Understanding the Clinical Significance of the Commensal Bacteria

Christine Stubbe, ND FABNO
Genova Medical Education Specialist
Patricia Devers, DO
Content Manager for Genova Diagnostics
Christine Stubbe, ND, FABNO
Genova Medical Education Specialist
Technical Issues & Clinical Questions

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Understanding the Clinical Significance of the Commensal Bacteria
Learning Objectives for This Presentation

• Learn about the 24 commensal bacteria measured on the GI Effects

• Recognize patterns commonly seen on the Commensal Balance and Relative Abundance charts

• Meet key players including *Akkermansia muciniphila*, *Methanobrevibacter smithii*, *Faecalibacterium prausnitzii*, and others

• Explore strategies for creating microbiome balance
What is a Commensal Bacteria?

- **Commensal**
  - Normal flora or normal microbiota
  - Microbiota that are expected to be present, and that under normal circumstances do not cause disease

- **Microbiota** – microorganisms in a defined environment (human GI microbiota)

- **Microbiome** – the entire habitat of microorganisms, their genomes, and the surrounding environmental conditions

- **Dysbiosis** – alteration in the composition of the commensal gut microbiota that can harm the host
Commensal Bacteria

- 24 microbes/groups assessed on GI Effects Comprehensive and GI Effects Microbial Ecology Profile
- Commensal Balance and Relative Abundance charts utilize algorithms to synthesize the information
PCR Probe Selection

• Clinical associations

• Validated genus and species level probes

• Reference ranges based on a healthy questionnaire-qualified cohort, with other strict exclusion criteria
Taxonomic Classification examples

Eukaryote (e.g. dogs)

- Domain: Eukarya
- Kingdom: Animalia
- Phylum: Chordata
- Class: Mammalia
- Order: Carnivora
- Family: Canidae
- Genus: Canis
- Species: lupus
  - Subspecies: familiaris

Prokaryote (e.g. E. coli)

- Domain: Bacteria
- Kingdom: (no kingdom)
- Phylum: Proteobacteria
- Class: y-proteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: Escherichia
- Species: coli
  - Multiple strains
### Gastrointestinal Microbiome

#### Commensal Bacteria (PCR)

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Result</th>
<th>1st</th>
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<tbody>
<tr>
<td><strong>Bacteroidetes Phylum</strong></td>
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<td>Bacteroides-Prevotella group</td>
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<td>3.4E6-1.5E9</td>
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<tr>
<td>Bacteroides vulgatus</td>
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<td>Bacteriales spp.</td>
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<td><strong>Clostridiales Phylum</strong></td>
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<td>Clostridium spp.</td>
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<td><strong>Prevotella Phylum</strong></td>
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<td><strong>Fusobacteria Phylum</strong></td>
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<tr>
<td><em>Subdoligranulum wadsworthii</em></td>
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<td>1.7E6-1.5E10</td>
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<td><em>Coprococcus ultimus</em></td>
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<td><em>Peptostreptococcus microsiae</em></td>
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<td><em>Vedderia</em> spp.</td>
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<td><em>Collinsella aerofaciens</em></td>
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<td>1.4E7-1.9E9</td>
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<td><strong>Proteobacteria Phylum</strong></td>
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<td><em>Desulfibacterus</em></td>
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<td><em>Escherichia coli</em></td>
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<td><em>Chelatobacter flagellatus</em></td>
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<td><em>Methanomethanoarshia</em></td>
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<td><strong>Fusobacteria Phylum</strong></td>
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<td><em>Verrucomicrobia</em> Phylum</td>
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<tr>
<td><em>Akkermansia muciniphila</em></td>
<td>3.1E7</td>
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<td>&gt;=1.2E8</td>
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</table>

#### Fungi/Bacteria (FB Ratio)

<table>
<thead>
<tr>
<th>Firmicutes/Bacteroidetes (FB Ratio)</th>
<th>Result</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>Reference Range</th>
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<tbody>
<tr>
<td><em>Firmicutes/Bacteroidetes (FB Ratio)</em></td>
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<td>12-620</td>
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</table>
SCFAs are Postbiotic Metabolites

- Ingestion of dietary fiber
  - Prebiotics
    - Specialized fiber that supports beneficial microbes present in the gut
- Fermentation by bacteria
  - Probiotics
    - Live microorganisms that provide health benefits
- Production of short-chain fatty acids
  - Postbiotics
    - Products made by bacteria that affect a range of physiological processes
# Short Chain Fatty Acids

<table>
<thead>
<tr>
<th>Short-Chain Fatty Acids (SCFA) (Total*)</th>
<th>81.3</th>
<th>&gt;=23.3 micromol/g</th>
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</thead>
<tbody>
<tr>
<td>Acetate, n-Butyrate, Propionate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-Butyrate Concentration</td>
<td>18.1</td>
<td>&gt;=3.6 micromol/g</td>
</tr>
<tr>
<td>n-Butyrate %</td>
<td>22.3</td>
<td>11.8-33.3 %</td>
</tr>
<tr>
<td>Acetate %</td>
<td>63.1</td>
<td>48.1-69.2 %</td>
</tr>
<tr>
<td>Propionate %</td>
<td>14.6</td>
<td>&lt;=29.3 %</td>
</tr>
</tbody>
</table>
FIG 1 Proposed model of metabolites and microbes that catalyze the flow of carbon from resistant polysaccharides to butyrate. There are cultivated strains from the gut microbiome that possess the metabolic activities proposed for the species listed.
What is the microbiome telling us?

Synthesizing the information
Can’t See the Forest for the Trees
It is important to perform data analysis on the unique set of biomarkers versus extrapolating from the literature:
- All studies do not have the same set of microbes - not an apples to apples comparison
- Different methodologies
- There is not a standardized way to assess the microbiome
- May reach different conclusions
- Literature provides a starting point

Data analysis is performed on Genova’s set of microbes:
- Patient questionnaires
- Analysis of biomarker associations with each other, even across different tests
- Creating meaningful data unique to this set of microbes
Commensal Balance Chart

*Healthy-Pattern Continuum*

**Reference Variance Score**
- Balanced: Represents 95% of healthy individuals
- Borderline: Represents 5% of healthy individuals
- Imbalanced: Represents 60% of unhealthy individuals

- Your Result

* A progressive ranking scale based on a Genova proprietary algorithm that differentiates healthy and unhealthy commensal patterns.

** The total number of Commensal Bacteria (PCR) that are out of reference ranges for this individual.
Bacterial Abundance

Lower commensal abundance is associated with inflammatory biomarkers and a higher Inflammation Associated Dysbiosis (IAD) score

<table>
<thead>
<tr>
<th>Cluster 1 (high commensal abundance)</th>
<th>Cluster 2 (low commensal abundance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-chain fatty acids</td>
<td>Calprotectin</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>EPX</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>IgA</td>
</tr>
<tr>
<td>Total SCFAs (butyrate + acetate + propionate)</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>% Butyrate of total SCFAs</td>
<td>% Acetate of total SCFAs</td>
</tr>
<tr>
<td>Pancreatic elastase 1</td>
<td>% Propionate of total SCFAs</td>
</tr>
<tr>
<td>Putrefactive SCFAs (valerate + isobutyrate + isovalerate)</td>
<td></td>
</tr>
</tbody>
</table>

Each group is associated with increased levels of certain fecal biomarkers

“In addition to the classic pathogenic species, we propose that another kind of pathogenicity exists in the gut: one in which the whole community is “pathogenic” when its emergent properties contribute to disease. In a “pathogenic community,” no single microbe is pathogenic alone. Instead, the community assemblage is an environmental risk factor that contributes to a disease state. A microbial community will be pathogenic within the context of other risk factors, such as host genotype, diet, and behavior.”
Conditions associated with gut dysbiosis

- Genova data analysis based on patient questionnaires
- Literature associations with these and other conditions such as autism, eczema, allergies, liver diseases, Parkinson's disease, SIBO, Multiple sclerosis, etc.
Spotlight on Individual Commensals
Bacteroides spp.

- Bacteroides enterotype correlated with diets rich in animal protein and fat
- Displays flexibility to adapt to many environmental conditions/diets
- Produces beta glucuronidase, secondary bile acids, acetate, propionate, products of protein breakdown, vitamins
- Associated with reduced microbial diversity, insulin resistance (B. vulgatus)

**Bacteroides spp.**

- **Increased**
  - Red wine, animal-based diets, high fat diets

- **Decreased**
  - Tart cherry juice, inulin, green tea, dates

**Prevotella** spp.

- Literature mixed on association with inflammation
- Produces acetate, propionate
- **Prevotella** enterotype richer in response to plant-based diets and complex carbohydrates
- Increased
  - Plant-based diet
- Decreased
  - Western Diet

**Clostridium spp.**

- *Clostridium* genus contains more than 100 species

- Genova’s *Clostridium* spp. probe is not meant to diagnose pathogenic *Clostridium* infections such as *C. difficile*.

- Produces SCFAs, hydrogen, beta-glucuronidase, secondary bile acids

- Necessary for immune homeostasis

Clostridium spp.

- Increased
  - Coffee, high beef diet

- Decreased
  - Mediterranean diet, FOS-, polydextrose, and AOS-based prebiotics

Faecalibacterium prausnitzii

- Decreased in intestinal disorders such as Crohn’s disease, ulcerative colitis and colorectal cancer
- Anti-inflammatory, supports healthy intestinal barrier function
- GDX found negative association with inflammatory biomarkers calprotectin, EPX, and sIgA
- Main butyrate-producer, B vitamins
Faecalibacterium prausnitzii

- **Increased**
  - Inulin-type fructan and FOS prebiotics, fiber, apple pectin, vegan/vegetarian diets, physical activity

- **Decreased**
  - Gluten-free diets and low FODMAPs diet, excess bile salts

Lactobacillus and Bifidobacterium

- Probiotic bacteria
  - Lactobacillus spp.
    - Produces acetate and lactate
    - Increased: Mediterranean diet, whole grain, pea protein, coffee, guar gum, polyphenols
    - Decreased: gluten-free diet, high saturated and trans fat
  - Bifidobacterium spp.
    - Produces SCFA, B vitamins
    - Increased: Mediterranean diet, inulin-type fructans, guar gum, pea protein, resistant starch, whole grain, polyphenols
    - Decreased: high-protein/low-carb diets, low FODMAP diet, gluten-free, high saturated and trans fat
Proteobacteria phylum

**Desulfovibrio piger**

- Hydrogen sulfide producer, decreases butyrate
- Increased risk of IBD, CRC, visceral nerve sensitivity
- Increased
  - Animal studies: High-fat/high-sugar diet, chondroitin sulfate
- Decreased
  - Human: *Lactobacillus plantarum*
  - In-vitro: lower pH

Escherichia coli

- This group has many strains, most do not cause disease
- Genova’s *Escherichia coli* probe is not meant to diagnose pathogenic *E. coli* infections such as shiga-like toxin *E. coli*
- Synthesizes vitamins, ferments amino acids
- Can produce ethanol and promote gut permeability
**Escherichia coli**

- **Increased**
  - Gluten-free diet
  - Consumes oligosaccharides and simple sugars

- **Decreased**
  - Plant-based diet, probiotic yogurt

- **E. coli** Nissle probiotic

**Oxalobacter formigenes**

- Key bacterium responsible for the degradation of oxalate, therefore reducing oxalate absorption, oxalate excretion in urine, and the risk of kidney stones
- Oxalate is in spinach, rhubarb, almonds, cashews, grits, cocoa powder, beets, baked potato with skin, wheat berries

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**Ticinesi A, et. al. Kid Intl. 2019:96;13-30.**
Oxalobacter formigenes

- Sensitive to and reduced with commonly used antibiotics

- *Lactobacillus* and *Bifidobacterium* are also capable of consuming oxalate
Methanobrevibacter smithii

- Hydrogen-consuming, methane-producing archaea (not a bacteria!)
- Methane associated with obesity, prediabetes, constipation
- Decreased
  - Rifaximin+Neomycin, statins, probiotics with Lactobacillus and Bifidobacterium strains, garlic oil (breath methane levels)
Methanobrevibacter smithii
**Akkermansia muciniphila**

- Low levels associated with obesity, diabetes, inflammation, insulin resistance, hepatic inflammation, gut permeability
- Mucin degrader, produces acetate and propionate
- Improves intestinal barrier integrity
- May limit toxicity of sulfate-reducing bacteria
- In development as a probiotic supplement for metabolic conditions

Akkermansia muciniphila

- **Increased**
  - Pomegranate, caloric restriction, resveratrol, polydextrose, inulin, sodium butyrate, physical activity

- **Decreased**
  - Low FODMAP diet
Strategies for Balancing the Microbiome
Conclusion: The “Western diet”, in particular a low-fiber high fat/high carbohydrate diet is one factor that can lead to severe dysbiosis. In contrast, “Mediterranean” and vegetarian diets that includes abundant fruits, vegetables, olive oil and oily fish are known for their anti-inflammatory effects and could prevent dysbiosis and subsequent inflammatory bowel disease.
Holistic Approach

- **A plant-based diet** appears to be beneficial for human health by promoting the development of a more diverse gut microbial system, or even distribution of different species.

- **Physical activity** can increase gut microbial diversity, as well as health-promoting bacterial species including *Faecalibacterium prausnitzii*, *Roseburia hominis* and *Akkermansia muciniphila*.

- **Stress**

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Holistic Approach

- **Medications**: Antibiotics, PPIs, NSAIDs, and others

- **Other toxic exposures**
  - Alcohol
  - Sugar
  - Tobacco
  - Western diet
  - Pesticides and chemicals in the home
  - Chlorinated drinking water
  - Artificial sweeteners
  - Food additives
  - Etc.
Fermented Foods

• Yogurt, kefir, kombucha, sauerkraut, kimchi, kvass, miso, tempeh
  – Contain probiotic strains including *Lactobacillus, Bifidobacterium, Streptococcus thermophilus* and others
Food for Microbiota

- Prebiotics: Indigestible (for humans) carbohydrate substrate that feeds bacteria
  - Resistant starch is an insoluble fiber (unripe bananas, cooked and cooled potatoes, seeds, legumes, whole grains) – **the fermentation of resistant starch produces SCFA**
  - Non-starch polysaccharides
    - Inulin foods include chicory, garlic, onion, Jerusalem artichokes, jicama
    - FOS naturally found in chicory, onions, asparagus, wheat, tomatoes, veggies, grains, seaweed
  - Soluble fiber (psyllium husk, acacia fiber); pectin (apples); beta glucan (oats, mushrooms, seaweed); beans

- Plant polyphenols: fruits, seeds, vegetables, tea, cocoa

- Special diets may lack prebiotics
  - Paleo, low-carb, ketogenic, grain-free, low FODMAPs
Supplements

• Prebiotic supplements: arabinogalactan, FOS, GOS, inulin, oligofructose, beta glucan

• Probiotic supplements: *Lactobacillus, Bifidobacterium, Strep thermophilus, Saccharomyces boulardii*, spore-based and others
  – No consensus on probiotics and dosing
  – Some clinicians rotate probiotics
  – Online databases that shows conditions and strains studied

• Postbiotic supplements:
  – Example: Butyrate for IBD
  – Butyrate naturally occurs in butter
  – Bacteria manufactures butyrate with the right substrate (prebiotic)
# EIA and Culture

## Add-on Testing

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<tr>
<th>Methodology</th>
<th>Result</th>
<th>Expected Value</th>
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</thead>
<tbody>
<tr>
<td>H.pSA - H. pylori</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Shiga toxin E. coli</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

### Microbiology Legend

- **NG**: No Growth
- **NP**: Non-Pathogen
- **PP**: Potential Pathogen
- **P**: Pathogen

### Gastrointestinal Microbiome

**Non-Pathogen**: Organisms that fall under this category are those that constitute normal, commensal flora, or have not been recognized as etiological agents of disease.

**Potential Pathogen**: Organisms that fall under this category are considered potential or opportunistic pathogens when present in heavy growth.

**Pathogen**: The organisms that fall under this category have a well-recognized mechanism of pathogenicity in clinical literature and are considered significant regardless of the quantity that appears in the culture.

#### Bacteriology (Culture)

- Lactobacillus spp.
- Escherichia coli
- Bifidobacterium

#### Additional Bacteria

- alpha haemolytic Streptococcus
- Yersinia enterocolitica
- Proteus mirabilis

#### Mycology (Culture)

- Candida species
- Candida albicans
Address other test findings
Key Points

• Organisms don’t act in isolation – it’s not a one bug, one treatment approach.

• Therapeutic interventions, such as dietary macronutrient content, fiber, prebiotics, probiotics, lifestyle modification, and the environment have been shown to modulate the individual microbiome.

• **Microbial diversity** is a good thing, therefore food diversity is a good thing.
  – “Diet continues to be the most important determinant in shaping the composition, diversity and richness even throughout adulthood. In general, intake of diet rich in fruits, vegetables and fibers is associated with a higher richness and diversity of the gut microbiota.”
  – “A majority of the studies noted that protein consumption positively correlates with overall microbial diversity.”

References:
Key Points

• Our microbiome performs numerous functions to keep us healthy.

• Our microbiome is an extension of us – take good care of it, by taking good care of yourself!
Questions?

Christine Stubbe, ND, FABNO

US Client Services: 800-522-4762
UK Client Services: 020.8336.7750

We look forward to hearing from you!
Upcoming LIVE GDX Webinar Topics

December 18, 2019

Fatty Acids: Making Clinical Sense of Lab Reports
Deanna Minich, Ph.D.

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Understanding the Clinical Significance of the Commensal Bacteria

Christine Stubbe, ND FABNO
Genova Medical Education Specialist