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The effect of adding radiotherapy to the administrated chemotherapy on infants' gut microbiome

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The Effect of Adding Radiotherapy to The Administrated Chemotherapy on Infants Gut Microbiome

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Abstract

The American University in Cairo

The Effect of Adding Radiotherapy to The Administrated Chemotherapy on Infants Gut Microbiome

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The gut microbiota has been described as the forgotten organ owing to its important roles in the human body, that includes but not limited to: digestion, immunity, homeostasis and response to some drugs such as, chemotherapy and immunotherapy. Its role has been also described in reflection to radiotherapy and associated gastrointestinal injuries, where dysbiosis and its associated side effects could be the driving force for dose determination or the complete suspension of the treatment plan. Linking the gut microbiota alterations to different cancer treatment protocols is not easy, especially in humans. However, enormous effort was exerted to understand this complex relationship. In the current study, we described the gut microbiota dysbiosis in infant sarcoma patients with regards to radiotherapy and antibiotics. Fecal samples were collected as a source of microbial DNA for which the gene encoding for 16S rRNA was sequenced, targeting V3-V5 regions. Two of the three patients understudy had experienced an increase in alpha diversity post treatment. Although phylum Firmicutes overall relative abundance was generally decreasing, six of its taxa increased in all patients. Our results indicate the possibility of radiosensitivity for the elevated taxa. Further studies are needed to describe the extent of radiosensitivity with regards to antibiotic resistance.

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List of Abbreviations

BF	Burkina Faso
BMT	Bone Marrow Transplantation
Bp	Base Pair
CAZymes	Carbohydrate active enzymes
CFU	Colony Forming Unit
CRC	Colorectal Cancer
CTX	Cyclophosphamide
DCA	Deoxycholic acid
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic acid
EAC	Esophageal Adenocarcinoma
FMT	Fecal microbiome transplantation
GF	Germ-Free
GI	Gastrointestinal
Gy	Gray
HCC	Hepatocellular Carcinoma
HMP	Human Microbiome project
HT	5-hydroxytryptamine
IBD	Inflammatory Bowel Disease
ICI	Immune checkpoint Inhibitors
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
PD-1	Programmed cell death protein-1
Pks	Polyketide-peptides
SASP	Senescence associated secretory phenotype
TBI	Total Body Irradiation
TLR	Toll-Like Receptor
WHO	World Health Organization

Chapter 1: Literature Review

The terms Microbiome and Metagenome are rapidly evolving and grasping more attention nowadays. Metagenome refers to the study of samples derived from an environment. While Microbiome refers to collection of microorganisms living in a specific environment (Stern et al., 2012). The human body includes different environments where the microbes can reside namely: skin, blood, liver, genital organs and the richest of all is the gut. Each of which has its own microbiome, i.e. collection of microorganisms that live and work in association. In 2008, the United States launched a five-years project to explore the microbiome of the human body, named "Human Microbiome Project- HMP". The project offered a catalogue that included a huge diversity of microorganisms living in association with the human body in different body parts such as: skin, vagina, nose, oral cavity, gastrointestinal tract, blood and others. Each of which has its own microbiome.

The gut is considered as the most diverse environment for a microbial collection. The gut alone is colonized by 10 to 100 trillion (10^{13}-10^{14}) bacteria; besides, other microorganisms like viruses (5.8%), archaea (0.8%) and eukaryotic microbes (0.5%) (Arumugam et al., 2011; Aziz, 2009). This number is approximately tenfolds the number of our own cells. It is thought that this microbiota encodes a surplus of 100-folds unique genes than we do; they have an immense impact on our physiology, nutrition, general health and body shape (Qin et al., 2010).

Metagenomics studies provide information about the abundance of bacterial species and the associated functions, which could indicate for their survival strategies in the human gut (Arumugam et al., 2011). It has been roughly calculated that 20%-60% of the human microbiome could not be cultivated (uncultivable) which in turn resulted in underestimation of their diversity and influence on the human development. The availability of such data offers novel approaches for both diagnosis and treatment of many diseases.

This chapter offers a review for human gut microbiome in different states of health and disease, the microbiome alterations as well as the effect of chemotherapy and radiotherapy prescription on the gut microbiota.

1. Microbial Composition of a Healthy Gut

The human gut is colonized by trillions of microorganisms that contribute to food digestion as well as shaping the state of the body being healthy or diseased. Unlike the human genome, that shares more than 90% similarity among different people, the human microbiome is highly variable among people in state of health. The state of health has been defined as "*a dynamic state of wellbeing characterized by physical, mental, and social potential, which satisfies the demands of a life commensurate with age, culture, and personal responsibility*" (Bircher, 2005). Healthy adults share a common core microbiome, a term that was addressed by Qin and colleagues, that shows to be constant among various people. They reported that 57 different bacterial species were shared among more than 90% of the people (Qin et al., 2010). The species constituting the core microbiota are highly abundant in fecal samples being necessary for performing defined functions within the body (Shade & Handelsman, 2012). The actual composition of the core microbiota depends on the inclusion and exclusion criteria that one is defining for a certain group (Bäckhed et al., 2012). The set of species in the core microbiome may vary among different geographical locations or ethnic groups (Yatsunenko et al., 2012). Some studies defined the human core microbiome itself as variable, being highly affected by external factors as: life style, diet and environmental factors (Figure 1) (Chase et al., 2015).

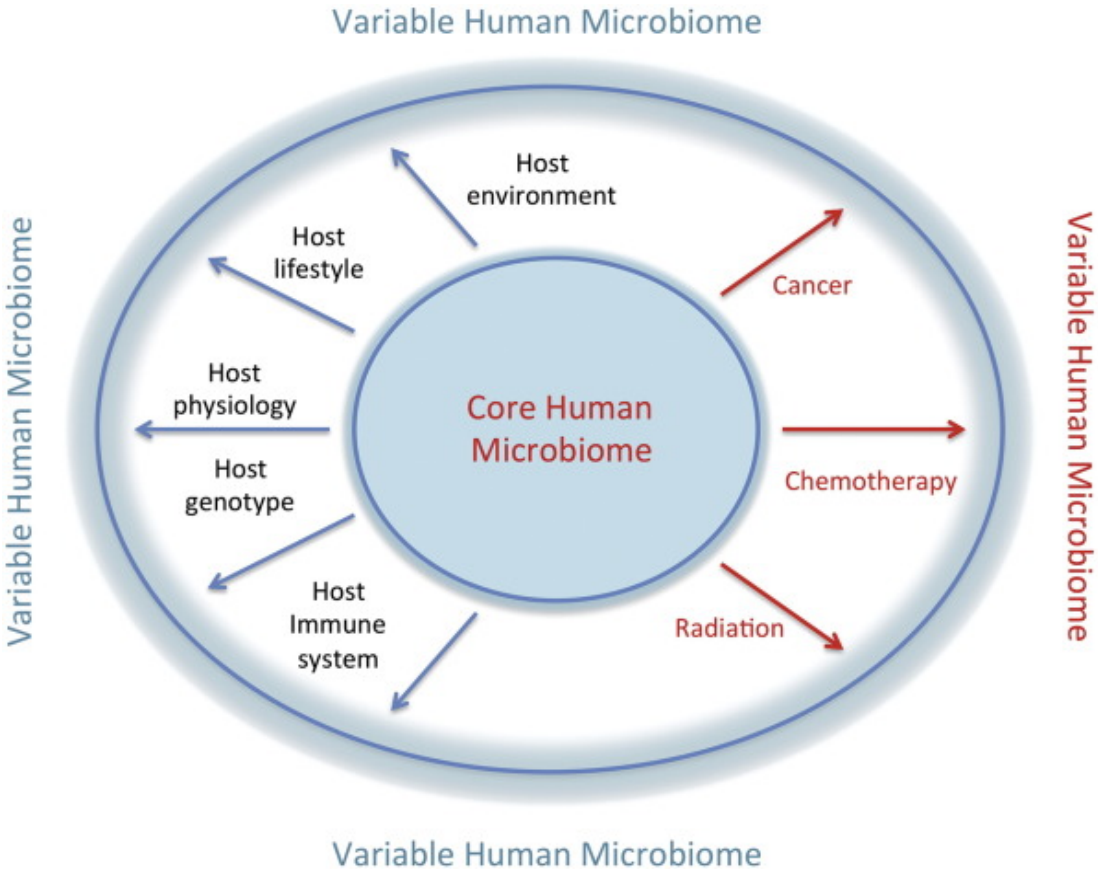

Figure 1: The Concept of Variable and Core Microbiome

Some bacterial species are found to be shared among different people, defining a set of core microbiome. The core microbiome could vary, being affected by different factors and remain normal (blue arrows) while other factors may alter the microbiome resulting in a diseased profile (red arrows) (Chase et al., 2015).

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2. Distribution of Microbial Species Along the Gastrointestinal Tract

The gastrointestinal tract is colonized mostly by two main phyla namely Bacteroidetes and Firmicutes (Huttenhower et al., 2012). The distribution and colonization of assorted microbial species varies along the GI tract from the

small intestine, caecum and large intestine (colon). Although, these parts are connected without barriers, each of which has its own characteristics such as the pH, set of active enzymes, level of oxygen and consequently the microbial composition.

The small intestine, which is characterized by an acidic pH and elevated oxygen levels, it is colonized by rapidly growing facultative anaerobes. The presence of bile acid at the proximal end of the small intestine limits its microbial diversity, since it acts as a bactericide to some species (O'Hara & Shanahan, 2006). However, limited number of species can withstand this extreme environment and still be able to survive (10^3–10^8 cells/g feces) (Eckburg et al., 2005). Two families were found colonizing the small intestine despite its harsh environment, those families are Enterobacteriaceae and Lactobacillaceae (Gu et al., 2013). Both families are almost saturated at the distal ends of the small intestines unlike the proximal ends where they inhabit in low amounts due to the extreme conditions mentioned earlier (Donaldson et al., 2015).

Unlike the small intestine with its acidic pH, the large intestine is colonized by much larger cellular densities reaching (10^{11} cells/g feces) (Eckburg et al., 2005). It is considered as the most inhabited, being colonized by the most diverse communities in the human body. The change in pH range from acidic (1.5-5) to alkaline (5-9) makes it more favorable for different bacteria to survive. It is worth mentioning that the oxygen content is also increasing in the large intestine compared to the small intestine, which enhances the probabilities of bacterial development. The large intestine is usually enriched in Bacteroidetes (Bacteroidaceae, Prevotellaceae, Rikenellaceae), Clostridiaceae, Firmicutes (Lachnospiraceae, Ruminococcaceae), Verrucomicrobia and *Akkermansia muciniphila* (Belzer & de Vos, 2012; Scheithauer, et al., 2016).

3. Functions Attributed to a Healthy Gut Microbiome

The gut microbiota is linked to many aspects in the human body, including digestion, body weight regulation, maintaining the homeostasis, modulation of brain development and shaping the host immune response. The microorganisms colonizing the gut in its state of health can extract nutrients from food that cannot be digested by human enzymes, as in case of dietary fibers. The dietary fibers are obtained from the daily intake of fruits and vegetables. They are digested mainly through fermentation and saccharification performed by the gut bacteria (Flint et al., 2012). As for the proteins, it was found that members of the gut Bacteroidetes phylum are responsible for their digestion, while the bacterium *Prevotella ruminicola,* of the same phylum, take care of the carbohydrates in diet (Wu et al., 2011). The carbohydrate degrading bacteria have carbohydrate active enzymes (CAZymes) encoding-genes in their genome. The CAZymes can degrade the plants polysaccharides such as pectin, xylan and cellulose (Flint et al., 2012) that cannot be digested or exhibited limited digestion by the human enzymes.

The monoamine serotonin 5-hydroxytryptamine (5-HT) is an important neurotransmitter in GI tract and central nervous system. It is a major regulatory factor activating numerous receptors in the GI tract along with other organs in the body. It modulates some feelings including mood and appetite. The Enterochromaffin cells in the human gut are considered the main source of the 5-HT, since 90% is synthesized in there (Baganz & Blakely, 2013; Chabbi-Achengli et al., 2012). The gut microbiome signals the Enterochromaffin cells to produce the 5-HT (Yano et al., 2015). It is still unclear which member of the gut microbiota contributes to the denovo synthesis of the 5-HT in gut. Accordingly, the host-microbial interaction between the residing microbiota and the human cells plays a major role in the regulation and the balance of the GI tract.

The intestinal bacteria shape the immune response of the host (Round & Mazmanian, 2009) by various mechanisms including inhibition of the growth

of pathogenic species by secreting bacteriocins (toxic peptides produced by the bacteria to inhibit the growth of other strains) (Hammami et al., 2013). Commensal bacterial populations can work in cooperation with the host Paneth cells of the host, triggering the expression of several antimicrobial factors. Thus, protecting the intestinal barrier from being penetrated or invaded by other pathogenic bacteria (Vaishnava et al., 2008).

In the developed countries and west Europe, it was noticed the extreme hygiene gave rise to several autoimmune diseases, inflammatory bowel disease, depression (Luna & Foster, 2015) as well as allergies (Azad et al., 2013; Blaser, 2006). Accordingly, a balanced gut microbiome plays a crucial role in maintaining the homeostasis.

4. Shaping the Profile of the Gut Microbiome

Different factors dictate the nature of the gut microbiome in each person. Several studies were conducted to determine the effect of genetic factors on the shape of the microbiota, in identical and non-identical twins as well as several family members. It was found that members of one family have highly similar microbiota, in comparison with unrelated people (Goodrich et al., 2014; Yatsunenko et al., 2012). Also identical twins share more similar microbiota compared to non-identical pairs (Hansen et al., 2011; Turnbaugh et al., 2009). This can only be partially associated with the common diet and life style they are sharing. However, similar microbial pattern was also identified among related individuals, thus increasing the possibility of genetic inheritance effect on the nature of the microbiota. It is still unknown which alleles in the human genome have a direct effect on shaping the gut microbiota (Goodrich et al., 2014).

Other factors shaping the microbiota include environmental differences and geographic locations that enforce a certain lifestyle or a certain diet. The diet itself being either the kind of food or the time of eating, in other words the circadian rhythm induced time of eating (Hall et al., 2017). An interesting study was conducted on the gut microbiota of the Japanese, who are famous for their sushi and diet containing seaweeds. Members of genus *Porphyra* are considered as the most important nutritional seaweed found in sushi and other traditional food. The porphyranase enzyme, originally found in marine Bacteroidetes, *Zobellia galactanivorans,* can act on the sulfated polysaccharide; porphyran that is found in the seaweeds from *Porphyra* genus. What grasped attention was a gene transfer phenomenon: the genes encoding for porphyranases were transferred to the gut bacterium *Bacteroides plebeius* isolated from Japanese individuals. Comparative gut metagenome analyses showed that genes encoding for porphyranases enzymes are frequent in the Japanese population and absent from the American individuals (Hehemann et al., 2010). Another study was conducted to compare the microbial profile of two populations of children (from 1 to 6 years old): one is from a rural African country: Burkina Faso (BF) and the other from Italy. Children in BF consume fibers in larger amounts compared to those from Italy. It was found that the high fiber intake was associated with increased Bacteroidetes and almost depleted Firmicutes. Fiber-based diet was also coupled with the presence of *Prevotella* and *Xylanibacter* genera. Members of those genera contain bacterial species having genes responsible for encoding the enzymes required for cellulose and xylan degradation, that were absent in Italian children (De Filippo et al., 2010). The two abovementioned studies showed a clear correlation between the geographic location, regular diet, life style and the gut microbiome.

Age also plays a major role in shaping the microbiota at various stages of life (Figure 2). The relationship between the gut microbiota and age starts at birth and depends on the mode of child delivery.

Cesarean section delivery affects the microbiota profile in comparison with the vaginal delivery. In case of cesarean section, the Actinobacteria and Bacteroidetes phyla were decreased; while Firmicutes phylum had higher richness and evenness. The cesarean section-delivered infants also a less abundance microbiota till they reached the age of one month (Rutayisire et al., 2016). On the other hand, vaginally delivered infants had higher colonization of *Bacteroides* genus (Ebihara et al., 2013; Grönlund et al., 1999). It is important to mention that several studies found that there is no differences in *Lactobacillus* genus abundance from day zero till the age of one month in both modes of delivery (Ebihara et al., 2013; Grönlund et al., 1999; Huurre et al., 2008).

After acquiring a primary gut microbiome profile, neonates start to develop their own microbiome till reaching the age of three years (Kundu et al., 2017). The shift to adult microbial profile occurs in parallel with the weaning process and the introduction of solid-food to the diet (Bergström et al., 2014). The microbial development at this stage relies on the food introduced and any medical treatment or antibiotics administrated during this period of development. Therefore, it is recomended to minimize the chemical intervention during the aforementioned developmental period (Koenig et al., 2011). A stable microbiome begins by the age of 3 years, consisting of six phyla, two of which are abundant (Bacteroidetes and Firmicutes) (Figure 3), while the other phyla are less abundant (Proteobacteria, Actinobacteria), and even less Fusobacteria, and Verrucomicrobia (Eckburg et al., 2005).

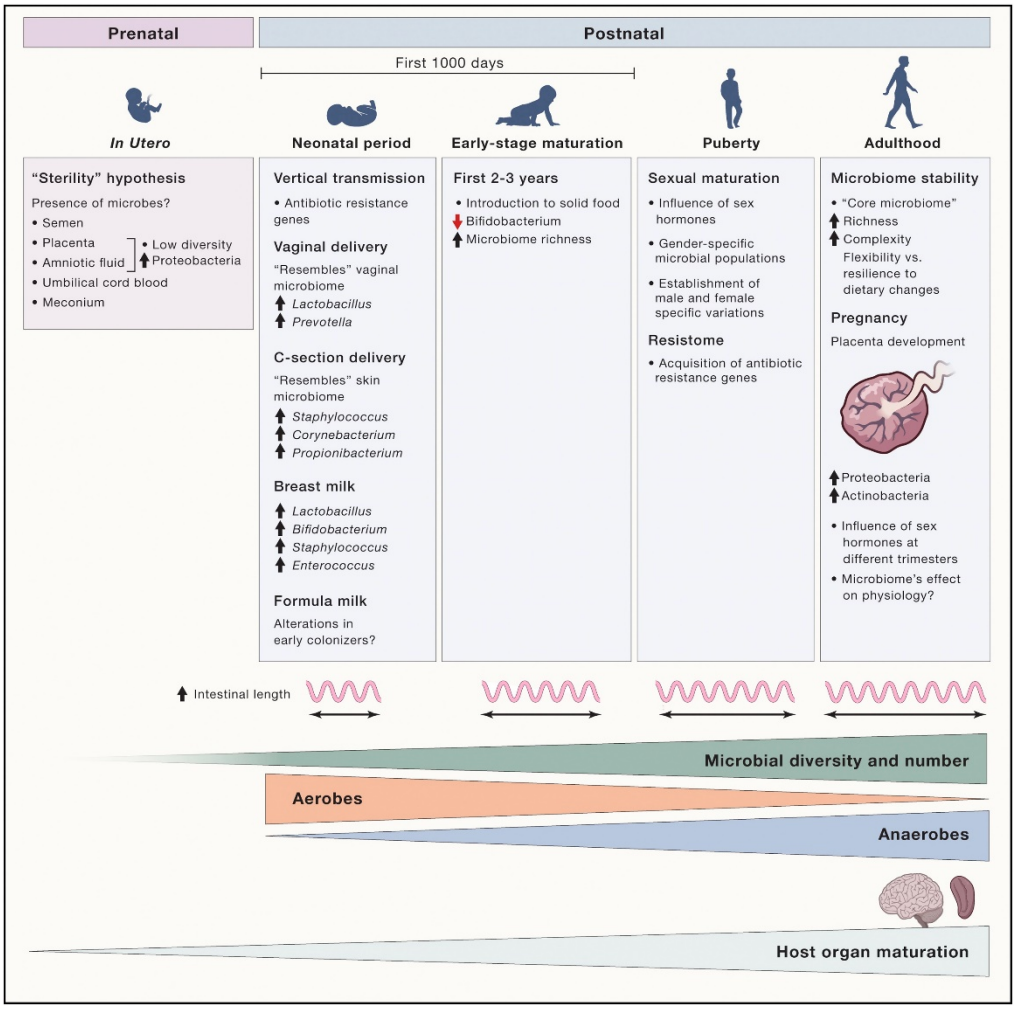

Figure 2: Gut Microbiota Profile Development with Age

The gut microbiota profile development from time of birth till reaching the adults' profile. During prenatal development, some theories support the prenatal is sterile and acquire the first microbiome from the mother during delivery; other theories support that prenatal start to acquire their microbiome in the womb. The neonates' microbiome depends on the mode of delivery and start to develop when the child food intake is changed. During early stage of development and puberty; the microbial diversity increase, the core microbiome becomes established, while the variable microbiome is shaped according to one's lifestyle and environment. The microbial development occurs in parallel with the organs development, elongation of the intestines, allowing more niches for the microbiome to develop, increase in number and increase in diversity. The adulthood microbiome is considered the most stable; although, changes are still acceptable according to state of health and disease and other factors as aforementioned (Kundu et al., 2017).

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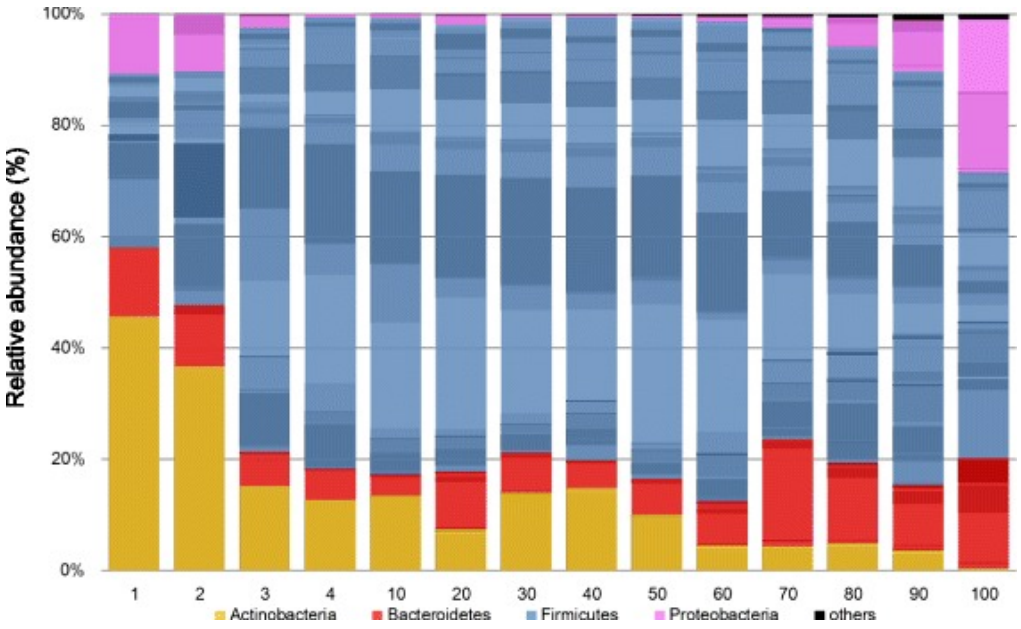

Figure 3: Variation in Microbiota Composition with Age

Actinobacteria (yellow), Bacteroidetes (red), Firmicutes (blue), Proteobacteria (pink). The numbers represent the age groups (from neonates to centenarians). The Firmicutes dominate the adult gut microbiome compared to the neonates' microbiome that is dominated by Actinobacteria. By increasing age groups (above 70 years old), the Bacteroidetes and Proteobacteria increase while the Actinobacteria decreases noticeably (Odamaki et al., 2016).

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5. Dysbiosis of gut microbiota

Dysbiosis is defined as a state of imbalance or shift from the normal microbiota profile. Gut microbiota dysbiosis has been linked to several diseases as inflammatory bowel disease (IBD), autism, stress, depression, immunity related diseases and several others. Many links were created to the gut microbiota, being either affected by the disease or the imbalance being the cause of the disease. The state of imbalance could occur as a result of several factors including: exposure to drugs, diet alterations, toxins and radiation. The imbalance could be either a change in the proportionality of the already existing bacteria (as in case of increased *Lactobacillus* in obese individuals) (Armougom, et

al., 2009) or could appear because of the introduction of undesired bacteria as *Clostridium difficile* (*C. difficile*) that may develop several complications.

5.1. Changing the Proportionality among the Symbiotic Bacteria

Symbiotic bacteria reside in the host in a well-balanced form that should be maintained to ensure a healthy state of life (Round & Mazmanian, 2009). Whenever the balance is interrupted, a shift from health state to disease occurs (Chow et al., 2010).

Obesity is a common disease linked with abnormal proportionality in the gut microbiota profile. Obese or overweight individuals usually consume excessive amounts of fats and carbohydrates in their diets; moreover, their lifestyle does not enforce much physical exercise. Accordingly, calories are stored in the body in the form of fats in adipocytes (Chakraborti, 2015). Since the gut microbiota plays a vital role in the food digestion and production of metabolic end-products, some phyla are directly linked to the production of certain metabolic end-products that govern the absorption of the digested food in the host body. Members of phylum Firmicutes are correlated with the production of butyrate, while acetate and propionate are usually correlated with Bacteroidetes and Actinobacteria (Chakraborti, 2015). Accordingly, the abundance of each phylum depends upon the availability of the target substrate in diet (Shoaie et al., 2013). In obese individuals, some studies reported an increase in Firmicutes over the Bacteroidetes (Abdallah Ismail et al., 2011; Bervoets et al., 2013; Hartstra et al., 2015). Members of phylum Firmicutes are more concerned with degradation of high energy food, thus raising the amount of lipid droplets leading to higher weight gain (den Besten et al., 2013; Semova et al., 2012). On the

other hand, was no observed difference in the proportion of Bacteroidetes at the phylum level between obese, non-obese and obese undergoing weight loss diet. Yet proportion of the Firmicutes was reduced in the group of obese undergoing weight loss diet (Duncan et al., 2008).

5.2. Invasion by Pathogenic Bacteria

Symbiotic bacterial strains are considered as a defense line, protecting the host from invading pathogens. Those symbiotic bacteria are perfectly occupying the available niches in the gut. Therefore, the invading pathogenic bacteria have to compete for space and food resources with the already existing and well-adapted symbiotic strains and escape the host immune response (Rohmer et al., 2011). Despite the high protection level in the gut, some pathogens succeed in the invasion and colonization of the gut niches.

Clostridium difficile is one of the gut pathogens that can escape the defense line and colonize in the intestine. Infection by *C. difficile* is symptomatic, appearing in the form of diarrhea that can even become more complicated resulting in pseudomembranous colitis and toxic megacolon, and might even lead to the death of the patient (Gerding, 2004). *C. difficile* is a gram positive, spore-forming bacterium (McDonald et al., 2006; Rupnik et al., 2009). The persistence of spores renders *C. difficile* as a major health issue, since the infection is usually associated with prolonged hospital-stays along with antibiotics prescription (Buffie et al., 2012). Long exposure to antibiotic treatment can alter the natural microbiota, decreasing its richness and diversity for a period of time (antibiotic perturbation) following the treatment, after which the host can retain the original microbiota profile. Clindamycin and cephalosporins are among the antibiotics associated with the possible development of *C. difficile*

infection. The *C. difficile* makes a great use of the dysbiosis occurring post antibiotic treatment, its spores in the surrounding environment can initiate the invasion (Lawley et al., 2010). In an experiment performed on mice, consumption of clindamycin antibiotic led to expected gut microbiota perturbation. In the absence of *C. difficile* spores, the mice were able to re-structure their original microbiota profile within 21 days after antibiotic treatment. On the other hand, in the same experimental set up, but in presence of *C. difficile* spores, the bacteria succeeded in the invasion. Moreover, the gut microbiota dysbiosis became persistent till 49 days post-clindamycin treatment (Lawley et al., 2012).

5.3. Differentiation Between Commensals and Pathogens

The gut can differentiate between symbiotic bacteria or beneficial commensals from intruding pathogens. Symbiotic bacteria are recognized by ones immune system once acquired in early developmental stage (Hooper et al., 2012). During the weaning period, the host Paneth cells secrete anti-microbial peptide that targets gram-positive pathogens while gram-negative commensals are not affected (Hooper et al., 2003). The host uses pattern recognition receptors, such as Toll-like receptors (TLRs), to identify different bacterial components (Ferreira et al., 2014). When TLRs recognize a symbiotic bacterial factor, homeostasis is induced. On the other hand, when pathogenic bacteria are recognized by the TLRs, inflammation is induced along with secretion of antimicrobial peptides to eradicate the pathogens (Figure 4) (Burdelya et al., 2008; Hsiao et al., 2008).

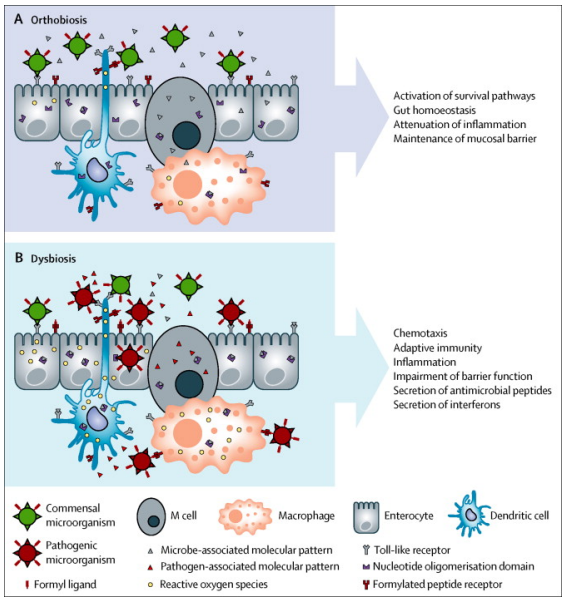

Figure 4: Host differentiation mechanisms between symbiotic and pathogenic bacteria

(4A): Identification of symbiotic bacteria in Orthobiosis (balanced beneficial symbionts in microbiota)
(4B): Dysbiosis accompanied with pathogenic bacteria identification by TLR (Ferreira et al., 2014).

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5.4. Dysbiosis Treatment

When a gut microbiota profile is identified as unhealthy or unfavorably altered, a treatment could be offered to shift it to a healthy profile. Such treatment relies on getting rid of pathogens or introducing missing taxa. One simple method for a desired microbial shift is following a healthy diet. The microbiota can undergo a rapid shift based on diet habits (David et al., 2014). Other ways for desired microbial shift will be addressed below, which includes the administration of probiotics, prebiotics, tailored drugs as Ecobiotics and fecal microbiota transplantation (FMT) from healthy donors.

Probiotics are defined by the World Health Organization as "*live microorganisms that can provide benefits to human health when administered in adequate amounts, which confer a beneficial health*

effect on the host" (WHO, 2001). The most common bacterial genera in probiotics are *Bifidobacteria* and *Lactobacilli*. Probiotics' beneficial effects have been linked to alleviation of traveler's diarrhea, antibiotic associated diarrhea and inflammatory bowel disease (Kaur et al., 2009). The probiotics mode of action is species specific. Some *L.bacilli* can produce defensins (antimicrobial peptides) that are normally produced by the host intestinal cells (Möndel et al., 2009). *Saccharomyces boulardii* can lessen ulcerative colitis by competing with *Citrobacter rodentium* which are pathogenic bacteria, for adherence to the host epithelial cells (Wu et al., 2008). Probiotics could be obtained either from food sources as yogurt and milk or administrated as drugs. The amounts of probiotics administrated are determined as colony forming unit (CFU). It is important to mention that the effect of probiotics is transient and diminishes in about 4 weeks after stopping probiotics intake (Gogineni et al., 2013).

Prebiotics are: "*A non-digestible food ingredient that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon thus improves the host health*" (Gibson & Roberfroid, 1995). They are found in some food sources including garlic, onion, wheat, soybean and asparagus (Van Loo et al., 1995). Prebiotics are mainly composed of carbohydrates although they might include some non-carbohydrate moieties. One major criterion to identify a food source as a potential prebiotic is its ability to resist hydrolysis by gastric enzymes, transfer to the intestine and undergo fermentation in the large intestine, thus enabling the intestinal microbiota to flourish (Xiao et al., 2014). Most of the microbiota residing in the large intestine are anaerobes; therefore, their energy uptake is based on fermentation. This reflects the indirect effect of prebiotics and its importance in enhancing the activity and growth of the intestinal microbiota.

Many pharmaceutical companies formulate drugs to supplement patients with useful microorganisms in a form of capsule

to shift the diseased profile to a healthy state, thus preventing or treating the microbiome-linked diseases. The formulated capsules could be probiotics, synbiotics or Ecobiotics. The synbiotics are formulations based on prebiotics and probiotics together. The choice of each should be in synergism, that is the prebiotics favoring the growth of the probiotics chosen. One example for a synbiotic drug could be *Bifidobacteria* and oligofructose, while the same prebiotic oligofructose cannot be grouped with the probiotic bacteria *Lactobacilli* for a synbiotic formulation (since the *Lactobacilli* requires Inulin as a substrate) (Schrezenmeir & de Vrese, 2001). The new trend of ecobiotic drugs is rapidly growing. Ecobiotics are based on the selection of desired bacteria from healthy stool and concentrating their spores in the form of capsule to shift certain diseased profiles to healthy ones (Hoffmann et al., 2017).

Several microbiome centers are being established worldwide, and many pharmaceutical companies started to benefit from the available data to develop various medications. SERES Therapeutics in the United Kingdom is working on several ecobiotic drugs, in clinical trials, that shift the microbiota from diseased states as recurrent of *C. difficile* (SER-109), primary *C. difficile* infection (SER-262) and ulcerative colitis (SER-287) to a health state. MicroBiome Therapeutics in the United States released its first product: NM504, in early 2017, targeting the GI dysbiosis in diabetic patients. Novartis has also teamed up with the University of California in San Francisco and funded $8.1 million to support microbiome research. Accordingly, this field of science became one of the basic research requirements for pharmaceutical developments and drug discovery.

Fecal Microbiota transplantation (FMT) is another possible solution for shifting the diseased microbiota profile to a healthy one or a healthier microbiota. FMT was first reported in late the 1950s in Colorado. Enemas aimed at treating patients with pseudomembranous colitis as a result of *C. difficile* infection (Borody & Khoruts, 2012;

Eiseman et al., 1958). Gustaffson and colleagues studied microbiota-associated characteristics (as interaction with antibiotics, symptoms ranging from diarrhea to membranous colitis) in 32 patients before and after FMT; the effect of FMT on the microbial shift was reported four days post transplantation (Gustafsson et al., 1999). It is important to mention that in case of *C. difficile* infection; the patients' microbiota is almost destroyed by the intensive use of antibiotics. Accordingly, implementation of a new healthy microbial profile is applicable (Borody & Khoruts, 2012). As for other diseases linked to microbiota disruption or imbalance as obesity, IBD or chronic fatigue, more studies are testing the effect of FMT on the recipient's health. In a study testing the effect of FMT between lean and obese men, the obese recipients had a noticeable decrease in fasting triglycerides level compared to self-recipients (placebo group) (Vrieze et al., 2012).

6. Relationship Between Gut Microbiota and Cancer

The relationship between the gut microbiota and Cancer development at various sites of the body is complicated, entering the dilemma of which came first (Figure 5). Is the gut microbiota dysbiosis the cause of cancer development? Or is it cancer and its associated cancer treatment protocols that caused dysbiosis? How will the response to treatment be in a cancer patient with an altered gut microbiota?

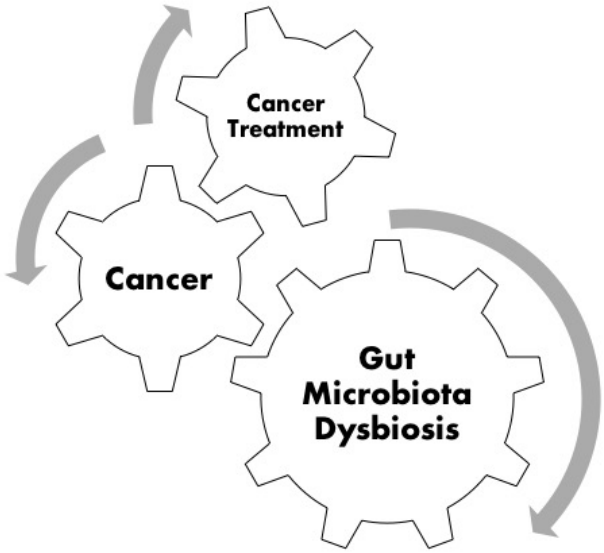

Figure 5: Relationship between dysbiosis, cancer and cancer treatment

A complicated relationship between dysbiosis, cancer and cancer treatment protocols. Gut microbiota dysbiosis might give rise to cancer and vice versa. While the common cancer treatment plans can result in gut microbiota dysbiosis.

6.1. Is the Gut Microbiota Dysbiosis Considered a Cause for Cancer Development?

Gut microbiota dysbiosis was linked to several cancer types including colorectal cancer (CRC), hepatocellular carcinoma (HCC) and gastric cancer. An observational study linked CRC and lymph node metastasis with the increased colonization by the pathogenic bacterium, *Fusobacterium nucleatum* (Castellarin et al., 2012). Cuevas-Ramos and colleagues have linked the development of CRC to the colonization of *Escherichia coli* of group B2 in mice (Cuevas-Ramos et al., 2010). The genomic DNA of 40%-60% of group B2 *E. coli* bacteria contains a pathogenicity island (54-kb) known as *pks* (McCarthy et al., 2015), the genes in *pks* island encode for the synthesis of a polyketide-peptide genotoxin named colibactin. The colibactin peptide has the ability to induce DNA double strand breaks in the host colon enterocytes (Nougayrede et al., 2006). The effect of

colibactin on DNA damage was assessed in mice and was comparable to the damage induced by 0.5 Gy of gamma radiation. Both colibactin and 0.5 Gy of whole body irradiation were able to induce DNA mutations, aneuploidy, chromosomal instability and consequently colon carcinogenesis (Cuevas-Ramos et al., 2010).

Gut microbiota dysbiosis has been linked to obesity as explained earlier. Another link was established between the gut microbiota dysbiosis, obesity and HCC. Yoshimoto and colleagues have described the complex link between high-fat diet, microbiota dysbiosis and HCC development in mice models (Yoshimoto et al., 2013). The high-fat diet can directly shift the microbiota profile, that in turns affect the microbial by-products produced, one of which is the deoxycholic acid (DCA). The DCA results from microbial bile-acid metabolism and adversely leading to DNA damage. Elevated DCA level induces the production of senescence-associated secretory phenotype (SASP) that is a group of proteases, chemokines and inflammatory cytokines. The elevation of SASP enhances the tumorigenesis. Liver diseases are associated with translocation of bacteria and bacterial components within the patients' body (Roderburg & Luedde, 2014). Another study highlighted the presence of the stomach pathogen *H. pylori* in the liver of patients with HCC (8 positive *H. pylori* out of 20 HCC) (Huang et al., 2004).

6.2. A Reciprocal Link Between Cancer Treatment Medications and Gut Microbiota

Common treatment protocols administrated to cancer patients are usually relying on chemotherapy, immunotherapy, radiotherapy or a combined treatment protocol. Each of which can cause a shift in the patients' gut microbiota. Moreover, treatment protocols are sometimes

combined with antibiotic administration, which adds a burden on the gut microbiota.

Zwielehner and colleagues studied the effect of 17 different chemotherapeutic agents in presence and absence of antibiotic administration and radiation (Zwielehner et al., 2011). Their study relied on PCR fingerprinting on denaturing gradient gel electrophoresis (PCR-DGGE), quantitative real-time PCR along with 454-sequencing for samples from only two patients. Accordingly, the results reflected overall species abundance with special emphasis on the potential pathogenic bacteria: *Clostridium* cluster IV. The study reported a steep drop in bacterial species richness, in comparison with healthy controls, after the first shot of chemotherapy. It also described a "rebound-effect" in the bacterial richness of the gut microbiota in their last time point (5-9 days after chemotherapy), but with a different composition. Another study focused on the effect of Cyclophosphamide (CTX) on the gut microbiota in mice models (Viaud et al., 2013). CTX did not alter the intestinal microbiota instantaneously (24 and 48 h after the first shot). However, the dysbiosis in the small intestine was only reported one week after the drug administration. Aside from the dysbiosis of gut microbiota, an important link was made between the absence of gut microbiota and resistance to CTX. It was found that the tumors in germ free mice were resistant to CTX treatment.

The link between the immunotherapies targeting the programmed cell death protein-1 (PD-1) and the gut microbiome was studied in Melanoma cancer patients (Gopalakrishnan et al., 2018) and patients suffering from epithelial tumors (Routy et al., 2018). The two studies have described a "favorable profile" of the gut microbiome was described in cancer patients, who responded to anti-PD1 drugs. Gopalakrishnan and colleagues described the favorable profile of the responders patients with higher alpha diversity and increased relative abundance of family Ruminococcaceae along with *Faecalibacterium*,

compared to the non-responders gut profiles (unfavorable) that has increased abundance of Bacteroidales and decreased alpha diversity (Gopalakrishnan et al., 2018). A favorable gut microbiome was also linked to a better antigen presentation and presence of higher level of effector T-cells in the tumor micro-environment. Routy and colleagues linked resistance to immunotherapies as immune check point inhibitors (ICI) with altered gut microbiome (the unfavorable profile described by (Gopalakrishnan et al., 2018)). They also found *Akkermansia muciniphila* to be common among responders to ICI. Accordingly, they concluded the possibility of manipulating the gut microbiome to decrease the resistance to ICI through FMT along with providing *Akkermansia muciniphila* bacterium in the form of oral supplementation (Routy et al., 2018).

The term pharmacomicrobiomics started to appear with the HMP. It was defined as "*The effect of microbiome variations on drug disposition, action, and toxicity*" (Rizkallah et al., 2010). The interdisciplinary field of pharmacomicrobiomics adds a great value to microbiome research, since determining the interaction between the gut microbes, in either health or disease, and various medications can offer a better response of the same drug by manipulating the patients' gut microbiome.

The TIMER (T: Translocation, I: Immunomodulation, M: metabolism, E: enzymatic degradation, R: reduced diversity and ecological variation) relationship between the patients' microbiome and chemotherapeutic drugs offered a better view for the complicated nature of this relationship (Alexander et al., 2017). The TIMER is a collective description for the modulation of the gut microbiota to different chemotherapeutic agents. Each of the TIMER component represents a different mechanism by which the gut microbiota can facilitate the antitumor effect of various chemotherapeutic agents. CTX chemotherapeutic drug induces the translocation of some gram-positive bacteria to reside in the lymphoid organs. The translocated

bacteria induce the production of pathogen T-helper 17 that in turn enhances the tumor response to the antitumor activity of CTX (Viaud et al., 2013). Iida and colleagues highlighted the importance of having intact commensal microbes in the tumor microenvironment that is required for a proper immunomodulation of myeloid-derived cells activated by immunotherapy and chemotherapy: CpG oligodeoxynucleotides and Oxaliplatin respectively (Iida et al., 2013), beacause the germ-free mice responded poorly to the aforementioned drugs.

Accordingly, it became necessary to understand the role of the commensal bacteria in antitumor effect of various chemotherapeutic agents, to enable the compensation for the commensal deterioration towards a better response to the therapy.

6.3. Link Between Radiotherapy and Gut Microbiota: Is It Only Dysbiosis?

Radiotherapy is administrated as a palliative or curative therapy to up to 50% of the cancer patients, either independently or in combination with chemotherapy (Abbasakoor et al., 2006). Focusing on the radiotherapy directed to the abdominal and pelvic regions, radiation enteropathy is the main concern in determining the coming therapeutic plan. Several symptoms arise after the first or second week of treatment as a side effect of radiation in abdominal region including: bloating, diarrhea, imperfect food absorption, abdominal pain and nausea (Bismar & Sinicrope, 2002). The side effects are usually the driving force for the dose limitation or discontinuation, thus affecting the patients' health. Radiation associated enteropathy is usually resulting from the effect of radiation on the intestinal region. Although the small intestine is not a common target for radiation, but its large surface area and the high sensitivity of its mucosal lining to

radiation are the main reason for the enteropathy side effects appearing on the patients (Packey & Ciorba, 2010).

An important study conducted on mice, has tested the role of the gut microbiota in determining the sensitivity to radiation (Crawford & Gordon, 2005). The mice in this study were subjected to a lethal dose (16 Gy) of total body irradiation (TBI) followed by bone marrow transplantation (BMT). All germ-free mice survived (more than 40 days) compared to the conventionally raised mice, where 52% died in 7 days post TBI. To further confirm the relation to the gut microbiota; the germ-free mice were inoculated with fecal samples from the conventional mice then subjected to the same dose (16 Gy) of TBI followed by BMT, 44% died in 7 days. A more recent study that was conducted on mice by Gerassy-Vainberg and colleagues (Gerassy-Vainberg et al., 2018) has focused on the relationship between post-radiation injuries, gut microbiota dysbiosis and the effect of dysbosis on increasing the liability to inflammations. Accordingly, a sequential effect of radiation was proposed as follows: radiation inducing gut microbiota dysbiosis, the altered microbiota induces a higher secretion of Interleukin-1β which consequently increases the susceptibility to tissue damage and appearance of proctitis.

The impact of radiation on gut microbiota was assessed in a group of gynecological cancer patients (45-64 years old) (Nam et al., 2013). The study reported a massive change in microbiota composition after receiving radiotherapy. Firmicutes phylum decreased by 10% while the Fusobacterium decreased by 3%. The post-radiotherapy samples collected in the follow-up period (one to three months after radiotherapy) showed a remold or reshaping of the gut microbiota.

In a nutshell, the relationship between gut microbiome, cancer development, chemotherapies, immunotherapies and radiotherapy are

ultimately complicated. The microbial balance during chemotherapy and immunotherapy may lead to a better response to treatment. While the presence of gut bacteria during radiotherapy has several unwanted side effects. On the other hand, the total absence (as in germ free mice) can render the patient resistant to lethal doses of radiation. Accordingly, the proper manipulation of gut bacteria during cancer treatment can increase the success rates of the prescribed treatment protocols. Therefore, this study has focused on understanding the link between infants' gut microbiota and radiotherapy treatment.

Chapter 2: Study Objective and Design

1. Study Objective

This study is an observational study, to determine the effect of radiotherapy on the infants' gut microbiome.

2. Study design

In the current study we analyzed 10 fecal samples, obtained from two healthy individuals and three cancer patients. It included two sets of controls: self-control (same patient before starting radiotherapy sessions) and healthy individuals. The healthy is defined as not consuming any chemotherapeutic agents or antibiotics (antibiotic free period was defined by at least 1 month before sample collection) and never exposed to radiotherapy before. All the participants in the study (patients and healthy individuals) are males, within the age range 3.5-7 years old.

Patients in the current study suffer from Rhabdomyosarcomas in the pelvic region. Such type of cancer receives a high dose of radiation. The patient

receives 50.4 Gy (180 cGy/fraction). The sample collection points were defined as follows: day 0: before starting radiotherapy sessions, mid-point: ranging from day 12 to day 16 and the last collection point: day 26-28. Patients with Rhabdomyosarcomas follow a combined treatment protocol including chemotherapy (Cyclophosphamide, Vincristine and Dactinomycin) and radiotherapy; 28 fractions of 50.4 Gy (180 cGy/fraction). The patients started the radiotherapy sessions either after 12 weeks of chemotherapy (Patient 1 and patient 3) or after 4 weeks of chemotherapy (patient 2). During the chemotherapy; a set of antibiotics (Table 1) is usually prescribed according to the patients' case or needs.

Table 1: Set of Antibiotics prescribed before and during radiotherapy

Antibiotic	Antimicrobial Spectrum	Patient (1)	Patient (2)	Patient (3)
Before Radiotherapy				
Amikacin	Gram-negative	NA	NA	Less than 1 months
Azithromycin	Enteric bacteria and other eubacteria and several pathogenic bacteria	2 months	NA	NA
Ceftriaxone	Broad spectrum	3 months	NA	Less than 1 months
Ciprobay (Ciprofloxacin)	Broad spectrum	NA	Less than 1 month	Less than 2 months
Meropenem	Broad spectrum	NA	NA	Less than 1 months
Rocephin	Broad spectrum	NA	NA	Less than 1 months

Sulfamethoxazole and trimethoprim	Broad spectrum and several pathogenic bacteria	4 months	Less than 1 month	Less than 1 month
Sutrim	Broad spectrum and several pathogenic bacteria	2 months	Less than 1 month	Less than 2 months
Tavanic	Broad Spectrum	NA	NA	Less than 2 months
Zithromax	Enteric bacteria and other eubacteria and several pathogenic bacteria	4 months	Less than 1 month	NA
During Radiotherapy				
Augmentin	Broad spectrum	NA	Day 2	NA
Ceftriaxone	Broad spectrum	NA	NA	Day 13
Levofloxacin	Broad spectrum	NA	NA	Day 6
Sulfamethoxazole and trimethoprim	Broad spectrum and several pathogenic bacteria	Yes	Day 2	Day 22
Sutrium	Broad spectrum and several pathogenic bacteria	NA	Day 15	NA

*Days and Months are calculated with respect to the start of radiotherapy sessions.

**Duration of antibiotic course ranges from 4 days to 3 weeks.

***Antibiotic spectrum information is retrieved from DrugBank Database (Wishart et al., 2018).

Chapter 3: Materials and Methods

1. Sample collection and informed consents

An institutional review board (IRB) approval was granted from the AUC (Approval of study #2016-2017-041) – Appendix 3. All participants have signed (child assent form) and the guardians have approved the participation in study and signed a parental permission form. Fecal samples were collected from the participants, by the help of their parents, in sterile falcon tubes, transferred on ice to the lab at Zewail City and stored in -80°C freezer until DNA extraction.

2. DNA extraction

Microbial DNA was extracted from the collected fecal samples by DNA extraction kit from stool, QIAamp DNA stool mini kit (Qiagen, USA), according to the manufacturer's instruction. The protocol used enhances the nonhuman DNA over the human DNA extracted from the sample, through optimization of the lysis conditions. The DNA was eluted in 50 µl elution buffer provided by the kit and stored in -20°C.

3. 16S rRNA sequencing

The extracted DNA was sent to Eurofins Genomics in Germany for sequencing of 16S rRNA. The sequence was performed on Illumina MiSeq platform, targeting V3-V5 variable regions of the 16S rRNA. The run was performed on 2x300 paired-end reads. The target region (V3-V5) length is approximately 700 bp; accordingly, the obtained reads do not overlap.

4. Data Analysis

The 16S rRNA sequencing data received from Eurofins Genomics were already demultiplexed (remove barcodes and assign each read to its original sample). The analysis was completed on Qiime2 pipeline (version 2017.12), q2cli command line interface (Qiime2 script in appendix 1). The denoise command was used followed by length trimming to 230 bp. Feature table was constructed on the same program (also referred as OTU table) using "Deblur" approach for OTU table construction, that is compatible with Hi-seq and Mi-seq Illumina results. The Deblur approach uses an error profile, operating on per-sample bases and depends on the read length and diversity in amplicon sequences. Thus, it offers a higher sensitivity and requires lower computational powers compared to other OTU clustering algorithms (Amir et al., 2017).

Taxonomy classes were assigned using the constructed feature table in comparison with SILVA database (Silva-119 99% OTUs full-length sequences). Unrooted phylogenetic tree was also constructed on Qiime2 (qiime phylogeny fasttree). The taxonomic classification was appended to the feature table and exported as a biom format file, the phylogentic tree was exported as (Newick tree format) and the sequences corresponding to the classified OTUs were exported as fasta file. All exported files from Qiime2 analysis were imported to phyloseq package (McMurdie & Holmes, 2013) on R-CRAN for figures plotting. It is important to mention that reads were not rarefied to an even sampling depth (McMurdie & Holmes, 2014)

Another OTU clustering algorithm was tested through USeacrh pipeline (Edgar, 2010), that resulted in clustering to 792 OTU. However, the taxonomy assignment on this platform was not accessible through the free version of USearch (Script used in Appendix 1).

Chapter 4: Results

1. Reads quality and optimization

A total of 10 fecal samples obtained from two healthy controls and three cancer patients (three samples on three-time points from each patient, except patient 1). Although, the original experimental design targeted a larger set of patients, only three could be reached due to the following constrains: (1) limitation of the number of patients with Rhabdomyosarcoma since it is a rare cancer type, (2) targeted age range; the patients were young and were not aware of participating in a study, (3) most of the patients were suffering from constipation before starting radiotherapy that made the sample collection at point zero (pre radiation) a challenging task. The patients with Rhabdomyosarcoma in the pelvic region underwent a combined treatment protocol of chemotherapy (Cyclophosphamide, Vincristine and Dactinomycin), radiotherapy (50.4 Gy on 28 fractions; 180 cGy/fr.) and a complex set of antibiotics as described previously in table 1. The microbial DNA was extracted and used for 16S rRNA sequencing on Illumina MiSeq platform, targeting V3-V5 region, paired-end reads (2x300).

The high throughput sequencing generated a sum of 904,685 reads, containing a yield of 510,237 bp (mean reads per sample = 42,407.9 and median reads per sample = 41,645.5) (Figure 6). The average read length obtained (Forwards reads ~ 280 bp, Reverse reads ~ 250 bp). All reads were trimmed to 230 bp after denoising on qiime2 (qiime deblur denoise-16S).

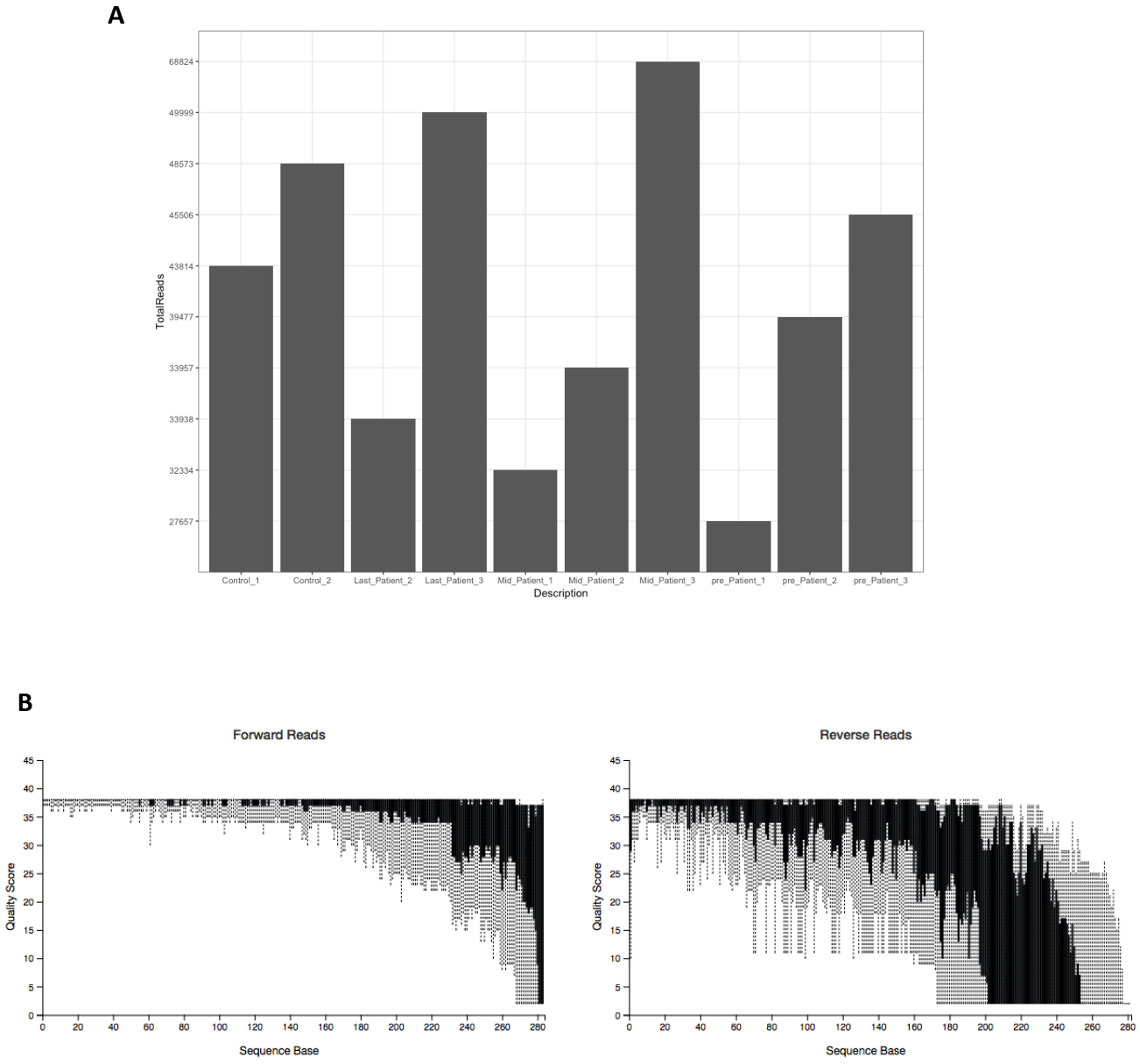

Figure 6: Validation of sequencing reads

Overview of sequencing reads (A): number of reads obtained from each sample. The least number of reads obtained were from sample (pre_patient_1) while the highest number of reads were from Mid_Pateint_3 (B) read length and quality score for both forward and reverse reads from each sample. The average reads length is 280 bp for all forward reads while the reverse read length ranges between 200 bp to 250 bp.

2. Alpha diversity analysis

All indices of alpha diversity measures (Observed OTU, Chao1, ACE, Shannon, Simpson, Inverse Simpson and Fisher) all showed a higher diversity in healthy controls compared to cancer patients in the three-time points collected (Figure 7). After completing the radiotherapy treatment along with the antibiotics courses (Last, blue) the alpha diversity has generally declined when compared to the mid-point (after 12-15 fractions) and pre (before radiation). The out layers at each time point are obvious, this could be attributed to the personal variations between the patients, along with the variation due to the set of antibiotics prescribed, antibiotic course duration and antibiotic course timing relative to the radiotherapy and sample collection time.

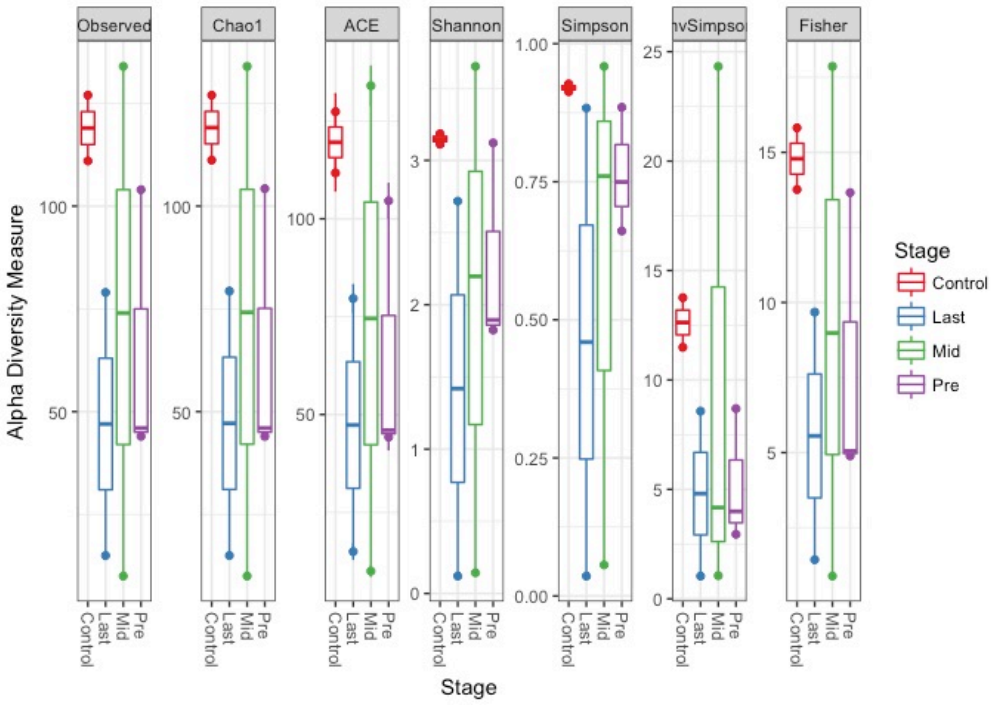

Figure 7: Alpha diversity analysis across different time points

Box plot showing different alpha diversity indices across the time points (Pre: before radiation, Mid: after 12-15 fractions and Last: after 26-28 fractions of radiation that is equivalent to 50.4 Gy) the control are healthy participants (never subjected to chemotherapy or radiotherapy before and at least one month free of antibiotics before the sample collection time).

3. Alpha diversity per sample

Alpha diversity measures (Chao1 and Shannon) per sample did not indicate a direct relationship between exposure to radiation, consuming intensive antibiotics courses and the relative abundance of all bacterial species per sample (Figure 8). Surprisingly, patients 1 and 2 experienced an increase in the alpha diversity after the two aforementioned exposures. Unlike patient 3 who experienced a massive drop in alpha diversity. On the other hand, a pattern or a relationship was inferred between high alpha diversity and response to treatment (Table 2). The decreased bacterial abundance was associated with a positive response to radiotherapy and vice versa. It is important to mention that the Chao1 index reflect the richness only (number of bacterial species per sample), while Shannon index reflects both richness and evenness (relative abundance of species that make up the richness).

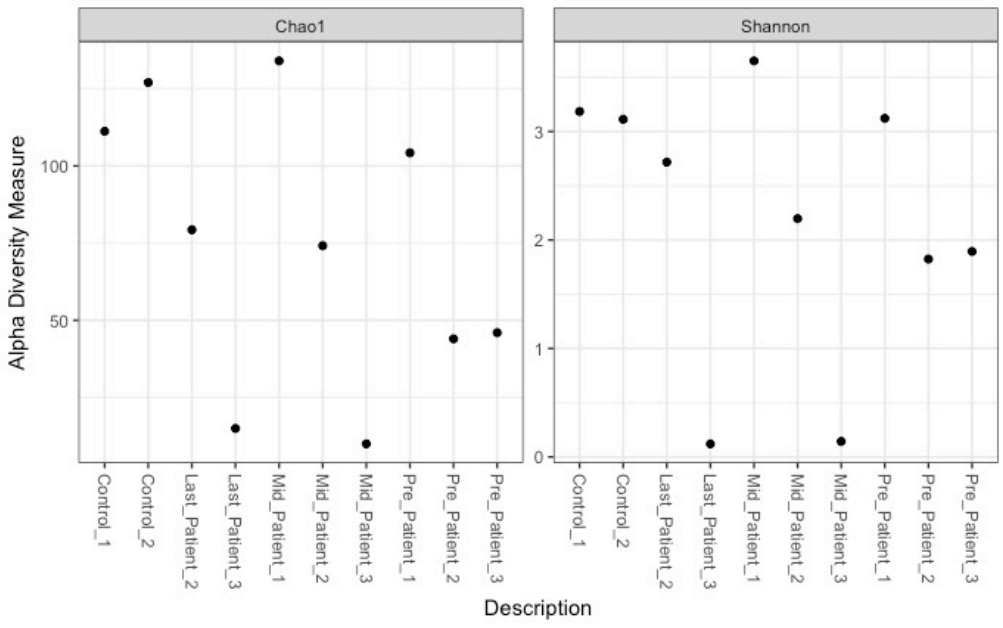

Figure 8: Chao1 and shannon indices per sample

Chao1 and Shannon indices of alpha diversity per sample. Neither index indicated a direct relationship between exposure to radiation and alpha diversity

Table 2:Alpha diversity with reflection to the response to radiation

Sample	Chao1	Shannon	Response to treatment
Control-1	111.2	3.184	NA
Control-2	127	3.11	NA
Pre-patient-1	104.25	3.121	NR
Mid-Patient-1	134	3.651	
Pre-Patient-2	44	1.82	R
Mid-Patient-2	74.167	2.19	
Last-Patient-2	79.333	2.717	
Pre-Patient-3	46	1.894	R
Mid-Patient-3	10	0.143	
Last-patient-3	15	0.12	

NA: Not Available

R: Responded to radiotherapy

NR: Not Responding to radiotherapy

4. Describing the variation in the bacterial abundance at different taxonomic levels

At the phylum level, the two healthy controls showed normal variation between the four most abundant bacterial phyla (Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria) (Figure 9), with domination of Firmicutes and relatively high abundance of Proteobacteria (Odamaki et al., 2016). Control-2 showed relative high abundance of Actinobacteria compared to Control-1. However, a related point to consider is that up to date, there is no database for the Egyptians' gut microbiota profile to which the controls could be compared to obtain a correct reflection with the Egyptian diet and life style.

After the exposure to different doses of radiation that ranged between (21.6 Gy and 50.4 Gy), along with the antibiotic courses (Table-1) the relative abundance of Firmicutes decreased while the Proteobacteria increased in the three patients. This comes in agreement with the previous results of Wang and colleagues (Wang et al., 2015). The phylum abundance frequency table (Table-3) showed the expected disturbance of microbial phyla in cancer patients when compared to the controls. However, when comparing each patient to himself (at the different time points collected), it was found that Actinobacteria, Bacteroidetes and Proteobacteria phyla increased after antibiotics and radiation while Firmicutes decreased. This can give a better insight for the increase in alpha diversity visualized earlier (Figure 8).

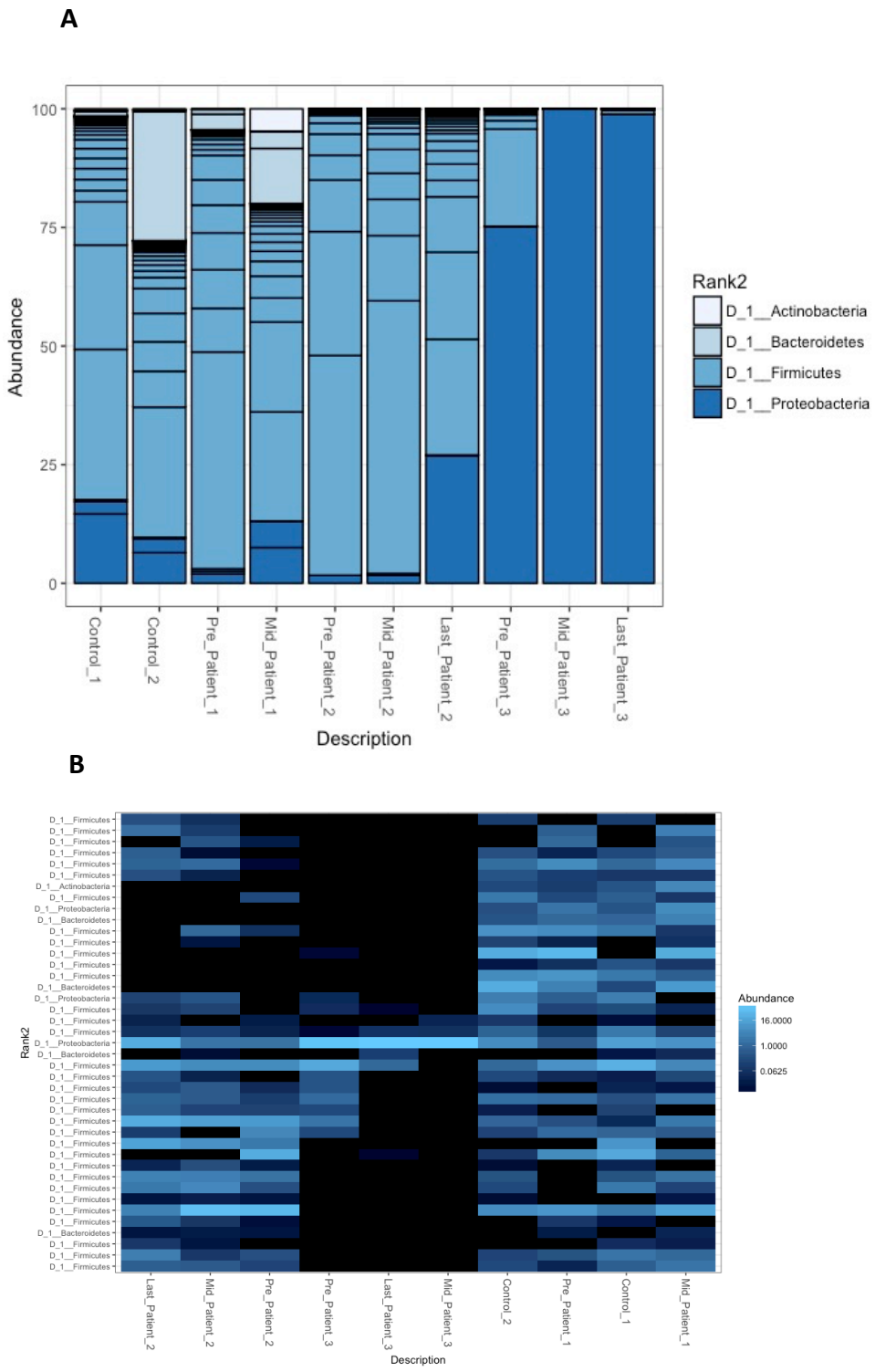

Figure 9: Relative abundance of bacterial species at the phylum level

The abundance of the four major bacterial phyla across different samples. (A) Bar plot showing the relative abundance of the four major phyla in controls and patients. (B) Heat map showing the abundance at the phylum level. The heat map showed that Firmicutes is the dominant phylum across all samples, in exception of patient 3, who has the Proteobacteria phylum dominating over all others. The two healthy controls and patient 1 (in both time points) are quite comparable, this could be due to the lessen exposure of patient 1 to antibiotics. However, the bar plot showed a relative decrease in Firmicutes and increase in Proteobacteria after his exposure to radiation (21.6 Gy)

Table 3: Frequency of each phylum

Phylum	Patient-1		Patient-2			Patient-3			Controls	
	Pre	**Mid**	**Pre**	**Mid**	**Last**	**Pre**	**Mid**	**Last**	**(1)**	**(2)**
Actinobacteria	153	736	0	2	21	4	21	2	211	134
Bacteriodetes	3958	7093	4	12	25	0	0	192	628	9161
Firmicutes	22860	19888	38875	33170	25832	15960	1938	692	34990	26391
Fusobacteria	0	0	0	0	0	0	0	0	0	34
Proteobacteria	607	4554	598	773	8060	29542	66865	49113	7944	9821
Verrucomicrobia	0	0	0	0	0	0	0	0	0	2977
Bacteria (unclassified)	79	63	0	0	0	0	0	0	41	55

The increase in bacterial frequencies in the presence of multiple broad spectrum antibiotic courses along with a high dose (50.4 Gy) of directed gamma radiation at the pelvic region is an abnormal phenomenon. Therefore, a higher resolution or an intuition of more specificity to the bacterial taxa was needed. The frequency table at the genus level was obtained for all samples from qiime2. The search criteria for the specific increasing genera was set as follows: (1) the frequency per patient is higher at each time point (fluctuating taxa were excluded), (2) frequencies per taxa is elevated in the three patients or completely absent in one patient. According to these criteria, eight different genera were identified (Table-4), six of which belong to the Firmicutes phylum (that had overall decreased): one belongs to Proteobacteria and the last is a member of Bacteroidetes phylum.

Table 4: Constantly increasing bacterial genera

Genus	Patient-1		Patient-2			Patient-3			Controls	
	Pre	**Mid**	**Pre**	**Mid**	**Last**	**Pre**	**Mid**	**Last**	**(1)**	**(2)**
Bacteroides	5	23	0	7	22	0	0	192	20	20
Streptococcus	13	22	14	46	72	NA	NA	NA	535	286
Defluviitaleaceae*	127	478	0	98	467	NA	NA	NA	12	0
Dorea	28	309	57	97	135	NA	NA	NA	95	119
Subdoligranulum	309	1434	0	7	79	NA	NA	NA	1909	112
Ruminococcaceae*	1120	2575	6	237	368	NA	NA	NA	444	468
Clostridiales**	25	647	0	3	23	NA	NA	NA	1225	0
Escherichia-shigella	95	1117	598	549	7977	25630	66865	49113	3330	2087

*Family
**Order
NA: Not Available

Full taxonomic classification:

1. Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; *Bacteroides*

2. Bacteria; Firmicutes; Bacilli; Lactobacillales; Streptococcaceae; *Streptococcus*

3. Bacteria; Firmicutes; Clostridia; Clostridiales; Defluviitaleaceae; uncultured

4. Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; *Dorea*

5. Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; *Subdoligranulum*

6. Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae

7. Bacteria; Firmicutes; Clostridia; Clostridiales

8. Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; *Escherichia-Shigella*

Chapter 5: Discussion

The gut microbiota is now being linked to various aspects in life rather than only its role in food digestion. It has been described as the "forgotten organ", owing to its diverse and effective roles (O'Hara & Shanahan, 2006). The relationship between microbiota and cancer has been intensively studied. Various links were created to describe the role of microbiota in developing cancer at different body sites as well as its role in determining the response to treatment, including chemotherapy, radiotherapy or immunotherapy (Gerassy-Vainberg et al., 2018; Gopalakrishnan et al., 2018; Nam et al., 2013; Routy et al., 2018; Viaud et al., 2013).

Crawford and colleagues reported that the GF mice are resistant to high dose (16 Gy) of TBI (Crawford & Gordon, 2005). The described resistance to TBI in GF mice was attributed to the role of the microbiota in inducing severe intestinal inflammation and increasing Interleukin-1β, that result in tissue damage and might lead to mice death. Several studies on human and mice models reported the microbiota dysbiosis after radiotherapy directed to the pelvic region, with a general decrease in Firmicutes and Bacteroidetes along with an increase in Proteobacteria and overall decrease in alpha diversity (Nam et al., 2013; Wang et al., 2015).

In the current observational study, we describe the effect of directing radiotherapy to the pelvic region on the microbial alpha diversity of infant cancer patients who were already on chemotherapy along with intensive doses of various broad-spectrum antibiotics. In comparison with two healthy controls; the patients' alpha diversity was generally lower. However, the extent of reduction was variable among the patients in study. This can be attributed to the different antibiotics courses prescribed for each, independent of the others.

On the phylum level, Firmicutes decreased post radiation while Proteobacteria increased. This agrees with previously described dysbiosis after radiotherapy in adults (Nam et al., 2013; Wang et al., 2015). Our results contradicted those by Nam and colleagues who reported an elevation in the relative abundance of Actinobacteria in gynecological cancer patients compared to healthy individuals (Nam et al., 2013). In their study, they selected gynecological cancer patients who did not take any antibiotics at the time of sample collection. However, excluding antibiotics from infants' treatment protocols was not possible. Therefore, the variation observed might be attributed to antibiotics. Moreover, by comparing actinobacterial abundance between patient-1 (at least 2 months between last antibiotic and point zero collection), patients 2 and 3 (last antibiotic course was on the same month of sample collection) we can find that Patient-1 has more Actinobacteria. In general, Patient-1 had higher alpha diversity (chao1: 104, Shannon: 3.121) that was comparable to the controls (chao1: 111, 127 and Shannon: 3.18, 3.11 respectively). This could be due to the long gap between the last antibiotic dose and point zero collection time, since it was previously reported that the microbiota can fully rebound in most patients after antibiotics courses within 90 days (Raymond et al., 2016).

By comparing the alpha diversity indices for each patient to himself at the three sample collection points, we found that Patient-3 experienced a drop in alpha diversity post radiation and antibiotics. On the contrary Patients 1 and 2 had surprisingly higher alpha diversity post treatment. We further defined the taxa that were constantly increasing at the three-time points and the three patients at the highest resolution that could be inferred from the sequencing reads. Accordingly, eight taxa were identified (*Bacteroides*, *Streptococcus*, Defluviitaleaceae, *Dorea,* *Subdoligranulum*, Ruminococcaceae, Clostridiales and *Escherichia-shigella*). Except for *Bacteroides* that belongs to Bacteroidetes phylum and *Escherichia-shigella* that

belongs to Proteobacteria phylum, all remaining six taxa belong to Firmicutes phylum that was generally decreasing.

When ionizing radiation is directed to the targeted body location, it primarily disrupts the cellular macromolecules including proteins, lipids and DNA resulting in mutations that might trigger the DNA damage response pathways or activate one of the cellular death mechanisms that include: apoptosis, autophagy or necrosis (Van Der Kogel et al., 2009). The damaging effect of ionizing radiation on the bacterial cells is reflected on DNA mutations as in eukaryotes. However, the radiosensitivity of bacteria differs greatly from one bacterial species to another. Some bacterial species are known to be radiosensitive, while others are extremely radioresistant, e.g. *Deinococcus radiodurans* that can withstand up to 5000 Gy of gamma irradiation (Slade et al., 2011). Goudarzi and colleagues studied the effect of 12 Gy of X-ray irradiation on the gut microbiome of mice; they reported an increase in families *Lactobacillaceae* and *Staphylococcaceae* while the *Lachnospiraceae*, *Ruminococcaceae* and *Clostridiacea* families have decreased in their relative abundance (Goudarzi et al., 2016). Direct comparison between those reported variations in relative abundance to the variations we have reported (all five families were increasing in relative abundance post radiation) is not rational because of the following reasons: (1) the reported changes were due to low dose X-ray irradiation, while we used 50 Gy of gamma irradiation, (2) our study was conducted in the presence of antibiotics and chemotherapy, which both affect the microbiota. Raymond and colleagues have studied the effect of antibiotics on microbiome using deep shotgun sequencing and reported the detection of resistant genes post antibiotic treatment, that might have been related to an increase in relative abundance of those species post antibiotics (Raymond et al., 2016). Accordingly, what could be inferred is the possible radiosensitivity of the fluctuating bacterial families. Further studies are needed to confirm with reflection to the

possible mutations in antibiotics resistant genes that might increase or decrease the antibiotics sensitivity to the target bacteria.

In conclusion, the relationship between the gut microbiome and cancer treatments is ultimately complicated. The increase in relative abundance of eight taxa (*Bacteroides*, *Streptococcus*, Defluviitaleaceae, *Dorea,* *Subdoligranulum*, Ruminococcaceae, Clostridiales and *Escherichia-shigella*) together with the previously reported fluctuation in the same taxa might indicate their radiosensitivity. However, further studies are needed to confirm. The gut microbiota profile of the patients' prior cancer treatment may predict the response, while the proper manipulation of the gut microbiome can improve the effect of cancer therapy.

Reference

A. Gustafsson, A. Berstad, S. Lund-, A. B. S. L.-T. T. M. E. N. (1999). The Effect of Faecal Enema on Five Microflora-Associated Characteristics in Patients with Antibiotic-Associated Diarrhoea. *Scandinavian Journal of Gastroenterology*, *34*(6), 580–586.

Abbasakoor, F., Vaizey, C. J., & Boulos, P. B. (2006). Improving the morbidity of anorectal injury from pelvic radiotherapy. *Colorectal Disease*, *8*(1), 2–10.

Abdallah Ismail, N., Ragab, S. H., Abd Elbaky, A., Shoeib, A. R. S., Alhosary, Y., & Fekry, D. (2011). Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. *Archives of Medical Science : AMS*, *7*(3), 501–7.

Alexander, J. L., Wilson, I. D., Teare, J., Marchesi, J. R., Nicholson, J. K., & Kinross, J. M. (2017). Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nature Reviews Gastroenterology & Hepatology*, *14*(6), 356–365.

Amir, A., McDonald, D., Navas-Molina, J. A., Kopylova, E., Morton, J. T., Zech Xu, Z., … Knight, R. (2017). Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. *mSystems*, *2*(2), e00191-16.

Armougom, F., Henry, M., Vialettes, B., Raccah, D., & Raoult, D. (2009). Monitoring Bacterial Community of Human Gut Microbiota Reveals an Increase in Lactobacillus in Obese Patients and Methanogens in Anorexic Patients. *PLoS ONE*, *4*(9), e7125.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D. R., … Bork, P. (2011). Enterotypes of the human gut microbiome. *Nature*, *473*(7346), 174–180.

Azad, M. B., Konya, T., Maughan, H., Guttman, D. S., Field, C. J., Sears, M. R., … Kozyrskyj, A. L. (2013). Infant gut microbiota and the hygiene hypothesis of allergic disease: impact of household pets and siblings on microbiota composition and diversity. *Allergy, Asthma, and Clinical Immunology : Official Journal of the Canadian Society of Allergy and Clinical Immunology*, *9*(1), 15.

Aziz, R. K. (2009). A hundred-year-old insight into the gut microbiome! *Gut Pathogens*, *1*(1), 21.

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., … Finlay, B. B. (2012). Defining a Healthy Human Gut Microbiome: Current Concepts, Future Directions, and Clinical Applications. *Cell Host & Microbe*, *12*(5), 611–622.

Baganz, N. L., & Blakely, R. D. (2013). A Dialogue between the Immune System and Brain, Spoken in the Language of Serotonin. *ACS Chemical Neuroscience*, *4*(1), 48–63.

Belzer, C., & de Vos, W. M. (2012). Microbes inside—from diversity to function: the case of Akkermansia. *The ISME Journal*, *6*(8), 1449–1458.

Bergström, A., Skov, T. H., Bahl, M. I., Roager, H. M., Christensen, L. B., Ejlerskov, K. T., … Licht, T. R. (2014). Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large

cohort of Danish infants. *Applied and Environmental Microbiology*, *80*(9), 2889–900.
Bervoets, L., Van Hoorenbeeck, K., Kortleven, I., Van Noten, C., Hens, N., Vael, C., … Vankerckhoven, V. (2013). Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut Pathogens*, *5*(1), 10.
Bircher, J. (2005). Towards a Dynamic Definition of Health and Disease. *Medicine, Health Care and Philosophy*, *8*(3), 335–341.
Bismar, M. M., & Sinicrope, F. A. (2002). Radiation enteritis. *Current Gastroenterology Reports*, *4*(5), 361–5.
Blaser, M. J. (2006). Who are we? Indigenous microbes and the ecology of human diseases. *EMBO Reports*, *7*(10), 956–60.
Bornschein, J., & Malfertheiner, P. (2014). Helicobacter pylori and gastric cancer. *Digestive Diseases (Basel, Switzerland)*, *32*(3), 249–64.
Borody, T. J., & Khoruts, A. (2012). Fecal microbiota transplantation and emerging applications. *Nature Reviews Gastroenterology & Hepatology*, *9*(2), 88–96.
Buffie, C. G., Jarchum, I., Equinda, M., Lipuma, L., Gobourne, A., Viale, A., … Pamer, E. G. (2012). Profound alterations of intestinal microbiota following a single dose of clindamycin results in sustained susceptibility to Clostridium difficile-induced colitis. *Infection and Immunity*, *80*(1), 62–73.
Burdelya, L. G., Krivokrysenko, V. I., Tallant, T. C., Strom, E., Gleiberman, A. S., Gupta, D., … Gudkov, A. V. (2008). An Agonist of Toll-Like Receptor 5 Has Radioprotective Activity in Mouse and Primate Models. *Science*, *320*(5873), 226–230.
Castellarin, M., Warren, R. L., Freeman, J. D., Dreolini, L., Krzywinski, M., Strauss, J., … Holt, R. A. (2012). Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Research*, *22*(2), 299–306.
Chabbi-Achengli, Y., Coudert, A. E., Callebert, J., Geoffroy, V., Cote, F., Collet, C., & de Vernejoul, M.-C. (2012). Decreased osteoclastogenesis in serotonin-deficient mice. *Proceedings of the National Academy of Sciences*, *109*(7), 2567–2572.
Chakraborti, C. K. (2015). New-found link between microbiota and obesity. *World Journal of Gastrointestinal Pathophysiology*, *6*(4), 110–9.
Chase, D., Goulder, A., Zenhausern, F., Monk, B., & Herbst-Kralovetz, M. (2015). The vaginal and gastrointestinal microbiomes in gynecologic cancers: A review of applications in etiology, symptoms and treatment. *Gynecologic Oncology*, *138*(1), 190–200.
Chow, J., Lee, S. M., Shen, Y., Khosravi, A., & Mazmanian, S. K. (2010). Host-bacterial symbiosis in health and disease. *Advances in Immunology*, *107*, 243–74.
Crawford, P. A., & Gordon, J. I. (2005). Microbial regulation of intestinal radiosensitivity. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(37), 13254–9.
Cuevas-Ramos, G., Petit, C. R., Marcq, I., Boury, M., Oswald, E., &

Nougayrede, J.-P. (2010). Escherichia coli induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proceedings of the National Academy of Sciences*, *107*(25), 11537–11542.

David, L. a, Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., … Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559–63.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J. B., Massart, S., … Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(33), 14691–6.

den Besten, G., van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D.-J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of Lipid Research*, *54*(9), 2325–2340.

Donaldson, G. P., Lee, S. M., & Mazmanian, S. K. (2015). Gut biogeography of the bacterial microbiota. *Nature Reviews Microbiology*, *14*(1), 20–32.

Duncan, S. H., Lobley, G. E., Holtrop, G., Ince, J., Johnstone, A. M., Louis, P., & Flint, H. J. (2008). Human colonic microbiota associated with diet, obesity and weight loss. *International Journal of Obesity*, *32*(11), 1720–1724.

Ebihara, K., Tachibe, M., Kaneko, N., Kishida, T., Kabeerdoss, J., Ferdous, S., … Ramakrishna, B. S. (2013). Hydroxypropylation of high-amylose maize starch changes digestion andfermentation-dependent parameters in rats / Development of the gut microbiota insouthern Indian infants from birth to 6 months: a molecular analysis – ERRATUM. *Journal of Nutritional Science*, *2*.

Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., … Relman, D. A. (2005). Diversity of the Human Intestinal Microbial Flora. *Science*, *308*(5728), 1635–1638.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461.

Eiseman, B., SILEN, W., BASCOM, G. S., & KAUVAR, A. J. (1958). Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery*, *44*(5), 854–9.

Ferreira, M. R., Muls, A., Dearnaley, D. P., & Andreyev, H. J. N. (2014). Microbiota and radiation-induced bowel toxicity: lessons from inflammatory bowel disease for the radiation oncologist. *The Lancet Oncology*, *15*(3), e139–e147.

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, *3*(4), 289–306.

Gerassy-Vainberg, S., Blatt, A., Danin-Poleg, Y., Gershovich, K., Sabo, E., Nevelsky, A., … Chowers, Y. (2018). Radiation induces proinflammatory dysbiosis: transmission of inflammatory susceptibility by host cytokine induction. *Gut*, *67*(1), 97–107.

Gerding, D. N. (2004). Clindamycin, Cephalosporins, Fluoroquinolones, and

Clostridium difficile –Associated Diarrhea: This Is an Antimicrobial Resistance Problem. *Clinical Infectious Diseases*, *38*(5), 646–648.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of Nutrition*, *125*(6), 1401–12.

Goodrich, J. K., Waters, J. L., Poole, A. C., Sutter, J. L., Koren, O., Blekhman, R., … Ley, R. E. (2014). Human genetics shape the gut microbiome. *Cell*, *159*(4), 789–99.

Gopalakrishnan, V., Spencer, C. N., Nezi, L., Reuben, A., Andrews, M. C., Karpinets, T. V, … Wargo, J. A. (2018). Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science (New York, N.Y.)*, *359*(6371), 97–103.

Goudarzi, M., Mak, T. D., Jacobs, J. P., Moon, B.-H., Strawn, S. J., Braun, J., … Li, H.-H. (2016). An Integrated Multi-Omic Approach to Assess Radiation Injury on the Host-Microbiome Axis. *Radiation Research*, *186*(3), 219–234.

Grönlund, M. M., Lehtonen, O. P., Eerola, E., & Kero, P. (1999). Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *Journal of Pediatric Gastroenterology and Nutrition*, *28*(1), 19–25.

Gu, S., Chen, D., Zhang, J.-N., Lv, X., Wang, K., Duan, L.-P., … Wu, X.-L. (2013). Bacterial community mapping of the mouse gastrointestinal tract. *PloS One*, *8*(10), e74957.

Hall, A. B., Tolonen, A. C., & Xavier, R. J. (2017). Human genetic variation and the gut microbiome in disease. *Nature Reviews Genetics*, *18*(11), 690–699.

Hammami, R., Fernandez, B., Lacroix, C., & Fliss, I. (2013). Anti-infective properties of bacteriocins: an update. *Cellular and Molecular Life Sciences*, *70*(16), 2947–2967.

Hansen, E. E., Lozupone, C. A., Rey, F. E., Wu, M., Guruge, J. L., Narra, A., … Gordon, J. I. (2011). Pan-genome of the dominant human gut-associated archaeon, Methanobrevibacter smithii, studied in twins. *Proceedings of the National Academy of Sciences*, *108*(Supplement_1), 4599–4606.

Hartstra, A. V., Bouter, K. E. C., Bäckhed, F., & Nieuwdorp, M. (2015). Insights Into the Role of the Microbiome in Obesity and Type 2 Diabetes. *Diabetes Care*, *38*(1), 159–165.

Hehemann, J.-H., Correc, G., Barbeyron, T., Helbert, W., Czjzek, M., & Michel, G. (2010). Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. *Nature*, *464*(7290), 908–912.

Hoffmann, D. E., Palumbo, F. B., Ravel, J., Rowthorn, V., & von Rosenvinge, E. (2017). A proposed definition of microbiota transplantation for regulatory purposes. *Gut Microbes*, *8*(3), 208–213.

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions Between the Microbiota and the Immune System. *Science*, *336*(6086), 1268–1273.

Hooper, L. V., Stappenbeck, T. S., Hong, C. V., & Gordon, J. I. (2003). Angiogenins: a new class of microbicidal proteins involved in innate

immunity. *Nature Immunology*, *4*(3), 269–273.
Hsiao, W. W. L., Metz, C., Singh, D. P., & Roth, J. (2008). The Microbes of the Intestine: An Introduction to Their Metabolic and Signaling Capabilities. *Endocrinology and Metabolism Clinics of North America*, *37*(4), 857–871.
Huang, Y., Fan, X.-G., Wang, Z.-M., Zhou, J.-H., Tian, X.-F., & Li, N. (2004). Identification of helicobacter species in human liver samples from patients with primary hepatocellular carcinoma. *Journal of Clinical Pathology*, *57*(12), 1273–1277.
Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., … White, O. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, *486*(7402), 207–214.
Huurre, A., Kalliomäki, M., Rautava, S., Rinne, M., Salminen, S., & Isolauri, E. (2008). Mode of Delivery – Effects on Gut Microbiota and Humoral Immunity. *Neonatology*, *93*(4), 236–240.
Iida, N., Dzutsev, A., Stewart, C. A., Smith, L., Bouladoux, N., Weingarten, R. A., … Goldszmid, R. S. (2013). Commensal Bacteria Control Cancer Response to Therapy by Modulating the Tumor Microenvironment. *Science*, *342*(6161), 967–970.
Islami, F., & Kamangar, F. (2008). Helicobacter pylori and esophageal cancer risk: a meta-analysis. *Cancer Prevention Research (Philadelphia, Pa.)*, *1*(5), 329–38.
K Gogineni, V., Morrow, L. E., & Malesker, M. A. (2013). Probiotics: Mechanisms of Action and Clinical Applications. *Journal of Probiotics & Health*, *1*(1), 1–11.
Kaur, I. P., Kuhad, A., Garg, A., & Chopra, K. (2009). Probiotics: Delineation of Prophylactic and Therapeutic Benefits. *Journal of Medicinal Food*, *12*(2), 219–235.
Koenig, J. E., Spor, A., Scalfone, N., Fricker, A. D., Stombaugh, J., Knight, R., … Ley, R. E. (2011). Succession of microbial consortia in the developing infant gut microbiome. *Proceedings of the National Academy of Sciences*, *108*(Supplement_1), 4578–4585.
Kundu, P., Blacher, E., Elinav, E., & Pettersson, S. (2017). Our Gut Microbiome: The Evolving Inner Self. *Cell*, *171*(7), 1481–1493.
Kusters, J. G., van Vliet, A. H. M., & Kuipers, E. J. (2006). Pathogenesis of Helicobacter pylori Infection. *Clinical Microbiology Reviews*, *19*(3), 449–490.
Lawley, T. D., Clare, S., Deakin, L. J., Goulding, D., Yen, J. L., Raisen, C., … Dougan, G. (2010). Use of Purified Clostridium difficile Spores To Facilitate Evaluation of Health Care Disinfection Regimens. *Applied and Environmental Microbiology*, *76*(20), 6895–6900.
Lawley, T. D., Clare, S., Walker, A. W., Stares, M. D., Connor, T. R., Raisen, C., … Dougan, G. (2012). Targeted Restoration of the Intestinal Microbiota with a Simple, Defined Bacteriotherapy Resolves Relapsing Clostridium difficile Disease in Mice. *PLoS Pathogens*, *8*(10), e1002995.
Luna, R. A., & Foster, J. A. (2015). Gut brain axis: diet microbiota interactions and implications for modulation of anxiety and depression. *Current Opinion in Biotechnology*, *32*, 35–41.

McCarthy, A. J., Martin, P., Cloup, E., Stabler, R. A., Oswald, E., & Taylor, P. W. (2015). The Genotoxin Colibactin Is a Determinant of Virulence in Escherichia coli K1 Experimental Neonatal Systemic Infection. *Infection and Immunity*, *83*(9), 3704–11.

McDonald, L. C., Owings, M., & Jernigan, D. B. (2006). Clostridium difficile infection in patients discharged from US short-stay hospitals, 1996-2003. *Emerging Infectious Diseases*, *12*(3), 409–15.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, *8*(4), e61217.

McMurdie, P. J., & Holmes, S. (2014). Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLoS Computational Biology*, *10*(4), e1003531.

Möndel, M., Schroeder, B. O., Zimmermann, K., Huber, H., Nuding, S., Beisner, J., … Wehkamp, J. (2009). Probiotic E. coli treatment mediates antimicrobial human β-defensin synthesis and fecal excretion in humans. *Mucosal Immunology*, *2*(2), 166–172.

Nam, Y.-D., Kim, H. J., Seo, J.-G., Kang, S. W., & Bae, J.-W. (2013). Impact of Pelvic Radiotherapy on Gut Microbiota of Gynecological Cancer Patients Revealed by Massive Pyrosequencing. *PLoS ONE*, *8*(12), e82659.

Nougayrede, J.-P., Homburg, S., Taieb, F., Boury, M., Brzuszkiewicz, E., Gottschalk, G., … Oswald, E. (2006). Escherichia coli Induces DNA Double-Strand Breaks in Eukaryotic Cells. *Science*, *313*(5788), 848–851.

O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO Reports*, *7*(7), 688–693.

Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J.-Z., … Osawa, R. (2016). Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiology*, *16*, 90.

Packey, C. D., & Ciorba, M. A. (2010). Microbial influences on the small intestinal response to radiation injury. *Current Opinion in Gastroenterology*, *26*(2), 88–94.

Parsonnet, J. (1993). Helicobacter pylori and gastric cancer. *Gastroenterology Clinics of North America*, *22*(1), 89–104. Retrieved from

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., … Wang, J. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, *464*(7285), 59–65.

Raymond, F., Ouameur, A. A., Déraspe, M., Iqbal, N., Gingras, H., Dridi, B., … Corbeil, J. (2016). The initial state of the human gut microbiome determines its reshaping by antibiotics. *The ISME Journal*, *10*(3), 707–720.

Rizkallah, M., Saad, R., & Aziz, R. (2010). The Human Microbiome Project, Personalized Medicine and the Birth of Pharmacomicrobiomics. *Current Pharmacogenomics and Personalized Medicine*, *8*(3), 182–193.

Roderburg, C., & Luedde, T. (2014). The role of the gut microbiome in the development and progression of liver cirrhosis and hepatocellular carcinoma. *Gut Microbes*, *5*(4), 441–445.

Rohmer, L., Hocquet, D., & Miller, S. I. (2011). Are pathogenic bacteria just looking for food? Metabolism and microbial pathogenesis. *Trends in Microbiology*, *19*(7), 341–348.

Round, J. L., & Mazmanian, S. K. (2009). The gut microbiota shapes intestinal immune responses during health and disease. *Nature Reviews Immunology*, *9*(5), 313–323.

Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P. M., Alou, M. T., Daillère, R., … Zitvogel, L. (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science (New York, N.Y.)*, *359*(6371), 91–97.

Rupnik, M., Wilcox, M. H., & Gerding, D. N. (2009). Clostridium difficile infection: new developments in epidemiology and pathogenesis. *Nature Reviews Microbiology*, *7*(7), 526–536.

Rutayisire, E., Huang, K., Liu, Y., & Tao, F. (2016). The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterology*, *16*(1), 86.

Scheithauer, T. P. M., Dallinga-Thie, G. M., de Vos, W. M., Nieuwdorp, M., & van Raalte, D. H. (2016). Causality of small and large intestinal microbiota in weight regulation and insulin resistance. *Molecular Metabolism*, *5*(9), 759–70.

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics--approaching a definition. *The American Journal of Clinical Nutrition*, *73*(2 Suppl), 361S–364S. Retrieved from

Schwabe, R. F., & Jobin, C. (2013). The microbiome and cancer. *Nature Reviews Cancer*, *13*(11), 800–812.

Semova, I., Carten, J. D., Stombaugh, J., Mackey, L. C., Knight, R., Farber, S. A., & Rawls, J. F. (2012). Microbiota Regulate Intestinal Absorption and Metabolism of Fatty Acids in the Zebrafish. *Cell Host & Microbe*, *12*(3), 277–288.

Shade, A., & Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. *Environmental Microbiology*, *14*(1), 4–12.

Shoaie, S., Karlsson, F., Mardinoglu, A., Nookaew, I., Bordel, S., & Nielsen, J. (2013). Understanding the interactions between bacteria in the human gut through metabolic modeling. *Scientific Reports*, *3*(1), 2532.

Slade, D., & Radman, M. (2011). Oxidative stress resistance in Deinococcus radiodurans. *Microbiology and Molecular Biology Reviews : MMBR*, *75*(1), 133–91.

Stern, A., Mick, E., Tirosh, I., Sagy, O., & Sorek, R. (2012). CRISPR targeting reveals a reservoir of common phages associated with the human gut microbiome. *Genome Research*, *22*(10), 1985–1994.

Suzuki, H., Iwasaki, E., & Hibi, T. (2009). Helicobacter pylori and gastric cancer. *Gastric Cancer*, *12*(2), 79–87.

Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., … Gordon, J. I. (2009). A core gut microbiome in obese and lean twins. *Nature*, *457*(7228), 480–4. https://doi.org/10.1038/nature07540

Vaishnava, S., Behrendt, C. L., Ismail, A. S., Eckmann, L., & Hooper, L. V.

(2008). Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(52), 20858–63.

Van Der Kogel, A., & Arnold, H. (2009). Basic Clinical Radiobiology.

Van Loo, J., Coussement, P., De Leenheer, L., Hoebregs, H., & Smits, G. (1995). On the presence of Inulin and Oligofructose as natural ingredients in the western diet. *Critical Reviews in Food Science and Nutrition*, *35*(6), 525–552.

Viaud, S., Saccheri, F., Mignot, G., Yamazaki, T., Daillère, R., Hannani, D., … Zitvogel, L. (2013). The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science (New York, N.Y.)*, *342*(6161), 971–6.

Vrieze, A., Van Nood, E., Holleman, F., Salojärvi, J., Kootte, R. S., Bartelsman, J. F. W. M., … Nieuwdorp, M. (2012). Transfer of Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome. *Gastroenterology*, *143*(4), 913–916.e7.

Wang, A., Ling, Z., Yang, Z., Kiela, P. R., Wang, T., Wang, C., … Wang, J. (2015). Gut Microbial Dysbiosis May Predict Diarrhea and Fatigue in Patients Undergoing Pelvic Cancer Radiotherapy: A Pilot Study. *PLOS ONE*, *10*(5), e0126312.

WHO. (2001). Probiotics in food Health and nutritional properties and guidelines for evaluation FAO FOOD AND NUTRITION PAPER.

Wishart, D. S., Feunang, Y. D., Guo, A. C., Lo, E. J., Marcu, A., Grant, J. R., … Wilson, M. (2018). DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Research*, *46*(D1), D1074–D1082.

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., … Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science (New York, N.Y.)*, *334*(6052), 105–8.

Wu, X., Vallance, B. A., Boyer, L., Bergstrom, K. S. B., Walker, J., Madsen, K., … Jacobson, K. (2008). *Saccharomyces boulardii* ameliorates *Citrobacter rodentium* -induced colitis through actions on bacterial virulence factors. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *294*(1), G295–G306.

Xiao, S., Fei, N., Pang, X., Shen, J., Wang, L., Zhang, B., … Zhao, L. (2014). A gut microbiota-targeted dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome. *FEMS Microbiology Ecology*, *87*(2), 357–367.

Yano, J. M., Yu, K., Donaldson, G. P., Shastri, G. G., Ann, P., Ma, L., … Hsiao, E. Y. (2015). Indigenous Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis. *Cell*, *161*(2), 264–276.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, (Ivic).

Yoshimoto, S., Loo, T. M., Atarashi, K., Kanda, H., Sato, S., Oyadomari, S., … Ohtani, N. (2013). Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature*, *499*(7456), 97–101.

Zwielehner, J., Lassl, C., Hippe, B., Pointner, A., Switzeny, O. J., Remely, M., … Haslberger, A. G. (2011). Changes in Human Fecal Microbiota Due to Chemotherapy Analyzed by TaqMan-PCR, 454 Sequencing and PCR-DGGE Fingerprinting. *PLoS ONE*, *6*(12), e28654.

Appendix 1- Scripts

```
Qiime2-2017.12

#Generate OTU table (feature table), Assign Taxonomy (SILVA full
length) and generate the phylogenetic tree

#Import sequence files to Qiime2
#manifest phred33

#create a manifest file
#rename file names as in the manifest

qiime tools import --type 'SampleData[PairedEndSequencesWithQuality]'
--input-path pe-33-manifest --output-path paired-end-demux.qza --
source-format PairedEndFastqManifestPhred33

#quality check

qiime demux summarize \
  --i-data paired-end-demux.qza \
  --o-visualization demux-summary-1.qzv

#The sequences are sorted inline from eurofins, sequences sorted by
barcode then trimmed.


#quality_filter

 qiime quality-filter q-score \
 --i-demux paired-end-demux.qza \
 --o-filtered-sequences demux-filtered.qza \
 --o-filter-stats demux-filter-stats.qza


#denoise_after_filtering

 qiime deblur denoise-16S \
  --i-demultiplexed-seqs demux-filtered.qza \
  --p-trim-length 230 \
  --o-representative-sequences rep-seqs-deblur.qza \
  --o-table table-deblur.qza \
  --p-sample-stats \
  --o-stats deblur-stats.qza


mv rep-seqs-deblur.qza rep-seqs.qza
mv table-deblur.qza table.qza


#create qiime visualizations

qiime metadata tabulate \
  --m-input-file demux-filter-stats.qza \
  --o-visualization demux-filter-stats.qzv

qiime deblur visualize-stats \
  --i-deblur-stats deblur-stats.qza \
  --o-visualization deblur-stats.qzv
```

```
#create a metadata file on google sheets and save as (.tsv)
#feature table and feature summary

qiime feature-table summarize \
  --i-table table.qza \
  --o-visualization table.qzv \
  --m-sample-metadata-file sample-metadata.tsv


qiime feature-table tabulate-seqs \
  --i-data rep-seqs.qza \
  --o-visualization rep-seqs.qzv


#Generate a tree for phylogenetic diversity analyses

qiime alignment mafft \
  --i-sequences rep-seqs.qza \
  --o-alignment aligned-rep-seqs.qza


#mask or filter

qiime alignment mask \
  --i-alignment aligned-rep-seqs.qza \
  --o-masked-alignment masked-aligned-rep-seqs.qza

#create a phylogenetic tree

qiime phylogeny fasttree \
  --i-alignment masked-aligned-rep-seqs.qza \
  --o-tree unrooted-tree.qza


#taxonomy_silva_full_length

qiime feature-classifier classify-sklearn \
  --i-classifier silva-119-99-nb-classifier.qza \
  --i-reads rep-seqs.qza \
  --o-classification taxonomy.qza


qiime metadata tabulate \
  --m-input-file taxonomy.qza \
  --o-visualization taxonomy.qzv


#bar_plot

qiime taxa barplot \
  --i-table table.qza \
  --i-taxonomy taxonomy.qza \
  --m-metadata-file sample-metadata.tsv \
  --o-visualization taxa-bar-plots.qzv


#export to biom hdf5


qiime tools export \
  table.qza \
  --output-dir exported-feature-table
```

```
#export the following files to be used in R-phyloseq:
feature table in biom format
taxonomy (export as tsv)
re_seq_file.fasta
tree.nwk
metadata.tsv

#append taxonomy classification to feature table and convert to biom
format

biom add-metadata -i feature-table.biom -o table.w_omd.biom --
observation-metadata-fp taxonomy.tsv --observation-header
OTUID,taxonomy --sc-separated taxonomy
```

Usearch Script

```
#fastq_info_command_in_fq_directory

mkdir -p ../fastq_info
for fq in *.fastq
do
  usearch -fastx_info $fq -output ../fastq_infotrim/$fq
done

#extract EE

cd ../fastq_infotrim
grep "^EE" *

#quality_chart

usearch -fastq_chars 16s_sample1_2_V3V5_FWD.fastq -log chars.log

#trimming_to_200bp
#Sample1

usearch -fastx_truncate 16s_sample1_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout PatA1_R1.fastq

#Sample2

usearch -fastx_truncate 16s_sample2_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout PatA2_R1.fastq


#sample3

usearch -fastx_truncate 16s_sample3_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout PatB1_R1.fastq

#sample4

usearch -fastx_truncate 16s_sample4_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout PatB2_R1.fastq

#sample5

usearch -fastx_truncate 16s_sample5_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout PatB3_R1.fastq

#sample6

usearch -fastx_truncate 16s_sample6_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout PatC1_R1.fastq

#sample7

usearch -fastx_truncate 16s_sample7_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout PatC2_R1.fastq

#sample8

usearch -fastx_truncate 16s_sample8_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout PatC3_R1.fastq
```

```
#sample9

usearch -fastx_truncate 16s_sample9_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout Cont1_R1.fastq

#sample10

usearch -fastx_truncate 16s_sample10_2_V3V5_FWD.fastq -trunclen
200 -label_suffix _R1 -fastqout Cont2_R1.fastq

#Sample1_REV

usearch -fastx_truncate 16s_sample1_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout PatA1_R2.fastq


#sample2_REV

usearch -fastx_truncate 16s_sample2_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout PatA2_R2.fastq


#Sample3_REV

usearch -fastx_truncate 16s_sample3_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout PatB1_R2.fastq


#sample4_REV

usearch -fastx_truncate 16s_sample4_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout PatB2_R2.fastq


#sample5_REV

usearch -fastx_truncate 16s_sample5_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout PatB3_R2.fastq

#sample6_REV

usearch -fastx_truncate 16s_sample6_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout PatC1_R2.fastq

#sample7_REV

usearch -fastx_truncate 16s_sample7_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout PatC2_R2.fastq

#sample8

usearch -fastx_truncate 16s_sample8_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout PatC3_R2.fastq

#sample9

usearch -fastx_truncate 16s_sample9_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout Cont1_R2.fastq

#sample10_REV

usearch -fastx_truncate 16s_sample10_2_V3V5_REV.fastq -trunclen
200 -label_suffix _R2 -fastqout Cont2_R2.fastq
```

```
#cat command to pool FWD and REV (applied to the 10 samples)

cat Cont1_R1.fq Cont1_R2.fq > Cont1_R.fq


#cat all reads in one file

cat samples.txt  | while read sample ; do cat $sample"_R2.fq" | paste
- - - - | awk -v sample=$sample 'BEGIN {FS="\t"} {print
"@"sample"."NR"\n"$2"\n"$3"\n"$4;}' > $sample.fastq ; done
cat *.fastq > tmp
mv tmp merged.fq

#filter_sequences

usearch -fastq_filter merged.fq -fastq_maxee 1.0  -fastaout
filtered.fa -relabel Filt

#finding_uniques

usearch -fastx_uniques filtered.fa -sizeout -relabel Uniq -fastaout
uniques.fa

#Cluster_OTU

usearch -cluster_otus uniques.fa -otus otus.fa -relabel Otu


#OTU table

usearch -otutab merged.fq -otus otus.fa -otutabout otutab_raw.txt -
biomout otutab.json -mapout map.txt -notmatched unmapped.fa -
dbmatched otus_with_sizes.fa -sizeout
```

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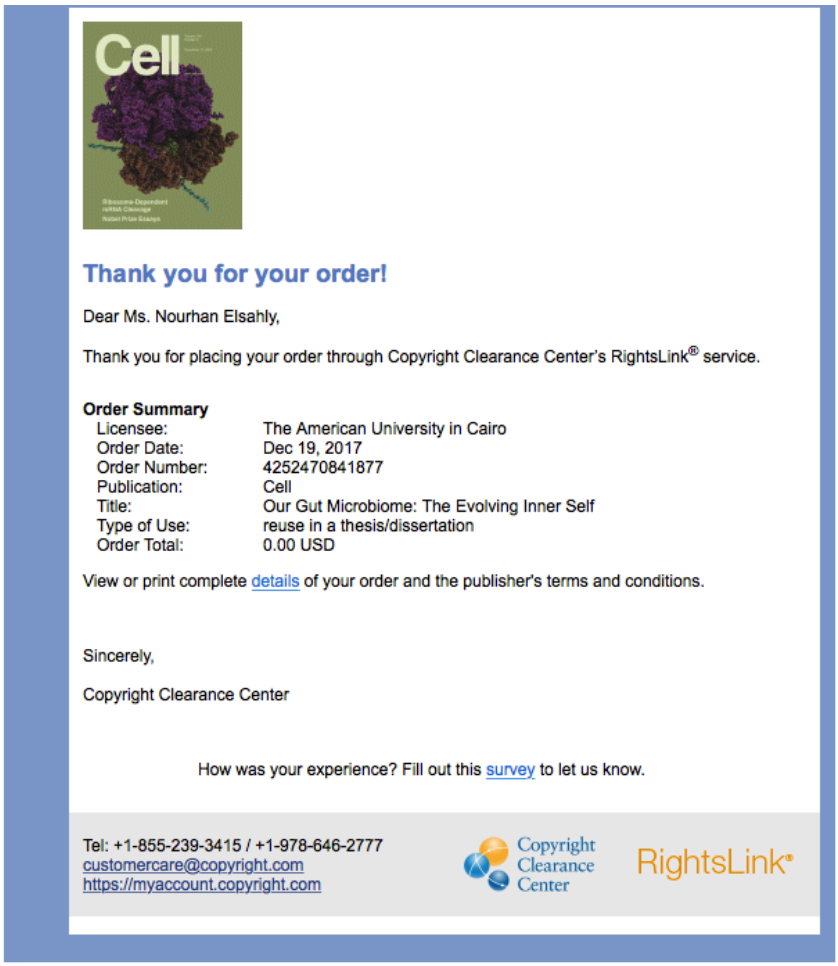

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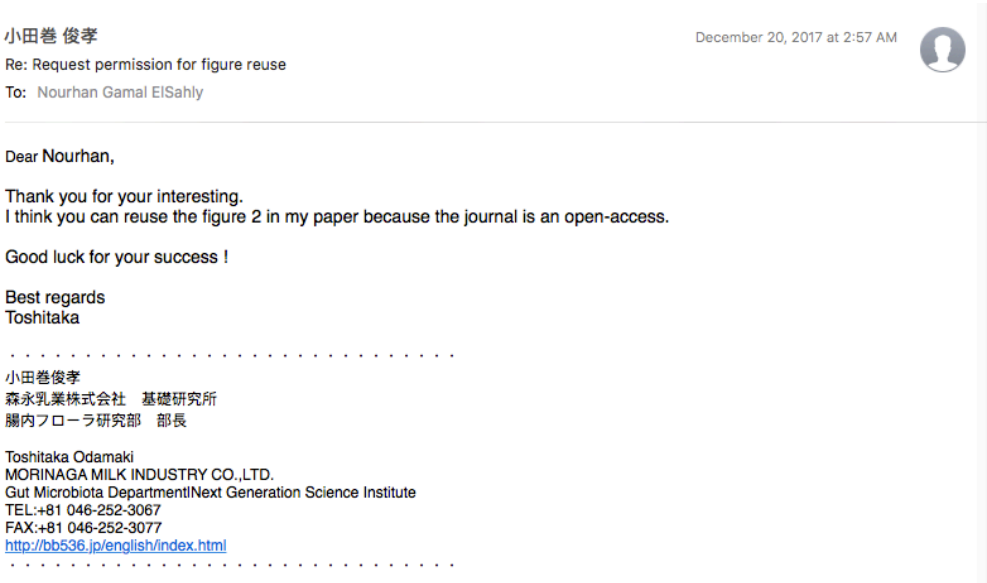

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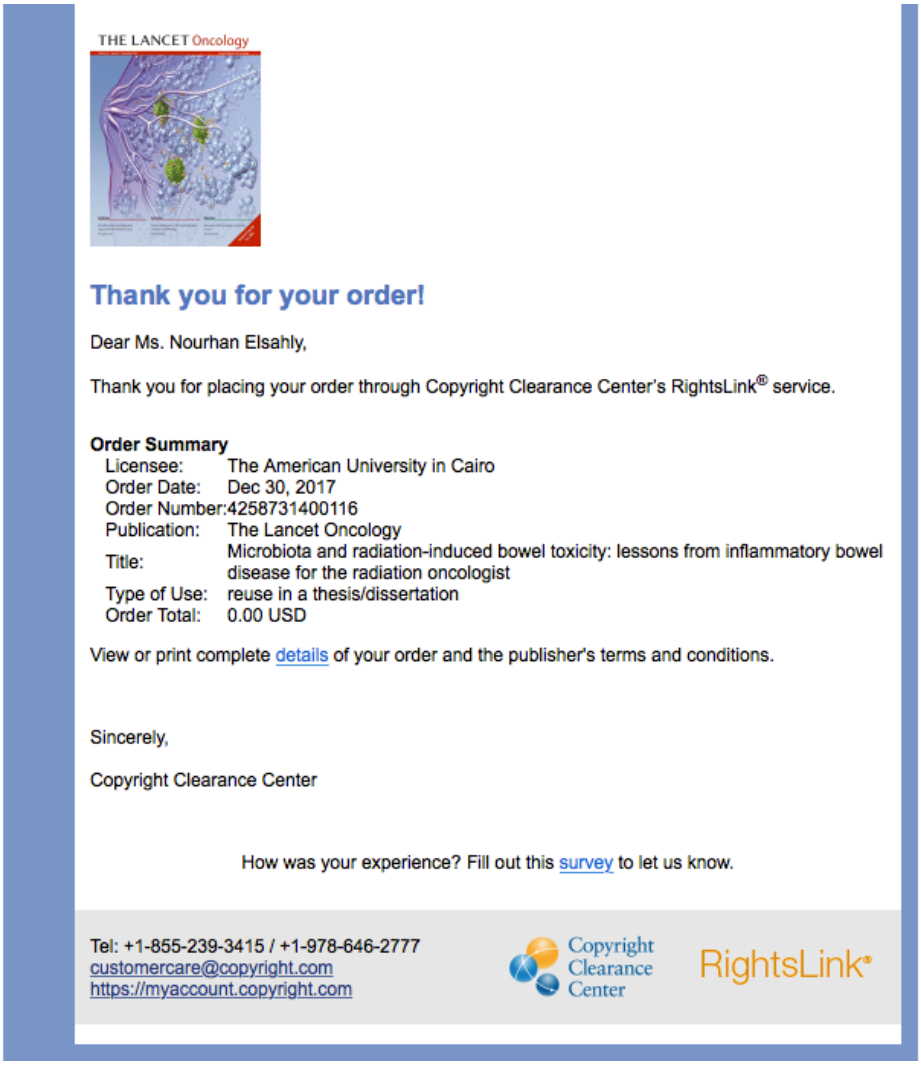

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Appendix 3- IRB Approval

CASE # 2016-2017-041

THE AMERICAN UNIVERSITY IN CAIRO
INSTITUTIONAL REVIEW BOARD

TO: Nourhan Gamal Elsahly

CC: Hind Al Helaly

FROM: George Marquis

DATE: 25 Nov 2016

RE: Approval of study #2016-2017-041

This is to inform you that I have reviewed your research proposal entitled **"The effect of adding radiotherapy to administered chemotherapy on the human gut microbiome"** and determined that it required consultation with the IRB under the "full-board" heading because of the inclusion of a vulnerable population. I have determined that the proposal design uses appropriate procedures to minimize risks and discomfort to human participants. I have also determined that adequate informed parental permission and child assent will be obtained.

This approval letter was issued under the assumption that you have not started data collection for your research project. Any data collected before receiving this letter may not be used since this would constitute a violation of the IRB policy.

Please note that IRB approval does not automatically ensure approval by CAPMAS, an Egyptian government agency responsible for approving some types of off-campus research. CAPMAS issues are handled at AUC by the office of the University Counselor, Dr. Amr Salama via an official letter from your School Dean. The IRB is not in a position to offer any opinion on CAPMAS issues, and takes no responsibility for obtaining CAPMAS approval.

This approval is valid for one year from the date of this letter. In case you have not finished data collection within a year, you will need to apply for an extension.

Thank you and good luck.

George Marquis
Acting IRB Chair, Fall 2016
T: 02-261-1598
Email: geomarq@aucegypt.edu

Institutional Review Board
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