Proposal: Kefir Supplement for the Prevention and Treatment of Clostridium Difficile

by

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ABSTRACT

Clostridium *difficile* infection (CDI) is one of the most prevalent causes of nosocomial diarrhea associated with an increased risk of mortality. Despite recent medical advances in diagnosing and treatment, CDI remains a significant challenge and economic burden to healthcare systems. The use of probiotics for the prevention and treatment of CDI is controversial. This thesis project is a research proposal for a controlled quasi-experimental clinical trial to assess the effectiveness of kefir probiotic for prevention and treatment of CDI. It is hypothesized that patients taking a kefir probiotic supplement during antibiotic treatment compared with Culturelle or no probiotic will prevent CDI and reoccurring CDI as well as aid antibiotics in treatment of patients diagnosed with CDI. The study primarily evaluates the efficacy of kefir supplement as a preventative measure for CDI and reoccurring CDI, the effectiveness of kefir supplementation with use of antibiotics as part of a treatment regimen of CDI, and the cost effectiveness of kefir supplement as prevention and treatment of CDI. Secondary outcomes include examining kefir supplementation and its relationship with weight loss and malnutrition status. This clinical trial will determine the impact of kefir supplementation on the occurrence and reoccurrence of CDI.

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CHAPTER 1: INTRODUCTION

Kefir Supplement for the Prevention and Treatment of Clostridium *Difficile*

Introduction

The incidence of clostridium *difficile* infection (CDI) has gradually increased over the last decade (Bakken, 2013). CDI is the most common microbial cause of healthcare-associated infections in hospitals (CDC, 2013). In acute care facilities, CDI costs about $4.8 billion per year in excess health care expenses (CDC News Room, 2015). Previous studies suggest probiotics have the means to prevent and treat CDI (Goldenberg, Ma, Saxton, et al, 2013). Probiotics are live organisms that inhabit the gut and provide healthful benefits, such as reducing the risk of pathogen colonization (NCCIH, 2015). Kefir, a specific probiotic supplement, has recently been studied as a preventive treatment for CDI. Kefir is a probiotic drink made by fermenting milk using kefir grains and lactobacillus bacteria.

Background

Clostridium *difficile* infection (CDI), defined by the Center for Disease Control and Prevention (CDC), is bacterium that affects the colon. The bacterium may cause inflammation of the inner lining of the colon, also known as colitis, which leads to diarrhea. People at risk for CDI include those with prolonged exposure to antibiotics and individuals greater than 65 years of age (CDC, 2015). Antibiotic associated diarrhea (AAD) is a complication of antibiotic treatment, and affects 5-39% of all individuals receiving up to 12 weeks of antibiotic treatment. Major risk factors for AAD include individuals greater than 65 years of age, hospital admission, and exposure to multiple antibiotics. CDI is also associated with AAD and accounts for 15-39% of AAD cases (Allen, Wareham, Wang, Bradley, Sewell, Hutchings,…Phillips, 2013). Incidence of AAD and CDI has risen over the years and are associated with the increased use of antibiotics (Pattani, Palda, Whang and Shah, 2013). Due to the increase in antibiotic-related infections, hospitals have been taking action with non-antibiotic therapy. The preventative intervention using probiotics with antibiotics has been studied and suggests reduction in both AAD and CDI outcomes.

Rationale

Kefir has recently been studied as a preventive treatment for CDI due to its beneficial probiotic properties. The kefir beverage is known for anti-microbial activity, anti-inflammatory activity, impact on gastrointestinal tract, and immune-modulating activity (Leite, Miguel, Peixoto, Rosado, Silva and Paschoalin, 2013). In a retrospective analysis study, conducted by Bakken (2014), daily administration of kefir probiotic supplement, combined with a staggered and tapered antibiotic withdrawal regimen, was found to be successful in preventing reoccurring CDI. However, there is limited research supporting kefir supplementation or the use of probiotics in the treatment and prevention of CDI, while simultaneously maintaining or improving a patient’s nutritional status, such as weight and malnutrition risk.

Potential significance

Kefir supplement may decrease the occurrence and reoccurrence of CDI, also improving quality of life for prospective patients. The results of this study will benefit the field of nutrition and dietetics in clinical practice by providing further evidence of the recommendation of kefir supplement use to prevent CDI and reoccurrence of CDI. This study may provide information on how kefir could potentially reduce healthcare cost with CDI interventions.

Hypothesis

It is hypothesized that patients taking a kefir probiotic supplement during antibiotic treatment compared with Culturelle, or no probiotic, will prevent CDI and reoccurring CDI as well as aid antibiotics in treatment of patients diagnosed with CDI.

Sub-problems

Does kefir probiotic supplement prevent or treat CDI and reoccurring CDI in adults receiving antibiotic treatment? The objectives of this study include assessing the: (1) efficacy of kefir supplement as a preventative measure for CDI and reoccurring CDI, (2) success of kefir supplementation with use of antibiotics as treatment regimen of CDI, and (3) cost effectiveness of kefir supplement as prevention and treatment of CDI.

Limitations

1. There is limited available research on kefir supplement in relation to CDI.
2. Recruiting a significant number of participants with reoccurring CDI will take a considerate amount of time.
3. The research project is a research proposal design. This is related to the fact that carrying out the actual study design would be time-consuming and costly.
4. There is limited research on kefir and effective dose for prevention and treatment of CDI.

Delimitations

1. The population will be adults (18-65 years of age) and older adult patients (greater than 65 years of age) undergoing antibiotic treatment at risk of developing CDI, adult and older adult patients with active positive CDI, and adult and older adult patients with history of CDI.
2. The facility will be Hospital Corporation of America (HCA) healthcare affiliated hospitals and outpatient clinics with older adult patient clientele.

Assumptions

1. Participants will comply with the intervention.
2. Participants will answer honestly in surveys/questionnaires and submit appropriate data.
3. Staff involved will document all appropriate data.

Definition of Terms

**Antibiotic associated diarrhea (AAD):** Diarrhea as a complication of antibiotic treatment.

**Anti-toxin:** An antibody that neutralizes a specific toxin.

**Assay:** The analysis of a toxin to determine the characteristics to label the toxin.

**Bifidobacteria:** A group of bacteria that resides in the intestines

**Cell rounding**: A process where the flat cell shape becomes spherical.

**Clostridium *Difficile*:** Bacterium that affects the colon causing inflammation that leads to chronic diarrhea.

**Colectomy:** Surgical removal of all or part of the colon.

**Colonocyte brush border membrane:** The microvilli-covered surface of epithelium cells.

**Cycloserine cefoxitin fructose agar (CCFA):** An enriched selective and differential medium for an isolation to identify clostridium *difficile*.

**Cytoskeleton:** The internal framework of a cell composed of actin filaments and microtubules that support, shape and move the cell.

**Cytotoxin:** A toxin with a destructive effect on cells.

**Enema:** A procedure where liquid is injected into the rectum to stimulate the bowels and produce a bowel movement.

**Enterotoxin:** A toxin specifically affecting the cells of the intestinal mucosa

**Epithelium:** One or more layers of cells that surface the gut or intestine.

**Fecal transplantation:** Taking fecal or stool sample from a healthy individual and inserting into a recipient.

**Filtrate:** A liquid that had passed through a filter.

**Gastroscopy:** An examination of the upper gestational tract using an endoscope.

**Glycoprotein:** A protein that contains oligosaccharide chains attached topolypeptideside-chains.

**H2-blocker:** A drug that inhibits gastric secretions.

**Ileus:** An intestinal obstruction with lack of peristalsis.

**Inflammable bowel disease:** Chronic inflammation of part or all of the digestive tract.

**Intestinal flora:** Naturally occurring symbiotic microorganisms that live in the intestine.

**Jejunostomy tube feeding:** Enteral nutrition feeding through a tube in the jejunum.

**Kefir:** A probiotic drink made by fermenting milk using kefir grains and lactobacillus bacteria.

**Lactobacillus:** A bacteria that produces lactic acid in the formation of carbohydrates.

**Lamina propria:** A thin layer of connective tissue beneath the epithelium, also referred to as “basement layer”. **Leukocyte counts:** A blood test to measure number of white blood cells.

**Maltodextrin:** A food additive

**Microbiota:** Microorganisms of a specific habitat (e.g. gestational tract).

**Neutrophil infiltration:** An inflammatory response diffusing neutrophilic white blood cells in cells and tissues.

**Pancreatitis:** Inflammation of the pancreas.

**Permeability:** The ability for a liquid substance to pass through a membrane.

**Probiotics:** Live organisms that inhabit the gut and provide healthful benefits

**Proteolytic effect:** To break down proteins.

**Pseudomembranous colitis:** A complication of antibiotic treatment with severe inflammation in the colon.

**Rho proteins:** A prokaryotic protein that regulate intracellular actin during the termination phase of transcription.

**Sepsis:** A fatal bacterial infection in the blood stream.

**Surface proteins:** Cell surface receptors that distinguish the cell from other defined cells and interact with what’s happening outside of the cell.

**Tight junctions:** When two cell membranes are joined and form an impermeable barrier.

CHAPTER 2: LITERATURE REVIEW

Introduction

Rates of Clostridium *difficile* infection (CDI), formally known as Clostridium *difficile*-associated diarrhea (CDAD), have been rapidly increasing over the past century, and hospitals are working to reduce CDI rates (Islam, Cohen, Chakravarthi, & Martin, 2012). Past studies indicate that within the United States, CDI is the most common microbial cause of healthcare-associated infections in hospitals. In acute care facilities, the cost of CDI is approximately $4.8 billion per year in excess health care expenses (CDC News Room, 2015).

Previous studies suggest probiotics have the mechanism to prevent and treat CDI (Goldenberg et al, 2013). Probiotics are live organisms that inhabit the gut and provide healthful benefits, such as reducing the risk of pathogen colonization (NCCIH, 2015). The purpose of this literature review is to critically analyze the research-based evidence on commonly used probiotics and newer products, including kefir supplementation, as a form of prevention and treatment of CDI.

Background

Clostridium *difficile* infection (CDI), defined by the Center for Disease Control and Prevention (CDC), are bacterium that affects the colon. The bacterium may cause inflammation of the inner lining of the colon, also known as colitis, which may cause diarrhea. People at risk for CDI include those with prolonged exposure to antibiotics and the elderly (CDC, 2015).

Antibiotic associated diarrhea (AAD) is a complication of antibiotic treatment and affects 5-39% of all individuals receiving up to 12 weeks of antibiotic treatment. Individuals greater than 65 years of age, hospital admission, and exposure to multiple antibiotics are major risk factors for AAD. CDI is also associated with AAD and accounts for 15-39% of AAD cases. (Allen et al, 2013). The incidences of AAD and CDI have risen over the years and are associated with the increased use of antibiotics (Pattani, Palda, Whang, & Shah, 2013). Due to the increase in antibiotic-related infections, hospitals have been taking action with non-antibiotic therapy. The preventative intervention of probiotics with antibiotics has been studied to reduce both AAD and CDI outcomes.

Antibiotics used for the treatment of CDI include vancomycin, metronidazole, and fidaxomin. However, the reoccurrence of CDI affects approximately 20% of patients treated with antibiotics (CDC, 2015). Surgical intervention in which the patient requires a colectomy is needed when medical management fails in cases of sepsis, ileus, or bowel perforation (Oldfield IV, Oldfield III, & Johnson, 2014). Fecal transplantation has been emerging as an alternative treatment option with a high success rate for patients suffering from reoccurring CDI (Oldfield IV et al, 2014).

Pathophysiology and Etiology of CDI

During antibiotic therapy, normal intestinal flora becomes disrupted. The disruption of flora permits C. *difficile* to colonize the intestinal lumen. The pathogen strains of C. *difficile* release two protein exotoxins: toxin A and toxin B. These toxins bind to glycoprotein receptors on the colonocyte brush boarder membrane then begin killing and shedding the epithelial cells (Castagliuolo & LaMont, 1999).

Surface proteins of C. *difficile* have a proteolytic effect, which contributes to the degradation of tissue integrity and the spread of infection. Toxin A is an enterotoxin and toxin B is a cytotoxin (Vaishnavi, 2010). Toxin A and toxin B work in conjunction with one another, as toxin B cannot access the underlying tissue until toxin A has caused the micro-ulcerations on the colonic mucosa. Toxin A induces cell rounding by altering the actin cytoskeleton. This damages the epithelial cells, which results in increased permeability of tight junctions. Toxin A causes micro-ulcerations of the colonic mucosa. Toxin B is unable to bind to receptors on the brush boarder membrane on its own. Once toxin A binds to the receptors initiating the destruction, toxin B is able to access the underlying tissue and also disrupt the function of Rho proteins (responsible for the maintenance of cytoskeletal structure and cell movement) and tight junctions, exposing the mucosa. Exposure of the mucosa and causing micro-ulcerations to the mucosa by both toxins causes neutrophil infiltration, which leads to a severe inflammatory reaction in lamina propria causing symptoms such as diarrhea (Vaishnavi, 2010).

Reoccurring CDI

While CDI is severe and can lead to complications, a more significant problem that has prevailed is reoccurrence of CDI after initial treatment (Oldfield et al, 2014). Reoccurrence of CDI occurs on average between 20-30% of initial CDI cases and 65% of patients with a history of reoccurring episodes. Adults greater than 65 years of age, comorbidities, hospital stays greater than 16 days, and additional courses of antibiotics increase the risk of CDI reoccurrence (Oldfield et al, 2014). A relapse of the initial infection or the patient becoming re-infected from a new strain of C. *difficile* may cause CDI reoccurrence as well. Reoccurring CDI results in repeated courses of antibiotic treatment as well as an increased risk for both adverse events and re-hospitalization. Based on systematic reviews, the total cost for recurrent CDI was estimated to be a three-fold increase from original episodes of CDI (Bouza, 2012). Preventing initial cases of CDI and reducing episodes of reoccurring CDI would decrease morbidity and mortality as well as associated healthcare cost.

Microbiome Effects on CDI

Differences in microbiome may also play a role in incidence of CDI. In one cohort study lead by Rondanelli et al (2015), specific genomes (the 16S rRNA gene, a sector of prokaryotic DNA found in bacteria, in this case intestinal microbiota) were studied among 178 elderly subjects not receiving antibiotic treatment. These subjects consisted of residents in an elderly community as well as frail residents of a long-term care center. Using 13 young adults as the control group, authors concluded microbiome compositions differ between healthy, older adults and hospitalized or institutionalized older adults due to health status, nutrition, living conditions, and medication use. Residents of the elderly community had higher numbers of Firmicutes and lower numbers Bacteroidetes compared to frail residents of long-term care center. This may be related to the reduction in gastric acid secretion, increased mucosal permeability, and reduction in efficiency of immune responses facilitated by B and T cells. (Rondanelli et al, 2015).

In addition, it has been found that antibiotics reduce Clostridium scindens’ bile acid-hydroxylating activity, which is essential for the protective effect against CDI. Antibiotics may increase bacterial translocation out of the gut also increasing risk of CDI (Langdon, Crook, & Dantas, 2016).

Severity of CDI and Role of Malnutrition.

Malnutrition is a serious condition that affects the health status of the elderly (>65 years), and can predict preterm death. Malnutrition is considered both under-nutrition and over-nutrition in which the body does not receive the amount of energy or nutrients it requires to support maintenance, growth, and specific functions. Malnutrition from under-nutrition is typically caused by poor or reduced dietary intake, thus, limited nutrients are provided for essential physiological functions. (Remond et al, 2015).

During a prospective study, Kyne et al (1999) evaluated the spectrum of disease severity caused by CDI. Over a four-month period, the authors monitored morbidity including dehydration, progression to toxic mega colon or death in relation to CDI, fecal incontinence, and malnutrition (>10% body weight loss) in patients diagnosed with CDI (confirmed with a positive stool cytotoxin test). Seventy-three patients had symptomatic CDI with a median age of 74 years old (range 17-91). Of the 73 patients, 24.7% had mild disease, 35.6% had moderately severe disease, and 39.7% had severe disease of prolonged symptoms. Of the patients with severe disease, six patients (8.2% of 73 patients) progressed to toxic mega colon, with four of these patients needing subtotal colectomy. Subsequently, all four died within three weeks after surgery (Kyne et al, 1999).

The authors found that age, hydration status, and malnutrition was not an indicator for disease severity. Debility and cognitive impairment at base line were significantly linked to prolonged or complicated disease severity. Nasogastric tube feeding and endoscopies were also associated with more severe outcomes. This may have been attributed by procedures performed on predisposed patients with gastrointestinal disease or by other mechanisms, such as high osmotic formulas (Kyne et al, 1999).

In a two-center retrospective cohort study, an inverse relationship was found between Vitamin D status (serum 25(OH)D) prior to hospital admission and risk of developing hospital acquired CDI. The number of patients with C. *difficile* toxin measure was highest in those with the lowest pre-hospital serum 25(OH)D measured between 7-365 days before admission. This may be explained by antibiotic usage for infection or low 25(OH)D levels as a marker of patient condition/health status (Quraishi et al, 2015). Further research is needed to determine the role of Vitamin D within the pathophysiology of CDI.

Relationship of Diet and CDI

Moore et al (2015), evaluated how severity of disease in a murine model of CDI may be altered by a protein-malnourished diet and how selected commensal microbiota is affected by a protein malnourished diet and burden of infection. Five different diets were used to assess different nutritional status in mice: a protein malnourished diet consisting of 2% protein by weight and its matched nourished control diet with 20% of protein, a malnutrition diet with 6.5% protein and its matched control with 19.5% protein, and a “traditional” natural ingredient chow diet. The diets were given to the mice 12-14 days before infection of CDI. The mice were given an antibiotic cocktail of vancomycin, colistin, gentamicin, and metronidazole starting 6 days prior to infection and discontinued three days prior to infection. Clindamycin was injected into the mice one day prior to infection. Weight, diarrhea, and disease severity were monitored (Moore et al, 2015).

After the mice were infected, the authors found that both groups of mice, fed either the the 20% or 2% protein diet, had delay onset of disease. The 20% protein diet group had 25% survival at post-infection day 14 (2/8 mice), the 2% protein diet group had 57.1% survival (4/7 mice) and the traditional diet fed mice all died by day four post-infection with 0% survival (0/7 mice). Mice fed the malnourished diet or the matched control also had higher survival rates compared to the traditional diet fed mice 7-days post-infection (100% (8/8), 87.5% (7/8), and 0% (0/4), respectively). There was no significant difference in survival or weight change between the 2% protein diet fed mice and the 20% protein diet fed mice. There was significant difference in survival and weight change between the traditional diet fed mice and 2% and 20% protein diet fed mice. This was likely due to the high fermentable fiber (100% fructo-oligosaccharides) content in the traditional diet compared to the 2% and 20% protein diets that contained no fermentable fiber. Fermentable fiber is fermented by anaerobic bacteria in the colon and in the process can cleave their (1-2) beta glyosidic bonds, which is responsible for promoting growth of anaerobic bacteria that enhance colonization resistance against CDI. Therefore, once the (1-2) beta glyosidic bonds are cleaved, the host becomes more susceptible to CDI (Moore et al, 2015).

While the authors found no relationship between protein malnourished diets in susceptibility or disease outcome of CDI, they did determine that diet does affect susceptibility and outcomes of CDI. Therefore, further studies are warranted for a more comprehensive analysis of the impact of specific nutrients on microbiota, immune response, and CDI (Moore et al, 2015).

Nutrition Utilization Profile of C. Difficile Strains

All bacteria require a source of nutrition (carbon, nitrogen, sulfur, phosphorus, magnesium, potassium, calcium, iron and vitamins) for survival and growth (Scaria et al, 2014). Microorganisms in the gut obtain these nutrients from the diet of the host, mucosal secretions, or other microbes. With more than 100 trillion microbes in the human gut competing for available nutrients and antibiotic treatment reducing the number of competing bacteria, colonization resistance weakens while the risk of CDI increases. C. *difficile* strains have a unique nutritional and chemical phenome and are capable of utilizing different nutrient sources along with the ability to survive the effects of chemicals, antibiotics and osmotic changes (Scaria et al, 2014).

Scaria et al (2014), conducted a study to complete the nutritional and chemical sensitivity profile with phenotype microarray (PM) of six C. *difficile* strains. Biolog PM was used to analyze the complete phenotype profile of six varying C. *difficile* strains. The authors found that all six strains metabolized simple sugars (maltose, maltotriose, D-fructose α-D-glucose, and D-mannose). Strain r20291 was found to utilize peptide nitrogen sources, which increased capacity to utilize nutrient sources and may allow for increased ability to colonize a host. The authors also found that acid pH is inhibitory to C. *difficile* (Scaria et al, 2014).

The results of the complete phenotype of six different C. *difficile* strains are important for future clinical trials when formulating supplements or enteral nutrition formulas for patients with an increased risk of CDI or reoccurring CDI (Scaria et al, 2014).

Laboratory Diagnosis of CDI

Laboratory testing for CDI is recommended only for symptomatic patients using only their diarrheal stool (Avila et al, 2016). Testing patients after treatment to verify “cure” or “clearance” is not recommended and deemed inappropriate use of laboratory testing. Patients may be asymptomatic after treatment and often continue to shed spores for several weeks up to months after treatment. CDI diagnostic tests are judged on specificity, sensitivity, cost, turnaround time, and availability. The clinical field currently uses the five accepted tests: Cell Culture Cytotoxicity Neutralization Assay (CCCNA), Toxigenic Culture (TC), Enzyme Immunoassay (EIA), Glutamate Dehydrogenase (GDH), and Nucleic Acid Amplification Tests (NAATs) (Avila et al, 2016).

NAATs detect toxigenic C. *difficile* strains by polymerase chain reaction (PCR) based assays and isothermal assays from DNA extraction of the stool. NAATs are highly sensitive and specific, widely available and have rapid turn-around times. However, NAATs may be considered too sensitive, as it detects the genes responsible for coding toxin production, leading to false positives. NAATs are also costly (Avila et al, 2016).

CCCNA was previously perceived as the “gold standard”. A filtrate from a stool sample is inoculated onto a single layer of a cell culture. Cytopathic effect will cause rounding of the cells identifying whether a toxin is present. Another filtrate from the same stool will be inoculated into a second cell culture with a C. *difficile* antitoxin. If no cytopathic effect occurs, the cytopathic effect in the first culture is confirmed as presence of C. *difficile* toxin. While CCCNA is specific for CDI, it is expensive and takes at least two days for results (Oldfield IV et al, 2014).

During TC, a stool sample is cultured for C. *difficile* on a selective medium of cycloserine cefoxitin fructose agar (CCFA) and tested for the ability to produce a toxin. TC is also considered highly sensitive, and does not identify the actual toxin present in the stool. The test is not specific and may cause false positives in asymptomatic carriers. TC also has a slow turn-a-round time, and is not widely available (Oldfield IV et al, 2014).

EIA detects the antigen or toxin by producing a color change triggered by the enzyme (Texas Department of State Health Services, 2010). EIA has a quick turn-a-round time, is convenient, and inexpensive. However, EIA is insensitive and non-specific to CDI. EIA is not recommended as a stand-alone test (Oldfield IV et al, 2014).

GDH detects cell-wall-associated antigen. GDH has a rapid turn-a-round time, is highly sensitivity, affordable, and widely available. However, GDH is non-specific as it also detects non-toxigenic strands (Avila et al, 2016).

Treatment and Management of CDI

Current treatment for CDI is oral antibiotics, such as metronidazole for mild/moderate CDI, oral vancomycin for severe CDI, and the combination of oral vancomycin and intravenous metronidazole for severely complicated cases. Severity of CDI is categorized based on leukocyte counts and renal function (Cohen et al, 2015).

Pharmacology for CDI

Antibiotic treatment is initially used for treatment of CDI. Previous studies have shown that all strains of C. *difficile* were resistant to bacitracin, ciprofloxacin, levofloxacin, and clarithromycin (Oldfield IV et al, 2014). Recent studies of C. *difficile* have shown no evidence of resistance to Metronidazole or Vancomycin. Both antibiotics have been proven to be effective with no significant difference between the two. Metronidazole is absorbed through the gestational tract and excreted through the biliary tract with about 14% of the drug excreted in the stool causing increase colonic inflammation. Metronidazole is more cost effective than Vancomycin in oral form. Vancomycin does not become absorbed in the colon, and has shown to be more effective in removing C. *difficile* from the stool then Metronidazole (Oldfield IV et al, 2014).

Culturelle probiotic (Lactobacillus rhamnosus GG) is one of the commonly used probiotics in hospitals. According to their website, Lactobacillus rhamnosus GG has been found to improve diarrhea in AAD and in CDI. However, according to the article cited by Goldin BR and Gorbach SL, Clinical indications of probiotics: an overview (2008), Lactobacillus rhamnosus GG showed beneficial effects of treating relapse of gastroenteritis induced by clostridium *difficile* toxin. For this statement, Goldin cited two uncontrolled clinical trials: one completed in 1987 and the other in in 1990, both of which were also considered inadequate in the number of patients and in the design of the studies (Gougoulias, Tuohy, & Gibson, 2007). These factors made their original statement weak. According to Goldin and Gorbach (2008), Lactobacillus rhamnosus GG does show beneficial effects on AAD in children; however, Goldin did not indicate a study in which reduced diarrheal symptoms in adults with AAD.

Fecal Microbiota Transplantation

Fecal microbiota transplantation (FMT), recently approved by the U.S. Food and Drug Administration, is used to improve functional intestinal microbiota in patients with reoccurring CDI (Cohen et al, 2015). Donors are selected and screened for risk of infectious diseases. Donors may be relatives, an intimate partner, or people may select an anonymous donor (Cohen et al, 2015). There are multiple methods used in FMT delivery. Lower gastrointestinal delivery is achieved via colonoscopy or enema, and upper gastrointestinal delivery involves using a nasogastric tube or nasojejunal tube and gastroscopy (Oldfield IV et al, 2014). Bowel preparation is completed by removing abnormal microbiota in the gut to allow the donor “healthy” microbiota to inhabit the gut more effectively. Samples are collected within a couple hours of passing, modified to a homogenous liquid by blending the stool with saline solution or water, and filtered to remove particulate matter. The fecal solution is then transplanted into the patient (Cohen et al, 2015). In 2015, a systematic review with meta-analysis of 18 observational FMT studies demonstrated a primary cure rate of 91.2% (n = 611), and the reoccurrence rate was 5.5% (Avila et al, 2016). These results indicate that FMT may be a highly successful treatment option for recurrent CDI.

While the majority of research indicates FMT as a successful treatment, there have been indications of adverse events. Short-term, minor adverse events that have reported by patients are constipation, diarrhea, nausea, vomiting, abdominal discomfort, and bloating. Short-term, serious adverse events that have been reported are perforation, bleeding, aspiration, and transmission of enteric pathogens. Long-term, potential adverse events are possible transmission of infectious agents, or development of disease/conditions related to changes in the gut microbiota such as hepatitis C and human immunodeficiency virus. Obesity, IBD, irritable bowel syndrome, diabetes, colon cancer, atherosclerosis, non-alcoholic fatty liver disease, autism and asthma have been linked to alterations in gut microbiota, suggesting a theoretical risk of inducing chronic disease via FMT (Kelly et al, 2015).

Medical Nutrition Therapy

There is no current standard of practice of medical nutrition therapy for treating CDI. The current purpose of medical nutrition therapy with regards to CDI is to decrease the outcome of dehydration with promotion of electrolytes and fluid status within CDI patients. According to Academy of Nutrition and Dietetics’ (AND) nutritional care manual (NCM), the nutrition prescription for diarrhea is to restore normal electrolyte, acid-base and fluid balance, decrease gastrointestinal motility, thicken consistency of stool, repopulate normal flora within the gastrointestinal tract, and introduce solid food slowly to stimulate the gastrointestinal tract without causing exacerbation of symptoms (Nutrition Care Manual, 2016).

Probiotics use with CDI

Probiotics exist as two bacterial species: exogenous and indigenous bacteria. These bacteria interact within the GI tract through biological signaling pathways with diverse cellular components. Intestinal Epithelial Cells (IECs) protect the host and intestinal microbes against pathogenic bacteria. Probiotics can increase IECs barrier function by increasing mucin production, modulating signaling pathways and cell survival, inducing antimicrobial and heat shock protein production, and disrupting pathogenic organisms (Thomas & Versalovic, 2010). Disruption of intestinal barrier function can decrease microbiota immune tolerance in the gut and cause abnormal immune response (Lee & Bak, 2011).

To be a functional probiotic, the bacteria must survive in the upper gastrointestinal (GI) tract. The bacteria must have the ability to survive during transit through the stomach and upper GI tract before providing probiotic effect in the distal part of the intestinal tract (Zheng et al, 2013).

Lactic acid bacteria (LAB) are microaerophilic, gram-positive organisms that produce lactic acid by fermenting glucose, galactose, or fructose. Lactobacillus species belong to the LAB group, and specific strains may possess potential therapeutic properties such as anti-inflammatory activity, which classifies it as a probiotic (Makarova et al, 2006).

*Saccharomyces boulardii* (*S. Boulardii*) is a strain of yeast with beneficial effects against enteric pathogens, (Rajkowska, Kunicka-Styczynska, & Rygala, 2012). *S. Boulardii* interferes with cellular signaling pathways modulating intestinal inflammation. The probiotic hasbeen shown to enhance the intestinal immune response of the host against C. *difficile* toxin A and reduce toxin A-receptor binding (Pothoulakis, 2008). In a prospective multicenter placebo controlled trial conducted by Mcfarland and Surawicz (1994), 124 people were enrolled to determine if *S. boulardii* paired with oral antibiotics would significantly decrease the reoccurrence of CDI. The study found that the rate of reoccurrence increased significantly in patients with an initial episode to those with a history of CDI episodes (24% to 65%). The combination treatment of S. boulardii and antibiotics was significantly more effective then antibiotics alone in preventing CDI reoccurrence in patients with a history of CDI (Mcfarland & Surawicz, 1994).

Current Use of Probiotics in Hospitals

Yi, Jernigan, and McDonald (2015) conducted both a cross-sectional study and a longitudinal study to assess and characterize the prevalence of probiotic use in 145 U.S. hospitals. The cross-sectional study was used to analyze the prevalence of probiotic use in 2012 and the longitudinal study was used to evaluate the use of probiotics among the sample of hospitals reporting yearly from 2006-2012. The cross-sectional study reported probiotics were use in 51,723 of 1,976,167 hospitalizations (2.6%) in 139 (96%) hospitals. Older adults were more likely to be prescribed probiotics when comparing median age 70 years old vs. 57 years old (*p*<0.0001), and were more likely to have a longer hospital length of stay when comparing mean length of stay 8.8 days to 4.4 days (*p*<0.0001). Inpatients prescribed probiotics were 21 times more likely to have a discharge diagnosis of CDI (95% CI, 20.4-21.7; *p*<0.0001). Four different probiotic strands were identified with *S. boulardii* marked as the most commonly used (32% of discharges in the probiotic group). The other three probiotic formulations were *L. acidiophilus* and *L. bulgaricus* (30%), *L. acidophilus* (28%), and *Lactobacillus rhamnosus* GG (11%) (Yi et al, 2015).

The longitudinal study reported 1.0% of discharges received 1 or more courses of probiotics in 2016, assessing 60 hospitals of the 145-hospital sample. There was a 1.2-fold usage increase annually to 2012 (95%, 1.1-1.3; *p*<0.0001). One or more courses of probiotics were received in 2.9% of discharges in 2012, an overall 2.9 fold increase between 2006-2012 (95%CI, 1.8-4.5; *p*<0.0001). The authors concluded that that use of probiotics as part of care in hospitalized patients is growing annually. However, sufficient data for the safety and efficacy of probiotic use in hospitalized patients is still needed (Yi et al, 2015).

Current Research on Probiotics, AAD and CDI

Meta-Analyses to assess probiotics on AAD and CDI

Pattani et al (2013) evaluated the efficacy of probiotics administered with antibiotics to reduce AAD and CDI in adult inpatients in a systematic review and meta-analysis. Systematic searches using MEDLINE (1946 to May, 2012), Embase (1980 to May, 2012), and Cochrane Central Register of Controlled Trials (May 31, 2012) were used. Reviewers screened articles for randomized control trials of adult inpatients treated with antibiotics, randomly assigned to co-administration of probiotics (intervention) or standard care (control) with or without a placebo that reported the prevention of AAD, CDI, or both as an outcome. Articles were excluded if they included patients with a history of CDI, the design were pilot trials of feasibility or tolerability, or had a non-comparable outcome definition or CDI or AAD. The reviewers assessed risk of bias including blinding, randomization, comparability of groups, use of intention-to-treat analysis, clarity of interventions, adequacy of follow-up and outcomes, and adequacy of study power. The studies were rated as good, fair, or poor based on identified criteria (Pattani et al, 2013).

The analysis included 16 studies, of which 15 examined AAD as the primary outcome while one examined CDI as the primary outcome. Twelve studies evaluated CDI as one of the outcomes. The meta-analysis showed that the studies had a statistically significant reduction in the risk of AAD with use of probiotics (RR 0.61, 95% CI 0.47 to 0.79; *I2*=39%)(*p*=0.0001). The studies were identified as heterogeneous in sample size and were found to have a moderate degree of publication bias. The reduction in risk of CDI was inconclusive due to a small sample size with available end points. Four studies were considered “good quality” based on clear inclusion criteria, interventions, and outcomes with long-term follow-up (between three and seven weeks). These studies also demonstrated that the use of probiotics reduced the prevalence and reoccurrence of AAD and CDI. The “fair-quality” studies showed the use of probiotics were not statistically significant for the reduction in AAD and CDI (Pattani et al, 2013).

When the results were pooled by probiotic, both Lactobacillus-based and a Saccharomyces boulardii-based probiotic formulas showed reductions in AAD and CDI. The analyses of Lactobacillus-based formulas had statistically significant lower incidence rates of AAD and CDI in the probiotic group (19% and 4%, respectively) compared to the control group (28% and 20%, respectively) (RR 0.64; 95% CI 0.48 to 0.84)( RR 0.33; 95% CI 0.18 to 0.60). Shorter follow-up periods (less than four weeks) compared to longer follow-up periods (four or more weeks) also showed statistically significant reductions in incidence of both AAD and CDI in the probiotic group (18% and 4%, respectively) compared to the control group (26% and 14%, respectively) (RR 0.57; 95% 0.41 to 0.84; RR 0.35; 95% CI 0.20 to 0.62) (Pattani et al, 2013).

The authors concluded that probiotics given with antibiotics reduce the risk of both AAD and CDI in adult inpatients requiring antibiotics. Stronger evidence exists for Lactobacillus-based probiotics; however, literature does not support the use of one probiotic over another.

One of the strengths of this study was that the articles evaluated had comparable outcome definitions used in the meta-analysis. The study had a clear inclusion and exclusion criteria for their meta-analysis. The study design was both a systematic review and a meta-analysis. The study used a specific patient population of adult inpatients and is comparable to other in-patient settings. The meta-analysis found that the use of probiotics with antibiotics was beneficial unrelated to study quality, type of probiotic used, and duration of follow-up. The results were considered statistically significant for the “good-quality” studies, studies analyzing lactobacillus-based formulas, and studies with follow-up of less than four weeks.

The review had limitations such as high baseline rates of AAD and CDI in the placebo arm of individual studies that would not be comparable to settings with lower baseline rates of AAD and CDI. Further limitations of the review include that some studies had evidence with moderate publication bias related to geographical location and multiple publication of research. Other studies were excluded because they were not in English, and a large number of patients assessed for AAD did not have CDI end points that could be measured.

Similar to Pattani et al (2013), Goldenberg et al (2013) used a systematic review and meta-analysis to assess the efficacy and safety of probiotics as the prevention of CDAD in children and adults receiving antibiotics. The studies included in the meta-analysis were randomized control trials (RCT) reporting incidence outcomes for CDI or CDAD. The participants included pediatric patients (ages 0-18) and adults (ages >18) receiving antibiotic therapy. The interventions in the studies compared probiotics of any strain or dose versus a placebo, alternative prophylaxis, or no treatment for the prevention of CDAD in children and adults. Studies using probiotics for the treatment of C. *difficile* were excluded. The primary outcome measured was the incidence of CDAD. Secondary outcomes were the incidence of C. *difficile* infection, antibiotic-associated diarrhea, length of hospital stay, and adverse events (Goldenberg et al, 2013).

Data was extracted from AMED (1985 to 2013), CENTRAL (2013, Issue 1), CINAHL (1982 to 2013), Pubmed (1966-2013), EMBASE (1966-2013), and ISI web of science (the first 500 citations of ISI’s large retrieval set). Data was also extracted using additional non-electronic resources (Goldenberg et al, 2013).

A total of 31 studies with 4,492 participants were included in the systematic review. Twenty-three studies (*n*=4,213 participants) assessed the incidence of CDAD. Using a complete case analysis, the results favored the use of probiotics, demonstrating a statistically significant reduction of 65% in the incidence of CDAD with 2.0% incidence of CDAD in the probiotics group compared to 5.5% in the control (RR 0.36; 95% CI 0.26 to 0.51; random effects), with moderate confidence of results. Heterogeneity was not statistically significant for this comparison (*p*=0.75; *I2*=0%). Thirteen studies assessed the incidence of C. *difficile* infection and the pooled results did not show a significant reduction in C. *difficile* infection with 12.6% in the probiotics group and 12.7% in the control group (RR 0.89; 95% CI 0.64 to 1.24; random-effects). Heterogeneity was not statistically significant for this comparison (*p*=0.84; *I2*=0%) (Goldenberg et al, 2013).

Twenty-six studies assessed adverse events (*n*=3,964 participants). The results used a complete-case analysis and indicated a statistically significant decreased in adverse events with 13.7% in the probiotics group compared to 18.7% in the control group (RR 0.80; 95% CI 0.68 to 0.95). Minimal heterogeneity was identified for this comparison (*p*=0.06; *I2*=37%) (Goldenberg et al, 2013).

Twenty-five studies assessed AAD (*n*=4,097 participants). Using a complete-case analysis the results indicated a statistically significant reduction in the incidence of AAD with 13% incidence of AAD in the probiotics group compared to 21% in the control group (RR 0.60; 95% CI 0.49 to 0.72). Due to missing data, a sensitivity analysis was used to compare plausible and worst plausible ratios of events to those with complete data. This sensitivity analysis indicated the result of incidence of AAD were weak to all assumptions (worst-plausible 5:1, RR 0.90; 95% CI 0.69 to 1.18). The evidence was also rated low due to potential publication bias. Heterogeneity was identified as statistically significant for this comparison (*p*=0.04; *I2*=36%). Using a priori defined subgroups suggested the heterogeneity identified was related to the adult versus pediatric subgroup effect (*p*=0.05) (Goldenberg et al, 2013).

The authors concluded evidence of “moderate quality” favors probiotics in preventing CDAD. However, probiotics is not favored in reducing the incidence of C. *difficile* infection. Evidence of “low quality” favors probiotics in preventing AAD. Although probiotics may be considered safe and effective, further research is needed to determine optimal strains of probiotics and dosages. Future trials should complete follow-ups and minimize missing data (Goldenberg et al, 2013).

There were three major strengths of this review. First, the study identified statistical heterogeneity using the *I2*statistic, as did Pattani et al (2013). However, Goldenberg identified that the study’s heterogeneity was related to the different ages between the pediatric age group and the adult age group. Second, the review evaluated subgroup effect including the risk of bias. Third, the authors determined the quality of evidence for each outcome.

Limitations of the review include missing data, sample size, and reporting of the results. The authors reported missing data from various trials. The overall quality of evidence was downgraded due to the total sample size (*n*=4,213), which did not match the calculated optimal information size (*n*=8,218). The authors did not report the results separately for children and adults, which could have been beneficial for determining implications for future research based on the differences between the age populations (children and adults).

Systematic reviews have provided some evidence that preventing AAD with probiotics could be effective. However, there is marked clinical heterogeneity between studies used in the meta-analysis causing statistical heterogeneity in the results (Allen et al, 2013).

Clinical and Cost-effectiveness of Probiotics

Systematic reviews, such as Goldenberg et al (2013) and Pattani et al (2013), have provided some evidence that preventing AAD with probiotics could be effective. However, there is marked clinical heterogeneity between studies used in the meta-analysis causing statistical heterogeneity in the results (Allen et al, 2013). Allen et al (2013) published a multicenter, randomized, double-blind, placebo-controlled, parallel arm trial to determine whether a high-dose, multi-strain probiotic in the prevention of AAD and Clostridium *difficile* diarrhea (CDD) is both clinically- and cost-effective for older adults in an in-patient setting. The purpose of this study was whether a probiotic should be included in health care routines for the prevention of AAD and CDD (Allen et al, 2013).

The study recruited patients aged 65 or older, exposed to one or more antibiotic within seven days or concurred with the intervention. Patients were excluded if they had diarrhea prior to intervention, were immunocompromised, of critical care, had CDD within three months prior, had inflammable bowel disease treatment in past 12 months, suspected for acute pancreatitis, known possible risk of compromised gut blood supply, receiving jejunal tube feeds, had a previous reaction to probiotics, or were unwilling to stop using all other probiotics (Allen et al, 2013).

Patients were screened by trained research nurses and divided randomly into either probiotic arm or placebo arm. The probiotic arm patients were given a high-dose, multi-strain preparation of both lactobacilli and bifidobacteria in capsule form. The placebo capsules contained maltodextrin powder and were identical in appearance to the probiotic capsule. Patients were instructed to take one capsule a day with food between antibiotic doses for 21 days. The nurses were blind to which patient received which intervention. Research nurses followed-up on each patient daily while in the hospital and weekly via telephone post discharge until eight weeks past initiation of intervention. The questionnaires were given at the initiation of the intervention, and again at four and eight weeks after the initiation of the intervention (Allen et al, 2013).

The probiotic arm consisted of 1,493 participants, and the placebo arm consisted of 1,488 patients. The frequency of AAD (including CDD) was not statistically significant to the probiotic arm (159 cases/1,470, 10.8%) compared to the placebo arm (153 cases/1,471, 10.4%; RR 1.04; 95% CI 0.84 to 1.28; *p*=0.72). AAD caused by CDD was not statistically significant in the probiotic arm (12/1,470, 0.8%) compared to the placebo arm (17/1,471, 1.2%) (RR 0.71; 95% CI 0.34 to 1.47; *p*=0.35). However, authors noted that the occurrence of CDD was slightly less in the probiotic arm versus placebo arm (Allen et al, 2013).

Total health-care cost per patient was relatively similar in both the probiotic arm ($11,570; 95% CI $11,000 to $12,150) and placebo arm ($11,560; 95% CI $10,970 to $12,150). The average hospital stay in both arms was 5.58 days (95% CI 2.78 to 8.39 days) (Allen et al, 2013).

There was no statistically significant evidence to suggest that administrating probiotics with antibiotics was effective in preventing AAD in older inpatient adults. Administering probiotics with antibiotics was proved not to be cost-effective (Allen et al, 2013).

Strengths of this study include that it had acquired a large sample size (*n*=2,981). The study was considered strong with a study design as a randomized, double-blinded, placebo-controlled study. The study used validated questionnaires.

Weaknesses of the study include cost-effectiveness when compared to control versus other preventive measures. Duration of the 12-week study may have curtailed outcome measures. Patient compliance for full treatment was low. Only 52.5% completed the full course of 21 days and 99.6% took at least one dose of the interventions within those 21 days, yet all participants were included in the study. The study lacks a protocol for patient compliance for completing all 21 days of treatment. The outcomes of the study would likely change greatly if there was a protocol to use only patients that complied with 100% of the 21 days or the patients that did not comply were controlled for during the data analysis. Similar to implications for future studies reported by Goldenberg et al (2013), compliance to intervention and follow-up report is crucial to avoid heterogeneity between studies.

Potential New CDI Treatments

Majority of cases of CDAD have prolonged in-patient hospital stays in health-care settings, increased medical cost, and increased risk of morbidity, resulting in high economic burden, and even more so when treating recurrent infections (Lenoir-Wijnkoop, Nuijten, Craig, & Butler, 2014). On average, length of hospital stay among hospitalized patients affected by CDAD is 7-21 days longer compared to hospitalized patients not affected by CDAD. Additionally, the cost of medical care during hospital stay is higher among patients with CDI due to need for isolation rooms in order to reduce transmitting infection. On average, the cost to treat each patient with CDAD is $43,823. This amount is expected to increase as the elderly patient population rises (Lenoir-Wijnkoop et al, 2014).

Lenoir-Wijnkoop et al (2014) conducted a health outcome analysis to assess the economic impact of fermented milk with probiotic in hospitalized patients to prevent incidence of AAD and CDI. Patients included in the study were 65 years of age or older treated with antibiotics. The cost was assessed based on costs associated with the probiotic administration and treatment of complications. Data was extracted from a randomized, double-blind placebo controlled trial of 135 hospitalized patients. The health economic analysis was based on a patient-based cost-effectiveness model (Lenoir-Wijnkoop et al, 2014).

The experimental group was given 100 grams (gm) of fermented milk with probiotic *Lactobacillus paracasei casei* CNCM I-1518. The control group received a placebo, which did not contain probiotics. Both probiotic fermented milk and placebo were administered twice a day from 48 hours after initiation of antibiotic therapy, which continued until one-week after the completion of antibiotic therapy. The probiotic group developed less AAD, 12% (7/57) compared to 34% (19/53) in the placebo group (*p*=0.007). Of the placebo group, 17% (9/53) developed CDI compared to 0% (0/57) in the experimental group (*p*=0.001). The authors determined that the fermented milk with probiotic saved $315 per case treated with antibiotics preventing AAD and $124 on average per case treated with antibiotics when preventing CDI. The mean cost of managing a case of AAD without the use of probiotics is $719 per patient. The mean cost of managing a case of AAD with the use of probiotics is $280 per patient with the potential total means cost savings of $439 per case. The authors concluded that providing a probiotic fermented milk drink as preventative treatment for AAD and CDI may potentially reduce costs associated with AAD and CDI (Lenoir-Wijnkoop et al, 2014).

*Lactobacillus* Rhamnosus GG Probiotic

*Lactobacillus*GG is prescribed widely in hospitals to patients diagnosed with positive C. *difficile* based on two studies by Bennett et al (1996) and Gorbach (1987) that promote the effective of the probiotic in treating CDI. Bennett et al (1996) completed a nonrandomized clinical trial to assess the effectiveness of *Lactobacillus* GG as treatment for relapsing C. *difficile* diarrhea. The study included 32 outpatient participants diagnosed with relapsing C. *difficile* diarrhea, nine of which were residents of nursing homes (Bennett et al, 1996).

Twelve participants were treated with one or two capsules containing 250 mg lyophilized *Lactobacillus* GG daily for ten days. Fourteen patients were treated with a dose of two capsules of *Lactobacillus* GG daily for 21 days. The nine participants in the nursing home were treated with a dose of four capsules for 14 days, and were followed for 60 days post-treatment. The non-nursing home group follow-up observation period ranged from 3-48 months, with median follow-up period of 12 months. The authors found that 27 of the 32 participants (84%) were cured after one course of treatment. Thus, the authors concluded that *Lactobacillus* GG is a safe and effective option for the treatment of relapsing C. *difficile* diarrhea (Bennett et al, 1996).

There were many strengths of the study. The inclusion criteria were specific and appropriate for the study, which strengthened the study by reducing possible confounding variables . The study used validated testing methods to diagnose CDI, and some of the follow up periods were considered a fair amount of time to prove that there was no reoccurrence of CDI (6-12 months).

Several limitations of this study are evident. The authors identified that the study was not placebo-controlled, which denotes that the authors are unable to state whether or not a placebo effect occurred. Another weakness of this study is its small sample size, which indicates a greater level of uncertainty. The study also had a poor design with varied follow-up periods and doses of probiotic among participants. The authors mentioned that four of the nine participants from the nursing home (44%) had reoccurring C. *difficile* diarrhea two to six months after treatment, which occurred after the 60-day follow-up period. However, the authors were unable to treat the patients with the probiotic due to study protocol, and all nine participants were considered “cured”. There was also no statistical analysis performed to indicate statistical significance.

Gorbach et al (1987) completed a non-controlled clinical trial to determine whether *Lactobacillus* GG is a reliable method to treat relapsing C. *difficile* colitis. Five patients with 2-5 relapsing episodes of C. *difficile* colitis over a 2-10 months were given a daily dose of 1010 *Lactobacillus* GG in 5 ml skim milk for 7-10 days. Three of the five patients started the intervention after completing a 10-day course of antibiotics while the remaining two patients started the intervention approximately two weeks after the last antibiotic treatment period and during the relapse phase of C. *difficile* colitis (Gorbach et al, 1987).

Four of the five patients’ diarrhea stopped with no further relapse after the intervention period. The fifth patient had relapsed three days after the intervention period, was given another round of antibiotic treatment followed by another intervention period, and had no further relapse after the second intervention. The authors concluded that *Lactobacillus* GG is effective in ending relapsing colitis from C. *difficile* (Gorbach et al, 1987).

The research is considered strong based on an adequate follow-up period in four of five patients (1-4 years) to determine no further relapse would occur. However, one patient was only followed for four months and was presumed to be cured. Another strength of the study includes the specific dose of the probiotic administered.

Weaknesses of the study include that the study had an extremely small sample size of 5 patients, the follow-up period was inconsistent between patients, and there was no control to compare data. The authors noted their conclusion should be viewed as tentative. However, as mentioned previously, this study is referenced based on its success of curing reoccurring CDI patients even though there are flaws to the design and methods.

Properties of Kefir Probiotic

A new probiotic supplement that has been recently examined as a possible use for prevention and treatment of CDI is kefir supplement drink. Kefir is a beverage that originated in the Caucasus Mountains, and has been traditionally consumed in Eastern Europe, Russia, and Southwest Asia. Kefir beverage is produced from milk inoculation with grains and fermented between 18-24 hours. The grains are sieved leaving the kefir beverage as the end product. These grains are unique due to their composition of microorganisms within the polysaccharide and protein matrix and their symbiotic relationship, which coexists between the bacteria and yeast. (Leite et al, 2013). Tibetan Kefir grains are composed of LAB (*Lactobacillus, Lactococcus*, and *Leuconostoc*) and yeasts (*Saccharomyces*, *Kluyveromyces* and *Torula*) (Zheng et al, 2013). Kefir beverages are known for anti-microbial activity, anti-inflammatory activity, impact on gastrointestinal tract, and immune-modulating activity (Leite et al, 2013).

Properties of Lactic Acid Bacteria and Yeast Isolated From Kefir

Three lactobacillus strain isolates from Tibetan kefir grains (*L. acidophilus, L. plantarum, and L. kefiranaofaciens*) were studied to identify various functional properties including adhesion ability and colonization ability (Zheng et al, 2013). These functional properties are essential for delivering their identified health benefits. The bacteria must have the ability of adhesion to colonize, stimulate the immune system and antagonize against enteropathogens. Adhesion ability of *L. plantaru’s* was significantly stronger than that of the reference strain LGG in vitro. *L. acidophilus and L. kefiranaofaciens* had similar adhesion ability to LGG (Zheng et al, 2013).

**Properties of Kefir-isolated Microorganism In Vitro**

Bolla, Carasi, Serradell, and De Antoni (2013) examined the ability of the isolated kefir bacteria and yeast strains to antagonize the C. *difficile* toxin effect on eukaryotic cells in vitro. Of the kefir-isolated microorganisms, three lactic acid bacteria (*Lactococcus lactis sub lactis* CIDCA 8221, *L. plantarum* CIDCA 83114, and *L. kefir* CIDCA 8348) and two yeast (*Kluyveromyces marxianus* CIDCA 8154 and S. cerevisiae CIDCA 8112) were examined individually and also all 5 microorganisms together as a microorganism mixture (MM). Spent culture supernatants (SCS) of C. *difficile* strain 117 with positive A and B toxin production were used in the study. *Lc. Lactis* CIDCA 8221 and MM supernatants were able to inhibit the cytotoxic effect of C. *difficile* SCS on Vero cells. However, *Lb. kefir* CIDAC 8384, *Lb. plantarum* CIDCA 83114, *Sac. Cerevisiae* CIDCA 8112, and *K. marxianus* CIDCA 8154 supernatants showed no ability to inhibit C. *difficile* SCS cytotoxic effect on Vero cells. The authors then tested the effect of different treatments on *Lc. Lactis* CIDCA 8221 supernatant ability to inhibit C. *difficile* SCS cytotoxic effect. As a result, the authors observed that the *Lc. Lactis* CIDCA 8221 supernatants secreted metabolites, responsible for the inhibitory ability, that are thermo-sensitive. The presence of proteases or proteases-inhibitors did not impede the protective effect (Bolla, 2013a).

**Properties of Kefir-isolated Microorganism in a Hamster Model**

After the authors determined the isolated microorganisms inhibitory abilities in vitro, they then aimed to determine the protective effect in an animal model. Bolla P., Carasi, Bolla M., De Antoni, and Serradell (2013) examined the protective effect of a mixture of kefir-isolated lactic acid bacteria (*Lactococcus lactis sub lactis* CIDCA 8221, *L. plantarum* CIDCA 83114, and *L. kefir* CIDCA 8348) and yeast (*Kluyveromyces marxianus* CIDCA 8154 and *S. cerevisiae* CIDCA 8112) on a hamster model of infection with vegetative forms of C. *difficile* (strain 117). Two different doses of the microbial mixture (MM) (diluted at 1/100 and 1/1000) or a placebo (0.3 M sucrose) were administered in drinking water for a course of 11 days prior to infection. Antibiotic treatment (ATB) of clindamycin 200μg was administrated in a single intra-gastric dose to animals. The animals were divided into five different treatment groups containing seven animals each: placebo + ATB, MM1/1000 + ATB, MM1/1000 + ATB + C. *difficile*, MM 1/100 + ATB + C. *difficile*, placebo + ATB + C. *difficile*. The treatment groups were compared to the control (placebo) group also containing seven mice (Bolla et al, 2013b).

Treatment of MM1/1000 was found to significantly reduce the fraction of animals with diarrhea after being infected with C. *difficile* (1 /7 vs. 6/7 hamsters developed diarrhea) and increase the percentage of survival (7/7 vs. 1/7 hamsters survived) in the animals compared to treatment with MM1/100. The animals treated with MM1/1000 +ATB and not infected with C. *difficile* also had no signs of illness (diarrhea or death) induced by administration of clindamycin. There was no significant difference between treatment of MM1/100 compared to the placebo + ATB + C. *difficile* for percent survival (1/7 vs. 2/7 hamsters) or reduction of diarrhea induced by infection of C. *difficile* (6/7 vs. 6/7 hamsters). The authors completed a histological analysis on all mice, and found that the hamsters treated with MM1/1000 and then infected with C. *difficile* had mild thickening of mucosa from mild inflammatory infiltrates of lymphocytes yet did not develop acute colitis. Hamsters that were infected with C. *difficile* or ATB alone both had signs of acute colitis with thickening of mucosa. The non-infected control and MM1/1000 + ATB groups had normal thickness of the mucosa and no signs of inflammation or colitis (Bolla et al, 2013b).

The results of the study suggest that an adequately dosed mixture of bacterial and yeast strains isolated in kefir-fermented milk reduces acute injury induced by antibiotic treatment and protects against infection with C. *difficile* in a hamster model. While this study used an animal model, demonstrating the effects on hamsters may increase understanding in humans because hamsters with CDI demonstrate many clinical symptoms observed in humans with CDI (Bolla et al, 2013b).

Effectiveness of Kefir Supplement as Probiotic Intervention

Of the few studies of kefir supplementation, Bakken (2014) addresses two imperative questions in a retrospective analysis research article. First, does daily administration of the kefir probiotic supplement combined with a staggered and tapered antibiotic withdrawal regimen prevented recurrent clostridium *difficile* infection? Second, does daily administration of kefir probiotic supplement combined with a staggered and tapered antibiotic withdrawal (STAW) regiment results compare to the success of fecal microbiota transplant (FMT) (Bakken, 2014)?

The Bakken study recruited patients from St Luke’s Hospital located in Duluth, Minnesota, with recurrent CDI, and patients were required to be on antibiotic therapy prior to recurrent CDI. Patients were instructed to drink five ounces of Lifeway Kefir with each meal at least three times per day. STAW regimen was initiated once normal bowel movements had been established and maintained for seven days. Antibiotic treatments were provided at 72-hour intervals over a six-week period, with gradual dose reduction every two weeks. Patients were instructed to continue drinking kefir for two-months post STAW. Reoccurring diarrhea was documented during post-treatment interviews via telephone or e-mail at 90 days post-STAW and quarterly intervals up to one year (Bakken, 2014).

The study was a retrospective analysis. Mean age of participants was 68 years (SD, 14 years). The mean number of CDI relapses prior to STAW was four (range 1-9). Median number of days of diarrhea from time of initial diagnosis of CDI until the initiation of STAW was 135 (range, 9-2,920 days). From the time of initial diagnosis of CDI to the initiation of STAW, the 25 patients submitted 103 stool samples for C. *difficile* testing, which amounted to an average of 3.6 (range, 1-9) positive samples per patient. Twenty-one of the 25 patients (84%) remained free from diarrhea for nine months post-treatment, and 20 patients remained free from diarrhea for greater than 12 months. Four patients (16%) relapsed with a positive C. *difficile* test between 24-45 days post STAW (Bakken, 2014).

The study reports an 84% success rate of STAW with Kefir supplement, and parallels the success rates reported for FMT. According to the author, one could argue that elimination of spore forms with STAW, combined with restoration of colon microbiota, using Kefir supplement will prevent recurrent CDI (Bakken, 2014).

Strengths of the study include that it was a human study with a one-year follow-up. This is a considerable amount of time for outcomes to occur. Another strength of this study is that there was no attrition in the number of participants at follow-up.

Limitations of this study include that the study was a non-controlled study. The study was retrospective analysis treated by a single provider. The number of cases studied is considered small. Similar to Allen et al (2013), the study did not indicate whether patients were compliant with kefir intake three times a day during the intervention, and full compliance may potentially skew results.

Conclusion

The incidence of CDI has gradually increased over the last decade (Bakken, 2013). CDI is the most common microbial cause of healthcare-associated infections in hospitals (CDC, 2013).

Probiotics have increasingly been prescribed in hospitals over the past century with inconsistent or outdated evidence supporting the use of probiotics to treat or prevent CDI. Probiotics have demonstrated beneficial properties, and different strains have suggested positive effects on CDI and AAD. From the studies reviewed, probiotics appear to be safe with minimal to no risk or adverse events. Overall, evidence suggests that probiotics have preventive effects on CDI. Allen et al (2013) found that there was no statistically significant evidence to suggest that the provision of probiotics was either clinically- or cost-effective in preventing AAD or CDD in older adult inpatients. However, the study had a poor compliance rate in which controlling and improving compliance could alter the outcomes of the study significantly.

The probiotic-containing kefir-fermented milk has shown to have LAB and yeast with protective properties against CDI both in vitro and in a hamster model. The Kefir-fermented milk has also presented beneficial properties in a recent human study. Kefir supplement, in the retrospective analysis by Bakken (2014) was found to be successful in preventing recurrent CDI when combined with a staggered and tapered antibiotic withdrawal regimen. However, there is limited sufficient research using kefir as a probiotic supplement in prevention and treatment in CDI. Future research using a probiotic supplement such as kefir may lead to changes in clinical practice for treatment and prevention of CDI. Implications for further research indicate the need for appropriate sample size, age, strain, dose, length of treatment, and probiotic supplements.

CHAPTER 3: METHODS

The purpose of this study is to determine the efficacy and cost effectiveness of kefir supplement as a preventative measure and treatment regimen with use of antibiotics for CDI and reoccurring CDI.

Study Design and Objectives

It is hypothesized that patients taking a kefir probiotic supplement during antibiotic treatment, compared with Culturelle or no probiotic, will prevent CDI and reoccurring CDI as well as aid antibiotics in treatment of patients diagnosed with CDI. This study will be a non-randomized controlled clinical trial examining kefir supplement as a preventive and treatment measure of CDI and reoccurring CDI. The primary objectives of this study include assessing: (1) the efficacy of kefir supplement as a preventative measure for CDI and reoccurring CDI, (2) the effectiveness of kefir supplementation with use of antibiotics as part of a treatment regimen of CDI, and (3) the cost effectiveness of kefir supplement as prevention and treatment of CDI. Secondary objectives include examining kefir supplementation and its relationship with weight loss and malnutrition status. IRB approval through Mount Mary University and HCA will be obtained prior to participant recruitment.

Target demographics

Target demographics include adults over the age of 18 years in an acute-care hospital setting.

Sampling procedure

Patients will be recruited through Hospital Corporation of America (HCA) healthcare inpatient facilities. There are 160 HCA healthcare-associated hospitals located in 20 states of the United States. This study aims to recruit a total of 1,440 participants, of which approximately 720 participants will be recruited to be in the control group and 720 participants recruited to be in the treatment group. Approximately 648 participants per group has been determined as an appropriate sample size to show statistically significant differences for the primary outcome of CDI prevention as well as reoccurring CDI among patients using kefir. Additionally, this sample size will ensure that a two-sided test with α =0.05 and β=80% power to detect 5% difference in the portion of patients with AAD who will develop CDI. A patient will be recruited when diagnosed with diarrhea and has met the inclusion criteria.

Inclusion criteria:

1. In-patient adults (ages 18-64 years) and older adults (age 65+).
2. Exposure to one or more oral or parenteral antibiotics within the last seven days administered/provided over a minimum of 72 consecutive hours.
3. Diagnosis of diarrhea defined as three or more loose or watery stools within the preceding 48 hours of starting intervention.
4. Patient may have a past medical history of CDI.
5. Patient has given written informed consent/assessment to take part in the study.
6. Patient must be able to take probiotic orally.
7. Inclusion in the study is approved by the admitting consultant and validated by a member of the trial team within 48 hours.

Exclusion criteria:

1. Patients had a recent gastrointestinal (GI) surgery or intervention within the last four weeks (e.g. colectomy).
2. Patients unable to take the supplement orally.
3. Patients not mentally able to complete the questionnaire.
4. Patients with an allergy or hypersensitivity to any component of the study product (i.e. milk proteins).
5. Patients enrolled in another clinical study during the intervention of this study.
6. Patients who are anticipated to be non-compliant with the clinical study.
7. Patients presenting with or a documented past medical history of infection of the gastrointestinal system such as inflammatory bowel disease, Crohn’s disease or ulcerative colitis, diverticular disease, or liver cirrhosis.
8. Patients with a condition affecting the pancreas including acute and chronic pancreatitis.
9. Immune-suppressed patients (e.g. HIV).
10. Post-transplant patients.

Study Protocol

Kingwood Medical Center of Kingwood, TX will be the central/primary site conducting the study. It is estimated that recruitment will last two years to recruit 720 participants into each group. A training video on implementing the experiment, documenting, and collecting data will be provided through the HCA intranet and required by all participating staff (nurses, dietitians, nursing assistants, and physicians) to watch/view prior to the intervention phase of the clinical study. To allow higher compliance with intervention, each participant that meets study criteria and provides voluntary consent will choose to be in either the intervention group or the control group. Patients will be instructed to sign a consent form informing that they will be able to opt out of the study at anytime and signing up for the study will entail completing questionnaires during their hospital stay and after discharge bi-monthly for one year post discharge date. Patients in the control group will not be permitted to use/ingest/consume Culturelle probiotic or any other probiotic during the study period.

Patients in the treatment group will be offered eight ounces of the kefir supplement daily during their hospital admission. Should a participant be diagnosed with CDI, daily supplementation of Lifeway™ kefir will continue in addition to antibiotic treatment. Patients that qualify to participate in the study and request no supplement will be followed as part of the control group. Three flavors of kefir will be offered (strawberry, blueberry, or vanilla). Each patient in the experiment group will be provided vouchers for free kefir supplements offered in stores or online to be shipped to their home. The vouchers will allow patients to continue the use of the oral probiotic until the end of the experimental phase. The patients will also be provided with instructions to continue drinking 8oz of kefir supplement daily until symptoms resolve and to restart daily supplementation if symptoms start to resurface/reoccur/resume/regress.

Inpatient Data Collection Process

Phone questionnaires will be completed by participants at the initiation of the study with a weekly follow-up, as an inpatient, until discharged from the hospital. Staff at the primary location of the study, Kingwood Medical Center, will conduct the phone questionnaires to all participants. Stool samples will be collected from patients with symptoms of CDI to determine diagnosis. Nurses and dietitians will be instructed to document compliance with daily supplement intake as well as bowel movement consistency and daily frequency using a study-specific form, which will be hung on the participant’s door inside the patient’s room in an effort to minimize contamination. Each study-specific form will be collected weekly until the participant/patient is discharged. All data collected by staff will be documented electronically and sent to the research team at the primary location, Kingwood Medical Center.

Post-discharge Data Collection Process

Questionnaires will be completed by participants bi-monthly after his or her discharge date for one year. Upon discharge from inpatient status, participants may choose whether or not to continue with the study. Based on the participant’s selected method of contact upon discharge, questionnaires will be completed bi-monthly via telephone or electronically via e-mail, both of which will be collected during the same bi-monthly data collection cycle. Self-reported anthropometric data including height, weight, and body mass index (BMI) will be collected over a one-year period to assess for malnutrition status and verified using hospital records.

Tools That Will Be Used

The questionnaires will be modified from existing validated questionnaires from the PROQOLID™ Database. PROQOLID™ Database provides access to the scientific community for Clinical Outcome Assessments (COAs) tools and Patient Centered Outcomes Research resources. The PROQOLID™ database can be accessed on the Internet at <http://www.proqolid.org> (PROQOLID, n.d.).

Questionnaires that will be conducted while inpatient will measure:

1. Dates of hospital admissions.
2. Length of hospital stay in measurement of days.
3. Average number of loose stools bowel movements per day.
4. Weight, percent body weight loss, BMI.
5. Occurrence and/or duration of CDI.
6. Number of days on antibiotics.
7. Number of days of documented diarrhea.
8. Reoccurrence and/or duration of CDI.
9. Number of days on antibiotics.
10. Number of days on Culturelle probiotic in the control group only.

Questionnaires that will be distributed bi-monthly post discharge will measure:

1. Date of last hospital admission.
2. Number of re-admissions.
3. Length of hospital stay in measurement of days.
4. Occurrence and/or duration of diarrhea.
5. Average number of loose stools bowel movements per day.
6. Occurrence and/or duration of CDI.
7. Number of days on antibiotics.
8. Reoccurrence and/or duration of CDI.
9. Number of days on antibiotics.
10. Weight, percent body weight loss, BMI.
11. Number of days on Culturelle probiotic in the control group only.

Cost Effectiveness

Length of hospital stay will be calculated using admission and discharge dates. Quality Adjusted Life Years (QALYs) is the measure of a person’s length of life weighted by health-related quality of life to evaluate cost-effectiveness of between the intervention and the control group. The QALY value is measured on a scale with zero (0) equaling death and one (1) equaling full health. Healthcare-related costs are hospital-related costs (hospital admissions and outpatient specialist care) that will be collected from hospital records. To evaluate cost-effectiveness, cost-effectiveness ratios (CERs) will be calculated for QALY. CERs are calculated by dividing the difference in the mean costs (between the intervention and control groups) by the differences in the mean outcomes (Sassi, 2006).

Data Analysis

All data collected will be analyzed using SPSS analysis software. Findings will be considered statistically significant if the *p*-value is less than or equal to 0.05. The intervention group will be compared to the control group. Baseline characteristics will be compared using *X*2 test. Pearson’s correlation coefficient will be applied to the correlation of nutritional status and number of days with active CDI symptoms. Primary comparison outcomes will include CDI occurrence, duration, and reoccurrence using repeated measures ANOVA. An independent *t*-test will be used to compare continuous variables (weight loss, malnutrition status, number of days of diarrhea, number of days of hospital stay, number of days on antibiotics, etc.). ANOVA will be used to analyze differences between the two studied age groups, adults (ages 18-64 years) and older adults (age 65+). Mann-Whitney will be used to compare differences between the means of continuous variables. Analyses will include the intent-to-treat population.

To test for cost effectiveness, the Mann–Whitney U tests will be used to test for statistically significant differences in means and standard deviations of costs between the intervention and control group. Compliance with study treatment will be calculated as the number of days that the participant consumed the kefir probiotic drink divided by the total number of intervention days.

CHAPTER 4: DISCUSSION

Repeated research needs to be executed to assess the role of probiotics in prevention and treatment of CDI and reoccurring CDI.

Potential Problems

There are several potential problems that may occur during the proposed study. Recruiting a significant number of participants for both groups may take substantial time to complete, in which recommended treatment of CDI could change. Other environmental or economic confounding variables, such as changes in health care cost or a new strain of CDI, could occur if the study were to last over multiple years. A solution to this would be to complete multivariate statistical analysis controlling for the confounding variables that may arise during this investigation.

The duration of the intervention phase is set at one-year post discharge date. The participants are instructed to consume the supplement during the initial hospitalization and during periods of occurring or reoccurring diarrhea symptoms until the intervention period is complete. Previous studies have indicated that diarrhea symptoms vary from three to 28 days and reoccurring diarrhea due to reoccurring CDI up to several years (McFarland et al, 1994). The one-year intervention phase post-discharge may present challenges to the effectiveness and strength of the study, including non-compliance or high attrition rate. This may have a negative impact on the statistical power to detect effects of the intervention. Removing these subjects from the analysis could also cause the two groups to be disproportionate, and comparison post-intervention may confound the effect of the intervention. A resolution would be to conduct a secondary analysis adjusting for these covariates.

Data collection will occur at the beginning of the initiation phase and at one-year post-discharge. The duration of time is considered adequate for follow-up on preventing and/or treating CDI. However, the response rate to kefir probiotic is unknown for reoccurring CDI. Replicating this study with longer follow-up periods would contribute data to understand the response rate of kefir probiotic for reoccurring CDI.

Anticipated Results

Resolution of CDI and reoccurring CDI using kefir supplement during antibiotic treatment of patients diagnosed with CDI compared to the control is anticipated to be of statistical significance based on a previous study (Bakken, 2014). CDI prevention is also expected as seen from previous studies with patients who develop antibiotic associated diarrhea (AAD) (Goldenberg et al, 2013). Compared to the control group, costs associated with CDI are projected to be less than patients treated with a probiotic (Lenoir-Wijnkoop et al, 2014). Additionally, a statistically significant difference is anticipated in both infection and response rates of patients’ ages 18-64 and 65+ years and between malnourish and non-malnourished patients (Allen et al, 2013).

Implications for Health Care

This will be the first controlled (non-randomized) clinical trial with an adequate sample size assessing the effects of kefir probiotic on CDI. If study results show an association between kefir supplementation and decreased occurrence/reoccurrence of CDI, nutritional recommendations could be warranted in the future for kefir probiotic supplementation in patients experiencing AAD or CDI. The study methodology is replicable, and thus, adding to the research and evidence to justify kefir supplementation. Clinical judgment is needed regarding the risk and benefits of kefir probiotic in populations at risk of being immunocompromised. The results of this study will benefit the field of nutrition and dietetics in clinical practice by providing further evidence of the recommendation of kefir supplement use to prevent CDI and reoccurrence of CDI. This study may provide additional information on how kefir could potentially reduce healthcare cost with CDI interventions.

Implications for Research

Results supporting kefir probiotic as a safe and effective intervention for prevention and treatment of CDI and reoccurring CDI may warrant repetitive studies to strengthen the evidence. Additional research is needed to gain a better understanding of how specific probiotic strains found in kefir affect CDI. This could be accomplished with a randomized double-blinded controlled trial using different kefir probiotics with different strands. More research is needed to address specific populations at risk of CDI and reoccurring CDI. Using a retrospective analysis to extract past medical history and demographic data of patients with CDI and reoccurring CDI may lead to identifying specific populations at risk. Additional research is also warranted to provide a further understanding of different response rates to kefir supplementation. This could be achievable with future trials involving genome sequencing of the gastrointestinal system to determine if there is a genetic difference between populations that are more acceptable to CDI and those that would respond better with kefir probiotic.

Further research is desired to determine the role of malnutrition in the risk of CDI and reoccurring CDI. Additional research is necessary to determine the most effective dose of kefir for preventing and/or treating CDI and reoccurring CDI. Leading a randomized double-blinded placebo control trial using different doses of kefir supplement will be able to provide further evidence on the most effective dose for preventing and/or treating CDI and reoccurring CDI.

APPENDIX A

**Mount Mary University**

**Institutional Review Board (IRB)**

**for the Protection of Human Subjects**

**Application for IRB Review**

***DATA COLLECTION CANNOT BEGIN***

***UNTIL THE IRB HAS APPROVED THIS PROJECT***

**Directions:**

* Faculty and student researchers, as well as student research advisors, should **read all relevant information on the University IRB page in My Mount Mary before initiating an application**. This includes full knowledge of the US Department of Health and Human Services Code of Federal Regulations Title 45 (Public Welfare), Part 46 (Protection of Human Subjects). <http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html>
* All applicants must verify completion of Human Subjects Training. See http://www.citiprogram.org
* The IRB application must be filed and approved by the IRB **prior** to any Mount Mary University faculty, staff, or student (undergraduate or graduate), initiating a research project/study.
* If there is a cooperating institution, attach a copy of their IRB approval.
* In the case of a student research project, the student may complete the IRB application but the student’s research advisor must sign and submit the application to the IRB for approval. It is the responsibility of the faculty research advisor to ensure that student applications and all attachments (e.g. informed consent forms and survey instruments) are in their final edited form. Even though a student research project may qualify as **exempt** from full IRB review, the research advisor may request the student to complete and submit a full IRB application.
* Complete this application using your word processing program (ex. Word), then print it out and obtain signatures from all investigators and advisors. (**Handwritten applications will not be accepted**.) For your benefit, save the completed application on your computer in case it needs to be revised and resubmitted.
* This is a professional document; please check spelling, grammar and punctuation.
* Submit a hard copy of the completed application with required signatures and attachments to Maureen Leonard, IRB Chair, Sciences Department. **(Emailed applications will not be accepted.)**
* Allow a **minimum of 10 working days** to process your application. Make sure this time frame is accounted for when considering initiation of data collection and due dates for student projects.
* For class projects you must submit IRB applications to the IRB Chair by October 31st of the fall semester and March 31st for the spring semester. For summer classes, please consult with the IRB Chair.
* Upon receipt of the IRB letter of approval, data collection may begin.

**I. Required Documentation (No action will be taken without these attachments.)**

Are the following attached to the IRB application?

|  |  |  |
| --- | --- | --- |
| Consent application | Yes | Applications should include explanation of procedures, risk, safeguards, freedom to withdraw, confidentiality, offer to answer inquiries, third party referral for concerns, signature and date. See Appendix.A. |
| Questionnaire/Survey Instrument(s) | Yes | If survey is being conducted verbally, a copy of the introductory comments and survey questions being asked must be attached to this application. If survey includes focus group questions, a complete list of the question should be attached. For research using a published/purchased instrument, a photocopy of the instrument will suffice. |
| Verification of Human Subjects Training | Yes | Copy of transcript, certificate or other evidence  . |
| Copy of cooperating institution’s IRB approval. | Yes | Not required if there is no cooperating institution.  . |

**II. Investigator(s):**

|  |  |
| --- | --- |
| Name: Sarah Anderson | Phone: 616-402-5316 |
| Affiliation with Mount Mary University (e.g. faculty, student, etc): Graduate Student  Email: anderssa@mtmary.edu |  |
|  |  |

|  |  |
| --- | --- |
| Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | Date: |

|  |  |
| --- | --- |
| Name: | Phone: |
| Affiliation with Mount Mary University:  Email: |  |

|  |  |
| --- | --- |
| Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ | Date: |

|  |  |  |  |
| --- | --- | --- | --- |
| **If student, list Research Advisor and complete Section II. Research Advisor must provide requested information and verify.** | | | |
| Research Advisor’s Name: Tara L. LaRowe | Department: Dietetics | | |
| Email: larowet@mtmary.edu | Phone: (414)-930-3292 | | |
| Research Advisor: Have you completed Human Subject’s Training?  **Research advisor’s signature indicates responsibility for student compliance with all IRB requirements.** | | Yes | No |
| Signature: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_  Research Advisor | Date: | | |

**III. Project Description**

**Instructions:** Briefly describe the proposed project including the sample and methodology (e.g. human subjects, data collection, data analysis and instruments).

1) Objectives (purpose of project):

The purpose of this study is to determine the efficacy and cost effectiveness of kefir supplement as a preventative measure and treatment regimen with use of antibiotics for CDI and reoccurring CDI.

2) Relevance to practice/body of knowledge:

Kefir has recently been studied as a preventive treatment for CDI due to its beneficial probiotic properties. The kefir beverage is known for anti-microbial activity, anti-inflammatory activity, impact on gastrointestinal tract, and immune-modulating activity (Leite, Miguel, Peixoto, Rosado, Silva and Paschoalin, 2013). In a retrospective analysis study, conducted by Bakken (2014), daily administration of kefir probiotic supplement, combined with a staggered and tapered antibiotic withdrawal regimen, was found to be successful in preventing reoccurring CDI. However, there is limited research supporting kefir supplementation or the use of probiotics in the treatment and prevention of CDI, while simultaneously maintaining or improving a patient’s nutritional status, such as weight and malnutrition risk. Kefir supplement may decrease the occurrence and reoccurrence of CDI, also improving quality of life for prospective patients. The results of this study will benefit the field of nutrition and dietetics in clinical practice by providing further evidence of the recommendation of kefir supplement use to prevent CDI and reoccurrence of CDI. This study may provide information on how kefir could potentially reduce healthcare cost with CDI interventions

3) Describe the research design (e.g. subject/participant selection and assignment, design, intervention, data analysis):

This will be a controlled (non-randomized) clinical trial the target demographics include adults over the age of 18 years in an acute-care hospital setting. Patients will be recruited through Hospital Corporation of America (HCA) healthcare inpatient facilities. There are 160 HCA healthcare-associated hospitals located in 20 states of the United States. This study aims to recruit a total of 1,440 participants, of which approximately 720 participants will be recruited to be in the control group and 720 participants recruited to be in the treatment group. . A patient will be recruited when diagnosed with diarrhea and has met the inclusion criteria. Kingwood Medical Center of Kingwood, TX will be the central/primary site conducting the study. It is estimated that recruitment will last two years to recruit 720 participants into each group. To allow higher compliance with intervention, each participant that meets study criteria and provides voluntary consent will choose to be in either the intervention group or the control group. Patients in the control group will not be permitted to use/ingest/consume Culturelle probiotic or any other probiotic during the study period.

Patients in the treatment group will be offered eight ounces of the kefir supplement daily during their hospital admission. Should a participant be diagnosed with CDI, daily supplementation of Lifeway™ kefir will continue in addition to antibiotic treatment. Patients that qualify to participate in the study and request no supplement will be followed as part of the control group. Three flavors of kefir will be offered (strawberry, blueberry, or vanilla). Each patient in the experiment group will be provided vouchers for free kefir supplements offered in stores or online to be shipped to their home. The vouchers will allow patients to continue the use of the oral probiotic until the end of the experimental phase. The patients will also be provided with instructions to continue drinking 8oz of kefir supplement daily until symptoms resolve and to restart daily supplementation if symptoms start to resurface/reoccur/resume/regress.

Phone questionnaires will be completed by participants at the initiation of the study with a weekly follow-up, as an inpatient, until discharged from the hospital. Stool samples will be collected from patients with symptoms of CDI to determine diagnosis. Nurses and dietitians will be instructed to document compliance with daily supplement intake as well as bowel movement consistency and daily frequency using a study-specific form, which will be hung on the participant’s door inside the patient’s room in an effort to minimize contamination. Each study-specific form will be collected weekly until the participant/patient is discharged.

Questionnaires will be completed by participants bi-monthly after his or her discharge date for one year. Upon discharge from inpatient status, participants may choose whether or not to continue with the study. Based on the participant’s selected method of contact upon discharge, questionnaires will be completed bi-monthly via telephone or electronically via e-mail, both of which will be collected during the same bi-monthly data collection cycle. Self-reported anthropometric data including height, weight, and body mass index (BMI) will be collected over a one-year period to assess for malnutrition status and verified using hospital records. Length of hospital stay will be calculated using admission and discharge dates. Quality Adjusted Life Years (QALYs) is the measure of a person’s length of life weighted by health-related quality of life to evaluate cost-effectiveness of between the intervention and the control group. To evaluate cost-effectiveness, cost-effectiveness ratios (CERs) will be calculated for QALY. CERs are calculated by dividing the difference in the mean costs (between the intervention and control groups) by the differences in the mean outcomes

All data collected will be analyzed using SPSS analysis software. Findings will be considered statistically significant if the *p*-value is less than or equal to 0.05. The intervention group will be compared to the control group. Baseline characteristics will be compared using *X*2 test. Pearson’s correlation coefficient will be applied to the correlation of nutritional status and number of days with active CDI symptoms. Primary comparison outcomes will include CDI occurrence, duration, and reoccurrence using repeated measures ANOVA. An independent *t*-test will be used to compare continuous variables (weight loss, malnutrition status, number of days of diarrhea, number of days of hospital stay, number of days on antibiotics, etc.). ANOVA will be used to analyze differences between the two studied age groups, adults (ages 18-64 years) and older adults (age 65+). Mann-Whitney will be used to compare differences between the means of continuous variables. Analyses will include the intent-to-treat population. To test for cost effectiveness, the Mann–Whitney U tests will be used to test for statistically significant differences in means and standard deviations of costs between the intervention and control group. Compliance with study treatment will be calculated as the number of days that the participant consumed the kefir probiotic drink divided by the total number of intervention days.

4) What measurement/data collection tools are being used?

Electronic medical records, site specific forms for documenting diarrhea, and validated questionnaires.

**Is the proposed project “research” as defined by Institutional Review Board requirements?**

* Research is defined as a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.
* A human subject is defined as a living individual about whom an investigator obtains either 1) data through intervention or interaction with the individual; or 2) identifiable private information.

**Does the research involve human subjects or official records about human subjects?**

Yes

No

***If NO STOP here and SUBMIT application*.**

**If the results will be available in the library, presented at a professional conference (includes any presentation to group(s) outside of the classroom), or published, please check the Yes box:**

Yes

No

***If the YES box is CHECKED, proceed to SECTION IV.***

***If the NO box is CHECKED, STOP here and SUBMIT application*.**

**IV. Exemptions**

Are you requesting exemption from IRB review in one of the federally approved categories?

If yes, please reference OHRP website <http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.html> and continue with application.

**1) Does the research meet the criteria for exempt category 1 (education)?** [45 CFR 46.101 (b) (1)]

|  |  |
| --- | --- |
| Is the research conducted in established or commonly accepted educational settings (e.g. schools, Universities or other sites where educational activities regularly occur)? | Yes |
| No |

|  |  |
| --- | --- |
| Does the research study involve only normal education practices (e.g. instructional strategies, techniques, curricula, or classroom management techniques)? | Yes |
| No |

*If* ***both*** *questions are answered* ***yes****, stop here, proceed to* ***Section I Required Documentation****, and* ***submit*** *application.*

**2) Does the research meet the criteria for exempt category 2 (specific procedures)?** [45 CFR 46.101 (b) (2)]

|  |  |
| --- | --- |
| Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior? | Yes |
| No |

|  |  |
| --- | --- |
| Is the information obtained recorded in such a manner that human subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B) | Yes |
| No |

*If* ***both*** *questions are answered* ***yes****, stop here, proceed to* ***Section I Required Documentation****, and* ***submit*** *application.*

**3) Does the research meet the criteria for exempt category 3 (public officials)?** [45 CFR 46.101 (b) (3)]

|  |  |
| --- | --- |
| Does the research involve only the use of educational tests, survey procedures, interview procedures or observation of public behavior? | Yes |
| No |

|  |  |
| --- | --- |
| Are the human subjects elected or appointed public officials or candidates for public office? **If no, proceed to Category 4.** | Yes |
| No |

|  |  |
| --- | --- |
| Does any federal statute require without exception that the confidentiality of the personally identifiable information will be maintained throughout the research and thereafter? (See Appendix B) | Yes |
| No |

*If* ***all*** *questions are answered* ***yes****, stop here, proceed to* ***Section I Required Documentation****, and* ***submit*** *application.*

**4) Does the research meet the criteria for exempt category 4 (existing data/specimens)?** [45 CFR 46.101 (b) (4)]

|  |  |
| --- | --- |
| Does the research involve only the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens? | Yes |
| No |

|  |  |
| --- | --- |
| Will the information be recorded by the investigator in such a manner that the subjects cannot be identified directly or through identifiers linked to the subjects? (See Appendix B) | Yes |
| No |

*If* ***both*** *questions are answered* ***yes,*** *stop here, proceed to* ***Section I Required Documentation****, and* ***submit*** *application.*

**5) Does the research meet the criteria for exempt category 5 (federal program research)?** [45 CFR 46.101 (b) (5)]

|  |  |
| --- | --- |
| Does the research involve studying, evaluating or examining federal public benefit or service programs? | Yes |
| No |

|  |  |
| --- | --- |
| Is the research conducted through a federal agency? | Yes |
| No |

*If* ***both*** *questions are answered* ***yes****, stop here, proceed to* ***Section I Required Documentation****, and* ***submit*** *application.*

**6) Does the research meet the criteria for exempt category 6 (taste and food quality)?**

[45 CFR 46.101 (b) (6)]

|  |  |
| --- | --- |
| Does the research involve a taste and food quality evaluation or consumer acceptance study? | Yes |
| No |

|  |  |
| --- | --- |
| Does the food consumed contain no additives, or a limited amount of food additives at or below a level approved by the FDA or EPA or the Food Safety and Inspection Service of the U.S. Department of Agriculture | Yes |
| No |

*If* ***both*** *questions are answered* ***yes****, stop here, proceed to* ***Section I Required Documentation****, and* ***submit*** *application.*

***If no exemptions apply, continue with application.***

**V. Additional Project Information**

1) What human subjects training has the researcher completed (e.g. course work, online certification)?

Online certification

2) What process is used for obtaining informed consent (attach the informed consent application)? See Appendix for consent application.

Informed consent application

3) Does the research include special populations?

|  |  |  |
| --- | --- | --- |
| Minors under 18 years of age? | Yes | No |
| Persons legally incompetent? | Yes | No |
| Prisoners? | Yes | No |
| Pregnant women, if affected by research? | Yes | No |
| Persons institutionalized? | Yes | No |
| Persons mentally incapacitated? | Yes | No |

4) If **YES,** describe additional precautions included in the research procedures.

5) Does the research involve any of the following procedures?

|  |  |  |
| --- | --- | --- |
| False or misleading information to subjects? | Yes | No |
| Withholds information such that their informed consent might be questioned? | Yes | No |
| Uses procedures designed to modify the thinking, attitudes, feelings, or other aspects of the behavior of the subjects? | Yes | No |

6) If **YES**, describe the rationale for using procedures, how the human subjects will be protected and what debriefing procedures are used.

7) Does the research involve measurement in any of the following areas?

|  |  |  |
| --- | --- | --- |
| Sexual behaviors? | Yes | No |
| Drug use? | Yes | No |
| Illegal conduct? | Yes | No |
| Use of alcohol? | Yes | No |

8) If **YES**, describe additional precautions included in the research procedures.

9) Are any portions of the research being conducted online?

|  |  |  |  |
| --- | --- | --- | --- |
| Survey posted on a website? | Yes | No | If yes, assure anonymity |
| URL for survey includes information that could identify participants? | Yes | No | If yes, assure anonymity |
| Invitation to participate sent by email? | Yes | No | If yes, assure anonymity |
| Items use drop-down box? | Yes | No | If yes, assure that items allow choice of “no response” |

10) If **YES**, describe additional procedures.

11) Describe the methods used to ensure confidentiality of data obtained.

Study codes will be used on data documents instead of recording identifying information and a separate document will be kept that links the study code to subjects’ identifying information locked in a separate location with restricted access to this document (e.g., only allowing primary investigators access). Identifiable data will be encrypted. Information obtained from data/documents will be properly disposed, destroyed, and deleted.

**Risks and Benefits**

1) Describe risks to the subjects and the precautions that will be taken to minimize them. (Risk includes any potential or actual physical risk of discomfort, harassment, invasion of privacy, risk of physical activity, risk to dignity and self-respect, and psychological, emotional or behavioral risk.)

There are no risks associated with this study proposal.

2) Describe the benefits to subjects and/or society. (These will be balanced against risk.)

Currently, there are no known benefits that might result from this research. Conducting this research may provide results that will lead to a better understanding of the role of kefir probiotic in prevention and treatment of CDI and reoccurring CDI. The results of this study will benefit the field of nutrition and dietetics in clinical practice by providing further evidence of the recommendation of kefir supplement use to prevent CDI and reoccurrence of CDI. This study may provide additional information on how kefir could potentially reduce healthcare cost with CDI interventions.

**Appendix A: Required Elements of Informed Consent**

Informed consent is the process of communicating to a prospective participant, in easy-to-understand language (usually sixth- to eighth-grade level), all that he or she needs to know about participating in a research project, and then obtaining the prospective participant's agreement to participate. The following ten elements of consent are widely recognized and, except under certain specific conditions, **must be included in all consent processes and forms**:

1. An explanation of the study, including goals, procedure, and a statement that the study is research.
2. A description of what participants are expected to do and expected length of participation.
3. A description of any likely risks or discomforts for the participants. Potential harm should be explained in language that participants can understand and that relate to everyday life.
4. A description of any likely benefits to the participant or to others.
5. A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the participant.
6. A statement describing the level of privacy assured for collected information (anonymous, confidential) and how private information and information security will be managed.
7. An explanation of whom to contact for answers to questions about the research. When a Mount Mary student is the principal investigator, the name and phone number of a supervising faculty member is required.
8. An explanation of whom to contact for concerns about the participant’s privacy and rights, which for Mount Mary University is its IRB Chair.
9. For research involving more than minimal risk, a statement describing any compensation for injuries and contact information. (Minimal risk is a risk of harm to the participant that is no greater than the risk encountered in normal, day-to-day activities or during routine physical or psychological examinations.)
10. A statement that research participation is voluntary and the participant may withdraw from participation at any time, without penalty or loss of benefits to which the participant is otherwise entitled. If the participant is a patient or client receiving medical, psychological, counseling, or other treatment services, there should be a statement that withdrawal from the study will not jeopardize or otherwise affect any treatment or services the participant is currently receiving or may receive in the future. Participants also should be told whether their data will be destroyed should they withdraw from the study. If a survey instrument or interview questions are used and some questions deal with sensitive issues, the participants should be told they may refuse to answer individual questions.

**Appendix B: IRB De-Identification Standard for Information**

Protecting the privacy of research participants is a general concern in the vast majority of research projects. The degree to which privacy needs to be ensured or maintained depends on the nature of the particular research, its setting, and the research participants. Researchers share a general obligation to design their research to reduce the risks of disclosure of collected information about individual research participants. Thus, the present standard for de-identification of information is useful as a guide to protecting privacy even when it is not required or fully required. In this regard, the researcher should consider the following question when collecting and handling data.

Does the information I am accessing, recording, and/or disclosing contain identifiers? Simple access to information may be without concern, for example when the researcher is an employee who routinely handles the records in carrying out his or her position. But, the presence of identifiers in any **recorded or disclosed** information in the research means the information is not anonymous and so does not meet the IRB de-identification standard, which in some cases may also disqualify the research from exemption from IRB review. The IRB de-identification standard includes all 18 direct identifiers specified in the HIPAA Privacy Rule de-identification standard—*45 CFR 164.514(b)*. Below are listed specific direct and indirect identifiers that lead to information not being anonymous.

**Identifiers: Direct; Indirect**

One way to distinguish between information that is truly anonymous and information that is simply being kept confidential is to determine whether the data set contains direct or indirect identifiers. Information in a data set with either direct or indirect identifiers is not anonymous.

***Direct Identifiers*** include:

* Names
* Addresses
* Telephone and fax numbers
* Email addresses, IP addresses, and URLs
* Social Security numbers
* Medical record numbers
* Account numbers, such as those associated with bank accounts or health plans
* License or certificate numbers, including driver's license numbers
* License plate numbers and other vehicle identifiers
* Fingerprints, voiceprints, or full-face photographic images
* Other unique characteristics or identification numbers (example student ID numbers)

***Indirect Identifiers*** can be combined with publicly available information to identify individuals. The determination of indirect identifiers depends on the nature of the research participants. For example, in a study of residents of the state of Wisconsin, the information that someone graduated from one of the UW system schools probably would not be a unique identifier. However, in a study of small business leaders in Racine, WI, the same information might well apply to only one individual. In general, if any single variable in a data set applies to fewer than five participants, it is considered a potential indirect identifier.

Examples of indirect identifiers include:

* Detailed geographical information, such as state, county, or census tract of residence
* Organizations to which participants belong
* Educational institutions from which participants graduated
* Exact occupations
* Places where participants grew up
* Many dates, e.g. birth dates, hospital admission dates, high school or University graduation dates, etc.
* Detailed income information
* Offices or posts held by participants.

APPENDIX B

**Consent Form for Participation in a Research Study**

**Mount Mary University**

**A Controlled (Non-Randomized) Clinical** **Trial Assessing the Efficacy and Cost Effectiveness of Kefir Supplement as a Preventative Measure and Treatment Regimen With Use of Antibiotics for CDI and Reoccurring CDI.**

**Description of the research and your participation**

You are invited to participate in a research study conducted by Sarah Anderson RD LD. Clostridium *difficile* infection (CDI) is one of the most prevalent causes of nosocomial diarrhea associated with an increased risk of mortality. Despite recent medical advances in diagnosing and treatment, CDI remains a significant challenge and economic burden to healthcare systems. The purpose of this study is to determine the efficacy and cost effectiveness of kefir supplement as a preventative measure and treatment regimen with use of antibiotics for Clostridium *difficile* infection (CDI) and reoccurring CDI.

**Risks and discomforts**

There are no known risks associated with this research.

**Potential benefits**

Currently, there are no known benefits that might result from this research. Conducting this research may provide results that will lead to a better understanding of the role of kefir probiotic in prevention and treatment of CDI and reoccurring CDI. The results of this study will benefit the field of nutrition and dietetics in clinical practice by providing further evidence of the recommendation of kefir supplement use to prevent CDI and reoccurrence of CDI. This study may provide additional information on how kefir could potentially reduce healthcare cost with CDI interventions.

**Protection of confidentiality**

Every effort will be made to maintain the confidentiality of your participation in this project. Confidentiality will be maintained within legal limits. Your identity will not be revealed in any publication resulting from this study.

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose to participate in this research project but not the treatment group, you will be offered the treatment that is routinely offered in this clinic/hospital. Patients in the treatment group will not be permitted to use/ingest/consume Culturelle probiotic or any other probiotic during the study period. Patients in the treatment group will be offered eight ounces of the kefir supplement daily during their hospital admission. Three flavors of kefir will be offered (strawberry, blueberry, or vanilla). Each participant in the experiment group will be provided vouchers for free kefir supplements offered in stores or online to be shipped to their home. The vouchers will allow participants to continue the use of the oral probiotic until the end of the experimental phase. We will provide you with instructions to continue drinking 8oz of kefir supplement daily until symptoms resolve and to restart daily supplementation if symptoms start to resurface/reoccur/resume/regress.

Your participation will require you to complete phone questionnaires at the initiation of the study with a weekly follow-up by a registered dietitian or registered nurse, as an inpatient, until discharged from the hospital. Stool samples will be collected from participants with symptoms of CDI to determine diagnosis.

Your participation will also require you to complete questionnaires bi-monthly after your discharge date for one year. Based on the participant’s selected method of contact upon discharge, questionnaires will be completed bi-monthly via telephone or electronically via e-mail, both of which will be collected during the same bi-monthly data collection cycle.

**Voluntary participation**

Your participation in this research study is voluntary. You may choose not to participate and you may withdraw your consent to participate at any time. You will not be penalized in any way should you decide not to participate or to withdraw from this study.

**Contact information**

If you have any questions or concerns about this study or if any problems arise, please contact Sarah Anderson of Kingwood Medical Center and Mount Mary University at 616.402.5316. If you have any questions or concerns about your rights as a research participant, please contact the Mount Mary University Institutional Review Board at 414.258.4810.

**Consent**

**I have read this consent form and have been given the opportunity to ask questions. I give my consent to participate in this study.**

Participant’s signature\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

A copy of this consent form should be given to you.

APPENDIX C

Phone Questionnaire During Hospital Stay (Example)

TODAY'S DATE: PARTICIPANT ID:

**PLEASE LISTEN CAREFULLY TO THE FOLLOWING MESSAGE**

DURING THE FOLLOWING PHONE CONVERSTATION YOU WILL BE READ STATEMENTS CONCERNING BOWEL PROBLEMS (ANTIBIOTIC ASSOCIATED DIARRHEA) AND HOW THEY AFFECT YOU.

FOR EACH STATEMENT, PLEASE ANSWER WITH THE RESPONSE THAT APPLIES BEST TO YOU.

IF YOU ARE UNSURE ABOUT HOW TO RESPOND TO A STATEMENT OR QUESTION, PLEASE GIVE THE BEST RESPONSE YOU CAN. THERE ARE NO RIGHT OR WRONG RESPONSES.

IF YOU HAVE ANY QUESTIONS PLEASE ASK FOR CLARIFICATION OR CONTACT:

SARAH ANDERSON RD LD 616.402.5316

1. Please state the date you were admitted into the hospital:
2. Average number of loose stools bowel movements per day:
3. Age:
4. Sex: (Circle one) Male Female
5. Height (inches):
6. Weight (lbs.):
7. Have you been diagnosed with Clostridium difficile infection (CDI) also known as C. diff prior to beginning this study? (Circle one) **YES NO**
   1. If **YES** please state number of times you have been diagnosed with CDI:
8. Have you experienced weight loss? (Circle one) **YES NO**
   1. If **YES** please state amount of weight loss (lbs.):
   2. If **YES** please state time frame of weight loss (lbs.):
9. Were you diagnosed with Clostridium difficile infection (CDI) also known as C. diff during this admission? (Circle one) **YES NO**
   1. If **YES** please state date of Diagnosis:
10. Have you been prescribed antibiotics? (Circle one) **YES NO**
    1. If **YES** please list the antibiotics you were prescribed:
    2. If **YES** please state dates you were prescribed to take them:

**Patients In the control group only:**

1. Were you prescribed Culturelle probiotic? (Circle one) **YES NO**
   1. If **YES** please state the date of first dose:
   2. If **YES** please state the last date you took a dose:

This questionnaire asks you about the severity of symptoms you may have related to your gastrointestinal infection. There are no right or wrong answers. Please answer each question as accurately as possible.

For each symptom, please *circle the number* that best describes how *severe* the symptom has been during the past **week**. If you have not experienced this symptom, circle 0. If the symptom has been very mild, circle 1. If the symptom has been mild, circle 2. If it has been moderate, circle 3. If it has been severe, circle 4. If it has been very severe, circle 5. Please be sure to answer every question.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | None | Very Mild | Mild | Moderate | Severe | Very Severe |
| Diarrhea | 0 | 1 | 2 | 3 | 4 | 5 |
| Loss of Appetite | 0 | 1 | 2 | 3 | 4 | 5 |
| Not able to finish a normal-sized meal | 0 | 1 | 2 | 3 | 4 | 5 |
| Feeling excessively full after meals | 0 | 1 | 2 | 3 | 4 | 5 |
| Nausea (feeling sick to your stomach as if you were going to vomit or throw up) | 0 | 1 | 2 | 3 | 4 | 5 |
| Vomiting | 0 | 1 | 2 | 3 | 4 | 5 |
| Stomach fullness | 0 | 1 | 2 | 3 | 4 | 5 |
| For each statement, please circle the response that applies best to you | | | | | | |
| Frequency of diarrhea bowel movements per day | 0 | 1 | 1-5 | 5-10 | 10-15 | >15 |
| Amount of weight loss (lbs.) | 0-1 | 2-5 | 5-9 | 10-14 | 15-20 | >20 |
| Does your diarrhea interfere with your daily activities?  (For example, inability to work or decrease in social events) | I have not experienced diarrhea | Never | Rarely | Sometimes | Often | Always |

APPENDIX D

Post Discharge Bi-monthly Questionnaires (Example)

TODAY'S DATE: PARTICIPANT ID:

**PLEASE READ THIS CAREFULLY**

ON THE FOLLOWING PAGES YOU WILL FIND STATEMENTS CONCERNING BOWEL PROBLEMS (ANTIBIOTIC ASSOCIATED DIARRHEA) AND HOW THEY AFFECT YOU.

FOR EACH STATEMENT, PLEASE WRITE THE RESPONSE THAT APPLIES BEST TO YOU.

IF YOU ARE UNSURE ABOUT HOW TO RESPOND TO A STATEMENT OR QUESTION, PLEASE GIVE THE BEST RESPONSE YOU CAN. THERE ARE NO RIGHT OR WRONG RESPONSES.

IF YOU HAVE ANY QUESTIONS PLEASE CONTACT:

SARAH ANDERSON RD LD 616.402.5316

1. Please state the date of last hospital admission:
2. Have you been readmitted to the hospital since the initiation of this study? (Circle one) **YES NO**
   1. If **YES** please state reason(s) of readmission:
   2. If **YES** please state date(s) of readmission:
3. Have you experienced diarrhea in the past two months?

(Circle one) **YES NO**

* 1. If **YES** please state the beginning date(s) of the episode(s):
  2. If **YES** please state the duration (days) of the episode(s):
  3. If **YES** please state the average number of loose stools bowel movements per day during episode(s) of diarrhea:

1. Age:
2. Sex: (Circle one) Male Female
3. Height (inches):
4. Weight (lbs.):
5. Have you experienced weight loss? (Circle one) **YES NO**
   1. If **YES** please state amount of weight loss (lbs.):
   2. If **YES** please state time frame of weight loss (lbs.):
6. Were you diagnosed with Clostridium difficile infection (CDI) also known as C. diff during your previous admission? (Circle one) **YES NO**
   1. If **YES** please state date(s) of Diagnosis:
7. Have you been prescribed antibiotics? (Circle one) **YES NO**
   1. If **YES** please list which antibiotic you were prescribed:
   2. If **YES** please state date(s) you were prescribed to take them:

**Patients In the control group only:**

1. Were you prescribed Culturelle probiotic? (Circle one) **YES NO**
   1. If **YES** please state the date of first dose:
   2. If **YES** please state the last date you took a dose:

This questionnaire asks you about the severity of symptoms you may have related to your gastrointestinal infection. There are no right or wrong answers. Please answer each question as accurately as possible.

For each symptom, please *circle the number* that best describes how *severe* the symptom has been during the past **two (2) months**. If you have not experienced this symptom, circle 0. If the symptom has been very mild, circle 1. If the symptom has been mild, circle 2. If it has been moderate, circle 3. If it has been severe, circle 4. If it has been very severe, circle 5. Please be sure to answer every question.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | None | Very Mild | Mild | Moderate | Severe | Very Severe |
| Diarrhea | 0 | 1 | 2 | 3 | 4 | 5 |
| Loss of Appetite | 0 | 1 | 2 | 3 | 4 | 5 |
| Not able to finish a normal-sized meal | 0 | 1 | 2 | 3 | 4 | 5 |
| Feeling excessively full after meals | 0 | 1 | 2 | 3 | 4 | 5 |
| Nausea (feeling sick to your stomach as if you were going to vomit or throw up) | 0 | 1 | 2 | 3 | 4 | 5 |
| Vomiting | 0 | 1 | 2 | 3 | 4 | 5 |
| Stomach fullness | 0 | 1 | 2 | 3 | 4 | 5 |
| For each statement, please circle the response that applies best to you. | | | | | | |
| Frequency of diarrhea bowel movements per day | 0 | 1 | 1-5 | 5-10 | 10-15 | >15 |
| Amount of weight loss (lbs.) | 0-1 | 2-5 | 5-9 | 10-14 | 15-20 | >20 |
| Does your diarrhea interfere with your daily activities?  (For example, inability to work or decrease in social events) | I have not experienced diarrhea | Never | Rarely | Sometimes | Often | Always |

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