

013 - Vaginal health and the microbiome

013.1 THE BROAD DIVERSITY OF CULTIVABLE MICROBIOTA IN PREGNANT WOMEN AND THE DETECTION OF NOVEL ORGANISMS

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Background Our objective was to assess the full range of cultivable microflora from pregnant women and to identify the proportion which represents novel genera or species.

Methods Vaginal swab samples from 451 pregnant women (median gestational age 25 weeks, range 9–39) were transported in an anaerobic transport device. Vaginal fluid was eluted from the swabs, serially diluted and inoculated onto 27 different pieces of culture media. Colonies were subcultured to purity and DNA was extracted. A combination of biochemical tests, 16S rDNA gene sequencing, and restriction fragment length polymorphism (RFLP) were used for identification. A vaginal smear was assessed according to the Nugent criteria.

Results A total of 5688 isolates were recovered, with a mean of 12.6 isolates per sample. Women having a Nugent score of 0–3 had a lower number of cultivable species per sample (2408/255 = 9.4) compared to women having Nugent scores of 4–6 (1168/76 = 15.4) or 7–10 (2112/120 = 17.6). A total of 305 unique bacterial species were recovered, with 62 (20%) representing novel organisms which may have previously been considered uncultivable, including *Megasphaera* phylotypes 1–3, novel *Prevotella* species, and new genera of anaerobic gram negative and positive rods.

Discussion Culture methods detect a greater diversity of microorganisms than has been previously recognised. Multiple novel bacteria will require further characterisation.

Disclosure of interest statement There are no conflicts of interest to disclose. This study was funded by the US National Institutes of Health.

013.2 HORMONAL CONTRACEPTION IS ASSOCIATED WITH STABILITY AND *LACTOBACILLUS*-DOMINANCE OF THE VAGINAL MICROBIOTA IN A TWO-YEAR OBSERVATIONAL STUDY

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Introduction Hormonal contraception (HC) has been associated with a reduced risk of bacterial vaginosis (BV). We conducted a prospective study to evaluate the relationship between vaginal microbiota and several HC methods.

Methods During 2-years of follow-up, 108 women provided 2,662 mid-vaginal samples. Participants reported three HC

methods [oral contraceptive pill (OCP), vaginal ring, implanon]. Controls not taking HC were also followed. Visits were scheduled at baseline, 2 weeks, 4 weeks, 3 months, 6 months, 12 months, 18 months, and 24 months. Additionally, participants self-collected mid-vaginal swabs twice-weekly in the two weeks before each visit. Vaginal microbiota composition was characterised on all samples by 16S rRNA gene analysis of the V3-V4 hypervariable regions. We identified four community state types (CSTs) which were dominated by *Lactobacillus* species. CST-IV-B was characterised by a low relative abundance of *Lactobacillus* spp. and higher proportions of BV-associated bacteria (*Gardnerella* and *Atopobium*). A multinomial model for dependence of proportions was used to evaluate the association between CST and HC. Jensen-Shannon distances between all pairs of samples were analysed to assess stability of the microbiota longitudinally.

Results Women on OCPs had more stable bacterial communities than controls during the 2-year follow-up ($p = 0.04$). HC (overall) also tended toward greater stability ($p = 0.10$). The low-*Lactobacillus* CST-IV-B had an 83% lower probability among OCP users and a 55% lower probability among implanon users. CST-II (*L. gasseri*-dominated) was significantly higher in all HC types compared to controls. Additionally, OCP users were more likely to be CST-III-A (*L. iners*-dominated) and CST-V (*L. jensenii*-dominated), while CST-III-A was also high among ring users. CST-I (*L. crispatus*-dominated) was associated with implanon. All reported findings were statistically significant ($p < 0.03$).

Conclusion Women on HC experienced greater stability of the vaginal microbiota over time compared to controls. OCPs and the vaginal ring were protective against transition to a *Lactobacillus*-depleted state.

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013.3 THE IMPACT OF VAGINAL BACTERIAL BIOFILM ON INTRAVAGINAL RINGS

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Introduction Intravaginal rings are used worldwide, mostly for contraception, and are now being investigated for pre-exposure prophylaxis for HIV and multipurpose technologies that combine antiviral products with contraception. Despite its ubiquitous use, little research has been done to study the effect of the vaginal microbiome on these rings.

Methods The amount of bacterial biofilm was assessed using crystal violet staining (CV) on 403 contraceptive intravaginal rings (CVR) worn for 21 days by 120 women participating in a CVR clinical trial in Rwanda. A subset of 22 CVRs was evaluated by confocal microscopy after Fluorescence *In Situ* Hybridization (FISH) with species-specific probes for *A. vaginae* (Av), *G. vaginalis* (Gv) and for the *Lactobacillus* genus (Lsp). At each CVR-removal visit, vaginal slides were made for Nugent scoring to diagnose bacterial vaginosis (BV) and FISH to assess the presence of Av and Gv biofilm.

Results A higher amount of biofilm on the CVR, according to CV, was associated with the presence of vaginal biofilm of Av ($p < 0.001$) and Gv ($p = 0.002$), but less with vaginal planktonic Av ($p = 0.026$) and not with dispersed Gv ($p = 0.189$), visualised with FISH. A higher amount of CVR-biofilm was also found in participants suffering from BV compared to women with a healthy vaginal microbiome ($p < 0.001$). FISH of the CVRs showed large areas of the ring surfaces covered with biofilm of vaginal epithelial cells and bacteria. BV-associated bacteria were included in the biofilm, as well as health-associated lactobacilli.

Conclusion Our study shows that biofilm is common on IVRs and consists of vaginal cells and microbes residing in the vagina: BV-associated bacteria and lactobacilli. The presence of biofilm of BV-associated bacteria in the vagina however leads to an increase of biofilm on the IVRs and might contribute to the persistence of the condition or could hamper the release of active product.

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013.4 CERVICOVAGINAL MICROBIOME DYSBIOSIS IS ASSOCIATED WITH PROTEOME CHANGES RELATED TO ALTERATIONS OF THE CERVICOVAGINAL MUCOSAL BARRIER

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Introduction Vaginal microbiome (VMB) dysbiosis is associated with increased acquisition of HIV and sexually transmitted infections (STIs). Cervicovaginal inflammation and other changes to the mucosal barrier are thought to play important roles but human data are scarce. In this study, we compared the cervicovaginal proteome among women with different VMB compositions.

Methods Cervicovaginal lavages of 50 Rwandan female sex workers with known VMB composition were selected for human proteome analysis using mass-spectrometry. These women were previously clustered into four VMB groups in order of increasing bacterial diversity: group 1 had a *Lactobacillus crispatus*-dominated VMB; group 2 a *L. iners*-dominated VMB; group 3 moderate dysbiosis; and group 4 severe dysbiosis. We compared relative protein abundances among these VMB groups using targeted (abundance of pre-defined mucosal barrier proteins) and untargeted (differentially abundant proteins among all human proteins identified) approaches.

Results With increasing bacterial diversity, we found: mucus alterations (increasing mucin 5B and 5AC), cytoskeleton alterations (increasing actin-organising proteins; decreasing keratins and cornified envelope proteins), increasing cell death (using LDHA/B as biomarkers of cell death), altered proteolytic activity (increasing proteasome core complex proteins/proteases; decreasing antiproteases), altered antimicrobial peptide balance

(increasing psoriasin, calprotectin, and histones; decreasing lysozyme and ubiquitin), increasing proinflammatory cytokines, and decreasing immunoglobulins IgG1/2.

Conclusion The VMB is strongly associated with the cervicovaginal human proteome in this cohort of Rwandan women at high risk of HIV and other STIs. Although temporal relationships cannot be derived, our findings support the hypothesis that dysbiosis causes cervicovaginal inflammation and other detrimental changes to the mucosal barrier that may lead to increased HIV/STI acquisition.

Disclosure of interest statement This work was funded by the Institute of Infection and Global Health of the University of Liverpool, the Aids Fonds Netherlands (project number 201102), European and Developing Countries Clinical Trials Partnership (project number CT.2005.33070.001) and the European Commission (CHAARM consortium). The authors declare no conflicts of interest.

013.5 ASSOCIATION BETWEEN DIETARY INTAKE AND DYSBIOTIC VAGINAL MICROBIOTA

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Background Suboptimal nutrition has been associated with an increased risk of bacterial vaginosis (BV). In this study, we examined the association between dietary intake and BV-associated vaginal microbiota.

Methods We analysed the baseline visit of the Hormonal Contraception Longitudinal Study, a cohort of reproductive-aged women who reported at enrollment intentions to initiate or cease hormonal contraception (HC). Dietary intake was estimated using the Block Brief 2000 Food Frequency Questionnaire. Vaginal microbiota composition was assessed using 16S rRNA gene analysis and categorised based on the types and relative abundance of bacteria (termed community state types (CSTs)). Nutrients were categorised into quartiles and the associations between nutrients and CST-IV, a low-*Lactobacillus* CST, were evaluated by logistic regression. Separate models were built for each nutrient controlling for demographics, tobacco use, behavioural factors, HC and dietary variables (total energy intake, and where appropriate, percent of calories from fat, protein, carbohydrates).

Results A total of 98 women were included in this analysis. The mean age of the women was 25.9, mean body mass index was 27.9, 29.6% were African American and 47.9% were on HC at enrollment. 26.5% of women had a low relative abundance of *Lactobacillus* spp. (CST-IV). In adjusted multivariate analyses, the highest quartile of vitamin E (OR: 0.01, 95% CI: 0.001–0.26), zinc (OR: 0.03, 95% CI: 0.18–0.03) and magnesium (OR: 0.06, 95% CI: 0.004–0.75) intake were associated with reduced risk of carrying a low-*Lactobacillus* CST-IV state.

Conclusion Higher intakes of vitamin E, zinc, and magnesium were associated with a decreased risk of having a dysbiotic vaginal microbiota. These findings concur with prior studies that have reported magnesium and zinc deficiencies associated with recurrent bacterial infections and inflammation, and vitamin E (an antioxidant) with anti-inflammatory properties. Dietary interventions targeted at improving intake of select micronutrients may decrease the risk of bacterial vaginosis and its sequelae.