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The human gut micro biome and future role of fecal microbiota transplants

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THE HUMAN GUT MICROBIOME AND THE FUTURE ROLE OF FECAL MICROBIOTA TRANSPLANTS

by

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ABSTRACT

With recent research being conducted in categorizing and analyzing the human microbiome, evidence has now linked the human microbiome to a range of diseases. Dysbiosis of the human gut microbiome exists in colon cancer, obesity, and *Clostridium difficile* infections. The use of fecal microbiota transplants has been proven effective in treating recurrent C.*difficile* infections by restoring gut microbiota. More needs to be done to establish fecal microbiota transplants procedures, effectiveness, and safety. Once established, fecal microbiota transplants may play a role in modulating other diseases linked to human gut microbiome dysbiosis.

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LIST OF ABBREVIATIONS

AMA.....American Medical Association

CDI ..... *Clostridium difficile* infection

CRC.....Colorectal Cancer

FMT ..... Fecal Microbiota Transplant

HMP ..... Human Microbiome Project

NIH .....National Institutes of Health

OTU..... Operational Taxonomic Unit

PRR..... Pattern Recognition Receptor

SCFA.....Short chain fatty acid

TLR.....Toll-like receptor

WGS..... Whole Genome Shotgun

INTRODUCTION

Around ten trillion microbial cells live inside a human being's gut (Mullard, 2008). This equates to ten times as many foreign cells than human cells (National Institute of Health, 2014). These microbial inhabitants, referred to as the human microbiome, harbor more than 100 times as many genes than the entirety of the human genome (Mullard, 2008). Back in 2005, an international conference was convened to discuss an effort to categorize the community of microbes in the human gut, as well as other epithelial linings, such as the skin, vagina, and oral mucosa (The NIH HMP Working Group et al., 2009). Shortly after, in 2008 the National Institute of Health (NIH) created the Common Fund Human Microbiome Project (HMP) with the mission of categorizing and analyzing the human microbiome in order to resolve the role it plays in human health and disease (National Institute of Health, 2014). HMP was designed to fund this cutting edge research. Similarly to the HMP, European MetaHIT consortium used the same techniques to investigate only the human gut microbiome (Arumugam et al., 2011).

The Human Microbiome Project has been planned out to conquer four different challenges of sampling and analyzing the HMB: analyzing 16S rRNA gene diversity in order to characterize the complex communities, using whole genome shotgun approach to analyze what genes are present and potentially active, isolating and mapping genomes in order to build a reference database,

and to then investigate the potential relationships between human microbiota and disease (Aagaard & Segars, 2014; The NIH HMP Working Group et al., 2009).

1.1 Analyzing 16S rRNA Genes

Recent advances in technology have allowed researchers to define and categorize bacterial species commonly found inhabiting in and on humans (Weng, Rubin, & Bristow, 2006). This commensal relationship is referred to as the human microbiome. Previous attempts at quantifying and analyzing the microbiome were foiled by the inability to culture all species that inhabited various locations of the human (Connon & Giovannoni, 2002; Pei et al., 2004; Verhelst et al., 2004) . In fact, culture based methods failed to identify up to 80% of organisms because of the organisms inability to grow in culture (Connon & Giovannoni, 2002; Hugenholtz, 2002; Pei et al., 2004; Verhelst et al., 2004; Weng et al., 2006). Because of this limitation, previous bacterial analyses have mostly investigated proverbial bacterial “weeds” that easily grow on culture medium (Hugenholtz, 2002).

The use of ribosomal RNA gene sequencing has allowed culture-independent identification of bacteria since these genes are sufficiently conserved across species, allowing PCR amplification, but variable enough to distinguish between different bacterial species (Weng et al., 2006). The most commonly used conserved sequence is the 16S rRNA gene (Hugenholtz, 2002; Mills, Entry, Voss, Gillevet, & Mathee, 2006; National Institute of Health, 2014).

By using PCR amplification or RNA cloning, one is able to bypass the need to culture a bacterial species before analysis as seen in Figure 1. The use of whole-cell fluorescence *in situ* can then be used to visualize and quantify the amounts of specific bacteria in a specific sample (Hugenholtz, 2002). In addition, recent developments have reduced the cost and sped up these processes so that analyzing the multitude of genes, which are in the order of millions, is no longer impossibly difficult (Mullard, 2008).

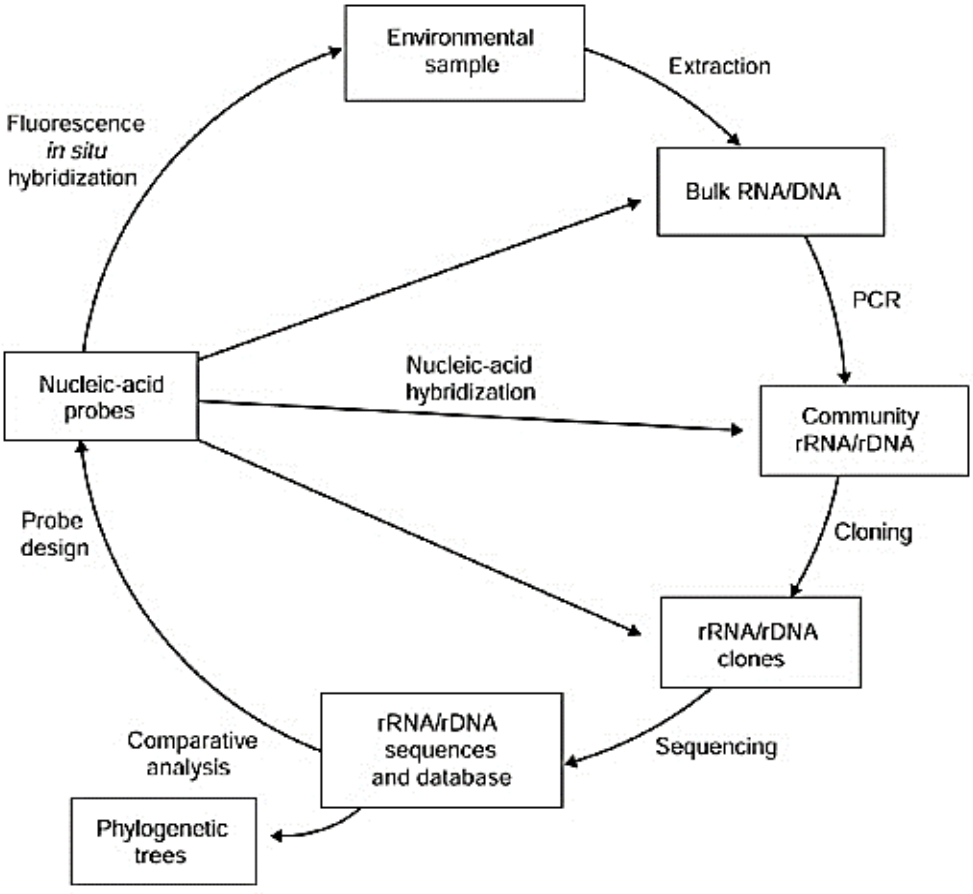

Figure 1. PCR and Cloning Eliminate Need for Culture

Nucleic acid probes are generated from existing databases of 16S rRNA genes. They are then applied to RNA/DNA samples extracted from environmental samples. Either PCR or Cloning is utilized to generate sufficient substrate for detection and sequencing of the sample (Hugenholtz, 2002)

Researchers have purified and analyzed over 3.5 terabases of genomic data to be able to quantify and categorize bacteria ("NIH Human Microbiome Project defines normal bacterial makeup of the body," 2013). In addition, by using 16S rRNA, researchers have been largely able to ignore human DNA sequences since the 16S rRNA sequences are primarily found in bacterial cells (The NIH HMP Working Group et al., 2009). Furthermore, new techniques for analyzing and obtaining this 16S rRNA data are ever improving the reliability and accuracy of their measurements (Schloss, Gevers, & Westcott, 2011)

1.2 Whole Genome Shotgun Approach

In addition to classifying specific bacterial populations through 16S rRNA, another focus is on identifying the various roles the bacterial genomes play in metabolite processing (Methe et al., 2012). While 16S rRNA can tell researchers what species of bacteria are present, they do little to identify the function of those bacterial species (Methe et al., 2012). Whole genome shotgun (WGS) techniques take the whole communities DNA into account (Methe et al., 2012; The NIH HMP Working Group et al., 2009). This approach is called "Metagenomics", which sequences genomic libraries directly from a mixed sample (The NIH HMP Working Group et al., 2009).

The human genome only carries around 22,000 protein-coding genes; however, the microbiome inhabiting humans contribute around 8 million unique protein coding genes ("NIH Human Microbiome Project defines normal bacterial

makeup of the body," 2013). That means that the microbes have the potential to contribute around 360 times more proteins towards the survival of the human host than the human host itself. Because of this, it is essential to investigate not only what species of microbe exist, but also what they do. For example, microbial genes in the human gastro-intestinal tract are responsible for digesting certain foods and nutrients for the human host that would otherwise be unavailable ("NIH Human Microbiome Project defines normal bacterial makeup of the body," 2013).

1.3 Reference Database

Through the use of a variety of methods, including sample dilution, micromanipulators, optical tweezers, filtration, centrifugation, and flow cytometry, hard to grow bacteria have been isolated and analyzed (Elsas, 1997). These methods have laid the groundwork in developing a database of genes used to classify and investigate the human microbiome.

In order to gain an insight into what a "normal" western microbiome constitutes, the HMP obtained samples from 242 volunteers from two distinct geographical locations in the United States, meeting an extensive list of inclusion and exclusion criteria defining "normal" from the United States of America; samples were taken from 15 body sites on both males and females (Figure 2), with an additional 3 sites for the female reproductive tract (The Human Microbiome Project Consortium, 2012). Gastrointestinal bacteria were analyzed using stool samples (The NIH HMP Working Group et al., 2009). Replicate

measures were taken 1 month to over a year after initial sampling. All samples were analyzed using 16s rRNA sampling, with a subset also analyzed using whole shotgun genome approaches.

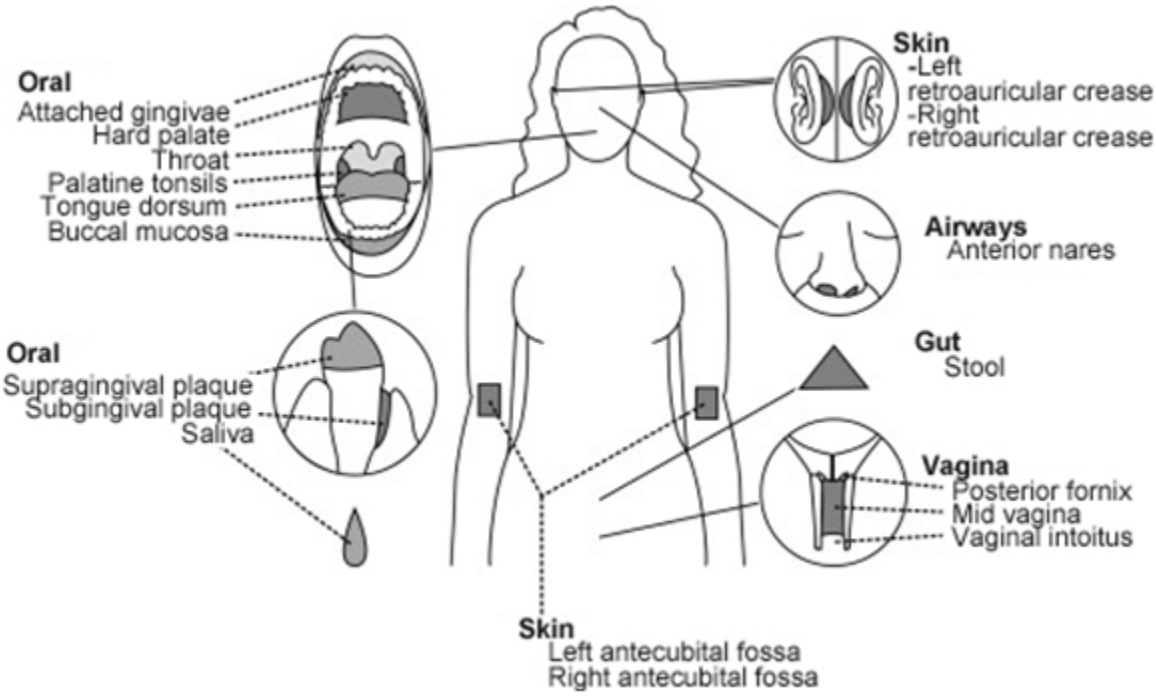

Figure 2. HMP Sample sites

Multiple sites were sampled and analyzed ("Human Microbiome Project DACC - Microbiome Analyses," 2014).

In addition, subjects were sampled up to two more times in the following months (The NIH HMP Working Group et al., 2009). Following the flow in Figure 3, the samples were analyzed and to increase the reference genome database (The NIH HMP Working Group et al., 2009). As of 2/1/2014, the human microbiome project (HMP) has 2673 reference genome projects in the works, spanning many human sample sites ("Human Microbiome Project DACC - HMP Project Catalog," 2014). Of these 2673 projects, there are over 1300 reference microbial genomes that have already been completed ("Human Microbiome

Project DACC - HMRGD," 2014). That's over 1.84 Terabytes of information that has been analyzed by the scientific community ("Human Microbiome Project (HMP) Metagenome Projects (ID 43017) - BioProject - NCBI," 2014). Reference genes can be obtained from both the National Center for Biotechnology Information's website NCBI.gov and the Human Microbiome Project's website HMPDacc.org.

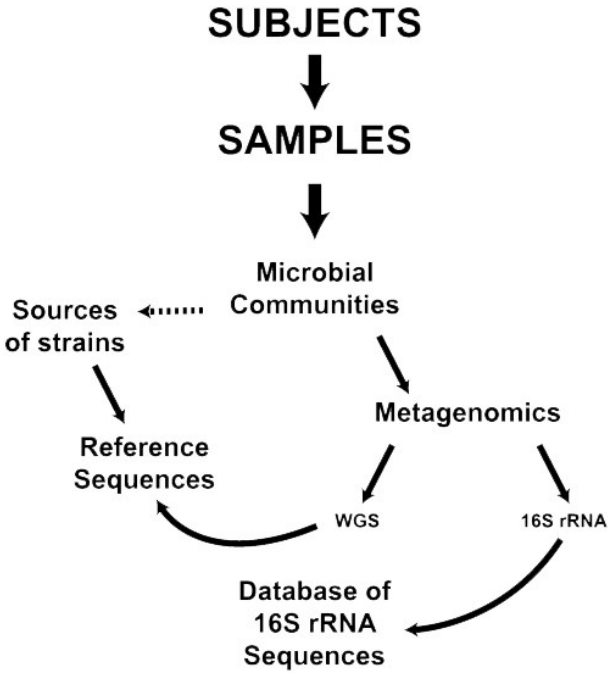

Figure 3. HMP Sample Analysis Pathway Flowchart

This flow chart represents the path the HMP has taken to analyze and sort subject samples into useful reference genomic material. Microbial communities are analyzed by both WGS and 16S rRNA methods to produce respective reference data. (The NIH HMP Working Group et al., 2009)

1.4 What Constitutes "Normal"

The work of many investigators and extensive analysis has laid the groundwork in establishing what is considered a "normal" microbiome (The Human Microbiome Project Consortium, 2012). These microbial communities

have been analyzed using reference genomic material obtained through the HMP (The Human Microbiome Project Consortium, 2012).

It was determined that the difference in microbial community makeup was highly variable when looking at a single subject across sampling sites, as in the bacterial composition varied vastly from skin to vaginal to oral to gastrointestinal sampling sites (The Human Microbiome Project Consortium, 2012). However, the difference between subjects when looking at the same sampling site was markedly less, with salvia having the highest diversity within a sample, but as a population shared a similar organism makeup (The Human Microbiome Project Consortium, 2012). Thus, an individual subject's saliva has a wide variety of organisms but is markedly similar to the saliva from another subject. The vagina had the lowest microbial diversity when looking at a single subject, as well as when comparing between subjects at the genus level; however, the bacterial composition between subjects had a very high diversity among operational taxonomic units (OTUs, approximately classify species, see http://hmpdacc.org/HMQCP) due to the presence of distinct lactobacillus species (The Human Microbiome Project Consortium, 2012). The diversity seen in microbial species could be roughly sorted into one of five groups: gastrointestinal, urogenital, nasal, oral, and skin samples (Figure 4). It has also been discovered that there is usually a predominant phyla in each of the sampling sites (Figure 5), and there is no universal microbe found across all body habitats (The Human Microbiome Project Consortium, 2012).

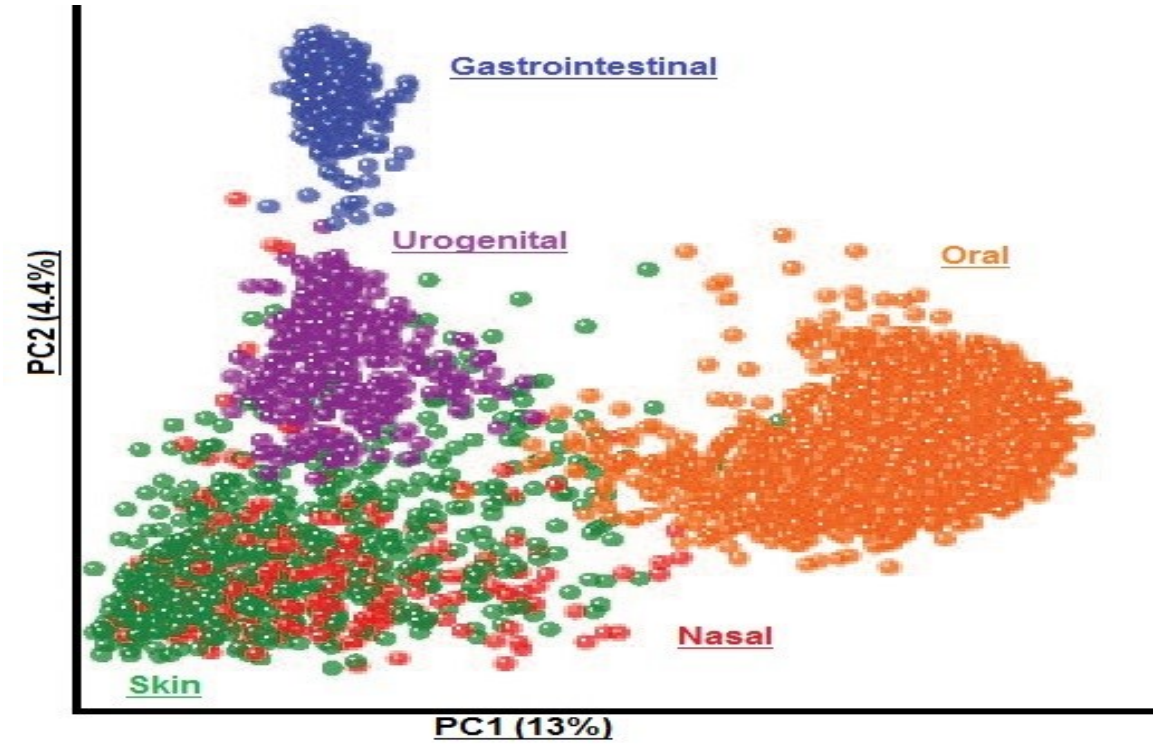

Figure 4. Diversity of Human Biome Roughly Grouped by Region

Principal coordinate plot showing the primary variation in the human microbiome is clustered by body regions. Nasal regions straddle skin and oral habitats. (The Human Microbiome Project Consortium, 2012)

Current distribution of genes surprised scientists in that the distribution of metabolic activities was more important than the distribution of microbial species ("NIH Human Microbiome Project defines normal bacterial makeup of the body," 2013). As shown in Figure 5, metabolic pathways are relatively stable across individuals when examining a single habitat. This is exemplified in stool where there is a high variability among phyla dominance with an inverse relationship between Bacteroidetes and Firmicutes (Figure 6) as the competing dominate phyla in stool, yet a relatively stable metabolic profile still emerged (The Human Microbiome Project Consortium, 2012).

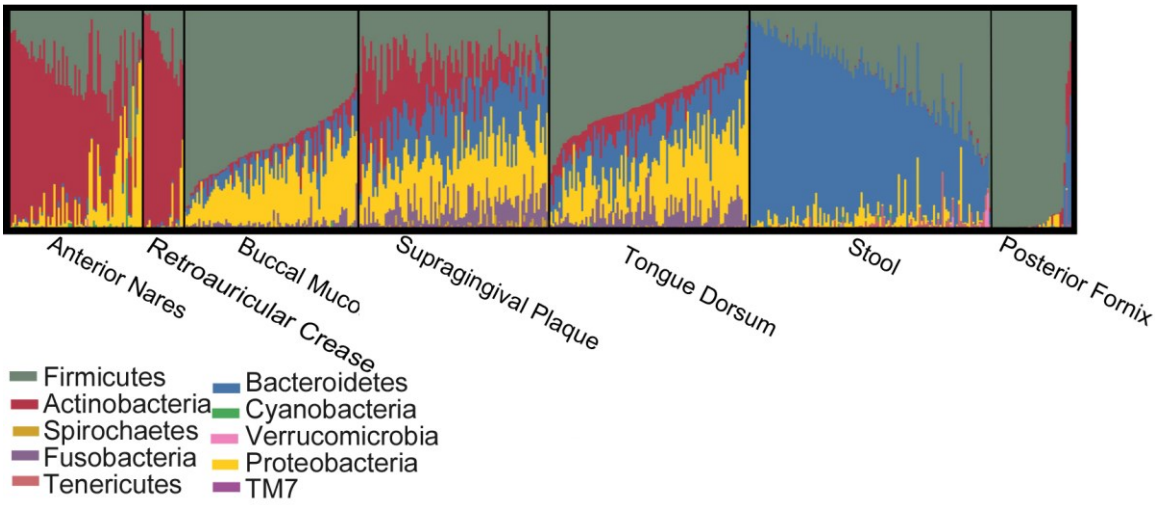

Figure 5. Phlya Distribution Varies Across Select Sampling Sites and Subjects

Relative average abundance of different phyla across subjects and their sample sites are shown by vertical bars. One phyla usually predominates the site, but this is not absolute (The Human Microbiome Project Consortium, 2012).

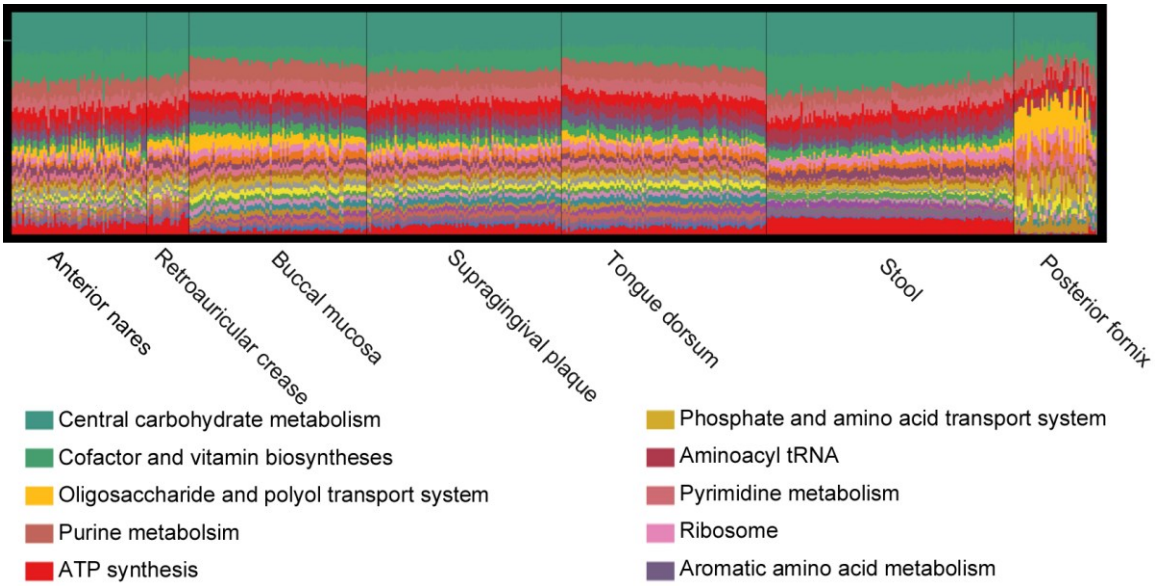

Figure 6. Metabolic Pathways Remain Consistent Across Subjects

Vertical bars represent varying average abundance of the most prevalent metabolic pathways across subjects and sampling sites. Metabolic pathways are much more stable across subjects than phyla (The Human Microbiome Project Consortium, 2012).

In addition, unlike the microbial taxa makeup, several metabolic pathways were omnipresent, with the most abundant being ribosome and translational machinery, nucleotide charging and ATP synthesis, and glycolysis, all of which are the fundamentals of host-associated microbe life (The Human Microbiome Project Consortium, 2012). Of note, up to 86% of the gut genes detected could not be prescribed a metabolic function (Li, Bihan, Yooseph, & Methe, 2012; The Human Microbiome Project Consortium, 2012). Low abundant taxa and metabolic pathways, while rare, are consistently found in more than 92% of samples, which suggest this is an important pool of genetic diversity in the human microbiome (Li et al., 2012; The Human Microbiome Project Consortium, 2012).

With repeated sampling taken from the same subjects over time, it was discovered that the variation of an individual's biome was consistently low, in both species classification and metabolic functions (The Human Microbiome Project Consortium, 2012). Microbial metabolism: remains relatively stable among individuals, can change to meet nutrient and metabolite availabilities, and vary depending on their environment (Abubucker et al., 2012; The Human Microbiome Project Consortium, 2012). There was also high variability dependent on subject ethnicity (The Human Microbiome Project Consortium, 2012).

It was noted that there was a particular absence of detrimental microbes, which supports that a healthy microbiome may be distinctly different than those in

disease states (The Human Microbiome Project Consortium, 2012). Data continues to suggest that carriage pattern is similar to that of genetic traits, in that high-risk detrimental pathogens (like genetic traits) are maintained in the population at miniscule to absent levels, whereas those that pose a moderate risk are present, but at a low rate (The Human Microbiome Project Consortium, 2012).

It was found that twins and their mothers had more similar microbiomes than when compared to strangers (Turnbaugh et al., 2009). This suggests that the microbiome may be partially inherited by vertical transmission from mother to child. Also Bacteroidetes were directly related to functional diversity (Turnbaugh et al., 2009). In fact, the microbiome commensally develops with the host, supporting the human body, and effecting the host's wellbeing by protecting the host from invasive pathogens as well as helping breakdown indigestible food for the host (Y.-T. Tsai, Cheng, & Pan, 2014)

1.5 Human microbiome implicated in health and disease

The HMP and the European metaHIT projects have increased our understanding and knowledge of what constitutes normalcy in the human microbiome. Now with these reference genes at the public's disposal, scientists are investigating how human health is affected by differing states of the human microbiome.

1.5.1 *Clostridium difficile* and the gut microbiome

The United States Centers for Disease Control and Prevention (CDC) has deemed *Clostridium difficile* infection (CDI) as one of the most significant hospital acquired infections (Dynamed, 2013). In fact, CDI causes such severe diarrhea that it can be associated with over 14,000 American deaths every year (Centers for Disease Control and Prevention, 2013). CDI is a prime example of gut bacterial dysbiosis leading to disease (Dynamed, 2013). *C. difficile* is an endospore forming anaerobic gram-positive large rod bacteria which produces two toxins that induce foul smelling watery diarrhea in patients (Dynamed, 2013). CDI is primarily a hospital-acquired disease in older patient populations in hospitals receiving antibiotic treatment (Dynamed, 2013). In about 20% of patients, CDI will resolve on its own when discontinuing the antibiotic that the patient was previously treated with (Centers for Disease Control and Prevention, 2013). On the other hand, in 12-20% of patients experiencing their first bout of a CDI will be plagued with recurrent CDI, and if this happens, 50-65% of those experiencing their second bout will continue to have recurrent episodes of CDI (Dynamed, 2013). Unfortunately, current antibiotic treatments are only around 30% effective at curing this type of recurrent CDI, and the more reoccurrences that happen, the less effective antibiotic treatments are (Dynamed, 2013).

The use of antibiotics can affect both the size and composition of the gut microbiome, with some bacterial genera gaining in proportion and others shrinking (Britton & Young, 2014). In contrast to patients who did not develop

CDI after antibiotic treatment, it has been shown that patients who acquire CDI after antibiotic use have decreased bacterial diversity, with susceptibility linked to a decrease in normal members of the gut microbiome with relative increases in others, mainly members of *Lachnospiraceae* and an increase in *Enterobacteriaceae* (Britton & Young, 2014). Furthermore, those that endured recurrent episodes of CDI have distinctly different microbiomes than those who did not (Rea et al., 2012). There seems to be a protective effect by *Lachnospiraceae* in mice, which limited the both the severity of symptoms and amount of colonization by *C. difficile* in susceptible mice (Reeves, Koenigsknecht, Bergin, & Young, 2012). In fact, 6 phylogenetically diverse bacterial species were identified that could restore colonization resistance and prevent chronic carriage of C. *difficile*, when administered together (Lawley et al., 2012). These studies show that the makeup of an individual's gut microbiome can heavily influence the susceptibility and outcome of a CDI. It has been shown that antibiotics affect the gut microbiome, allowing opportunistic infection by C. *difficile*, which can be modulated by various other microbiota (Britton & Young, 2014; Lawley et al., 2012; Reeves et al., 2012).

The exact mechanism by which C. *difficile* overwhelms the native microbiome is not entirely known (Dynamed, 2013). However, there have been mouse experiments to investigate these mechanisms (Ng et al., 2013; Stiemsma, Turvey, & Finlay, 2014). Ng *et al.* (2013) identified that the use of antibiotics reduced host gut bacteria allowing an increase in "free" host carbohydrates.

These free carbohydrates were then utilized by the pathogenic *C*. *difficile* bacteria to establish an infection. However, Ng *et al.* (2013) continued to show that *C*. *difficile* infections were also modulated by the composition of the underlying microbiota. Gnotobiotic mice with only *Bacteroides theatatiotaomicron*, which contain a sialidase used to free sialic acid from the gut mucosa, showed an increased colonization by *C*. *difficile,* which can utilize sialic acid as an energy source, relative to germ-free mice (Ng et al., 2013); therefore, other bacteria may provide beneficial growth conditions for *C.difficile.* Furthermore, Ng *et al.* (2013) also showed that mutant *C*. *difficile,* which lacked the genes to utilize sialic acid as a carbon source, resulted in a fourfold decrease in colony forming units per gram of feces. Antibiotic usage disrupts the gut microbiome, freeing previously consumed nutrients, such as sialic acid, allowing the opportunistic *C. difficile* to utilize this and proliferate into a pathological causing infection (Britton & Young, 2014; Ng et al., 2013; Stiemsma et al., 2014).

Theriot et al. (2014) also showed that the use of antibiotics in mice created susceptible states marked by higher concentrations of primary bile acids, taurocholate and other tauro-conjugate bile acids, a decrease in secondary bile acids such as, deoxycholate, and a marked increase in sugar alcohols, most notably mannitol and sorbitol. The increase in free carbohydrates also occurred simultaneously with a decrease in free short-,medium- and long-chain fatty acids (Theriot et al., 2014). All of these substrates increase *C*. *difficile* spore germination in *in vitro* growth studies (Theriot et al., 2014).

Colonization resistance to CDI depends on specific microbiome community structure and not the overall community size (Theriot et al., 2014). Interestingly enough, in experiments with hamsters, it was shown that being colonized by nonpathogenic *C. difficile* conveyed resistance when hamsters were subsequently introduced to a pathogenic strain of C. *difficile* (Merrigan, Sambol, Johnson, & Gerding, 2003; Sambol, Merrigan, Tang, Johnson, & Gerding, 2002). This parallels previous findings in humans, where earlier non-symptomatic *C. difficile* colonization reduced the chances of developing a CDI (Shim, Johnson, Samore, Bliss, & Gerding, 1998). CDI is clearly caused by bacterial dysbiosis. New treatments exploring how to treat and perhaps even prevent CDI would be a boon to the human population and those currently suffering from the uncomfortable and sometimes deadly symptoms of CDI.

1.5.2 Colon cancer and the gut microbiome

There are multiple types of cancer that are caused by infectious agents, and these malignancies are often in tissues that experience a high level of contact with microbes (Zhu, Gao, Wu, & Qin, 2013; zur Hausen, 2009). Well-known instances include cervical cancer and gastric cancer, often caused by human papillomaviruses and the bacteria *Helicobacter pylori*, respectively (Zhu et al., 2013; zur Hausen, 2009). It is interesting to note that there is an approximately 12 fold increased risk of cancer of the large intestine (colon), which has ~10^{12} cells/ml, compared to that of the small intestine, which has ~10^{2}

cells/ml (Proctor, 2011). Considering that there are previous examples of infectious agents causing cancer and that the colon is in contact with trillions of potentially infectious microbes, it is no surprise that in recent studies scientists are confirming that there is a strong relationship between the gut microbiome and colon cancer (Gold JS, Bayar S, & Salem RR, 2004; Moore & Moore, 1995; Nakamura et al., 2002; Peek & Blaser, 2002; Zhu et al., 2013).

Colon cancer is the third most common cancer, but second leading cause of mortality in men and women, with over 131,000 incidences and 57,000 deaths in 2010 (Centers for Disease Control and Prevention, 2014). Colorectal cancer (CRC) is cancer of the large intestine or rectum (Centers for Disease Control and Prevention, 2014). Sporadic colorectal cancer (that which cannot be explained by inherited genetic deficiencies) normally progresses from healthy gut mucosa to an adenoma (benign tumor of the epithelial tissue) and onto carcinoma (Al-Sohaily, Biankin, Leong, Kohonen-Corish, & Warusavitarne, 2012). Non-inherited colorectal cancer is dominated by the chromosomal instability pathway, which accounts for 65-70% of all random CRC (Al-Sohaily et al., 2012). This pathway is characterized by either entire chromosome loss or partial chromosome loss followed by progressive deleterious mutations and cellular damage, following a slow but progressive path from healthy mucosa to adenoma to carcinoma (Al-Sohaily et al., 2012). Due to the slow progression, if caught early, most patients will be cured of their cancer (Centers for Disease Control and Prevention, 2014).

In previous research, *Streptococcus bovis,* the genus *Bacteroides,* the class *Clostridia*, and *H. pylori* have been implicated in cancer pathogenesis (Gold JS et al., 2004; Moore & Moore, 1995; Nakamura et al., 2002; Peek & Blaser, 2002). On the other hand, some bacterial strains, including *Bifidobacterium longum* and *Lactobacillus acidophilus*, have been shown to inhibit carcinogen-induced colon tumor development (McIntosh, Royle, & Playne, 1999; Rowland, Bearne, Fischer, & Pool-Zobel, 1996). These results suggest that the balance between “good” and “bad” bacteria may affect the progression of cancer (Zhu et al., 2013).

High concentrations of bacteria were apparent in 90% of biopsy specimens from patients with colorectal cancer, with bacteria also apparent in 93% of samples collected from patients with colonic adenomas, which is in direct contrast to finding no bacteria in asymptomatic controls (Swidsinski et al., 1998). Furthermore, *Escherichia coli* strains that were adherent or invasive were present in the colonic mucosa of patients with colorectal carcinoma and adenoma, but not in normal colonic mucosa (Cuevas-Ramos et al., 2010). *S. bovis* has been linked to colonic neoplasia: this bacterial strain, as well as its bacterial cell wall antigens, have been shown to initiate the transformation of normal mucosal crypts into hyper-proliferative abnormal colonic crypts and also augment the expression of proliferation markers in rats treated with carcinogens (Ellmerich, Djouder, Schöller, & Klein, 2000). Taken together, these results suggest that bacteria may be directly responsible for some types of CRC.

Human colonic dysbiosis in patients with CRC was reported in high-resolution maps by multiple investigators (Castellarin et al., 2012; W. Chen, Liu, Ling, Tong, & Xiang, 2012; Kostic et al., 2012; Marchesi et al., 2011). These studies obtained samples from late stage CRC tumors and healthy tissue. Higher levels of *Fusobacterium spp*. were observed in cancerous lesions than matched healthy controls (Castellarin et al., 2012; W. Chen et al., 2012; Kostic et al., 2012). Histological analysis showed that *Fusobacterium* can invade tumor cells, which may explain why a positive correlation was found between Fusobacterium and metastases to lymph nodes (Castellarin et al., 2012). In addition to *Fusobacteriacium, Bacteroidaceae, Peptostreptococcaceae, Streptococcaceae, Veillonellaceae*, and *Pasteurellaceae* have been found to be enriched in cancerous tissue compared to the intestinal lumen, with *Luminococcaceae, Lachnospiraceae,* and *Lactobacillaceae* being relatively depleted in cancerous tissue compared to the intestinal lumen (W. Chen et al., 2012). When comparing bacteria attached to the mucosa of cancerous lesions to matched healthy controls, *Bifidobacterium, Blautia*, and *Faecalibacterium* were depleted in CRC individuals, whereas *Porphyromonas, Fusobacterium, Peptostreptococcus*, and *Mogibacterium* were increased (W. Chen et al., 2012). On the phylum level in patients with CRC, Proteobacteria had higher concentration while Bacteroidetes were lower when compared to matched controls (Shen et al., 2010). At the genus level CRC patients had increased *Dorea spp., Faecalibacterium spp.,* but had

fewer *Bacteroides spp.* than controls (Shen et al., 2010). These results continue to support that bacterial dysbiosis is associated with CRC development.

With these associations one may ask: What if there were no bacteria present? Would CRC still develop? In only 20% of germ-free rats did carcinogenic chemicals generate CRC, in contrast to 93% of germ-filled rats (Zhu et al., 2013). Multiple animal studies have continued to show that having microbiota present in the intestine presents a significantly larger CRC risk than having a sterile microbiome (Balish & Warner, 2002; Engle et al., 2002; Erdman et al., 2003; Gold JS et al., 2004; Kado et al., 2001; Marteau, Vrese, Cellier, & Schrezenmeir, 2001; Takaku et al., 1998; Zhu et al., 2013). These data continue to support that the gut microbiome plays a crucial part in the development of CRC.

There are multiple possible ways bacteria may influence the development of CRC. In addition to the enzymatic conversion of primary bile acids into secondary bile salts, increased carcinogen production associated with anaerobic bacteria may contribute to the progression of CRC (Owen, 1997). Chronic inflammation induced by bacteria, either through innate immune responses to the bacteria itself and its products may cause inflammatory responses that release pro-carcinogenic cytokines, pro-tumorigenic cytokines, or carcinogenic metabolites, or the enzymatic activity of the bacteria causing the creation of pro-carcinogenic byproducts that can increase the genesis of colorectal cancer, see Figure 7 (G. Y. Chen, Shaw, Redondo, & Núñez, 2008; Zhang & Ghosh, 2001;

Zhu et al., 2013). Therefore, the gut microbiome may induce CRC through causing chronic inflammation and creating DNA damaging metabolites and/or carcinogens (Hope, Hold, Kain, & El-Omar, 2005). It has also been suggested that there may be specific ‘super-bad’ bacteria that both activates certain immune pathways and creates carcinogenic byproducts such as hydrogen peroxide, a potent DNA damaging reactive oxygen species, which may contribute significantly to CRC development (Sears & Pardoll, 2011). Two bacterial enzymes, β-glucuronidase and 7α-dehydroxylase, may also contribute to CRC risk, as well as nitrate reductase activity (de Kok & van Maanen, 2000; Deschner, Cohen, & Raicht, 1981; Gill & Rowland, 2002; Gråsten et al., 2000; D.-H. Kim & Jin, 2001; Lee, Kim, Yim, & Joo, 2004; Reddy, Mangat, Weisburger, & Wynder, 1977).

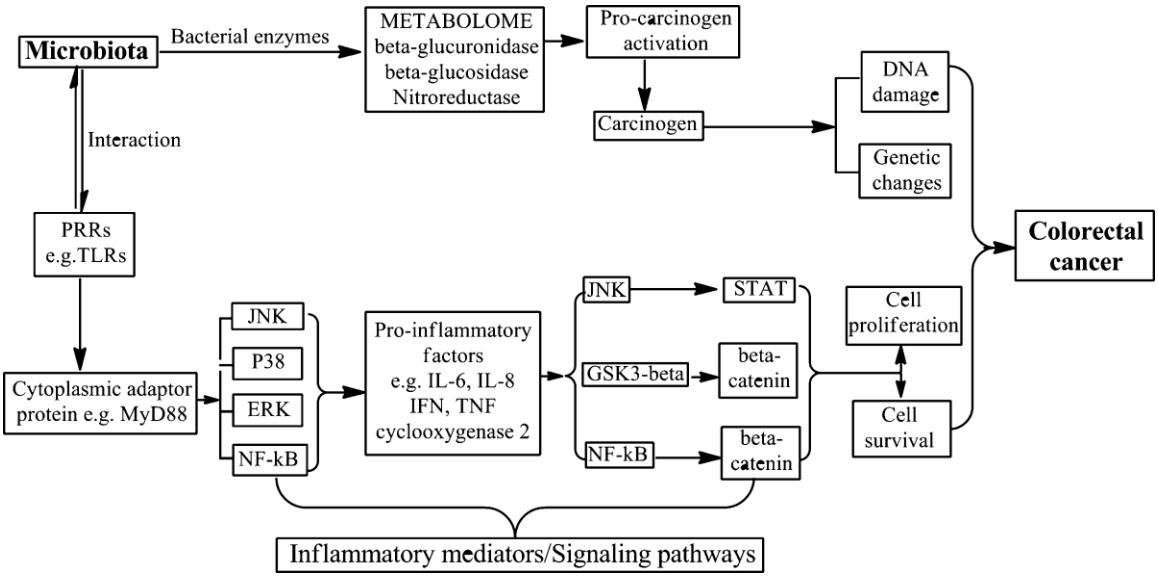

Figure 7. Proposed Methods That Microbiota May Cause Cancer

Microbes interacting with pattern recognition receptors (PRRs) and toll like receptors (TLRs), which are part of the innate immune system, signal pro-inflammatory signaling pathways that may increase the risk of developing CRC. The enzymatic activity of the microbiome may also create carcinogenic byproducts from pre-carcinogenic molecules (Zhu et al., 2013).

It is evident that the human gut microbiome influences the development of colorectal cancer. Colon cancer's significance warrants investigation into factors that may ameliorate its mortality or reduce the incidence. There have been a multitude of animal studies that have shown specific bacteria cause CRC. It has been shown that dysbiosis exists in patients with CRC. While it may be specific bacteria that influence CRC, or only the ratio or proportion that these bacteria are present in the gut, it is imperative that we investigate whether changing the microbiome can help the prognosis of CRC or prevent it all together.

1.5.3 Obesity and Microbiome

According to the World Health Organization, the rate of obesity has nearly doubled worldwide since 1980 (World Health Organization, 2013). Overweight adults, 20 years of age or older surpassed 1.4 billion in 2008, with 500 million of those individuals categorized as obese (World Health Organization, 2013). Sadly, over 40 million children younger than 6 were overweight in 2011 (World Health Organization, 2013). The United States leads the world, with two-thirds of its adult population being overweight, half of whom are considered obese (World Health Organization, 2013). This is an alarming trend that has an enormous impact on the public health of a nation (World Health Organization, 2013).

Obesity, now officially recognized as a disease by the American Medical Association (AMA), entails a host of comorbid diseases, including a fatty liver, hypertension, hyperlipidemia, insulin resistance and the buildup of surplus intra-abdominal adipose tissue, in conjunction with a chronic low-grade whole body inflammation, which is the main contributing factor to many types of chronic diseases (Poirier et al., 2006; Shoelson, Herrero, & Naaz, 2007). Being overweight or obese as an adult has significant comorbidity with a multitude of diseases, such as cardiovascular diseases, hypertension, diabetes, osteoarthritis and some types of cancer (endometrial, breast and colon) (Poirier et al., 2006; Shoelson et al., 2007; World Health Organization, 2013). In children, obesity is linked to difficulty breathing, vulnerability to fracture, hypertension, psychological stress, and continues into adulthood (Poirier et al., 2006; Shoelson et al., 2007).

Globally, being overweight or obese is the fifth leading cause of death, with an annual 2.8 million adult deaths (World Health Organization, 2013). Clearly obesity is a force to be reckoned with; many scientists are investigating why there has been such a recent explosion in obesity (Kverka & Tlaskalova-Hogenova, 2013; Riley, Raphael, & Faerstein, 2013; F. Tsai & Coyle, 2009; Żak-Gołąb, Olszanecka-Glinianowicz, Kocełak, & Chudek, 2014). Recently, focus has turned to the gut microbiome and its possible role in obesity (Frazier, DiBaise, & McClain, 2011; F. Tsai & Coyle, 2009).

Using mice, several researchers have demonstrated some possible effects of altering the intestinal microbiome on obesity outcomes (Bäckhed et al., 2004; Bäckhed, Manchester, Semenkovich, & Gordon, 2007; Turnbaugh et al., 2006; Turnbaugh, Bäckhed, Fulton, & Gordon, 2008). Compared to germ free mice, conventionally raised mice exhibited an increase of 40% in body fat content and 47% higher gonadal fat, despite eating less (Bäckhed et al., 2004).

To further demonstrate that this was due to the activity of the microbiome, the microbiota of the conventional mice were transplanted to the previously germ free mice (Bäckhed et al., 2004). The previously germ free mice then experienced an 57% increase in total body fat, a 61% increase in gonadal fat, and developed insulin resistance two weeks post-transplant (Bäckhed et al., 2004). All of this occurred with the previously germ free mice continuing to eat the same amount of food and continuing similar exercise (Bäckhed et al., 2004). The conventionalized mice showed increased monosaccharide uptake compared

to their germ free counterparts, as well as increased signaling to the liver to generate triglycerides (Bäckhed et al., 2004). This suggests that the transferred microbiota not only helps intestinal epithelial cells absorb nutrients, but can also change host metabolism (Bäckhed et al., 2004). Furthermore, these microbiota also suppressed the intestine from producing circulating lipoprotein lipase inhibitor, which increased deposition of triglycerides in adipocytes as well as heart muscle (Bäckhed et al., 2004). These experimental data help display that the microbiome can have profound impacts on the host metabolism and metabolic profile. In fact, it has been shown that germ free mice fed a diet that mimics much of the western world, i.e. high in fat and sugar, failed to develop obesity or insulin resistance (Bäckhed et al., 2004). This continues to provide evidence that the microbiome is crucial to the development of obesity.

Furthermore, using a leptin deficient mouse model for obesity, Turnbaugh et al. (2006) demonstrated similar results when transferring microbiota from the obese mice's ceca to germ free mice. Recipients of the obese mice's cecal contents gained 74% more body fat two weeks post transplantation than their counterparts who received lean mice cecal contents, despite eating less than pre-transplantation (Turnbaugh et al., 2006). Since the transplant recipients' cecal contents continued to mirror their donors post transplantation, this shows that obesity can be transferred through microbiota (Turnbaugh et al., 2006).

When investigating the actual makeup of the mice cecal contents, it was discovered in multiple studies that the phylum Firmicutes were enriched and

Bacteroidetes were concordantly reduced in obese mice when compared to lean mice (Ley et al., 2005; Turnbaugh et al., 2006). Furthermore, when investigating the effect of diet on mice, it was found that the respective increase/decrease in Firmicutes/Bacteroidetes was primarily due to the type of diet eaten, independent of their obese state (Hildebrandt et al., 2009). This helps explain that the disease state is not solely responsible for these changes in the microbiome, but rather the microbiome is independent of this disease state (Hildebrandt et al., 2009).

When investigating humans, the trend that obese people have enriched levels of Firmicutes with a corresponding drop in Bacteroidetes is more controversial, with multiple studies conflicting or failing to confirm this observation (Collado, Isolauri, Laitinen, & Salminen, 2008; Cotillard et al., 2013; Duncan et al., 2008; Kalliomäki, Collado, Salminen, & Isolauri, 2008; Le Chatelier et al., 2013; Ley, Turnbaugh, Klein, & Gordon, 2006; Santacruz et al., 2009; Schwiertz et al., 2010). However, the contradicting studies by Duncan et al. (2008), Kalliomäki et al. (2008), and Santacruz et al. (2009), had varying methodologies with only Schwiertz et al. (2010) making the direct pre-intervention comparisons between obese and lean individuals' microbiomes. Schwiertz et al. (2010) also summed *Bacteroidetes* and *Prevotella* genera to obtain their total for the phylum Bacteroidetes, potentially leaving out bacteria belonging to other genera that belong to the Bacteroidetes phylum; thus, conclusion that Bacteriodetes and Firmicutes are not related to obesity may not be entirely accurate (Million et al., 2012).

Despite the controversy over the role that the ratio of Firmicutes to Bacteroidetes plays in obesity, there have been more specific observations on which species of bacteria may be implicated (Million et al., 2012; Schwiertz et al., 2010). A decrease in *Methanobrevibacter* was associated with obesity (Le Chatelier et al., 2013; Million et al., 2012; Schwiertz et al., 2010). In addition *Bifidobacterium* was negatively correlated with BMI in obese subjects (Schwiertz et al., 2010). *Lactobacillus paracasei* and *Bifidobacterium animalis* was associated with leanness (Million et al., 2012). However, overall increased numbers of the Lactobacillus genus, notably *L. reuteri*, was associated with obesity (Million et al., 2012).

In contrast to trying to classify what bacteria exist in the gut microbiome of obese individuals, some researchers have investigated the metagenomics through WGS (Cotillard et al., 2013; Le Chatelier et al., 2013; Schwiertz et al., 2010; Turnbaugh et al., 2009). It was found that phosphotransferase systems, systems involved in microbe carbohydrate usage, were increased in obese individuals (Turnbaugh et al., 2009). Obese individuals also had increased Actinobacteria genes whereas increased Bacteroidetes genes were associated with lean individuals (Turnbaugh et al., 2009). While methods have varied there is clearly a difference in the microbiomes between those with obesity and not.

Some researchers have linked obesity and comorbid diseases with low microbial genetic diversity (Cotillard et al., 2013; Le Chatelier et al., 2013). Individuals with low microbial genetic diversity had increased gene density for

metabolic pathways suggesting increased capability to neutralize reactive oxygen species as well as increased capability to produce procarcinogens (Le Chatelier et al., 2013). In addition, there were noted decreases in butyrate producing bacteria, potentially increased mucus breakdown due to decreases in *Akkermansia* to *R.torque/gnavus* ratio, potential decreases in hydrogen and methane production with increases in hydrogen sulfide, increases in *Campylobacter* and *Shigella*, and increased ability to handle oxidative stress using peroxidase (Le Chatelier et al., 2013). These data suggests that low genetic diversity microbiomes are pro inflammatory (Le Chatelier et al., 2013). Indeed, obese individuals with low microbial gene diversity had higher BMIs, less insulin sensitivity, higher fasting serum triglycerides, and higher LDL cholesterol and inflammation than their high gene diversity microbiome counterparts (Cotillard et al., 2013). Pro inflammatory states may play a role in metabolic endotoxemia, which may be significant in determining future complications and a poor prognosis of obesity (Frazier et al., 2011). With yet another way to investigate the gut microbiome of humans it may be hard to decipher what is important and what is not, with future studies it may be helpful to do both 16S rRNA, WGS and gene diversity measurements so studies can be analyzed across the board.

Yet another investigation into obesity and the human gut microbiome revealed that irrespective of microbial makeup, it was really increases in short chain fatty acid concentration (SCFA) that predicted obesity in humans

(Schwiertz et al., 2010). Obese volunteers had 20% increases in SCFA concentration in stool samples than their lean counterparts, with the greatest increase in propionate (Schwiertz et al., 2010). SCFA's are a primary fuel for epithelial cells of the colon and can be used in other host metabolic processes for energy (Schwiertz et al., 2010). Stool SCFA concentration may play a role in controlling host metabolism and is the subject of future studies (Puertollano, Kolida, & Yaqoob, 2014). However, Schwiertz et al. (2010) did not take into account diet, exercise, and other lifestyle habits which may be correlated with SCFA production and obesity. So while SCFA concentration in stool correlates with obesity, causative effects have yet to be determined (Million et al., 2012).

Distal gut microbiota are responsible for utilizing calories from food that human enzymes alone cannot process, storing those calories into host fat for later use, as well as providing necessary nutrients for microbial self-preservation and growth (Frazier et al., 2011). The hypothesized mechanisms by which the microbiota may exert their influence on obesity include: inhibition of circulating lipoprotein lipase inhibitor, increased gut permeability leading to increased nutrient absorption, increased nutrient breakdown and concordant availability to the host, and host immune system interactions may all be potential mechanisms that influence the development and course of obesity (Bäckhed et al., 2007; Bashir, Louie, Shi, & Nagler-Anderson, 2004; Frazier et al., 2011; Ley et al., 2006; Million et al., 2012; Schwiertz et al., 2010).

Obesity is a worldwide problem (World Health Organization, 2013). The microbiome has been implicated in the genesis of obesity and potentially poses a good venue for therapeutic interventions (Fukuda & Ohno, 2014). With this in mind it is imperative that we explore possible therapeutic strategies while keeping patient safety in mind.

SPECIFIC AIMS

It is clearly evident that the human microbiome is strongly implicated in a variety of human diseases. While there have been multiple studies on ways to potentially alter the intestinal microbiome (Butel, 2013; Fallucca, Porrata, Fallucca, & Pianesi, 2014; K.-A. Kim, Gu, Lee, Joh, & Kim, 2012; Matuchansky, 2014; F. Tsai & Coyle, 2009; Xu et al., 2014), this study aims to address the possible use of fecal microbiota transplants to alter the human intestinal microbiome in order to alleviate or cure diseases, while also assessing cost, feasibility, and safety.

PUBLISHED STUDIES

2.0 What is a fecal microbiota transplant?

A fecal microbiota transplant (FMT) has also been known as fecal bacteriotherapy, fecal transplantation, transfaunation and human probiotic transplantation (Bakken et al., 2011). The concept behind FMT is that bacterial dysbiosis exists in the patient, usually due to some type of antibiotic insult, which leads to pathogenesis of certain disease states, notably *C. difficile* (Bakken et al., 2011). By taking microbiota from a healthy donor and transplanting it into the recipient, the healthy flora can replace the diseased dysbiotic state (Bakken et al., 2011). Recipient preparation usually includes an abbreviated course of antibiotics followed by a colonoscopy flush (Bakken et al., 2011). Donor stool is usually collected fresh, blended into a suspension with saline, milk, or water, and then infused into the patient through nasogastric/nasoduodenal tubes, colonoscope, or using a retention enema (Bakken, 2009).

2.1 Fecal microbiota transplants in *Clostridium difficile* infection (CDI)

Two recent publications, a systematic-review of FMT and an overview of FMT for practitioners, provide an introduction to the use of FMT to treat CDI (Brandt & Aroniadis, 2013; Cammarota, Ianiro, & Gasbarrini, 2014). These sources were combined with searches on PubMed and Web of Science to look for any publications regarding FMT treating CDI with the last search being 3/March/2014. Four new hits were added to Table 2 to describe the current

summary of research done on FMT and CDI. There were 41 studies that directly described the use of FMT to treat CDI, with 40 being case studies and only 1 being a randomized controlled trial (Bowden, Mansberger, & Lykins, 1981; Brace, Gloor, Ropeleski, Allen-Vercoe, & Petrof, 2014; Brandt et al., 2012; Collins, 1960; Eiseman, Silen, Bascom, & Kauvar, 1958; Fenton, Stephenson, & Weder, 1974; Fløtterød & Hopen, 1991; Friedman-Moraco, Mehta, Lyon, & Kraft, 2014; Gallegos-Orozco, Paskvan-Gawryletz, Gurudu, & Orenstein, 2012; Garborg, Waagsbø, Stallemo, Matre, & Sundøy, 2010; Gustafsson, Berstad, Lund-Tønnesen, Midtvedt, & Norin, 1999; Hamilton, Weingarden, Sadowsky, & Khoruts, 2012; Härkönen, 1996; Hellemans, Naegels, & Holvoet, 2009; Jorup-Rönström et al., 2012; S. A. Kahn, Young, & Rubin, 2012; Kassam, Hundal, Marshall, & Lee, 2012; Khoruts, Dicksved, Jansson, & Sadowsky, 2010; Kleger et al., 2013; Lund-Tønnesen, Berstad, Schreiner, & Midtvedt, 1998; MacConnachie, Fox, Kennedy, & Seaton, 2009; Mattila et al., 2012; Nagy, Várvölgyi, & Paragh, 2012; Neemann et al., 2012; Nieuwdorp et al., 2008; Paterson, DL, 1994; Persky & Brandt, 2000; Polák et al., 2011; Quera, Espinoza, Estay, & Rivera, 2014; Rohlke, Surawicz, & Stollman, 2010; Rubin, Gessert, Aas, & Bakken, 2013; Russell, Kaplan, Ferraro, & Michelow, 2010; Schwan, Sjölin, Trottestam, & Aronsson, 1983; Shahinas et al., 2012; Silverman, Davis, & Pillai, 2010; Trubiano et al., 2013; Tvede & Rask-Madsen, 1989; van Nood et al., 2013; Yoon & Brandt, 2010; You, Franzos, & Holman, 2008).

2.2 Analyzing the fecal microbiota transplant studies

These studies were analyzed for numbers of patients with CDI treated with FMT, the average age and range of patients, whether or not the patients were experiencing recurrent bouts of CDI or if this was their first episode, the method used to deliver the FMT, the number of patients whose diarrhea resolved post FMT, the number that tested negative for *C. Difficile* toxin post FMT, whether or not the patients had received previous therapy (usually oral antibiotics) prior to FMT, and whether or not any adverse events were reported. These data were put into Table 1.

The average patient age was 64 years old, with an age range of 1 to 94 years old. There were studies that involved children (S. A. Kahn et al., 2012; Russell et al., 2010). The 41 studies spanned the globe but reflected an emphasis on Western countries, with the most number coming from the USA at 15 (Table 1). A total of 569 patients have been treated with FMT in the selected literature.

2.3 Methods of FMT used

The method of infusing the FMT was varied between studies (Brandt & Aroniadis, 2013; Cammarota et al., 2014). 17 studies utilized colonoscopies, 13 used enemas, 11 did some variety of naso-gastric/duodenal or endoscopy procedure, with only 2 using gastrostomy lines (Table 1). Colonoscopy was preferred by some for the ability to visualize the entire colon for other diseases,

as well as being able to access the entire colon for transferring the FMT (Cammarota et al., 2014). However, distal enema and nasogastric (NG) tubes were considered cheaper and less invasive than colonoscopy procedures (Bakken, 2009; Brandt & Aroniadis, 2013; Kassam et al., 2012). There has been no study that has compared the efficacy of using different routes of inoculation.

Hamilton et al. (2012) were the only researchers who used frozen donor samples, with every other study using fresh stool samples. Polyethylene glycol bowel preparation was used in a minimum of three studies, including the randomized controlled study (van Nood et al., 2013). Observationally, there was a strong trend where patients would stop antibiotic treatment in preparation for their FMT (Bakken, 2009; Brandt & Aroniadis, 2013; Cammarota et al., 2014).

There was no consensus amount of donor sample and infusion volume used between the studies, but Bakken (2009) does suggest quantities at 25-30 g of stool for upper gastrointestinal tract infusions and 200-300g for lower gastrointestinal infusions.

Table 1: Results from reviewing data on FMT related studies.
Nr is not reported, diarrhea resolved is the number of people cured by FMTs. Appended from (Cammarota et al., 2014).

Investigator	Location of Study	Patients Treated with FMT (n)	Mean Age	Age Range	Patients CDI history	FMT Delivery method	Diarrhea resolved (n)	Average time to follow up, post FMT	Did Patients receive prior therapies	Serious Adverse Events
Aas et al. (2003)	USA	18	81	58-88	Recurrent	Nasogastric tube	15	3 mo	Yes	No
Bowden et al. (1981)	USA	16	56	NR	Recurrent	Enema	13	up to 5 yr	nr	No
Brace et al. (2014)	Canada	1	87		Recurrent	Colonoscopy	1	5 d	Yes	No
Brandt et al. (2012)	USA	77	65	22-87	Recurrent	Colonoscopy	72	17 mo	Yes	Possible
Collins et al. (1960)	USA	12	NR	NR	Recurrent	Enema	10	nr	nr	No
Eiseman et al. (1958)	USA	4	56	45-68	Recurrent	Enema	4	10 d	nr	No
Fenton et al. (1974)	Canada	1	56		Recurrent	Enema	1	nr	No	No
Flotterod and Hopen (1991)	Norway	1	64		Recurrent	Nasoduodenal tube or Upper endoscopy	1	3 d	nr	No
Gallegos-Orozco et al. (2012)	USA	1	71		First episode	Colonoscopy	1	1 mo	Yes	No
Garborg et al. (2010)	Norway	40	75	53-94	Recurrent	Upper endoscopy or Colonoscopy	31	80 d	Yes	No
Gustafsson et al. (1999)	Norway	9	53	29-83	Recurrent	Enema	9	18 mo	nr	No
Hamilton et al. (2012)	USA	29	65	62-68	Recurrent	Colonoscopy	25	2 mo	Yes	No
Harkonen (1996)	Finland	1	71		Recurrent	Colonoscopy	1	8 mo	Yes	No
Hellemans et al. (2009)	Belgium	1	59		Recurrent	Colonoscopy	1	4 mo	nr	No

Table 1 (continued): Results from reviewing data on FMT related studies. Appended from (Cammarota et al., 2014).

Investigator	Location of Study	Patients Treated with FMT (n)	Mean Age	Age Range	Patients CDI history	FMT Delivery method	Diarrhea resolved (n)	Average time to follow up, post FMT	Did Patients receive prior therapies	Serious Adverse Events
Quera et al. (2014)	Chile	1	61		Recurrent	nr	1	5 mo	Yes	Bacteremia
Rohlke et al. (2010)	USA	19	49	29-81	Recurrent	Colonoscopy	19	27.2 mo	Yes	No
Rubin et al. (2013)	USA	74	63	Jun-94	Recurrent	Nasogastric/ percutaneous gastrostomy/ upper endoscopy	59	12 wk	Yes	No
Russell et al. (2010)	USA	1	2		Recurrent	Nasogastric tube	1	6 mo	Yes	No
Schwan et al. (1983)	Sweden	1	67		Recurrent	Enema	1	9 mo	Yes	No
Shahinas et al. (2012)	Canada	4	70	56-84	Recurrent	Enema	3	NR	Yes	No
Silverman et al. (2012)	Canada	7	65	30-88	Recurrent	Enema	7	14 mo	Yes	No
Trubiano et al. (2013)	Australia	1	75		Recurrent	Upper endoscopy	1	1 mo	Yes	No
Tvede and Rask-Madsen (1989)	Denmark	2	59	59-60	Recurrent	Enema	1	6 mo	Yes	No
van Nood et al. (2013)	Nether-lands	34	70	52-85	Recurrent	Nasoduodenal tube	30	2.5 mo	Yes	No
Yoon and Brandt (2010)	USA	12	66	30-86	Recurrent	Colonoscopy	12	3wks to 8 yr	Yes	No
You et al. (2008)	USA	1	69		First episode	Enema	1	NR	Yes	No

Table 1 (continued): Results from reviewing data on FMT related studies. Appended from (Cammarota et al., 2014).

Investigator	Location of Study	Patients Treated with FMT (n)	Mean Age	Age Range	Patients CDI history	FMT Delivery method	Diarrhea resolved (n)	Average Followup post FMT	Did Patients receive prior therapies	Serious Adverse Events
Jorup-Rönström et al. (2006)	Sweden	5	83	79-88	Recurrent	Enema	5	12 mo	Yes	No
Jorup-Rönström et al. (2012)	Sweden	32	75	27-94	Recurrent	Enema / Colonoscopy	22	26 mo	Yes	No
Kahn, S.A. et al. (2012)	USA	1	1	5-Jan	Recurrent	Colonoscopy	1	20 mo	Yes	No
Kassam et al. (2012)	Canada	27	69	26-87	Recurrent	Enema	25	14 mo	Yes	No
Khoruts et al. (2010)	USA	1	61		Recurrent	Colonoscopy	1	6 mo	Yes	No
Lund Tonnesen et al. (1998)	Norway	18	64	nr	Recurrent	Colonoscopy / Gastrostomy	15	3 d	nr	No
MacConnachie et al. (2009)	UK	15	81	68-95	Recurrent	Nasogastric tube	12	4 mo	Yes	No
Mattila et al. (2012)	Finland	70	73	22-90	Recurrent	Nasoduodenal tube	66	12 mo	Yes	No
Nagy et al. (2012)	Hungary	1	59		Recurrent	Colonoscopy	1	5 mo	Yes	No
Neemann et al. (2012)	USA	1	21		First episode	Nasojejunal tube	1	2 mo	Yes	No
Nieuwdorp et al. (2008)	Nether-lands	7	67	nr	Recurrent	Colonoscopy	7	6 mo	Yes	No
Paterson (1994)	Australia	7	56	nr	Recurrent	Colonoscopy	7	24 mo	Yes	No
Persky and Brandt (2000)	USA	1	60		Recurrent	Colonoscopy	1	60 mo	nr	No
Polak et al. (2011)	Czech Republic	15	82		Recurrent	Nasojejunal tube	13	3 mo	Yes	No

2.4 FMT safety

Most studies described their procedures for screening donors, often testing for communicable diseases such as human immunodeficiency virus, Hepatitis A, B, C, sexually transmitted diseases, as well as common stool parasites such as *Cryptosporidium* and *Giardia* (Brandt & Aroniadis, 2013; Cammarota et al., 2014). In addition, donors usually were required not to have any current known digestive problems or have taken antibiotics in previous months (Brandt & Aroniadis, 2013; Cammarota et al., 2014). With such interest in FMT, a guide has been published on how FMT's should be conducted, including minimum safety considerations that should be in place (Bakken, 2009).

Common side effects reported were diarrhea, cramping, fatigue and belching (Brandt et al., 2012; van Nood et al., 2013). These symptoms often went away quickly, from a couple of hours to days (Brandt et al., 2012; van Nood et al., 2013). These symptoms were well tolerated (Brandt et al., 2012; van Nood et al., 2013).

There has only been one serious adverse reaction to an FMT in published papers (Quera et al., 2014). A 61 year old patient with recurrent severe CDI, Crohn's disease, and acute diverticulitis was evaluated for FMT after hospital admission due to his CDI relapse (Quera et al., 2014). After safety screening and following pre FMT protocol, the patient received a FMT, but within 24 hours developed a high grade fever and bacteremia (Quera et al., 2014). The patient

was treated with a course of antibiotics that cured his bacteremia, and 6 months post FMT he is *C. difficile* free when measured by PCR (Quera et al., 2014).

In the only published long term FMT follow up study, there were four cases of patients reporting new adverse medical conditions after receiving a FMT (Brandt et al., 2012). These new conditions were unexplained and included peripheral neuropathy, Sjogren's disease, idiopathic thrombocytopenic purpura, and rheumatoid arthritis (Brandt et al., 2012). In addition two patients reported improvement in their allergic sinusitis and arthritis post FMT (Brandt et al., 2012).

2.5 Does fecal microbiota transplantation work in patients with CDI?

Almost all patients that underwent FMT were those who suffered from recurrent bouts of hard to treat CDI (Table 1). These patients are the hardest to treat with conventional therapeutic strategies, as each bout of CDI decreases the chances that a course of antibiotics will work (Dynamed, 2013). Antibiotic effectiveness drops to a 30% success rate in patients with recurrent CDI (Brandt & Aroniadis, 2013; Cammarota et al., 2014; Dynamed, 2013). However, the use of FMT has an average success rate of around 88% (Table 2).

Table 2: FMT success rates per infusion method used.
Resolution of CDI was categorized by absence of diarrhea post FMT.

Infusion Method	**Number of Treated Patients**	**CDI Resolved**	**Percent success**
Colonoscopy	249	218	88%
Naso-gastric/duodenal/jejunal Tube	230	199	87%
Enema	89	80	90%
All Methods	**569**	**498**	**88%**

Observationally, there seems to be no difference among success rates between the intervention groups. The only randomized controlled trial comparing the efficacy of FMT to vancomycin was stopped short at its interim analysis because the FMT was so effective it would have been unethical to continue treating patients with vancomycin (van Nood et al., 2013). FMT was seen as having a 94% overall cure rate compared to 31% for vancomycin (van Nood et al., 2013) In the long term follow up study, Brandt et al. (2012) reported that 97% patients were willing to undergo FMT again if they experienced another bout of CDI. Furthermore, 53% stated they would want FMT as a first line treatment instead of antibiotics (Brandt et al., 2012). This suggests that not only is FMT effective, but also very well tolerated among patients.

2.6 FMT in patients with ulcerative colitis

While CDI is the basis for the bulk of FMT studies, there have been investigations into whether FMT can affect the outcome of ulcerative colitis, an inflammatory bowel disease (Borody, Warren, Leis, Surace, & Ashman, 2003). Six subjects with ulcerative colitis aged 25-53, who had suffered from ulcerative colitis less than 5 years with consistent recurrent symptoms, underwent FMT (Borody et al., 2003). Donors were healthy adults screened for most common pathogens and communicable diseases (Borody et al., 2003). Polyethylene glycol and oral antibiotics were used to prepare subjects before undergoing FMT by retention enema (Borody et al., 2003). The process was repeated for 5 days

straight, but by 1 week post FMT some patients were experiencing fewer symptoms of their ulcerative colitis (Borody et al., 2003). Four months post FMT, all six patients were free from all ulcerative colitis symptoms (Borody et al., 2003). During follow up 1-13 years after FMT there is no evidence of the treated patients having ulcerative colitis (Borody et al., 2003).

2.7 FMT in patients with metabolic syndrome

Metabolic syndrome is a disease of discordant energy utilization and storage ("Metabolic syndrome," 2014). Diagnosis criteria requires both central obesity (waist circumference greater than 94 cm for European men) and at least two of the following: high serum triglycerides, low levels of high density lipoprotein cholesterol, high blood pressure, and high blood glucose levels (R. Kahn, Buse, Ferrannini, & Stern, 2005).

In a randomized controlled study, nine men with metabolic syndrome were selected to receive FMT from either lean donors or autologous donations (Vrieze et al., 2012). Subjects were blinded to which sample they were receiving (Vrieze et al., 2012). Insulin sensitivity was measured pre and 6 weeks post FMT using a "hyperinsulinemic euglycemic clamp", the gold standard when measuring insulin sensitivity (Vrieze et al., 2012). Additionally, fecal samples and duodenal samples were taken pre and post FMT to reflect possible changes in the microbiota located in large intestine and small intestine locations (Vrieze et al., 2012).

Six weeks post FMT, there was a statistically significant improvement in insulin sensitivity in lean donor recipients when compared to the autologous recipients (Vrieze et al., 2012). There was no difference in resting energy expenditure, diet composition, or hormonal profile to explain this cause (Vrieze et al., 2012). When analyzing the diversity of the microbiota of participants post FMT, it was discovered that the gut microbial diversity increased drastically after receiving lean donor sample; however, there was no statistically difference in autologous sample recipients (Vrieze et al., 2012).

DISCUSSION

It is clear that fecal microbiota transplants are extremely effective, relatively cheap to do, and well tolerated by patients. After getting over the hurdles of having a donor screened and then provide the patient with a fresh sample, all a practitioner needs is some saline, a nasogastric tube with syringe, a strainer and a dedicated blender (Bakken, 2009). While it may be a little uncomfortable for the patient undergoing the colonoscopy cleanse before FMT, the resulting procedure is quick and the effects are almost immediate (Anderson, Edney, & Whelan, 2012; Bakken, 2009; Brandt & Aroniadis, 2013; Neemann et al., 2012). When comparing the alternative of having recurrent bouts of horrible diarrhea that may land a patient in the hospital versus one thirty minute procedure, it is clear that FMT should be at the forefront of treating CDI, and not a last resort.

However, caution must be exercised in interpreting the available data. To date, there has yet to be one well designed experiment that investigates what types of FMT are better others. There have been no systematic evaluations on whether colonoscopy, naso-gastro/duodenal/jejunal tubes, or retention enemas are any better than the other. In addition, there is no research on exactly how much fecal bacteria need to be transplanted to be effective, or whether a pre FMT colon cleanse is necessary, or beneficial. Furthermore, there was only one study that investigated the use of frozen donor samples in FMT, and it showed to be efficacious (Hamilton et al., 2012). It would be helpful for practitioners and donors alike, if screened healthy samples could be frozen and prepared ahead of time, so patients were not at the mercy of a donor's digestive timetable. If preparing samples ahead of time is feasible, then effort should be made to find a single donor or a set of specific donors to potentially help control the variability in donor samples to allow conclusions on the effectiveness of specific donor FMTs.

Furthermore, with the microbiome's recent implication in a host of possible diseases such as diabetes, obesity, colon cancer, rheumatoid arthritis and autoimmunity, ulcerative colitis, metabolic syndrome, multiple sclerosis, fibromyalgia, and allergies, it should be imperative that practitioners do not rush into treating one disease while causing another (Abrahamsson et al., 2012, 2013; Brace et al., 2014; Butel, 2013; Fukuda & Ohno, 2014; Kverka & Tlaskalova-Hogenova, 2013; Vrieze et al., 2012). It is extremely important that science and practice stay mindful of these possible connections and be extra vigilant in the

screening process of donors. One only needs to remember the large amounts of hemophiliacs infected with Human Immunodeficiency Virus during blood transfusions in the late 1970's simply because the medical community did not know any better ("Contaminated haemophilia blood products," 2014). It should also be of note that many of the diseases that are possibly linked to the microbiome are insidious in nature, with no clear cause or methodology (Brandt & Aroniadis, 2013; Hornig, 2013). In essence, while FMTs have few to no recorded adverse events in published literature to date, this does not mean there won't be repercussions down the road (Brandt & Aroniadis, 2013; Cammarota et al., 2014; Quera et al., 2014). As the previous United States Secretary of Defense, Donald Rumsfeld, once said "… as we know, there are known knowns; there are things we know that we know. There are known unknowns; that is to say, there are things that we now know we don't know. But there are also unknown unknowns – there are things we do not know we don't know" ("There are known knowns," 2014).

This warning is also aimed towards antibiotics. Now that science knows the microbiome is not static and can change with differing insults, it is imperative that practitioners limit our use of antibiotics to true medical emergencies (Huse, Ye, Zhou, & Fodor, 2012; Shahinas et al., 2012; Theriot et al., 2014). Since bacteria are clearly big players in the host's wellbeing, antibiotic genocide of its commensal organisms should not be taken lightly. Similar to the need of

investigating FMT further, studies involving antibiotics should look at the long term effects of Western cultures exuberant use of them (“Antimicrobial,” 2014).

However, this is not to say that science should stop because it’s scared. With the multitude of disease states that have been associated with the human digestive microbiome, such as ulcerative colitis, irritable bowel disease and syndrome, *C. difficile* infections, Crohns disease, and esophageal cancer as well as the possible interplay with the immune system, which implicates the microbiome in rheumatoid arthritis, allergies, Parkinson’s disease, multiple sclerosis and chronic inflammation, we now have a new therapeutic approach, FMT, that is proven to change the microbiota of a recipient (Abrahamsson et al., 2013; Borody et al., 2003; Brandt & Aroniadis, 2013; Critchfield, van Hemert, Ash, Mulder, & Ashwood, 2011; Crouzet et al., 2013; Kverka & Tlaskalova-Hogenova, 2013; Shahinas et al., 2012). We now have more knowledge than we ever did, thanks to the scientists working on the Human Microbiome Project and metaHIT

CONCLUSION

The human microbiome is an exciting new frontier that has the potential to open a completely new avenue of treatment and therapeutic targets for treating a wide range of potentially related diseases. The strides scientists have made in mapping out the genomes of the microbiota has been significant. With this new data, a range of disease states are being implicated with human microbiota dysbiosis. Fecal microbiota transplants are an easy, cheap, safe, and powerful

tool in altering a recipient's microbiome. While research needs to be done to standardize and solidify FMT's and prove its long term safety in treating CDI, the ability to use FMT to alter both CDI and other disease courses is an exciting possibility.

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