Microbe-managing: Manipulating the Human Gut Microbial Ecosystem to Enhance Health

Emma Allen-Vercoe
AMMI CANADA – CACMID ANNUAL CONFERENCE

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Microbes – on us, in us and all around us

- We are each colonized by millions of microbes
- Every surface of our bodies is a niche for an organized community of bugs
- Humans are 90% bacteria, 10% human!
- At least 100x more microbial genes associated with us than our own human genes
- Humans are the ‘spaceships’ operated by their microbes
There are more bacteria in your gut than there are people on the planet...
Gut bug diversity

• We each have around 500-1000 different bacterial species living in our guts
• Just as we each have unique DNA, fingerprints and iris patterns, we all have unique collections of microbial species in our guts
Remarkably...

The bacterial community in your gut remains stable from

• weaning...

• ...to old age

And we are only just starting to understand this homeostasis
It’s all about Balance!

http://www.gbposters.com
Maintaining the equilibrium

High diversity of species:
• Healthy ecosystem
• Functional redundancy
• Resistance to disease

Low diversity of species:
• Sick ecosystem
• Functional disability
• Susceptibility to disease
Our microbes are vitally important…

• But we are working very hard to exterminate them!
‘Extinction events’ may impact health

- **Hygiene hypothesis**
  - We are preventing proper colonization by being too clean

- **Missing microbiota hypothesis**
  - We are disturbing proper colonization across generations through e.g. antibiotic use, & poor diet

- **Antibiotic use (especially in early childhood) may be particularly problematic**
The United States is among the most intensive users of antibiotics in the world

- FRANCE
- GREECE
- ITALY
- BELGIUM
- UNITED STATES
- CANADA
- AUSTRALIA
- SPAIN
- CZECH REPUBLIC
- UNITED KINGDOM
- GERMANY
- NETHERLANDS

**Sources:** United States and Canada (McManus, Hammond et al. 1997), Australia (National Prescribing Service 2005), European countries (Goossens, Ferech et al. 2003).

**Note:** DDD = defined daily doses, a standardized measure of antibiotic consumption.
Stop the killing of beneficial bacteria

Concerns about antibiotics focus on bacterial resistance — but permanent changes to our protective flora could have more serious consequences, says Martin Blaser.

Consequences of collateral damage

• Several studies have shown:
  – Gut microbial flora changes significantly with antibiotic use
  – Takes a long time afterwards to return to baseline
  – Sometimes does not return to baseline at all
  – Repeated ‘hits’ cause vast changes from which the ecosystem does not recover

The additional impact of the Western diet

- Average Western diet – rich in refined foods, low in fermented foods, complex carbohydrates, fibre

- Refined foods are easily broken down in the upper GI tract
  - Thus very little left-over food makes it to the colon

- Colon is the site of most beneficial gut microbial activity
  - Starvation of this community can lead to ecosystem damage
  - ‘extinction events’ and reduced diversity
Save our Rainforests

Save our gut microbiota
Examples of diseases associated with reduced gut microbiota diversity (published research)

Infant colic
Autism
Allergic asthma
Neonatal necrotizing enterocolitis
Irritable Bowel Syndrome

Inflammatory bowel diseases
Eczema
Colorectal cancer
Celiac disease
Obesity

Clostridium difficile infection
• Lack of microbial diversity
• Loss of ‘keystone’ species
• Overgrowth of opportunistic pathogens
• Poor diet/lifestyle
• Drug interactions

Looking inside the black box is the key to understanding disease

“Dysbiosis”
To understand disease, we need to understand health

- What are the microbes that make up a ‘healthy’ gut microflora?
- What jobs do these microbes do for us?
- What happens if they are missing?
- Can we replace missing gut microbes?
  - If so, how?
The good, the bad and the ugly

• The Good
  • Lactic Acid Bacteria (LAB)
    – E.g. *Bifidobacterium* and *Lactobacillus* spp.
  • Butyrate-producing bacteria
    – E.g. *Faecalibacterium prausnitizii*, *Roseburia* spp.

• The Bad
  • Opportunistic pathogens
    – E.g. *E.coli*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Bacteroides fragilis*
  • Sulfate-reducing bacteria
    – E.g. *Desulfovibrio* spp.

The Ugly: it really is not that clear-cut!!
Everyone is different

- Gut microbial ecosystems are highly variable in composition and abundance profiles
• However…
• Ecosystem function is preserved across individuals
  – It’s not about what species are there, but about what the species are doing
• To understand health, we need to study gut ecosystems
  – We have only accessed the biology of ~30-50% of the gut microbiota
  – Remainder are ‘unculturable’
Why don’t we know more than we do?

- Most of the microbes in the gut are strict anaerobes
- Require specific conditions (and equipment) to culture them
- Even then, many species refuse to grow in the lab
Microbes in nature…
• Almost always exist as part of microbial communities
• Benefit from their microbial friends (& host)
• Rarely grow logarithmically
• Rarely have access to rich nutrient sources

Microbes in a microbiology lab…
• Almost always exist on their own as part of a pure culture
• Usually have to adapt to survive this way
• Are often grown logarithmically
• Are usually given access to rich nutrient sources
Just like teenagers: Microbes behave differently when on their own

…They are usually happier when with their friends!
The human gut microbiota is a complex microbial ecosystem. Function and behaviour of this ecosystem is best studied as a whole.
Growing microbes in communities

The human gut is a type of ‘chemostat’

Rate of medium in = rate of medium out

Temperature probe
pH probe
Stirrer
Bubbler
Our “Roboguts”

- Seeded with fresh feces and set to model the distal gut ecosystem
- Host-free system
- Can be used to support growth of fastidious gut anaerobes
“Liquid gold”
Different people’s microbiota responds to antibiotic disturbance in different ways.
Liquid gold derived from different donors produces metabolic profiles unique to the respective hosts.
So, if human health depends on microbiota health...

...how can we modulate the gut microbiota to improve health?
Why not just use existing probiotics?

**Pros**
- ‘Generally regarded as safe’
- Many naturally ferment foodstuffs
- May have beneficial effects as they transition through the intestine
- Currently very popular

**Cons**
- Not policed well
  - Many do not live up to their claims
- Can be very expensive
- No ‘one-size fits all’ probiotic
  - But often marketed this way
- Do not colonize; no lasting effects
The layperson’s view of probiotics...
The microbial ecologist’s view of probiotics

Normal gut microbiota vs. Probiotic
Microbial Ecosystem Therapeutics, MET

• Dysfunctional ecosystems have been associated with many diseases
  – Cause or effect not yet fully understood

• Can we cure disease by replacing a damaged microbiota?

• One disease we know is caused by gut ecosystem disturbance is Clostridium difficile infection, CDI
Ecosystem damage and CDI

- Lack of diversity in the gut ecosystem allows overgrowth of *Clostridium difficile* in the niche
  - Toxin production ↑, colitis ensues
A healthy gut microbiota is like a healthy lawn:
Lush growth, no room for weeds
The healthy lawn analogy

When the lawn is stressed, e.g. during drought, damage ensues
The healthy lawn analogy

If you’re unlucky, weeds can move in before the lawn recovers from the stress.
The healthy lawn analogy

Applying more damage to the lawn is one way to get rid of the weeds.
Another approach is to replace the damaged turf with new, healthy growth.
Fecal transplant/fecal bacteriotherapy (aka “re-turfing”)

- Donor selected
- Usually close family member
- Screened for range of diseases that are potentially passed on through stool
- If ‘pass’, donation time coordinated with patient drug taper
• Fresh homogenate instilled into patient within 6 hrs of preparation
  • Rectal enema
  • Colonoscopy
  • Nasoduodenal tube
  • “Poop pills”

• Results in cure of the patient in >90% of cases
• Rapid resolution of CDI
• Only rare recurrence of disease
Pros and cons of fecal transplants

• Pros:
  • They work! ~90% of patients are cured of CDI
    • Van Nood et al., NEJM 2013
  • They’re comparatively cheap

• Cons:
  • Somewhat primitive
  • Undefined; will vary donor to donor
    • How do you know who’s healthy?
  • Despite screening, still much potential for spread of pathogens
  • They’re gross – lots of psychological stigma
Can we use cultured microbes to make ‘fake poop’?

- Collaboration with Dr. Elaine Petrof, Queen’s University
- Plan: to develop the fecal transplant concept further by using pure bacteria – ‘probiotics’
  - But not your average probiotic: “RePOOPulate”!
- By doing so should mitigate fears about:
  - Safety
  - Reproducibility
  - Delivery
  - Shelf-life
- “Microbial Ecosystem Therapeutics” (MET)

- Not really a new idea
  - But in the past, barrier to this was perceived unculturability of gut bacteria
Our healthy donor

• Healthy female in her early 40s
• Average BMI
• Very healthy lifestyle
• Very few or no antibiotic exposures in childhood
• 1 reported exposure to antibiotics in the last 10 years
• Cultured >70 strains from poop sample using Robogut…
  • Formulated RePOOPulate (33 strains)
“RePOOPulate”

- *Acidaminococcus intestinalis*
- *Bacteroides ovatus*
- *Bifidobacterium adolescentis* (x2)
- *Bifidobacterium longum* (x2)
- *Collinsella aerofasciens*
- *Dorea longicatena* (x2)
- *Escherichia coli*
- *Eubacterium eligens*
- *Eubacterium limosum*
- *Eubacterium rectale* (x4)
- *Eubacterium ventriosum*

- *Faecalibacterium prausnitzii*
- *Lactobacillus casei*
- *Lactobacillus paracasei*
- *Parabacteroides distasonis*
- *Raoultella* sp.
- *Roseburia faecalis*
- *Roseburia intestinalis*
- *Ruminococcus torques* (x2)
- *Streptococcus mitis*
- Likely novel species (x5)
- Likely novel genus & species (x1)

(Closest species by full-length 16S alignment)

Formulation tested for ecosystem stability in our Robogut
“RePOOPulate”

- Acidaminococcus intestinalis
- Bacteroides ovatus
- Bifidobacterium adolescentis (x2)
- Bifidobacterium longum (x2)
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‘Lachnospiraceae’ family species
RePOOPulate proof-of-principle trial

• 2 elderly ladies with severe, recurrent *C. diff* infections were treated (April and June 2011)

• RePOOPulate made fresh at Guelph, driven to KGH, and administered via colonoscopy
  – 1 dose, 100mLs

• Both patients recovered within 2 days and have remained *C. diff*-free ever since (despite numerous subsequent antibiotic exposures)
Petrof et al. *Microbiome*, 2013
For patient 1, chemostat 16S rRNA gene profile after 12 days (steady state) closely matched patient profile after 14 days.

The chemostat represents a good surrogate for the *in vivo* environment.

This therapeutic ecosystem *colonized* our patients.
Fake Feces To Treat Deadly Disease: Scientists Find They Can Just Make Sh*t Up

By Christie Wilcox | January 10, 2013 | 4
I foresee a time when...

- Gut microbial ecosystem functional screening will be a critical component of all comprehensive medical check-ups.
- It will be possible to enhance ecosystem functionality to maintain health by manipulating the microbiota and supporting these ecosystems with a tailored diet.
- Broad spectrum antibiotics will not be used without measures to protect the microbiota.
- "Symbiontology" will become a new medical specialty:
  - A merger of Gastroenterology, infectious disease, microbial ecology and nutrition science (and many other specialties!)
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I found the problem, Mr. Smith. Instead of probiotics, you have been taking amateur biotics.