

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.