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Case study

The first fungi: mode of delivery determines early life fungal colonization in the intestine of preterm infants

Dr Jannie Henderickx, Wageningen University, The Netherlands.

Research objective:

To determine the composition and diversity, as well as the effect of clinical variables, of the gastrointestinal mycobiome of preterm infants within the first six postnatal weeks.

Sample collection:

Samples were available from the EIBER study collected at Isala Women and Children's Hospital in Zwolle, The Netherlands. The EIBER study was an observational study with the objective of investigating the effect of antibiotic treatment on gut microbiome development in preterm and full-term infants. Faecal samples had been taken during postnatal weeks 1, 2, 3, 4, and 6. These samples were reused for the present study and selected based on the following criteria:

- A gestational age of between 24 and 40 weeks
- Mothers did not receive antibiotic treatment during labour or for 6 weeks thereafter
- Infants received at least one antibiotic treatment

These criteria resulted in 116 faecal samples from 57 infants for DNA extraction.

Sequencing strategy:

ITS2 amplicon-based metagenomic sequencing was performed on the NovaSeq6000 platform with 250 paired-end reads with a sequencing depth of 30,000 raw tags per sample.

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Background:

The bacterial part of the microbiome and its link to health or disease has been studied extensively, however the need to study the microbiome beyond its bacterial component is becoming evident, as interactions between the various kingdoms within the intestine can affect ecosystem dynamics and immune homeostasis. The initial colonisation of the gut occurs during birth and is influenced thereafter by many clinical and environmental variables such as gestational age, mode of delivery, hospital environment, and diet.

Start of life for pre-term infants is very different to that of full-term infants, as they are usually exposed to a lot of clinical interventions such as antibiotic treatment, adapted feeding modes, and respiratory support. The gut mycobiome of preterm infants is often dominated by a single species, in contrast with the more diverse mycobiome of full-term infants. *Candida spp*. are typically one of the most predominant species in preterm infants up to the age of 6 months. Within this genus, many opportunistic pathogens are persistent in pre-term infants. Fungal overgrowth of normally commensal and symbiotic fungi can also lead to infections associated with considerable morbidity and mortality rates. Systemic candidiasis affects approximately 10% of preterm infants, and this susceptibility has been linked to the aforementioned clinical factors, including the presence of a naïve immune system.

Results:

The first and second most abundant fungal phyla were Ascomycota and Basidiomycota, respectively. Within the Ascomycota phylum, *Candida spp*. was the most abundant genus in the first 6 weeks in all preterm and full-term samples. Relative abundance increased gradually with both gestational age and postnatal age. Relative abundance of *Candida spp.* increased from 0.39 in the first week to 0.56 in the sixth week in extremely preterm infants, whereas it increased from 0.02 in the first week to as high as 1.00 in full-term infants. Phylogenetic diversity was stable in late preterm and full-term infants in the first two post-natal weeks, and this diversity decreased significantly in the fourth post-natal week in late pre-term infants. Although none of the changes were statistically significant, a decrease in phylogenetic diversity was observed in the first week in preterm infants.

The variation observed in mycobiome composition was shown to be down to a high level of individual variability and mode of delivery. The influence of individual variability was suggested by the random hierarchical clustering results, indicating the need for a larger number of samples per gestational age category. The clinical variable investigated for their effect on mycobiome composition were, amongst others, mode of delivery, gestational age, birth weight, individuality, and duration of the second and third antibiotic treatment. After automatic stepwise model selection, individuality and mode of delivery were identified as predictors significantly explaining variation in the mycobiome composition. However, after adjusting the P-value, both predictors lost their significance.

Mode of delivery was hypothesised to influence mycobiome seeding, due to its initial significance in the redundancy analysis. Each type of delivery mode – vaginal, emergency c-section and planned c-sections – was characterised by specific taxa. The mycobiome of infants delivered vaginally was enriched with the vaginal-like Candida genus.

Vaginally delivered infants and those delivered by emergency c-section shared fungi within the Saccharomycetes class. Interestingly, there was no overlap in taxa characteristic for planned and emergency c-sections. Those delivered by emergency c-section were characterised by the Malasseziomycetes class, while infants delivered via planned c-section were characterised by the Microascales order and the Cladosporium genus.

Discussion & Conclusions:

This study was the first to show the impact of clinical variables on the gut mycobiome composition of preterm infants. It showed that the gut of preterm infants is colonised by various eukaryotic kingdoms, of which fungi are the most prominent. Ascomycota was the most abundant taxa, within which the Candida genus was the most abundant. The abundance of this genus increased with gestational age, however samples did not cluster based on gestational age. Within the Candida genus, *C. albicans* was identified as the most dominant species. Interestingly, the abundance of *C. albicans* reported within this study was lower than that reported by a similar study (James et al., 2020). The infants studied by James et al. did not receive antibiotic treatment, which suggests that antibiotic treatment may have enriched *Candida spp.* in the infants described in this present study.

Within this study, it was additionally shown that vaginal delivery promotes colonisation with *Candida spp*. Although commensal in most cases, *Candida spp.* can cause fungal overgrowth and systemic candidiasis in immunocompromised hosts. Preterm infants often experience such overgrowth after antibiotic treatment. Candidiasis is often caused by *C. albicans,* the most abundant species found within this cohort, however no infants within this cohort suffered from candidiasis. This suggests that the mycobiome may act as a reservoir of opportunistic pathogens in immunocompromised hosts, which can be triggered by events such as antibiotic treatment.

The varying colonisation dependent on mode of delivery observed within this study aligns with previous research highlighting the vertical transmission of the mother's microbiome during labour and delivery. This study confirmed for the first time that vaginal-like *Candida spp*. is observed at a higher abundance in infants delivered vaginally than in infants delivered via emergency c-section, which are usually characterised by *Malassezia spp*., a species commonly found on the skin of adult and infants. These findings support the hypothesis of vertical fungal transmission from previous studies, however *Malassezia spp*. was not characteristic of infants delivered by planned c-section. It is possible that infants delivered via emergency c-section are characterised by a more vaginal-like mycobiome due to the mother having undergone a substantial amount of labour before the procedure is performed. It is difficult to draw any conclusions from this however, as previous studies have not created a clear distinction between the type of caesarean delivery they are referring to.

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Future work:

The authors of this study acknowledge the relatively small sample size and lack of longitudinal data for some of the infants described in this study. A larger cohort is currently being established to study the effect of pre-term microbiome development on human milk digestion and samples from this study may be used in the future to add to the work of the current study. Lacking from this study, due the low levels of genetic material it was possible to extract, qPCR was not carried out. This will be an important addition to the study in the future to provide perspective on inter-kingdom interaction within the microbiome with absolute abundance values, as well as relative abundance values.

With regards to the sequencing of the mycobiome, current technologies and resources are limited. Many species remain unannotated in available databases, resulting in many "unknown" hits during taxonomic assignment. Fungi also exist in multiple forms, and often two forms of the same species will be identified as two different species. Study of the mycobiome is gaining traction however, with many researchers acknowledging its importance in health and disease. Future studies should focus on the development of reliable and standardised sequencing and analysis methods to allow for scalability.

Dr. Jannie G.E. Henderickx obtained a bachelor's (cum laude) and master's education in Nutrition and Health at Wageningen University & Research in the Netherlands. She specialized in Molecular Nutrition and Toxicology and developed a keen interest for the interaction between nutrition, health and the human microbiome.

Jannie started her academic career as a doctorate student at the Laboratory of Microbiology (Wageningen University & Research) under guidance of prof. dr. Jan Knol and dr. Clara Belzer. In her PhD research — titled "The immature gut: microbes and nutrition orchestrate maturation of the preterm gastrointestinal tract" — she investigated the maturation of the gastrointestinal tract and of the intestinal microbes in preterm infants with their implications for infant growth, development and health.

Having obtained her doctorate and being eager to learn more about the microbiome in health and disease, Jannie now works as a postdoctoral researcher and study coordinator at the Center for Microbiome Analyses and Therapeutics at Leiden University Medical Center in the Netherlands.