O13 - Vaginal health and the microbiome

O13.1 THE BROAD DIVERSITY OF CULTIVABLE MICROBIOTA IN PREGNANT WOMEN AND THE DETECTION OF NOVEL ORGANISMS

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10.1136/sextrans-2015-052270.147

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* phytophylotypes 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.

O13.2 HORMONAL CONTRACEPTION IS ASSOCIATED WITH STABILITY AND LACTOBACILLUS-DOMINANCE OF THE VAGINAL MICROBIOTA IN A TWO-YEAR OBSERVATIONAL STUDY

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10.1136/sextrans-2015-052270.148

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, implant]. 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-L. *Lactobacillus* CST-IV-B had an 83% lower probability among OCP users and a 55% lower probability among implant 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. jenseni-dominated), while CST-III-A was also high among ring users. CST-I (L. crispatus-dominated) was associated with implant. 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.

Disclosure of interest statement The study was funded by the US National Institute of Allergy and Infectious Diseases (NIAID) R01-A1089878. No pharmaceutical grants were received in the development of this study.

O13.3 THE IMPACT OF VAGINAL BACTERIAL BIOFILM ON INTRAVAGINAL RINGS

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10.1136/sextrans-2015-052270.149

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 (Lap). 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.

Sex Transm Infect 2015;91(Suppl 2):A1–A258

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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 persistence of the condition or could hamper the release of active product.

This work was supported by European and Developing Countries Clinical Trials Partnerships (EDCTP), by Combined Highly Active Anti-Retroviral Microbicides (CHAARM) and by Donmeur Investment Service. No pharmaceutical grants were received in the development of this study.

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

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 VMB; group 2 a

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