**What Defines a Healthy GIT Microbiome in the USA?**

**Healthy:** A population of \(10^{14}\) bacteria of at least 1000 species and belonging to four major phyla, the majority (90%) of which are anaerobes (e.g. Bacteriodetes)

**Unhealthy:** A low diversity, i.e. < 300 species, in e.g. patients after extensive antibiotic therapy

An increase in the Firmicutes:Bacteriodete ratio (e.g. an increase from 0.5 to 1.6)

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Metabolism</th>
<th>Key Genera</th>
<th>Healthy GIT</th>
<th>Unhealthy GIT</th>
<th>Proportion (%)</th>
</tr>
</thead>
</table>
| Actinobacteria  | Facultative anaerobes | *Bifidobacteria*  
*Actinobacillus*  | > 10         | 1     | 5             |
| Firmicutes      | Facultative anaerobes | *Clostridium*  
*Enterococcus*  
*Ruminococcus*  
*Lactobacillus*  | 40          | 30    | 40            |
| Proteobacteria  | ("pathobionts")  | *E. coli*  
*Salmonella*  
*Staphylococcus*  
*Shigella*  | 25          | 6     | 30            |
| Bacteriodetes   | Gram negative anaerobes | *Bacteriodes*  
*Prevotella*  | 25          | 63    | 25            |
The Constituency of the Microbiome Changes with Age

Mouse studies show that a microbiome is individually specific and established very early in life.
Culture and Ethnicity may affect the Constituency and Definition of a Healthy Microbiome

The GIT microbiome of children in Burkina Faso resembles the adult GIT in America (high Bacteriodetes).

In Siberia, Italy, Venezuela, and Tanzania, the adult GIT microbiome is dominated by Firmicutes, not Bacteriodes.

Neonates in Luxembourg are dominated by Proteobacteria, not Bacteriodes.

The GIT of adults in Papua New Guinea resembles that of human infants in America.

Aboriginals from Venezuela have hundreds more taxa than 157 Colorado families to which they were compared.

Conclusion: A “standard” healthy microbiome may be culturally determined.
The Concept of “Good” (Eubiotic) versus “Bad” (Dysbiotic) Microbiomes

The concept derives largely from studies of unhealthy individuals, e.g. patients with inflammatory bowel disease (IBD), the very old and infirmed, unhealthy infants and the obese.

“Bad” or dysbiosis features a microbiome with:

1. An increase in the Firmicutes to Bacteriodete ratio (shift from 0.5 to 1.6; see first slide) which means more Clostridiales, Enterococcus and Ruminococci
2. A decrease in *Bacteroides fragilis* which in healthy people stimulate anti-inflammatory T-cells (Tregs) and generates SCFA needed for mucosal integrity
3. More Proteobacteria (pathobionts) and less of Bacteriodes in the elderly (>80 years old) of poor health
4. Colonies of *Staphylococcus* instead of Bifidobacteria in unhealthy C-section infants
5. Higher Clostridiales (Firmicutes) in patients with CVD
6. Higher Actinobacteria in patients with periodontal disease (PD)

A “Good” or eubiotic microbiome appears to correlate with a higher proportion of Bacteriodetes that thrive on and convert complex fibers to SCFA. However, “Good” versus “Bad” may depend on the circumstances since, children with an enrichment of Clostridia (Firmicutes) resolve their allergies faster while those with higher levels of Enterobacteria (*E. coli; Salmonella*)

*Lactobacillus plantarum* in symbiotic combination given to newborns in India can reduce infant sepsis by 40%

[6.3 million die per year of sepsis worldwide]

**Problem:** Of the >1000 species, only 2% can be cultured so we do not know what most of the microbiome does!
The Microbiome and Cardiovascular disease (CVD)

Important correlations

“Western” diets are linked to Type II diabetes, CVD and “fishy breath”
Vegans and types on a “Mediterranean” diet have less CVD
“Western” diets have less complex fermentable fiber than in “Mediterranean” diets

Trimethylamine (TMA) and TMAO (a TMA oxide)

Dietary carnitine and phosphatidyl choline from red meat and lecithin (eggs) is converted to TMA by the gut microbiome
Blood levels of TMA are strongly linked to vascular plague formation and CVD
Vegans and herbivores have lower TMA and TMAO levels than omnivores

TMA, CVD and the microbiome

Less carnitine and more complex fiber results in more Bacteriodetes
Probiotics (Lactobacilli) are associated with weight loss but not lowering of TMA
Short ester forms (acetyl-1-carnitine) are being tested as a competitive dietary substitute to lower TMAO
How do probiotics help in the “good” versus “bad” bacteria circumstances?

Two semi-distinct motives are behind the use of probiotics

1. Use in healthy subjects to maintain good health
2. Therapeutic restoration of the microbiome

Probiotics make changes in the population dynamics of the GIT microbiome

1. Daily ingestion of $10^8$ to $10^{10}$ probiotic bacteria results in a ratio of one probiotic bacterium to $10^3$ to $10^7$ resident bacteria
2. The same treatment for patients with an antibiotic-depleted GIT microbiome shifts downward to a ratio of $10^1$ to $10^3$.

Diet may have a greater effect on the microbiome than the use of probiotics

1. High fiber diets encourage Bacteriodetes (e.g. *Prevotella*) that degrade cellulose to SCFA. [See profile for Burkina faso]
2. SCFA-generating bacteria lower the pH which discourages pathobionts
3. High fat diets shifts GIT microflora to favor Firmicutes and Enterobacteria

The Microbiome-Gut-Brain Axis

The linkage between gut and brain is old, i.e. “having a gut feeling” and napping after a nice Sunday dinner. Patients with irritable bowel syndrome are indeed anxious and irritable.

*Lactobacillus rhamnosus* (but not all *Lactobacilli* or *E. coli*) activate GABA, the main CNS inhibitory neuro transmitter. Alterations in GABA are correlated with irritable bowel syndrome. *L. rhamnosus* lowered stress-induced corticosteroid levels, but not in germfree mice or after cutting the vagus nerve in colonized mice.

Antibiotic disruption of the microbiome reduces Proteobacteria and Bacteriodes but increases Actinobacteria. This increases the hippocampal factor that controls the “exploring” behavior of mice and is reversible by antibiotic withdrawal. Antibiotic disruption had no effect in germfree mice suggesting the microbiome is needed for the gut-brain axis.

EAE (Experimental autoimmune encephalomyelitis) is a mouse model for muscular dystrophy (MS). *Prevotella histicola* used as a probiotic reduces MS. *P. histicola* reduces the blood-brain-permeability barrier (BBB). *P. histicola* results in a 3-fold increase in Tregs (Section 3; Lecture 1).

Autism and the Microbiome??????

Various speculative studies

I prefer not to touch it since I would not welcome a lawsuit.
Probiotics can help after disruption of the microbiome

Pathobionts or potential pathobionts are always “lurking” among the 10^14 bacteria that comprise the GIT microbiome. These include e.g. *C. difficile*, *S. aureus* and pathogenic *E. coli*

*E. coli* exists in many forms and specializes in the exchange of plasmids that carry virulence and antibiotic-resistance genes

Most antibiotic resistances genes “hide” in plasmids carried by “good “ bacteria.

*Salmonella* and *Shigella* can act as a “Trojan horse” and breach the mucosal barrier by entering through M-cells.

Disruption of the microbiome can give advantages to pathobionts

Viral infections, e.g. TGE in piglets, erodes the mucosal epithelium by shearing off villi and allowing pathobionts a site for translocation

Antibiotic therapy can result in massive losses of the “good “ bacteria that comprise the normal GIT microbiome allowing *C. difficile* to proliferate
Ulcerative Colitis (type of IBD) is associated with an unhealthy microbiome and is increasing in developed Western cultures

Possible Villains

Dietary Changes?
Cultural Changes?
Use of Antibiotics?

IBD is multifactorial

Before 1960
1960-1979
1980-2008

[Discussed by David Elliott; Session 3]
Is Antibiotic Therapy Involved?

Does antibiotic therapy parallel the increase in IBD?

- Overall this is true
- Regionally it is **not true** since IBD is lower in Italy and Spain where antibiotic use is high compared to Scandinavia
- Withdrawal of short-term antibiotic therapy usually allows the microbiome to return to normal

Continuous use of antibiotics **in early life** is correlated with:

- Development of childhood asthma and milk allergy
- Development of IBD in mouse models
- Obesity in mouse models
- Permanent alterations in the microbiome of treated mice

**Caution**: Correlations when considered alone, can get you into trouble!
Acquiring the Microbiome

Many newborns are “fecalphagus”, i.e. they eat 🍗

Cockroaches   Birds   Rodents & Carnivores
Piglets       Termites  Rabbits

Placental mammals obtain their microbiome from various sources

Birth canal (vagina) favor *Lactobacillus*
The location of the birth canal and anus favor inoculation from

- Fecal material
- Vagina
- Skin

Favors mother’s microbiome

Suckling favor bacteria in breast milk and from the skin
e.g.. *Bifidobactor* and *Staph aureus*

Kissing and licking favors mothers oral microbiome and that on the skin
*Actinobacteria*  &  *Proteobacteria*

Amniotic fluid and the meconium favors *Lactobacillus*

Suggested TV viewing:  “Call the midwife” on IPTV
The conditions seen in adults are not the same at birth

The stomach and oral cavity pH changes: Compared to adults, the stomach is less acidic and duodenum less alkaline to allow more of the maternal antibodies obtained through suckling to survive.

Enterocyte receptors delay their development until after suckling so attachment by e.g. *E. coli* is discouraged.

Bifidobacteria in human breast milk and *Lactobacillus* discourage expansion of *Bacteroidetes*. Lactic acid bacteria (*Lactobacillus*) lowering the pH of the GIT to favor their own kind (other Firmicutes) and discourages *Bacteroidetes*.

Total microbiome levels in infants are several logs lower than is characteristic for adults (see figure on the left).