Gut Goodness: The Microbiome: Impact on Neonatology

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Disclosure

• I am not a microbiologist.
• My interests stems from my desire to learn for the care of my patients.

Objectives

• Review differences in microbial colonization and immune function of gastrointestinal tract of healthy term newborns as compare with preterm infants.
• Review implications for development of disease when there is dysbiosis.
• Review the Brain-gut axis and implications for neurodevelopment.

Did you know?

• Human body has 10X more bacteria than human body cells
  – 100 trillion organisms
• Bacteria has 100X more genes than human genome.
• 20% of small molecules in human blood are microbiota products
Evolution

- “past decade has produced a magnificent and still rapidly evolving toolbox of experimental and computational techniques for culture-independent identification of the microorganisms that comprise our body habitat-associated microbial communities (microbiota), as well as their genes (microbiome) and gene products”

Definitions

- **Microbiota** – all the microbes in a given environment.
  - Microbial super organ residing symbiotically within mucosal tissues and integumentary system of human host
  - Difficulties of culturing complex samples with fastidious or un-culturable organisms
  - Influences all other organ systems and communicates via metabolites (metabolome)
- **Holobiont** – individual vertebrate composed of multiple microbes and macrobes in symbiotic relationship
- **Metagenomics**
  - Application of modern genomics technique to study of communities of microbial organisms directly in their natural environments
  - Utilize analytical instruments that define molecular signatures
- **Microbiome** – organisms identified by DNA sequence
  - Sum of all microbial life living in or on human body
- **Operational Taxonomic Units (the thing being studied)** – applied to next-generation marker gene sequencing studies, organisms not directly observed.
  - Cluster of reads (genes) with 97% similarity correspond approximately to species.
- **Dysbiosis**
  - Perturbation within ecosystem of microbiome
Molecular Technique

- Exploits ubiquitous and evolutionarily conserved 16S rRNA
- Samples undergo DNA extraction before universal PCR primers to allow for amplification of intervening hypervariable regions.
- Amplicons are differentiated into similar groups to be classified into operational taxonomic unit
- Bacterial classification determined by open access sequence databases
- Fungal, archaeal, protozoal, and viral microbiomic studies exist.

Archaea - Domain

- no cell nucleus or any other membrane-bound organelles in their cells
- Live at high temperatures and produce methane
- Ubiquitous – interacts with viruses and bacteria

Methodologies

- First Generation Techniques
  – Physical means separation, denaturing or temperature gradient gel electrophoresis
  – Fingerprinting
- Next Generation sequencing (NGS)
  – Combine enzymology, chemistry, high-resolution optics, hardware, software engineering
  – Enormous data and sequence to great depth
  – 454 pyrosequencing, HiSeq and bench top MiSeq platforms
- Third Generation Platforms
  – Reads >400 base pairs, facilitate id to species

Berrington et al, Current Opinion Inf Dis., 2014

Data handling

- Large and complex datasets
- Richness (constituents)
- Evenness (dominance)
- Diversity (combination richness and evenness)
- Ordination analyses and constrained or redundancy analysis – exploration of potential mediators of structure changes
• Human Microbiome project – NIH
  • http://hmpdacc.org/overview/about.php
    – Integrative Human Microbiome Project – 2nd generation
      • Pregnancy and preterm birth
      • Inflammatory bowel disease
      • Prediabetes
• Metagenomics of Human Intestinal Tract – European Commission

• Neonatal Microbiota study
  – Longitudinal prospective study assessing importance of GI microbiota in relation to NEC and sepsis

Cradle to Grave
• Microbiome changes much more than human genome over lifetime and contributes to plasticity.
• Constantly fine-tuning to maintain homeostatic balance with host’s immune system
• Evolution governed by:
  – Mother
  – Adaptive and innate immune system
  – Diet
  – Medication
  – Toxin exposure
  – Illness

GI microbiota
• Majority populate distal ileum and colon
• 99% are anaerobes
• Individual microbiome is populated by only 15% of 1000+ species described
• 13,344 prokaryotic rRNS sequences from intestinal mucosal tissue and fecal samples of healthy adults
  – 395 phylotypes identified, 244 novel, 80% sequences from species not yet cultivated.

Functions of gut microbiota
• Defense against pathogen colonization
  – Production antimicrobial substances
  – Nutrient competition
  – Reinforcement intestinal barrier function
  – Enhancement IgA secretion
• Metabolism of macronutrients require coordination of processed encoded by human genome and microbiome.
  – Cholesterol oxidized in liver to conjugated bile acids, released into small intestine - metabolized by microbiota into hydrophobic bile acids to be excreted.
  – Both bile acids - agonists for host receptors, GPCR and nuclear hormone receptors, important host metabolism regulators

Eckburg PB, et al. Science, 2005
Newborn

- Crucial window of opportunity
- Appropriate and diverse microbiota need to be present for metabolic and immune system pathways to develop optimally for long-term health.

How does a newborn acquire microbiome?

- Evidence suggest that process occurs before delivery.
- Aagaard et al – placental microbiome profile
  - Non-pathogenic commensal microbiota: Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria phyla
  - Similar to oral microbiome
  - Low numbers, large number of species
  - Theories as to meaning of placental microbiome, not always harmful, helpful in terms of priming immune system, colonization fetal gut

Shifting Paradigm: Bacteria are not always harmful to the fetus

- 27% of 195 investigated placenta, intracellular bacteria in placental basal plate
- Microbiome of preterm and term placentas identified bacterial community of low numbers of organisms from large number of species
  - Nonpathogenic commensals – phyla Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, Fusobacteria

Possible mechanism for bacteria to reach placenta

Aagaard et al, Sci Transl Med, 2014
Development of GI microbiota

- Microbes begin competition to establish niche as soon as baby's oral mucosa is exposed to environment
- Reach intestines, facultative anaerobes first to dominate in colon
  – Obligate anaerobes flourish after O2 decreases during first week due to bacterial metabolism.

Maternal Intrapartum Antibiotics

- Vertical transmission of Lactobacillus, 45 mother-newborn pairs
- 90% vaginal samples had Lactobacillus-dominant mixed flora; 28% neonatal oral cultures (31% transmission rate).
- Maternal intrapartum antibiotics and duration of ROM was significantly associated with decreased transmission rate of Lactobacillus.
**Mode of delivery**

- Term C/S fecal microbiome  
  - Enterobacter, cancerogenus, Hemophilus, Staphylococcus, Streptococcus  
  - Skin and oral microbes, surrounding environment  
- Vaginal delivery  
  - Bacteroides, Bifidobacterium, Parabacteroides, Escherichia/Shigella (earlier sample)  
- Difference gradually decreased at 4 months and 12 months  
- C/S infants remain more heterogeneous  
- Bacteroides less prevalent or missing in C/S delivered even up to 12 months.


**Breastfeeding**

- Breast-fed infants receive mix of nutrients, fatty acids, lactoferrin, sIgA to affect milieu of development of microbiota  
- Oligosaccharides, glycoconjugates also prevent attack of enteropathogens and stimulate growth of Bifidobacterium  
- Interleukin-10, EGF, TGF-B1, erythropoietin are mediators in inflammatory response against pathogens in gut.  
- Microbiota – Staphylococcus, Streptococcus, Bifidobacterium, Lactobacillus  
  - Enteromammary pathway  
  - Genotyping mother milk and infant fecal samples identical strains.
Human Milk Oligosaccharides Exhibit Antimicrobial and Antibiofilm Properties against Group B Streptococcus

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ACS Infect. Dis. 2017

Formula feeding

• Microbiota – E coli, Clostridium difficile, Bacteroides, Prevotella, and Lactobacillus
• Mixed feeding shifts pattern to that of formula feeding.

Symbiotic Relationship

• Microbiota benefit from warm, nutrient-rich environment of the GI tract
  – Optimal growth within stable ecosystem
• Infant benefits from microbiota activities
  – Increased digestive capacity
  – Harvesting nutrients
  – Limit nutrient resources to pathogens
  – Development of barrier function, integrity, and immune function
  – Tolerant state
Goal

• Innate and adaptive immune system interact with microbiota to establish normal digestion, gut motility, immune tolerance to foods and microbial antigens, and protection against pathogens.

Prematurity – Factors impact Microbiome

• PPROM
• Maternal infection
• C/S delivery
• Perinatal and postnatal broad-spectrum antibiotic exposure,
  • Gut immaturity with dysmotility
• Fasting
• Intensive care infection control standards selecting for resistant microbes
• Exposure to formula
• Gut modifying medications such as H2 blockers.

Preterm infants

• Microbiota of decreased diversity coupled with colonization of pathogenic organisms.
  – Mode of delivery less contributory
  – Increased populations of facultative anaerobes, Enterococcus, Enterobacter, Lactobacillus, Staphylococcus
  – Decreased Bifidobacterium, Bacteroides, Atopobium

Berrington et al, Curr Opin Infect Dis, 2014

Patterned Progression Premature Gut

• 58 preterm infant, 922 stool samples pyrosequenced 16S rRNA gene
• 33-36 weeks PMA (3rd to 12th weeks of life), gut colonized by anaerobes
• Antibiotics, mode delivery, diet, age influence pace but not sequence of progression
• 3 bacterial classes – Bacilli, Gammaproteobacteria, Clostria

La Rosa PS, et al, PNAS, 2014
Antibiotics

- Use in infants associated with higher proportions enterobacteria and enterococci and lower bifidobacteria
- Use coincides with decrease diversity
- Maternal use (prenatal or during lactation) associated with lower Bacteroides, Atopobium, lower sum.

Impact Postnatal Antibiotics

- 29 infants 24-32 wks, predominately human milk, fecal sample at 10 day and 30 day.
- Decrease diversity index in 10 d samples from those who received more antibiotics.
- 30 d sample had more Proteobacteria and Actinobacteria
- Process of bacterial acquisition perturbed by antibiotics.

Dysbiosis
- Shifts in microbial composition or diversity
- Settings – dietary changes, antibiotic exposure, infection
- Favor invasion and growth of pathogenic species
- Disrupt finely tuned regulatory circuits of immune system that keeps pro- and anti-inflammatory checks and balances
- Neonatal microbiome is dynamic, fragile and impressionable.

Is Preterm Birth a dysbiosis?
- Vaginal Microbiome Consortium team
  - VA Commonwealth University, Seattle Children’s
  - Better understand how microbiome and host profiles change throughout pregnancy and influence nascent microbiome in neonates
  - Samples from up to 2000 women and neonates to be collected throughout pregnancy, delivery, and postpartum.
  - 2nd generation NIH Microbiome project

Necrotizing enterocolitis
- Multifactorial, devastating, poorly understood
- Link to infection – outbreaks, pneumatosis likely by product of bacterial fermentation
- Perturbation of intestinal immune homeostasis, disturbance of normal colonization patterns
- Berrington et al showed great variability in proposed dysbiotic growth patterns.
Late onset Sepsis

- Mai et al. analyzed 10 preterm infants with LOS and 18 preterm controls and showed association between microbiomic signatures 2 weeks before diagnosis
- Gut dysbiosis with preponderance of Proteobacteria and Firmicutes have been associated as well as less diversity and preponderance of Staphylococci.


Neonatal Intestinal Dysbiosis precedes Necrotizing Enterocolitis and Late-onset sepsis

Interventions

- Given instability and impressionability of developing gut microbiome and the association of disease prediction, detection, its logical to explore avenues that evolution and maintenance of healthy gut milieu can be promoted.
- Gold standard is mimic the term vaginal delivered exclusively breastfed infant.

Breast milk

- Complex immune-protective and growth factors
- Bioactive immune-modulatory cells
- Immunonutrients: amino acids, fatty acids, lysozyme, lactoferrin, minerals, metals, oligosaccharides (prebiotic)
- Live bacteria – probiotic
Transforming growth factor - β
- Induce production IgA AB
- Direct anti-inflammatory and maturational effects on immune and intestinal epithelial cells
- Establishing and maintaining immune tolerance
- TGF-β2 abundant in breastmilk; TGF-β1 produced by intestinal immune cells

Prebiotics
- Non-digestible substances that selectively stimulate colonic growth or activity of anaerobic/microaerophilic flora.
- May improve intestinal motility and gastric emptying.
- Inulin, lactulose, short chain fructo-oligosaccharides and galacto-oligosaccharides are some well-studied prebiotics.
  - Use, efficacy, and safety is not established in preterm

Human Milk Oligosaccharides
- Correlated milk oligosaccharides with fecal microbiota composition.
- 22 healthy full-term vaginally delivered, no antibiotics; 16 exclusively breastfed and 6 formula fed.
- Breastmilk and stool collected at 3 months of age.
- Partial least squares regression of HMO and microbiota showed several infant fecal bacterial genera could be predicted by their mothers’ HMO profiles.  
  
Probiotics

• Live microorganisms that confer health benefit on host (WHO).
• Recent Cochrane review (2014)
  – 25 RCT (n=5895)
  – Probiotics reduced the time to full enteral feeds in preterm neonates. Additional research is necessary to assess the optimal dose, duration, and probiotic strain or strains used specifically for facilitating enteral nutrition in this population.


Lactoferrin

• Antimicrobial, immunostimulatory, and immunomodulatory properties
  – Promote colonization with favorable bacteria
• Recent Cochrane review:
  – 6 RCTs
  – Low quality evidence that lactoferrin supplementation with/out probiotics decreases late-onset sepsis and NEC II/III in preterm infants without adverse effects.
  – Completion of ongoing trials will provide evidence from >6000 preterms may enhance the quality of the evidence.
  – Clarifications regarding optimum dosing regimens, type of lactoferrin (human or bovine), and long-term outcomes is needed.

Human Microbiome Project

• Intestinal dysbiosis as mediator of inflammatory bowel disease, obesity, and neurodevelopmental disorders in adults.
  – Metagenomics implicate gut microbiota and diet as key modulators of bidirectional signaling pathways between gut and brain.
• How does microbiome affect neurodevelopmental process?

Brain-gut Axis

• GI motor and sensory components send messages to the CNS and the return response to the intestine.
• Brain, central component, connections between cerebral cortex, limbic system, hypothalmic-pituitary axis, brain stem
• Peripheral components communicate with CNS via enteric, autonomic, and sympathetic nervous system
• ENS resides within intestinal wall; gut microbes modulate neural signaling to alter brain development and function.
  – Communicates with brain via vagus nerve and dorsal root and nodose ganglia
Intestinal Components of GBA

Loss of Gut Barrier Protection and Increased Permeability

Some Burning Questions

- Is prematurity a dysbiosis?
- Is dysbiosis of the microbiome cause or effect of NEC and LOS?
- What is the contribution of the host genes to alterations to the microbiome in disease states?
- Can we manipulated a gut microbiota reflective of a FTVDBF infant?
- Can the host immune response be enhanced to promote the growth of organisms seen in health states rather than in disease?
• Can probiotic use, dose, strain(s), and duration be optimized given the different types of babies at risk?
• What is the role of prebiotics either instead of probiotics or in combination (synbiotics)?
• Can ‘intelligent’ use of antibiotics help harness the microbiome to promote health?
• What role do non-bacterial members have individually and in community interactions with other microorganisms?

Bibliography
