.

Results Urethral equivalents consisted of a fully differentiated urethral epithelium on a urethral fibroblast populated collagen hydrogel. The epithelium consisted of multiple differentiated cell layers resembling native urethral tissue. We successfully infected urethral equivalents with a Ct genovar D strain. Ct invasion and expansion was detected in the epithelial layer, but not in the underlying collagen matrix, at 2, 4 and 6 days post infection. Morphological changes of the urethral equivalent could be observed at 2, 4 and 6 days post infection compared to non-infected urethral equivalents, whereby it appeared that the epithelial layer grows around the invaded Ct bacteria.

Conclusion We were able to construct a urethral equivalent resembling native urethral tissue. Moreover, these urethral equivalents could successfully be infected by a Ct genovar D strain, making this a promising life model to investigate the human pathogenesis of urogenital Ct infections.

O06.6 THE URETHRAL MICROBIOTA IN NONGONOCOCCAL URETHRITIS

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Introduction There is no known aetiology for up to half of all nongonococcal urethritis (NGU) cases. We sought to characterise the bacterial communities in men with (NGU+) and without (NGU-) NGU among men who have sex with men (MSM) and men who have sex with women (MSW), to determine if the urethral microbiota is associated with NGU status.

Methods From December 2014 to December 2015, urine samples were collected from 49 MSM (23 NGU+; 26 NGU-) and 48 MSW (21 NGU+; 27 NGU-) attending the Seattle STD Clinic. NGU was defined as urethral symptoms and/or visible discharge, and >5 PMNs per high powered field. *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) were detected by transcription mediated amplification (TMA). The urethral microbiota was characterised using broad-range 16S rRNA gene PCR with deep sequencing. Bacterial diversity was calculated using the Shannon index.

Results Mean urethral bacterial diversity in NGU+ MSM (0.82) was lower than in NGU- MSM (1.48), *Streptococcus* (n=7), *Corynebacterium* (n=5), *Haemophilus* (n=3), *Mycoplasma* (n=3), and *Lactobacillus iners* (n=2). Urethral microbiotas with dominant taxa were more common in NGU+ MSM (95.7%), while NGU+ MSW (61.9%) had microbiotas characterised by the presence of several vaginal bacteria. Among NGU- men, 53.8% MSM and 48.1% MSW had microbiotas with dominant taxa, including *Streptococcus* (n=19), *L. iners* (n=5), and *Gardnerella vaginalis* (n=4).

Conclusion The urethral microbiota in NGU is heterogeneous. NGU- MSM had more diverse urethral bacterial communities than NGU+ MSM, and presence of vaginal bacteria in MSW suggests sharing or acquisition from female partners. Future longitudinal studies may help inform if key bacteria predict incident NGU.