Human Milk Microbiome

Mother Nature’s Prototypical Probiotic Food?

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Outline

• Milk – why do we care what’s in it?

• Historical perspective and definitions
  – Paradigm shift

• What factors are related to microbial community structure?

• What’s normal? Do they vary around the globe?

• Are the bacteria living? Where do they come from?

• Practical implications (including risk/benefit of pasteurization)?

• Critical next steps...
Milk – Why do we care what’s in it?

• The only food ever “designed” specifically to feed humans.

• Human milk composition used as gold standard for:
  – estimating nutrient and energy requirements for infants (Institute of Medicine, DRI), and
  – manufacturing infant formulas.

• Bottom line: understanding what’s in human milk and what it all does may improve human health more than understanding any other food!
Does human milk contain bacteria?

- Human milk has long been considered sterile unless contaminated or collected from “infected” gland
  - Human milk banks pasteurize milk.
    - No one would have ever suggested adding bacteria back to this milk.
    - Until recently, formula manufacturers wouldn’t dream of adding bacteria to their products....

- Why the paradigm shift?
  - Evolution of methods
  - Nothing is sterile...

What about the mammary glands?

NIH HUMAN MICROBIOME PROJECT
Microbes vs. Microbiome? – An issue of semantics

- **Microbe (micro-organism)**
  - Very small, organisms
    - Bacteria, protozoa, viruses, fungi
    - Often called “microflora” although they should technically be called “microfauna”

- **Microbiome**
  - All of our microbes’ genes
Our interest in milk microbiota... “accidental microbiologists...”

- Milk lipids and mastitis?
  - Many lipids halt growth of *Staphylococcus aureus*, a “pathogen” associated with mastitis in humans.

- What about conjugated linoleic acid (CLA)?
  - Dairy fat, actively made in bovine and human mammary gland
Our interest in milk microbiota... “accidental microbiologists...”

**FINDINGS**
- Of all fatty acids tested, CLA was most inhibitory
- At the lowest dose!

**NEXT QUESTION**
- Does CLA modulate host-pathogen interactions in mammary gland?

### TABLE 1

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<th>Lipid[^]</th>
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<td>NI</td>
</tr>
<tr>
<td>C10:0 Capric acid</td>
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</tr>
<tr>
<td>Glycerol trilinoate</td>
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<tr>
<td>18:2 cis-9, trans-11 CLA[^]</td>
<td>50</td>
<td>25</td>
<td>25</td>
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<tr>
<td>18:2 trans-10, cis-12 CLA[^]</td>
<td>100</td>
<td>50</td>
<td>75</td>
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</tbody>
</table>

[^] Numbers reported represent the mean of duplicates. NI = no inhibition observed at any dose tested.
[^] All FA tested were in FFA form. FA were esterified at the sn-1 position for all monoacylglycerols and at the sn-1 and sn-3 position for dicylglycerols.
[^] CLA = conjugated linoleic acid.

Kelsey et al, 2006
CLA and mammary inflammation?

• Human CLA intervention trial (CLA supplements vs. placebo)
  – Milk collected from healthy lactating women several times over lactation.
  – Looked at milk “smears” to quantify immune cell types
  – Sent milk samples to mastitis lab for culture-dependent analysis.

• No effect of CLA, but...
  – Saw bacteria in many of the slides *even though culture-dependent analysis suggested there were no bacteria* → *Great Plate Count Anomaly*
Characterizing microbial communities in human milk

- Characterized bacterial communities in milk using culture-independent methods
- Diverse bacterial genera identified in every sample
- High level of variability among women
- "Bacterial fingerprints" within a woman
Paradigm Shift: Milk, even when produced by healthy women, contains bacteria; and many of these bacteria would/could be considered as potentially pathogenic!
Outline

• Milk – why do we care what’s in it?
• Historical perspective and definitions
  – Paradigm shift

• **What factors are related to microbial community structure?**
  • What’s normal? Do they vary around the globe?
  • Are the bacteria living? Where do they come from?
  • Practical implications (including risk/benefit of pasteurization)?
  • Critical next steps...
What factors are associated with variation in the milk microbiome?

• Time postpartum?
• Delivery mode?
• Maternal diet?
• Other milk components?
  – Complex sugars?
  – Immune cells?
• Childcare?
• Maternal genetics
• Environmental microbial exposure
Milk Microbial Community Structure Relatively Stable Over Time

• Prospective, longitudinal human study
  – 21 mothers and infants
  – Milk collected from 0-6 mo postpartum (9 collection times)
• Major finding: Only minor shifts in milk microbiome (at least statistically)
  – In early lactation
  – *Gemella* most affected

• Really need to have larger sample size and more colostrum samples!
Shifts in “core” genera over first 3 months of lactation

- Week 1
- Week 3
- Week 6
- Week 12

- Streptococcus
- Lactobacillus
- Pseudomonas
- Stenotrophomonas
- Staphylococcus
- Brevundimonas
- Elizabethkingia
- Chryseobacterium
- Variorae
- Enterobacter
- Bifidobacterium
- Other
- Flavobacterium

Murphy et al., 2017
Effect of delivery mode?

• Mixed and limited findings.

• C-section associated with
  – ↓diversity in milk (Cabrera-Rubio et al., 2016)?
  – ↑total bacteria (Khodayar-Pardo et al., 2014)?
  – we found trend toward higher relative abundance of Propionibacterium (unpublished)
  – No difference (Urbaniak et al., 2016)
  – Verdict is still out on this.

Cabrera-Rubio et al., 2016
Effect of delivery mode?

- Elective vs. nonelective c-section?

Cabrera-Rubio et al., 2012
Delivery mode

Kumar et al., 2016
Relationship with maternal adiposity?

• Yes
  – Cabrera-Rubio, 2012

• No/Not so much
  – Williams (unpublished) – no relationship with pre-preg BMI, but $\uparrow$ Granulicatella in overweight/obese women

Cabrera-Rubio et al., 2012
Relationship with maternal diet?

- ↑Firmicutes related to ↑ total energy, lipids, carbohydrates, fiber in diet
- Many other relationships...

Williams et al., in review
Fatty acids

Eicosapentaenoic acid (EPA)
Docosahexaenoic acid (DHA)
Amino acids and vitamins

Clear patterns here!

Opposite patterns from energy-yielding macronutrients

Williams et al., in review
Maternal probiotic intake and milk microbes

- When women consume probiotic bacteria, they find their way to the milk.
  - Local fermented Syrian foods (Albesharat et al., 2011)
  - Can nearly “cure” mastitis (Arroyo et al., 2011)
  - More effective than antibiotic treatment
Needed!

Relationships among maternal nutrient intake, milk nutrient content, and milk microbial community structure.
What factors are associated with variation in the milk microbiome?

• Time postpartum?
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• Other milk components?
  – Complex sugars?
  – Immune cells?
• Childcare?
• Maternal genetics
• Environmental microbial exposure
Milk oligosaccharides stimulate growth of milk-derived *Staphylococcus* species

- Isolated 2 *Staph* species from milk
  - confirmed causal relationship between total human milk oligosaccharides (HMO) and bacterial growth
  - HMO are considered protective; what’s this say about *Staph* species common in milk?
- Assumed that bacteria were metabolizing HMO
  - HMO didn’t disappear from media
  - But some amino acids did
  - HMO somehow signaling bacteria to metabolize amino acids for growth

*S. epidermidis*

*S. aureus*
Back to FIRST milk sample set analyzed for microbiome...also personalized for immune cell distributions.

Williams et al., in review
Relationship between immune cells in milk and microbial community in milk (chicken?/egg?)

Maternal cells: MSE, neutrophils, lymphocytes, eosinophils
Genera: *Streptococcus, Staphylococcus, Corynebacterium, Serratia, Pseudomonas, Propionibacterium*

Williams et al., in review
What factors are associated with variation in the milk microbiome?

• Time postpartum?
• Delivery mode?
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• Other milk components?
  – Complex sugars?
  – Immune cells?
• Childcare?
• Maternal genetics
• Environmental microbial exposure
Relationship with childcare practices?

- PRELIMINARY DATA
  - Milk collected from women living in the Central African Republic
  - ↑ milk microbiome diversity related to ↑ social networks
  - We are currently looking at this among American families
Summary so far...

- Very little is known about what factors influence variation in milk microbes.
- Seems to be related to...
  - Delivery mode?
  - Chronic, maternal nutritional status and nutrient intake
  - Maternal probiotic intake
  - Other milk constituents
  - Childcare practices (social networks)
- Will require controlled intervention trials to figure some of this out!
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• Practical implications (including risk/benefit of pasteurization)?
• Critical next steps...
Human milk from Central African Republic
US vs. Finland

- Is this difference real or due to methodologic variation?
- If it’s real, what’s driving it?
- Many other studies have now suggested differences amongst locations.

US (WA/ID) vs. Finnish milk microbiomes

Cabrera-Rubio et al., 2012
International differences – controlled for methods!

Kumar et al., 2016
Is there such thing as a “normal” human milk microbiome?

Can “normal” shift to support optimal health in particular biosocial, environmental constructs?

What is Normal Milk? Sociocultural, Evolutionary, Environmental, and Microbial Aspects of Human Milk Composition

- Funded by the National Science Foundation’s INSPIRE mechanism
Overarching Rationale for Project

• Primary hypothesis: Environmental and social constructs have acted as selective pressures to drive variation in milk composition to optimize maternal/infant fitness for a particular location

• Our focus: Milk microbiota and oligosaccharides (HMO)
  – Appear to be “fingerprints” in both within a woman.
    • HMO profiles are, in part, genetically driven
  – Is there really international variation?
  – Are these fingerprints related to each other?
  – How much can we explain by sociocultural, environmental, and biological variables?
Basic Experimental Design

• ~40 mother/infant dyads in 11 locations
  – 440 total moms and babies
  – 1-3 mo postpartum; HEALTHY women and babies
  – All samples collected and analyzed similarly

• Detailed information on sociocultural, childcare, dietary, and environmental factors

• FIRST RESULTS: Milk oligosaccharides vary greatly around the world.
  – We expect the same for milk microbes.
Milk oligosaccharides: First results from INSPIRE study

Eco-homeorhesis?

McGuire et al., AJCN in press
Summary

• Milk microbiome likely varies geographically.
  – Genetics?
  – Cultural practices?
  – Diet?
  – Environmental exposures?

• MUCH to learn!
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• Practical implications (including risk/benefit of pasteurization)?

• Critical next steps...
Origin of milk microbiota?

- Skin
- Infant mouth
- Environment
- Maternal GI tract (via lymphatic)
How similar are microbes in milk to other sites?
Are there bacteria in the nonlactating breast?

- Yes
- And bacteria in healthy tissue differ those in malignant healthy tissue.

Urbaniak et al., 2014
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• Practical implications.
  – Risk vs. benefit of pasteurization?
• Critical next steps...
Major warning: We need to conduct randomized controlled trials to assess correlation vs. causation!
Do we really know what bacteria to add to formula? Are there risks associated with adding just 1 or 2?
What about banked milk?

“Human milk samples are taken during the pasteurization process and cultured to check for bacterial growth. Any contaminated human milk is discarded.”
Effects of pasteurization on human milk composition

- Kills bacteria, of course
  - Might there still be benefit of bacterial pieces? Antigens?
- Denatures (lowers) protein, enzymes and immunoglobulins

Table 2. Total Protein, Lysozyme, and Immunoglobulin Concentrations in Colostrum*

<table>
<thead>
<tr>
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<th>&lt; 32 wk (n = 36)</th>
<th>32-36 wk (n = 32)</th>
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<tr>
<td></td>
<td>Raw</td>
<td>Pasteurized</td>
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<tr>
<td>Total proteins, g/dL (x ± SD)</td>
<td>8.9 ± 2.4</td>
<td>7.7 ± 2.1</td>
</tr>
<tr>
<td>Lysozyme, μg/L (x ± SD)</td>
<td>705.6 ± 847.1</td>
<td>242.7 ± 343.3</td>
</tr>
<tr>
<td>Immunoglobulin A, g/L (x ± SD)</td>
<td>3.102 ± 1.360</td>
<td>2.032 ± 1.115</td>
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<tr>
<td>Immunoglobulin G, g/L (x ± SD)</td>
<td>0.076 ± 0.038</td>
<td>0.018 ± 0.026</td>
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<tr>
<td>Immunoglobulin M, g/L (x ± SD)</td>
<td>0.017 ± 0.038</td>
<td>0.000 ± 0.000</td>
</tr>
</tbody>
</table>

*Values reflect x ± SD concentrations of proteins in raw and pasteurized colostrum samples associated with difference effect P value; P = pasteurization effect P value; P = gestational age effect P value.

Koenig et al., J Hum Lact 2015
Effects of pasteurization on antibacterial properties of human milk

S. aureus

E. coli

Gysel et al., Eur J Peditr 2012
Do the benefits of pasteurizing banked milk outweigh the risks of not getting these bacteria?

• Shift in balance between high- vs. low-risk situation?

• This will take careful, interdisciplinary, objective study.
Let the science drive the policy...
Next Steps – Tip of the Iceberg

- Next steps (before we can really start looking at implications for health and weighing risks against benefits)
  - Understand variability
    - Milk nutrients?
    - Maternal probiotics?
    - Delivery mode?
    - Antibiotic use?
    - Genetics?
  - Relationship with maternal immune system
  - Other microbial taxa (protozoa, viruses, fungi)?
  - Elucidate origins
Thank you...Questions?

- Mark McGuire, PhD
- Janet Williams, PhD
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