Hormonal contraception is associated with stability and *Lactobacillus*-dominance of the vaginal microbiota in a two-year observational study

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Hormonal contraceptives (HC) and bacterial vaginosis (BV)

- With BV ~ STIs and treatments for BV sub-optimal, any additions to the BV treatment toolkit are welcomed.
- The data suggest that HC use, particularly OCP, DMPA and implants, are associated with decreased BV recurrence in some women.
  - Data are sparse on how intrauterine devices (copper & hormonal) and contraceptive vaginal rings affect the vaginal microbiota.
- HC use is common.
  - 17% of U.S. women aged 15-44 years (~18 million) use HC.
- **Why does HC use decrease the risk of BV recurrences in some women but not others?**
- We sought to determine how HC use affects the vaginal microbiota in an observational cohort.

Bradshaw *CID* 2013
Riggs *Sex Transm Dis* 2007
Shoubnikova *Contraception* 1997
Rifkin *Contraception* 2009
Vodstrcil *PloS ONE* 2013
Baeten *Am J Obstet Gynecol* 2001

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How would HC reduce BV?

- HC, especially those containing progestins, inhibit uterine bleeding and reduce menstrual loss. Menses ~ abnormal vaginal microbiota.
- Adequate estrogen levels increase glycogen production in vaginal epithelial cells, and glycogen is broken down into metabolites used by *Lactobacillus* spp. to thrive and produce lactic acid.
- HC may also promote a favourable anti-inflammatory cytokine milieu that facilitates BV clearance
- Despite >50 years of widespread use, the effect of HC on the vaginal microbiota are still incompletely understood and have not been widely studied.

Achilles and Hillier, *AIDS* 2013– **an excellent review**
Vodstrcil, *PloS ONE* 2013– **a meta-analysis**

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Study design- HCL

Pre-screen   Enroll   2wk   4wk   3m   6m   12m   18m   24m

- 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.
- Daily behavioral diary collected in 2 weeks before each visit.
- Blood, urine, vaginal swabs collected.
- STI screening at baseline and follow-up as necessary.

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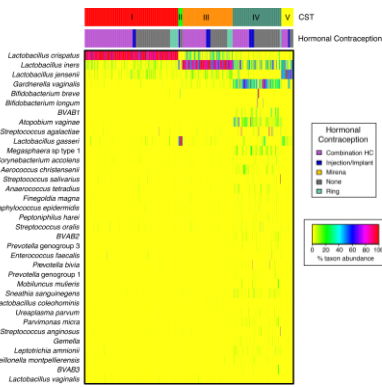INSTITUTE FOR GENOME SCIENCES

Methods

- The study is ongoing.
- To date, we have analyzed 112 women (>2,800 mid-vaginal samples)
- This analysis is based on the three most commonly chosen HC methods: oral contraceptive pill (OCP), vaginal ring, implanon.
- Controls not taking HC were also followed.
- Microbiota analysis:
  - Vaginal microbiota composition was characterized by 16S rRNA gene analysis of the V3-V4 hypervariable regions.
  - We identified community state types (CSTs) which are categories based on the diversity and relative abundance of taxa detected.
  - 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 analyzed to assess stability of the microbiota longitudinally.

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Study Population

- 16 to 35 year old women initiating or planning to discontinue HC (mean 26 years)
- 64% white and 25% African American
- Age-matched control women presenting for routine gynecological care who were not interested in starting HC.
- Time from last HC use has been at least 3 months for OCP and 6 months for the longer acting formulations (e.g. DMPA).
- All women initiating HC undergo clinical evaluation to ensure safety, and to determine the most appropriate HC formulation.
- *Exclusion criteria included*: any contraindications to HC, hysterectomy, known condition altering sex hormone cycles (e.g. polycystic ovarian syndrome, premature ovarian failure, etc), known immunodeficiency, diabetes mellitus, contraindications for HC use (also applied to the control group)
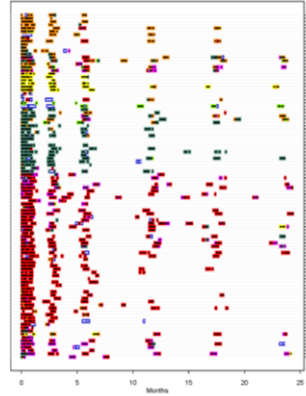
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Slide 1

Relative abundance of bacterial taxa in the HCL study (n=2801 samples)

- CST assignment algorithm uses a machine learning algorithm called Support Vector Machine (SVM) and was trained on over 4000 samples.

- Combination HC: OCP, patch, ring

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Slide 2

Longitudinal profile of all women on HCL study

- Sorted by proportion of CSTs

- 112 women

- 2,801 samples

- We see some fluctuation within the 2 week period prior to clinical visit

- Overall, a lot of stability

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Slide 3

Stability of the vaginal microbiota

- 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).

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Slide 4

Association between hormonal contraception and community state type (CST)[†] compared to women on no HC in a longitudinal study

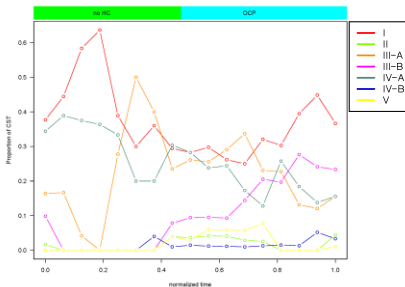| CST	dominant bacteria	OCP[§]	Ring	Implant
I	*L. crispatus*	-	-	+
II	*L. gasseri*	+	+	+
III	*L. iners*	+	+	-
IV-A*	Low-*Lactobacillus*	-	-	+
IV-B*	Low-*Lactobacillus*	-	0	-
V	*L. jensenii*	+	-	-

[†] CSTs reflect the clustering of samples based on bacterial composition and abundance.

* **CST IV-A** is characterized by various species of anaerobic bacteria including *Anaerococcus, Peptoniphilus* and *Prevotella* spp., whereas **CST IV-B** had higher proportions of bacteria from the genera *Gardnerella, Atopobium* and *Megasphaera* among others.

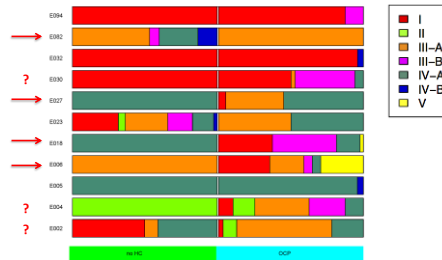[§] The "+" is increased, "-" is decreased and 0 is not statistically significant.

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Slide 5

Change in CST over time with OCP initiation

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Slide 6

Proportion of CSTs for women initiating OCP, n=11 women and 385 observations

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Difficulties in HC studies

- Overall, where data on HC and BV are conflicting, it is most likely due to a number of factors including:
  - heterogeneity of approaches used to diagnose BV and assess vaginal microbiota
  - observational nature of the studies:
    - sexual behaviors, and particularly condom usage, may change with HC
    - regimen compliance
    - switching of HC types
    - the availability of various formulations of HC with differences in progestational activity

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