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First Trimester Vaginal Microbiome as Pregnancy Outcome Predictor

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Background

- The human microbiome can play a protective or harmful role during a woman's pregnancy.
- The non-gravid vaginal microbiome fluctuates in diversity depending on hormonal changes, menses, contraception, etc.[1-2], but the vaginal microbiome during pregnancy is more stable and dominated by fewer organisms[3].
- *Lactobacillus* spp. are the predominate species in the gravid vaginal microbiome and inhibit colonization of pathogenic species such as *Gardnerella vaginalis, N. gonorrhea*[4]*, "Lachnospiraceae* BVAB1," and *Sneathia* spp. The prevalence of these pathogenic microorganisms increases the susceptibility to infections such as bacterial vaginosis, which has been linked to premature rupture of membranes (PROM) and preterm birth[5-6].
- Previous studies have attempted to link certain organisms and microbiome patterns to clinical outcomes. Furthermore, most studies have been observational rather than investigating how these microbiome characterizations can be used as a potential screening tool for early intervention.
- We will adopt the opposite approach, starting with clinical outcomes and then examining the microbiomes for patterns. By comparing microbiomes at different trimesters in women with uncomplicated, healthy pregnancies and those who had adverse outcomes, we are looking to identify a microbial signature associated with complications such as preterm premature rupture of membranes (PPROM), premature rupture of membranes (PROM), gestational diabetes (GDM), gestational hypertension (GHTN), pre-eclampsia, and chorioamnionitis.

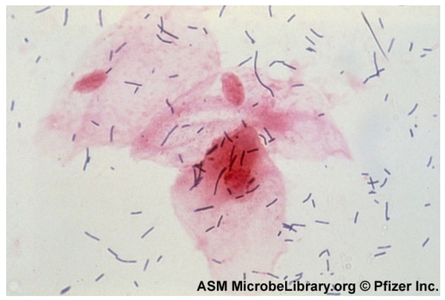


Figure 1: Squamous epithelial cell with lactobacillus. *Buxton, Rebecca. University of Utah Pathology Department*

Methods

1. Subjects were pregnant women enrolled in VCU's Vaginal Human Microbiome Project (VaHMP) and delivered at VCU. Vaginal swabs were obtained during an antenatal visit and microbiome analysis by 16s gene rRNA was performed. Clinical outcomes were abstracted from medical records.
2. Exclusion criteria included: multipara pregnancies (twins, etc.), immunosuppression (HIV, etc.), fetal demise (miscarriage, intrauterine fetal demise, etc.)
3. Complicated pregnancies was defined as: preterm delivery (<37 weeks), PPROM, PROM, GHTN, CHTN, pre-eclampsia, chorioamnionitis. Healthy pregnancies was defined as: term delivery (≥37 weeks), *without* any pregnancy or labor complications
4. Healthy vs. complicated pregnancies were case-control matched based on demographics and gestational age at sampling and the microbiome taxa were compared by LEfSe linear discrimination analysis (LDA).

Results

Table 1: Demographics of subjects in study

	First Trimester		Second Trimester		Third Trimester	
	Healthy n=127	Complicated n=94	Healthy n=112	Complicated n=76	Healthy n=142	Complicated n=61
Mean age (range)	27.20 (18-28)	26.89 (18-38)	25.29 (18-37)	26.46 (18-39)	26.28 (18-42)	27.18 (19-43)
Ethnicity/Race						
Caucasian	33 (26%)	16 (17%)	6 (5%)	8 (11%)	20 (14%)	4 (7%)
African American	45 (35%)	61 (65%)	49 (44%)	43 (57%)	32 (23%)	23 (38%)
Asian	2 (2%)	0 (0%)	1 (1%)	1 (1%)	2 (2%)	2 (3%)
Hispanic or Latino	39 (31%)	14 (15%)	52 (46%)	21 (28%)	82 (58%)	32 (52%)
American Indian or Alaska Native	3 (2%)	0	0	1 (1%)	1 (1%)	0
Native Hawaiian (or other Pacific Islander)	0	0	0	0	1 (1%)	0
Other/mixed	5 (4%)	3 (3%)	4 (4%)	2 (3%)	3 (2%)	0
Income						
Less than 15K	54 (43%)	45 (47%)	57 (51%)	46 (61%)	85 (60%)	32 (52%)
15K-20K	14 (11%)	11 (12%)	20 (18%)	11 (14%)	19 (13%)	14 (23%)
20K-40K	11 (9%)	14 (15%)	9 (8%)	7 (9%)	14 (10%)	4 (7%)
40K-60K	11 (9%)	7 (7%)	2 (2%)	3 (4%)	1 (1%)	2 (3%)
60K-80K	9 (7%)	2 (2%)	1 (1%)	0	1 (1%)	2 (3%)
More than 80K	12 (9%)	8 (9%)	0	1 (1%)	3 (2%)	1 (2%)
N/A	16 (13%)	7 (7%)	23 (21%)	8 (11%)	19 (13%)	6 (10%)
Education						
Less than high school	27 (21%)	13 (14%)	34 (30%)	17 (22%)	45 (32%)	19 (31%)
High school	45 (35%)	41 (44%)	53 (47%)	38 (50%)	62 (44%)	25 (41%)
Some college	17 (13%)	16 (17%)	16 (14%)	16 (21%)	26 (18%)	7 (11%)
Two year college	3 (2%)	10 (11%)	3 (3%)	1 (1%)	4 (3%)	3 (5%)
Four year college	16 (13%)	11 (12%)	2 (2%)	2 (3%)	2 (1%)	3 (5%)
Masters degree	12 (9%)	2 (2%)	1 (1%)	0	2 (1%)	2 (3%)
Doctoral degree	7 (6%)	0	0	1 (1%)	0	0
N/A	0	1 (1%)	3 (3%)	1 (1%)	1 (1%)	2 (3%)
Marital Status						
Single, never married	47 (37%)	47 (50%)	54 (48%)	45 (59%)	54 (38%)	23 (38%)
Cohabitating, unmarried	29 (23%)	15 (16%)	25 (22%)	13 (17%)	42 (30%	19 (31%)
Married	40 (31%)	28 (30%)	20 (18%)	9 (12%)	28 (20%)	13 (21%)
Divorced	2 (2%)	1 (1%)	0	0	3 (2%)	0
Separated	5 (4%)	0	9 (8%)	0	0	4 (7%)
N/A	4 (3%)	3 (3%)	4 (4%)	4 (5%)	3 (2%)	2 (3%)
OB history: Prior pregnancies						
Never pregnant	13 (10%)	14 (15%)	12 (11%)	16 (21%)	12 (8%)	2 (3%)
1-2	58 (46%)	34 (36%)	44 (39%)	27 (36%)	61 (43%)	34 (56%)
3-4	39 (31%)	33 (35%)	41 (37%)	22 (29%)	52 (37%)	18 (30%)
5 or more	17 (13%)	13 (14%)	15 (13%)	11 (14%)	17 (12%)	7 (11%)

Table 2: Pregnancy Clinical Outcomes based on trimester sampled

	First Trimester (n=221)	Second Trimester (n=188)	Third Trimester (n=203)
Healthy	127 (57%)	112 (80%)	142 (70%)
Complicated	94 (43%)	76 (20%)	61 (30%)
Preterm	29	23	17
PPROM	16	10	5
PROM	8	9	14
CHTN	27	13	12
GHTN	25	22	15
Pre-eclampsia	6	16	10
GDM	17	13	8
Chorioamnionitis	12	16	12

Figure 2: First Trimester Vatigypes

Vagitypes of subjects sampled in first trimester. Vagitype is defined by the microorganism that was ≥30% predominance in the vaginal sample. Controls = healthy pregnancies sampled in first trimester

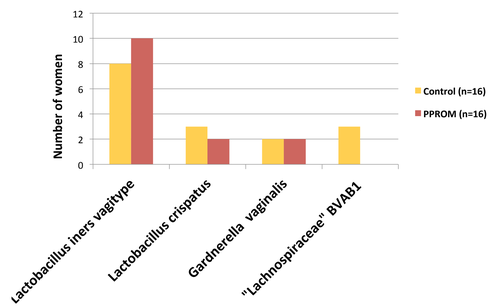
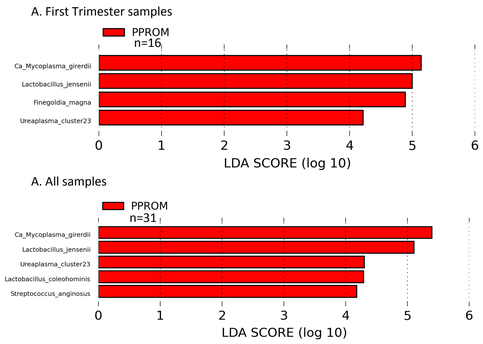


Figure 3: Significant microorganisms associated with PPROM

These LEfSe plots reveal microorganisms from A) first trimester samples and B)all samples overall that are significantly associated with pregnancies that ended in PPROM. An LDA score>2 is significant.



Conclusion

- We have developed clinical definitions of healthy and complicated pregnancies based on pathologies that will be used in future VaHMP studies.
- Although one study[7] found dysbiotic vagitypes in all three semesters of women who had PPROM, none of our PPROM subjects had a BVAB1 vagitype, and there were equal *Gardnerella vaginalis* vagitypes in both the controls and subjects who had PPROM (Fig. 2).
- While there were more PPROM subjects with *Lactobacillus iners* vagitypes, this *Lactobacillus* is less protective as it can coexist with pathogenic anaerobic bacteria.
- *"Candidatus* Mycoplasma girerdii", *Lactobacillus jensenii*, and *Ureaplasma* were significantly associated with PPROM in the first trimester samples as well as in all samples collected (Fig. 3). "*Candidatus* Mycoplasma girerdii" is strongly linked with *Trichomonas vaginalis* and elicits a strong pro-inflammatory response[8] which could explain the etiology of preterm delivery associated with trichomoniasis.

Future Study

- Little is known about "*Candidatus* Mycoplasma girerdii," and data from this study suggests further investigation is necessary. Perhaps treating this microorganism early on in the pregnancy could prevent outcomes such as preterm delivery and PPROM.
- We hope to use this approach to further analyze other clinical outcomes for possible vaginal microbiome signatures.

Resources

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