P06.05 DYNAMICS OF VAGINAL IMMUNE CORRELATES AND MICROBIOTA IN WOMEN FROM SUB-SAHARAN AFRICA

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Introduction Alterations in vaginal microbiota (VMB) have been shown to increase HIV acquisition and transmission in women. We carried out a longitudinal characterisation of the VMB, soluble cervicovaginal immune mediators and their determinants in women from Sub-Saharan Africa.

Methods Cervicovaginal lavages from two cohorts of sexually active women from Kenya, South Africa and Rwanda were analysed for IL-1α, IL-1β, IL-6, IL-12(p70), MIP-1β, IP-10, IL-8, GM-CSF, G-CSF, Elafin, SLPI, IL-1RA and total protein. qPCR was used to quantify total *Lactobacillus*, *L. crispatus, L. iners*, L. jensenii, *L. gasseri, L. vaginalis, A. vaginae*, *G. vaginalis*, *P. bivia* and *E. coli* in vaginal swab samples. Cohort A had 40 women with a healthy VMB (Nugent score < 4) at all five bi–weekly visits. Cohort B consisted of 40 women with incident bacterial vaginosis (BV) (Nugent score > 7) in the course of their visits.

Results Cohort A: Individual *Lactobacillus* species were consistently present or absent within each woman over five study visits. Sexual activity was associated with reduced counts of total *Lactobacillus*, *L. iners* and *Prevotella bivia* but increased concentrations of IL-6, IL-12(p70) and IP-10. pH was positively associated with IL-1RA and IL1RA/IL1(α+β) ratio but negatively associated with total protein and SLPI. The amount of total *Lactobacillus* was significantly lower and total soluble immune mediators, MIP-1β and IL-8 higher in 14 women on progesterone-only contraception compared to those with a cycle (20 not on any contraceptives and 6 on combined pill). Cohort B: Total *Lactobacillus*, *L. crispatus*, IP-10, GM-CSF, Elafin, SLPI and total protein were all reduced during the first visit with BV. Conversely, *G. vaginalis*, A. vaginae, E. coli and IL-1β were increased with incident BV.

Conclusion Sexual activity, progesterone, clinical symptoms of pathology and BV alter vaginal mucosal immunity in Sub-Saharan African women potentially increasing their susceptibility to HIV infection.

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P06.06 AZYTHROMYCIN TREATMENT FOR *CHLAMYDIA TRACHOMATIS* IS ASSOCIATED WITH VAGINAL MICROBIOTA LACKING PROTECTIVE *LACTOBACILLUS* SPP

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Introduction Recurrence rate of *Chlamydia trachomatis* genital infection is frustratingly high (~25%). While re-exposure is thought to be the main reason. We hypothesised that after and because of azithromycin treatment, the vaginal microbiota is not optimally restored to a protective *Lactobacillus* spp. dominated state, resulting in enhanced susceptibility to *C. trachomatis* re-infection.

Methods We characterised the composition, structure and metagenome of the vaginal microbiomes in a cohort of 129 *C. trachomatis*-positive (CT+) women followed longitudinally before and after azithromycin treatment. We established *in vitro* susceptibility patterns to azithromycin and doxycycline of vaginal bacteria, including *Lactobacillus* crispatus, *L. iners*, *L. gasseri*, *L. jensenii*, and *Gardnerella vaginalis*.

Results Before treatment, CT+ women harbour communities that comprised either a complex assemblage of strict anaerobes, including *G. vaginalis*, with low proportions of *Lactobacillus* spp. or a high abundance of *L. iners*. After azithromycin treatment, we observed an increased proportion of women with communities dominated by high abundance of *G. vaginalis* and other strict anaerobes, or dominated by *L. iners*. Antibiotic resistance assays showed that certain types of *L. iners* and *G. vaginalis* are highly resistant to azithromycin and to lesser extents to doxycycline. Analysis of *L. iners* genomes reconstructed from vaginal microbial communities metagenomes showed that multiple phylogenetic clades of *L. iners* exist. One of these clades is not associated with CT+ women, and is characterised by low number of phage genes as well as unique secondary metabolites gene clusters, all of which could contribute to their resilience.

Conclusion These findings suggest azithromycin treatment is likely to restore a vaginal microbiota with low protective properties, increasing the risk to *C. trachomatis* re-infection.

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P06.07 THE EFFECTS OF CONTRACEPTION ON THE VAGINAL MICROBIOTA

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Introduction The human microbiota plays important roles in immune system development and resistance to infection.

However, factors that influence vaginal bacterial community composition and dynamics are not well understood. There have been conflicting reports of altered vaginal microbiota and infection susceptibility with contraception use. The objective of this study was to determine if contraception use altered the vaginal microbiota.

Methods Vaginal swab samples were obtained from over 400 women during their first year of using hormonal contraception (levonorgestrel intrauterine system (LNG-IUS), depot medroxyprogesterone acetate (DMPA), combined oral contraceptive, contraceptive patch and etonogestrel implant) or a non-hormonal copper intrauterine device. Samples were obtained at baseline, 6 months and 12 months as part of the Contraceptive CHOICE study. The V4 region of the bacterial 16S rRNA-encoding gene was amplified from the vaginal swab DNA and sequenced with an Illumina MiSeq. The 16S rRNA gene sequences were processed and analysed using the software package mothur. After clustering the sequences into operational taxonomic units (OTUs) based on sequence similarity we calculated several ecological metrics including θ_{YC} distances (a metric that takes relative abundances of both shared and non-shared OTUs into account) between communities.

Results The vaginal microbiota in this study clustered into 3 major vaginal bacterial community types: one dominated by *Lactobacillus* iners, one dominated by *Lactobacillus* crispatus and one more diverse community type. Initial analysis indicates differences between the microbiota at baseline and after LNG-IUS use.

Additionally, specific OTUs were enriched with the use of certain contraceptive methods. For example, higher levels of 2 *Prevotella* OTUs were associated with DMPA use.

Conclusion Alterations of the vaginal microbiota are associated with the use of certain contraceptives. Further studies and analysis will be needed to verify these findings and determine the implications for infection susceptibility.

Disclosure of interest statement We did not receive any commercial contributions for this study.

P06.08 THE ASSOCIATION BETWEEN FREE GLYCOGEN IN THE VAGINAL FLUID AND COLONISATION BY LACTOBACILLI

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Introduction Glycogen is both an energy source and metabolic product of lactobacilli. Our objective was to assess the association between free glycogen in the cervicovaginal lavage (CVL) and vaginal microbiota as assessed by Nugent score, quantitative polymerase chain reaction (qPCR) for *Lactobacillus* crispatus and *L.iners*, and quantitative culture detection of lactobacilli.

Methods Healthy women (n = 55) aged 18–45 without clinical bacterial vaginosis, gonorrhoea, chlamydia or trichomoniasis were enrolled. A 10 mL CVL sample was collected and tested for glycogen using a fluorometric assay (BioVision) and protein was assessed using the Lowry assay. A vaginal smear was interpreted using the Nugent criteria. Separate vaginal swabs were used for vaginal culture and qPCR. Differences in median levels of glycogen were evaluated using the Kruskal-Wallis test.

Results Glycogen concentrations (ng/ug protein) were significantly higher in women having a Nugent score of 0–3, compared to those having scores of 4–6, or 7–10 (457 vs 398 vs 128, P = 0.049). Glycogen content was higher among women colonised by *L crispatus* vs other lactobacilli (*L. jensenii, L. gasseri, L. iners*) vs no lactobacilli (426 vs 280 vs 36, p = 0.013) based on culture. Similarly, the 38 women having *L. crispatus* dominant ($>10^5$) flora by qPCR had higher glycogen levels vs the 15 women who had dominant *L iners* (413 vs 201, P = 0.036).

Conclusion Increased levels of free glycogen in the CVL are associated with flora dominated by *L. crispatus*. It is unknown whether glycogen enhances *L. crispatus* colonisation, or whether *L. crispatus* synthesises glycogen, increasing the glycogen content.

Disclosure of interest statement Nothing to declare.

P06.09 *LACTOBACILLUS* CRISPATUS INHIBITS GROWTH OF *GARDNERELLA VAGINALIS* AND *NEISSERIA GONORRHOEAE* ON A PORCINE VAGINAL MUCOSA MODEL

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Introduction The vaginal microbiota affects susceptibility to bacterial vaginosis (BV) and sexually transmitted infections (STIs). BV is characterised by depletion of *Lactobacillus* spp., an overgrowth of anaerobes (usually dominated by *Gardnerella vaginalis*) and a pH > 4.5. BV is associated with an increased risk of acquiring STIs such as chlamydia and gonorrhoea. An *ex vivo* porcine vaginal mucosal model (PVM) was developed to explore the mechanistic role of *Lactobacillus* in affecting vaginal colonisation by *G. vaginalis* and *Neisseria gonorrhoeae*.

Methods Explants (5 mm) of freshly collected PVM were placed in transwells over various media, including *Lactobacillus* culture supernatant, inoculated with bacteria and incubated under aerobic or anaerobic conditions. Colonised explants were processed for CFU enumeration and presence of biofilm (via confocal microscopy) at indicated times. Lactic acid produced by a clinical isolate of L. crispatus growing on PVM was also quantified.

Results All isolates tested could colonise and grow on PVM. *G. vaginalis* and *N. gonorrhoeae* form biofilms on PVM. L. crispatus produces lactic acid on PVM and inhibits the growth of *N. gonorrhoeae* and *G. vaginalis* in a pH-dependent manner. Finally, L. crispatus produces a secreted factor that kills *N. gonorrhoeae* on PVM at an otherwise permissible pH.

Conclusion These data demonstrate that PVM is a useful model for studying the interactions of commensal vaginal microbes with pathogens on the vaginal mucosa. The data confirm a role for lactic acid in inhibiting growth of *G. vaginalis* and *N. gonorrhoeae*. The discovery of an L. crispatus secreted factor that kills *N. gonorrhoeae* is intriguing and future work will identify this compound and explore its mechanism of action.

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