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

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Disclosure of interest statement

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AYZITHROMYCIN TREATMENT FOR CHLAMYDIA TRACHOMATIS IS ASSOCIATED WITH VAGINAL MICROBIOTA LACKING PROTECTIVE LACTOBACILLUS SPP


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 phyllogenetic 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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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 similarities, we calculated several ecological metrics including pDC (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.

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**P06.08 THE ASSOCIATION BETWEEN FREE GLYCogen IN THE VAGINAL FLUID AND COLONISATION BY LACTOBACILLUS**

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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. crispsatus dominant (>10³) 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.

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**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 *in 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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