

The many facets of the vaginal microbiota

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Microbial communities analyses

Several levels of analyses:

- **Who is there? Define the microbiota**
 - Survey of the microbial species composition and abundance in a samples by sequencing taxonomic marker genes (i.e. 16S rRNA gene) – relative abundance
 - Cultivation – Quantitative, access to isolates but more time consuming
 - Targeted qPCR – Quantitative, no discovery, more expensive
- **What can they do? Metagenomics**
 - Determine the microbial community genes and genomes content by sequencing total genomic DNA
- **What are they doing? Metatranscriptomics**
 - Determine the suite of gene expressed by the microbial community (and host) by sequencing total cDNA

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Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. *Microbiome* 2015, 3:31.

The normal human vaginal microbiota

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Vaginal community composition

- Well-powered study of 400 asymptomatic reproductive age women, equally representing 4 ethnic groups
- Five major **community state types** that differ in their microbial composition and abundance
- Community state type IV lack significant number of *Lactobacillus* - higher diversity

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Ravel et al. The vaginal microbiome of reproductive age women. *PLoS*. 2011, 108 Suppl 1, e460-4607.

Community State Types - Large sample size

- ~5,000 samples from over 800 women
- Illumina MiSeq - 300 bp PE - V3-V4 regions of the 16S rRNA gene
- Seven community state types (CST III and CST IV sub-divided)

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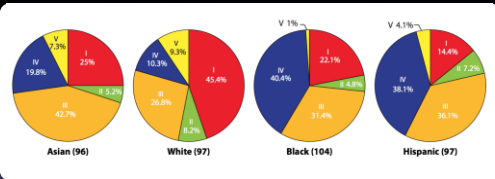
Misconceptions about Community State Types

"They forced their results in community state types generated by [previous] datasets" "CST are determined by the dominant *Lactobacillus* species"

- How are CSTs generated?
 - CSTs are useful in reducing the complexity of the dataset and allowing epidemiological investigations.
 - Generated by clustering of distance matrix - "Not done by eye"
 - Assignments of vaginal CSTs do not depend on the method used, the signal is so strong that multiple metrics often produce the same results. However assignments can be dependent on the dataset.
- Support Vector Machine CST assignments
 - SVM approach affords consistent assignments independent of the dataset

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Frequency of CSTs in ethnic groups



Community abundance differ among ethnic groups ($\chi^2 = 36.8$ on 10 df $P < .0001$) when comparing community proportions on the five most common communities



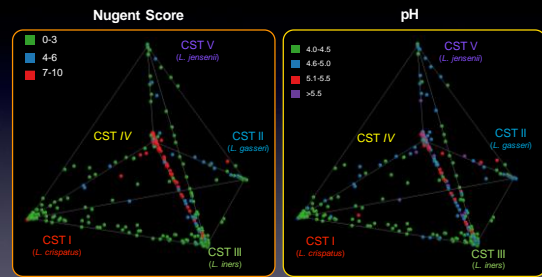
Ravel et al. The vaginal microbiome of reproductive age women. PNAS. 2011, 108 Suppl 1, 4680-4687

CST IV: an common state that carry risks?

- At any given time, >25% of women are in a non-*Lactobacillus* dominated state.
- This state is associated with higher Nugent scores (Nugent-BV) and higher pH



pH, Nugent score, community state types



Ravel et al. The vaginal microbiome of reproductive age women. PNAS. 2011, 108 Suppl 1, 4680-4687

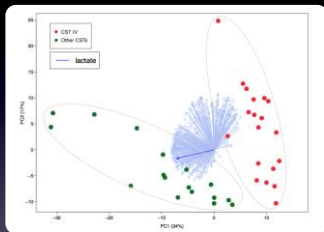
CST IV: an common state that carry risks?

- At any given time, >25% of women are in a non-*Lactobacillus* dominated state.
- This state is associated with higher Nugent scores (Nugent-BV) and higher pH
- Nugent-BV is associated with increased risk of sexually transmitted infection acquisition and transmission, including HIV
- When asymptomatic and apparently healthy women with CST IV are at increased risk of STI



Wiesenfeld HC, et al. Bacterial vaginosis is a strong predictor of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection. *Clinical Infectious Diseases* : an official publication of the Infectious Diseases Society of America 2003, 36(5):663-669.
 Taha TE, et al. Bacterial vaginosis and disturbances of vaginal flora: association with increased acquisition of HIV. *AIDS (London, England)* 1998, 12(13):1699-1706.
 Cohen, C. R., et al. (2012). Bacterial Vaginosis Associated with Increased Risk of Female-to-Male HIV-1 Transmission: A Prospective Cohort Study. *Journal of Infectious Diseases*, 205(10):1507-1515.

CST IV metabolic signatures



- Significant differences in lactate production, polyamine production, and metabolites associated with redox homeostasis and inflammation



Srinivasan S, et al. Metabolic signatures of bacterial vaginosis. *Mbio* 2015, 6.
 Yoonessi G, et al. A multi-omic, systems-based approach reveals metabolic markers of bacterial vaginosis and insight into the disease. *PLoS ONE* 2013, 8:e61111.
 Ravel et al. Nugent-BV is associated with specific metabolic profiles with implications for STI susceptibility. Unpublished.

CST IV: an common state that carry risks?

There are windows of higher risk that open and close on a temporal scale


- How long does CST IV persist over time? How frequently does the vaginal microbiota of a women is in CST IV?
- Do duration and frequency CST IV better represent risk?

Longitudinal studies of the vaginal microbiota



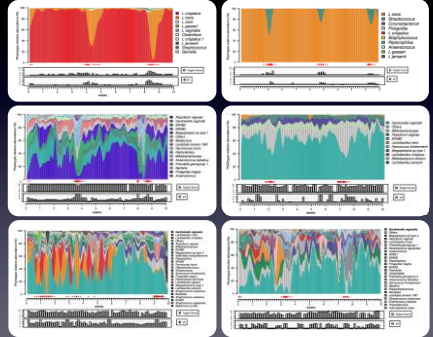

The dynamics of the vaginal microbiota

A longitudinal study



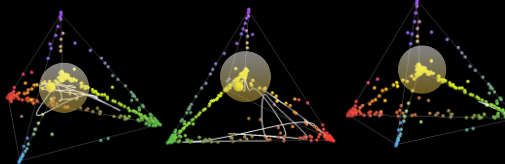
Longitudinal profiles - stability/instability

- 160 women
- Daily sampling
- 10-week study


Vaginal community dynamics

Windows of higher risk that open and close on a temporal scale




Low resilience = increased risk

Understand the molecular basis of the association between stability and susceptibility

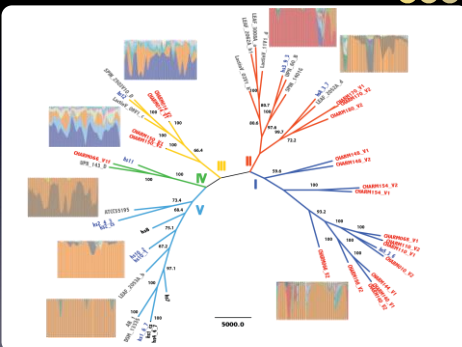



Stability and the community genome

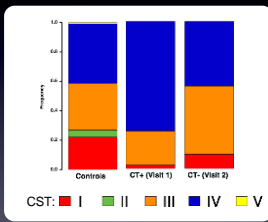
- Is there a correlation between the gene content of some of the species and community stability?
- Use metagenomics analysis of microbial communities
- Establish community composition and assemble the genomes of community members
- Perform comparative genome analysis with known genomes




L. iners and community stability

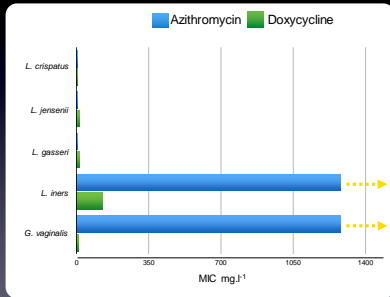
CHARM vaginal microbiota V1(CT+) and V2 (CT-)



CST: I II III IV V



Vaginal Bacteria Antibiotics Resistance



Poster P.06.06

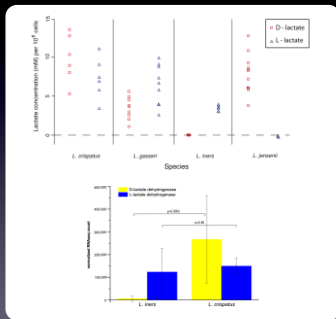
Conclusions

- It appears that some strains of *L. iners* are unable to dominate the vaginal microbiota, are associated with increased instability, are selected for by antibiotic treatment and are associated with susceptibility to *C. trachomatis*.

Why does *L. iners* appear to be less protective than others *Lactobacillus* ?



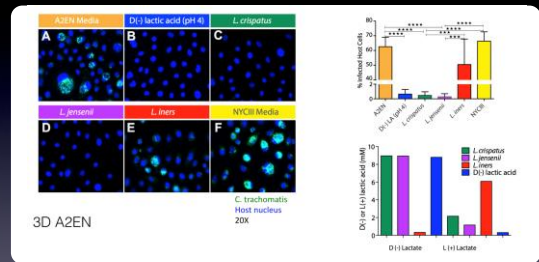
D/L lactic acid production - *Lactobacillus* spp.



Wilson, S. S., Mendes-Goncalves, H., Johnson, L. M., Jayaram, A., Ledger, W. J., & Forney, L. J. (2013). Influence of vaginal bacteria and D- and L-lactic acid isomers on vaginal extracellular matrix metalloproteinase inducer: implications for protection against upper genital tract infections. *mSystems*, 8(6).



Lactic acid isomers affect *C. trachomatis* infection



Culture supernatants from *Lactobacillus* spp. inhibit *C. trachomatis* infection according to the concentration of D(-) and L(-) lactic acid present



Poster P.08.06

Gaps and Challenges

- Our understanding of the interactions between the vaginal microbiome and the host is incomplete and fragmented - Mostly from studies that are difficult to compare to others - Need for harmonization of the methods
- We need larger studies that use Systems Biology approach to model these interactions *in vivo* over time in health and disease, so that predictive models and rationale interventions be developed - *in vitro* validations
- Development of rationally designed, cheap and easy to use/deliver product, such as vaginal probiotics and/or prebiotics to manage/restore a protective vaginal microbiota - More personalized approach - Understand strain-level differences



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