Human Milk Microbiota and Mycobiota in the CHILD Cohort Study

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Brief overview of work (2015-2020)

• Background, Objectives, Methods
• Results
  – Ch3 - Microbiota & Mycobiota profile
  – Ch4 - Culture-enriched molecular profiling
  – Ch5 - Determinants of milk microbiota (bacteria)
  – Ch6 - Milk components and milk microbiota (bacteria)
  – Ch7 - Determinants of milk mycobiota (fungi)
  – Ch8 - Comparison of data processing
• Summary & Discussion
Breastfeeding is a major factor shaping the infant microbiome development.

- **Intra-uterus exposure to bacteria (?) or bacterial products**
- **Initial colonisation depending on birth mode: surgical vs. vaginal**
- **Acquiring microbes depending on feeding and home environment**
- **Transition to adult-like stable microbiota**

**Time (y)**
-1 0 3

**Breastfeeding & HMOs**

**Role for other milk components?**

**Infant Microbiome Development**
Does mode of breastfeeding modify the role of breastfeeding on infant health?

Breastfeeding

Mode of Breastfeeding

Direct at the breast vs. some pumped

Asthma

Potential mechanisms?

Potential microbiological impacts of pumping breastmilk

Direct Contact to the Infant Mouth

Introduction of Environmental Microbes

Change in Bioactive Compound during Storage

Milk Microbiota & Mycobiota

Infant Microbiota & Mycobiota
Milk serves as a niche for these microbes
Question: are milk components associated with milk microbiota?

Do HMOs impact milk microbiota?

HMOs constitute 20% of milk carbohydrates > 100 different HMOs identified

Do milk fatty acids impact milk microbiota?

Milk lipids are the main source of energy for the infant
Primarily consist of triglycerides, fatty acids bound to glycerol

Probiotic effect on infant gut microbiome
Same effect on milk microbiota?

Inhibitory effect on bacteria in vitro and associated with gut microbiota composition
Any association with milk microbiota?

Milk contains a diverse community of bacteria and fungi.

Some factors associated with the composition.

Previous studies have been limited by:
- Small sample size
- Low sequencing depth
- Lack of control for confounding factors.

METHODS

Objectives

Amplicon Sequencing

PROFILE

DETERMINANTS

Early life
Infant
Mother
Breastfeeding
Milk
Environment

DATA PROCESSING

Culture-enriched molecular profiling

PROFILE

Different culture conditions and O₂ levels
METHODS
Canadian Birth Cohort
N=3,455 mother-infant dyads

Representative Nursing Mothers

Direct & Pumped Breastfeeding

Initial milk sample
1 ml

gDNA extraction

Amplification of 16S rRNS/ITS2

Sequencing Illumina MiSeq

N=393 (bacteria)
Milk sample collected at 3m postpartum

N=271 (fungi)
Analysis Workflow

Sequencing → Sequencing Data → Quality Control → ASV Identification → Taxonomy Assignment

Data Analysis → Threshold Filtering → Contaminant Identification

Relative Abundance → Discriminant Analysis → Causal Modelling → Network Analysis

**CFI = 0.985
RMSEA (90% CI) = 0.019 (0.00 – 0.042)
SRMR = 0.040
Χ² P-value = 0.247
df = 40**
Theoretical framework based on the origins of milk microbiota

<table>
<thead>
<tr>
<th>Maternal microbiota</th>
<th>Exogenous microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary gland colonisation</td>
<td>Intra-mammary milk inoculation</td>
</tr>
<tr>
<td>Entero-mammary Oro-mammary</td>
<td>Breast microbiota</td>
</tr>
</tbody>
</table>

Theoretical framework based on the origins of milk microbiota

Maternal factors
- BMI
- Age
- Ethnicity
- Diet

Breastfeeding factors
- Mode
- Exclusivity
- Time to the last feed

Early life factors
- Mode of delivery
- Antibiotics

Infant factors
- Sex
- Older siblings
- Birth weight

Milk environment
- HMOs
- Lipids
- Cytokines
- etc.

Maternal Gut microbiota

Milk microbiota

Moossavi et al. Cell Host Microbe (2019)

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Milk microbiota composition variability

What factors are associated with the variability in the milk microbiota composition?
Determinants of milk microbiota overall composition

Overall Composition

- Infant sex -
- Maternal age -
- Infant secretor status -
- Milk leptin -
- Atopy -
- Total HMO concentration -
- Mother antibiotics at the time of sample collection -
- Milk insulin -
- Intrapartum antibiotics -
- Maternal secretor status -
- Child antibiotics at the time of sample collection -
- Prenatal smoking -
- Maternal BMI -
- Lactation stage -
- Birth mode -
- Exclusive breastfeeding -
- Ethnicity -
- Number of older siblings -
- Mode of breastfeeding -
- HMO compositional profile -
- Milk fatty acid profile -

Variability (%) in RDA

- Breastfeeding
- Infant and Early Life
- Maternal
- Milk components
Causal modeling

Mode of breastfeeding is associated with milk microbiota composition

Milk environment is not associated with milk microbiota composition

Edges
- Negative association

Nodes
- Unobserved variable
- Observed variable

** p<0.01

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Associations of individual components

Microbiota

HMOs

Fatty acids

Pairwise correlation

Associations are more numerous and stronger within components

HMOs
Fatty acids
Microbiota
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Theoretical framework for assessment of milk mycobiota (fungi)

Representative Nursing Mothers

Available Milk Microbiota (N=393)

Milk Fungi Profiling (N=271)

Initial milk sample
1 ml

gDNA extraction
Amplification of 16S rRNA/ITS2
Sequencing Illumina MiSeq

Theoretical Framework

Environment
Season
City
Vegetation

Maternal Health
Antibiotics
Atopy/Asthma

Skin
Oral cavity
Milk
Bacteria
Fungi
HMOs
Inter-kingdom interactions
Positive
Negative
Presence/absence of milk fungi defined based on the depth of sequencing per sample

Fungi absent  Fungi present
\( n=210 \)  \( n=58 \)

\( n=25 \)  \( n=33 \)

Moossavi et al. BMC Microbiol (under revision)
Home environment and milk bacterial composition are associated with presence of milk fungi

Univariate Associations with Presence/Absence of Fungi

- Secretor vs. Non-secretor
- Prenatal smoking
- History of atopy
- Male infant
- Oral thrush
- Caesarean section
- Multiparity
- Intrapartum antibiotics
- Child antibiotics at 3 months
- Some pumping
- Exclusive breastfeeding
- Total HMO (mg/mL)
- LNH relative abundance (CLR)
- DSLNT relative abundance (CLR)
- High mould level
- High moisture level
- High dust level
- Dog ownership
- Furry pet ownership
- Spring (vs. other seasons)
- High population density
- Moderate green residential space (vs. grey)
- Green residential space (vs. grey)
- Vancouver (vs. other cities)
- High bacterial richness
- Bacterial taxonomic clusters C2 (vs. C1)
- Bacterial taxonomic clusters C3 (vs. C1)
- Bacterial taxonomic clusters C4 (vs. C1)
- Bacterial composition outlier

Crude OR (95% CI) for breastmilk fungi
SUMMARY & DISCUSSION
Summary

Representative Nursing Mothers → Milk Bacteria & Fungi

Milk Bacteria

Mode of breastfeeding

Other important factors


Milk Fungi

Home Environment

Other important factors

Moosavi et al. BMC Microbiol (under revision)
**FUTURE DIRECTION**

**Scientific questions**
- What is the functional significance?
- Does vertical transmission occur?
- Milk virome?

**Translational implications**
- Best practices for the use of breastfeeding aids.
- Bacterial reconstitution in human donor milk.

**VISION**

- Infant Microbiota
- Asthma
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