Group B *Streptococcus* and the vaginal microbiome

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Background

Streptococcus agalactiae (Group B *Streptococcus*; GBS) is an important cause of neonatal sepsis and meningitis. Acquisition of the organism generally occurs in the peripartum period, and maternal rectovaginal colonization with GBS is the major risk factor for neonatal disease. Sustained, population-level control of GBS disease will require understanding the determinants of maternal colonization as well as the pathogenesis of invasive disease. Roughly 25% of adult women are colonized with GBS, but little is known about the ecologic interactions between GBS and other members of the vaginal microbiome. Bacterial vaginosis (BV) is a vaginal dysbiosis involving loss of the resident *Lactobacillus*-dominant microbiome and replacement by *Gardnerella vaginalis*, BVAB1-2, *Megasphera*, and other anaerobic species. Interactions between BV and GBS carriage have been hypothesized but are inconsistent across studies.

New computational tools have been developed to assess ecological interactions among members of the microbiome. We sought to identify potential positive and negative correlations between other vaginal microbes and GBS.

Hypothesis

The composition of the vaginal microbiome influences GBS colonization.

Methods

The BV-IDEAS (**B**acterial **V**aginosis: **I**mproved **D**iagnosis by **E**LISA **a**nd **S**equencing) cross-sectional study enrolled non-pregnant women (n=437) from a racially/ethnically diverse urban clinic population to evaluate a new diagnostic test for BV and to elucidate BV risk factors. BV status was determined by Nugent scoring of vaginal swab samples (0-3, negative; 4-6, intermediate; 7-10, positive). GBS status was assessed by culture and quantitative PCR of vaginal lavage specimens. The vaginal microbiota was profiled by broad-range PCR of the V1 and V2 regions of 16S rDNA followed by Illumina MiSeq sequencing.

Sequences were aligned to a vaginal reference set (Srinivasan et al. 2012) using cmalign (Nawrocki et al. 2013). pplacer (Matsen et al. 2010) was used to place the sequences on a reference tree. guppy was used to classify the reads. Mubiomics (Smith et al. 2012) and in house (https://github.com/geoffrosen/vaginal-microbiome) scripts were used for data processing and operational taxonomic unit (OTU) table creation. LEfSe (Segata et al. 2011) was used to look for differential features correlated with GBS status. LEfSe first performs the Kruskal-Wallis test to test whether the mean abundance of each feature is the same between groups. Next, a pairwise Wilcoxon test is performed. Finally, the linear discriminant analysis (LDA) score of each feature is calculated.

Results

Figure 1. LEfSe analysis of vaginal microbiome data from the BV-IDEAS cohort reveals individual species more (green) or less (red) frequently associated with GBS status independently determined by culture and PCR.

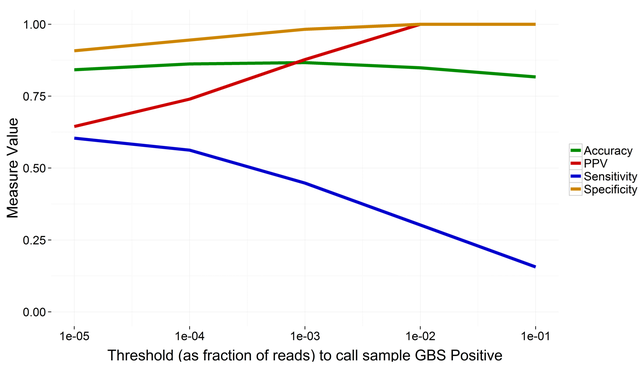


Figure 2. Accuracy (fraction of GBS status calls correct), positive predictive value (PPV), sensitivity, and specificity of our algorithm to call GBS status at different *Streptococcus agalactiae* read thresholds.

	GBS Positive (%)	GBS Negative
Nugent Score 0-3	22 (25%)	66
Nugent Score 4-6	29 (22%)	103
Nugent Score 7-10	44 (21%)	167

Table 1. No relationship between GBS colonization and BV as assessed by Nugent scoring was found in the BV-IDEAS cohort (χ^2 for trend, *P*=0.45).

	GBS Positive (%)	GBS Negative
CST I	13 (21%)	49
CST II	7 (44%)	9
CST III	24 (21%)	89
CST IV	51 (21%)	188
CST V	1 (8%)	12

Table 2. No relationship between GBS status and vaginal community state type (Ravel, 2011) was found in the BV-IDEAS cohort. χ^2 proportion, *P*=0.19. When comparing CST II (7/16) to all others (89/427), χ^2 proportion, *P*=0.06.

Figure 3a. Alpha (within sample) diversity analysis, rarefied to 5000 reads, with Shannon diversity shows no difference between GBS Positive and GBS Negative Groups (nonparametric t-test with 1000 Monte Carlo permutations, P=0.55).

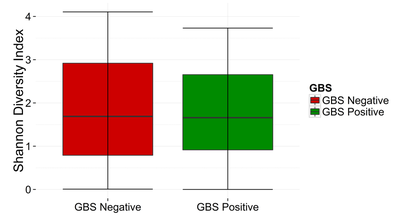
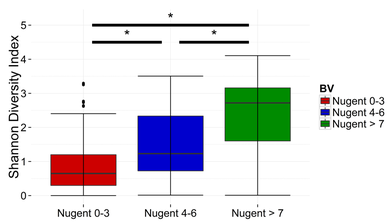


Figure 3b. Alpha (within sample) diversity analysis, rarefied to 5000 reads, with Shannon diversity shows that high Nugent score is associated with high alpha diversity (nonparametric t-test with 1000 Monte Carlo permutations, * indicates P<0.05).



Additional analyses

LEfSe failed to find any significant differential features when using CST as a subclass. Some of the CSTs were small. Our largest were CST III and CST IV. GBS was the only consist differential feature in CST III and CST IV.

We looked at PICRUSt (Langille et al. 2013) imputed metagenomes and metagenomics pathways inferred from this data with HUMAnN (Abubucker et al. 2012). There appears to be a positive relationship between GBS positive status and tRNA biosynthesis and an association between chemotaxis and GBS negative status. PICRUSt requires taxonomy assignment against the greengenes database (DeSantis et al. 2006) as input. This does not include many important vaginal species.

Conclusions

Streptococcus agalactiae was very strongly associated with GBS positive status (**Figure 1**). We are able to achieve accuracy >85% in predicting GBS status based on *Streptococcus agalactiae* calls alone (**Figure 2**). This will allow us to extend our microbial and metagenomic analysis to publicly available datasets.

Prevotella bivia is strongly associated with GBS positive status. *Prevotella bivia* is believed to have a symbiotic relationship with *Gardenerella vaginalis* (Pybus et al. 1997), a bacterium associated with BV and with CST IV. BV associated bacteria are strongly associated with GBS negative status. *Megasphaera sp* and the BVABs are very strongly correlated with BV (Fredricks et al. 2007, Marazzo et al. 2008). There does not appear to be a relationship between GBS and BV by Nugent score in our sample (**Table 1**). High alpha diversity is correlated with BV (Liu et al. 2013). Alpha diversity was not related to GBS status in our sample (**Figure 3a**), but the trend noted in Liu et al. remained (**Figure 3b**)**.** These results together suggest that previously hypothesized relationships between GBS and BV are not the predominant factors influencing GBS colonization. Instead, we propose that there are bacteria that are related to both GBS and to BV driving the correlation.

There does not appear to be a relationship between GBS and CST (**Table 2**)**.** There may be a protective influence of CST II, and further investigation is warranted. GBS was the only consistently differential feature in both CST III and CST IV. Further analyses at the microbe level may have to control for CST to allow for direct comparison. We hypothesize that metagenomics relationships will hold across CST.

Future Directions

Because detection of GBS 16S sequences in microbiome datasets correlates well with GBS status by PCR/culture (**Figure 2**), we will use the techniques described here to analyze data from the Human Microbiome Project and the Vaginal Human Microbiome Project in order to validate the ecological relationships in **Figure 1** and to further explore the reliability of our imputed metagenome data and the potential protective influence of CST II. *In vitro* and *in vivo* studies are planned to elucidate potential mechanisms of competition and/or cooperation among specific groups of vaginal microbes.

Acknowledgements

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