Spatial characterisation of the reproductive tract microbiome in preterm prelabour rupture of fetal membranes (PPROM)

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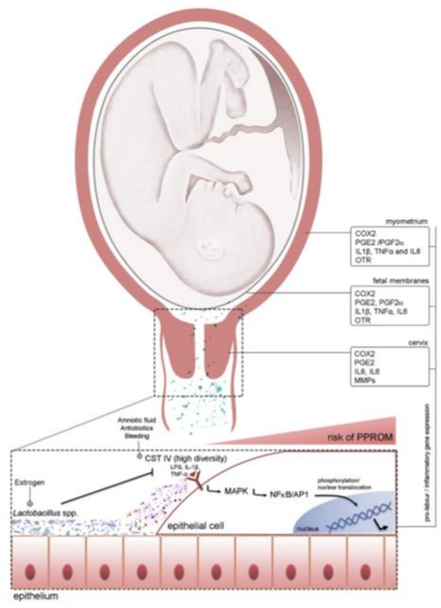
INTRODUCTION

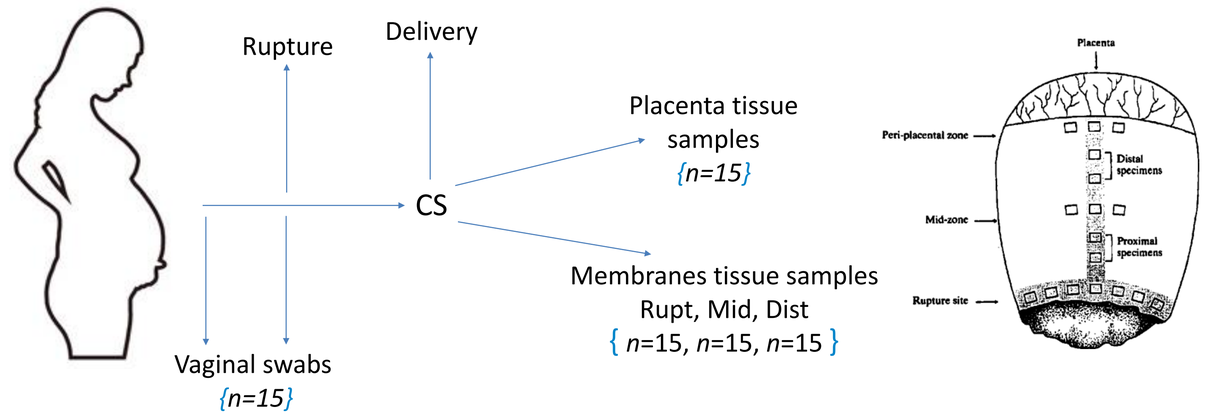
- Preterm birth (PTB) is the primary cause of death in children under 5 years of age worldwide and survivors often suffer life-long morbidities.
- Around 30-40% of all cases are associated with intrauterine infection. A common pathway of infection is thought to be ascension from the vagina to the upper reproductive tract (RT) (1).
- PPROM precedes 30% of spontaneous preterm birth cases. We have recently shown that specific pathogenic bacteria in the vagina increase risk of PPROM whereas *Lactobacillus spp*. dominance, can offer protection against PPROM and preterm birth (2, 3). Further more, *Lactobacillus iners* dominance early in pregnancy is also associated with increased risk of preterm birth (4).



- Previous studies have shown that neutrophil invasion of the fetal membranes is observed most frequently at the site of membrane rupture, close to the cervix and vagina (5) and in twin pregnancies, microbial invasion of the amniotic cavity is most commonly observed in the presenting and first born twin (6). However, direct evidence supporting vaginal bacteria ascension in infection driven PPROM is lacking.

References
1. Bennett PR, et al.,2020., Vaginal Microbiome in Preterm Rupture of Membranes. Obstet Gynecol Clin North Am. 2020 Dec;47(4):503-521.
2. Brown RG, et al., Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of the fetal membranes. Transl Res. 2019;207:30-43.
3. Brown RG, et al., Vaginal dysbiosis increases risk of preterm fetal membrane rupture, neonatal sepsis and is exacerbated by erythromycin. BMC Med. 2018;16(1):9.
4. Kindinger LM, et al.,. The interaction between vaginal microbiota, cervical length, and vaginal progesterone treatment for preterm birth risk. MICROBIOME. 2017 19;5(1):6.
5. Romero R, et al., Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. Am J Obstet Gynecol. 1989;161(3):817-24.
6. Romero R, et al., Infection and labor. VI. Prevalence, microbiology, and clinical significance of intraamniotic infection in twin gestations with preterm labor. Am J Obstet Gynecol. 1990;163(3):757-61.

STUDY DESIGN & METHODS



- Bacterial DNA was extracted from matched vaginal, amnion and placental tissue samples taken close to and/or at the time of caesarean section delivery following PPROM.
- Kit (Qiagen) and environmental path lab controls were collected and processed to enable detection of contaminates.
- MiSeq-based sequencing region V1-V2 (28f-YM mixed primer set) of 16S rRNA gene amplicons and shallow shotgun sequencing was performed to profile bacterial composition.

AIM

1. To characterise and compare the bacterial signatures associated with PPROM across reproductive tract sites.
2. To determine if bacteria detected in fetal membranes and/or placenta following PPROM are detected in the vagina prior to rupture.

RESULTS

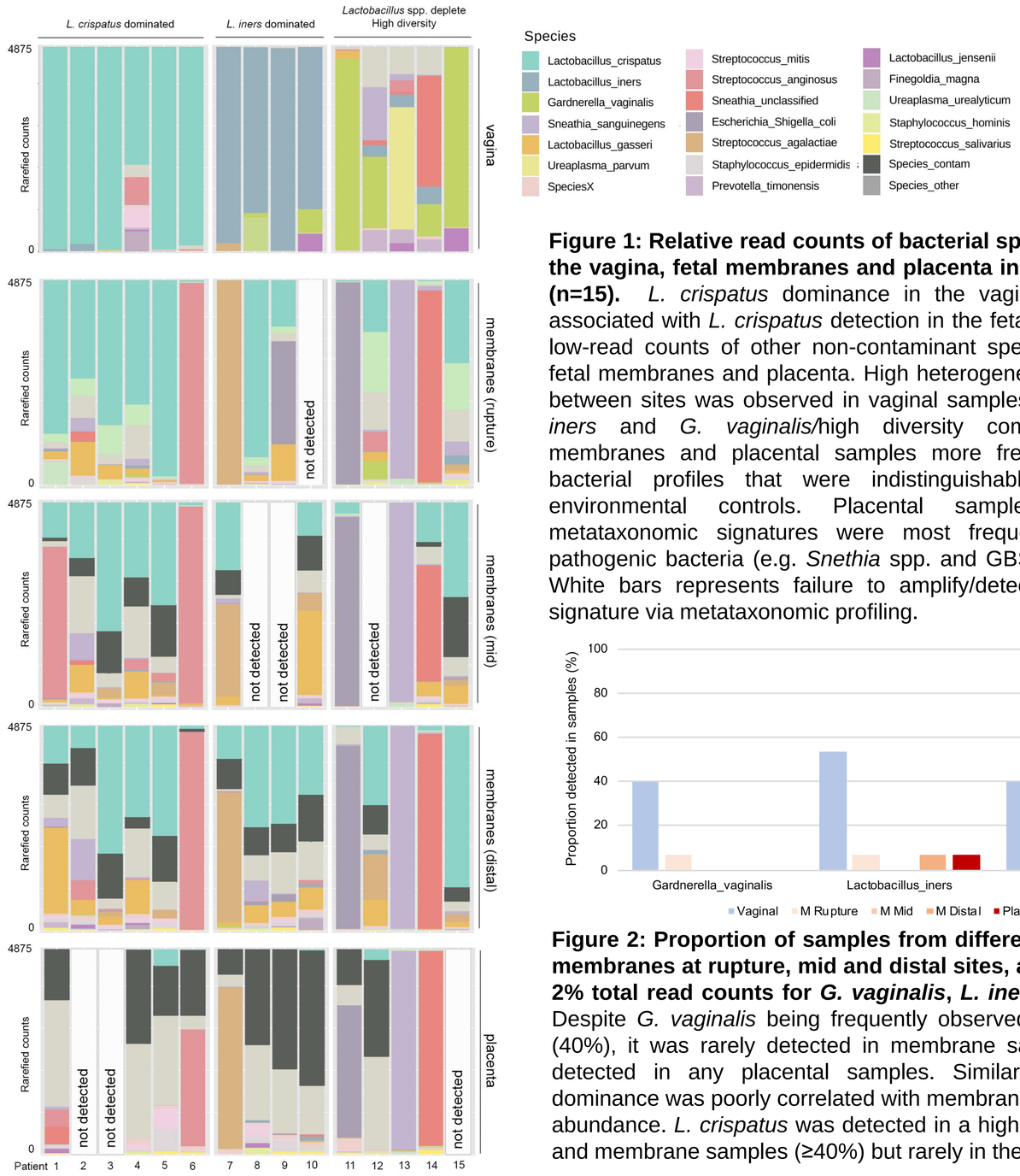


Figure 1: Relative read counts of bacterial species detected in the vagina, fetal membranes and placenta in PPROM patients (n=15). *L. crispatus* dominance in the vagina was generally associated with *L. crispatus* detection in the fetal membranes and low-read counts of other non-contaminant species in the upper fetal membranes and placenta. High heterogeneity in composition between sites was observed in vaginal samples dominated by *L. iners* and *G. vaginalis*/high diversity compositions. Distal membranes and placental samples more frequently produced bacterial profiles that were indistinguishable from kit and environmental controls. Placental samples with robust metataxonomic signatures were most frequently mapped to pathogenic bacteria (e.g. *Snethia* spp. and GBS (*S. agalactiae*)). White bars represents failure to amplify/detect any microbiota signature via metataxonomic profiling.

Figure 2: Proportion of samples from different RT sites (vagina, membranes at rupture, mid and distal sites, and placenta) with > 2% total read counts for *G. vaginalis*, *L. iners* and *L. crispatus*. Despite *G. vaginalis* being frequently observed in vaginal samples (40%), it was rarely detected in membrane samples and was not detected in any placental samples. Similarly, *L. iners* vaginal dominance was poorly correlated with membrane or placental relative abundance. *L. crispatus* was detected in a high proportion of vaginal and membrane samples (≥40%) but rarely in the placenta.

Figure 3: Longitudinal profiling of vaginal bacteria using shallow shotgun sequencing prior to PPROM in a case study. A) *S. anginosus* was detected in the vagina 5 weeks prior to rupture, however high proportion reads for *S. anginosus* were observed in the membranes and placenta limiting the ability to speculate on directionality of colonisation. Doted lines represent rupture event. ***B)*** Shotgun sequencing data showing that the vaginal *S. anginosus* bacterial strain, DORA-7, detected prior to rupture was the same strain detected in fetal membranes and placenta following PPROM and delivery.

CONCLUSION

- Our data supports ascending vaginal colonisation as a likely route of infection in some cases of PPROM. However, in other women vaginal microbiota composition is not always reflective of upper gestational tissue composition at delivery suggesting alternative colonisation pathways (e.g., haematogenous spread to the placenta).
- *L. iners* and *G. vaginalis* are rarely detected in upper RT samples of PPROM patients, whereas *L. crispatus* is detected in the vaginal and fetal membranes samples.
- Detailed understanding of how vaginal microbiota contribute to PPROM is fundamental for the development of predictive and individualised therapeutic strategies to control microbial infection in the reproductive tract, leading to improved pregnancy outcomes.

CURRENT & FUTURE WORK

- We are currently undertaking strain-tracking approaches to determine directionality of pathogenic colonisation and validation in a larger cohort.
- Analysis of the immune mediators (chemokines and cytokines) present in the matched samples will be undertaken to test the hypothesis that the presence or absence of certain bacteria in the reproductive tract and gestational tissues influences the local inflammatory environment, which may be a driving mechanism of PPROM and preterm labour.
- Imaging techniques (e.g. fluorescence in situ hybridization) will also be performed to localise bacteria in the fetal membranes and placenta.