Prevention of *Clostridium difficile*-associated diarrhea by daily intake of kefir

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Research Question

This study is being conducted to investigate the potential benefits of probiotic intake for preventing antibiotic associated diarrhea and *Clostridium difficile* infection (CDI) in patients undergoing a systemic antibiotic treatment. **Primary research question:** can daily kefir consumption reduce the incidence of diarrhea and CDI in patients during antibiotic treatment?

Specific Aims

- Enroll a total of 1190 patients into three, randomized study groups:
  Patients prescribed antibiotics and receive no kefir
  Patients prescribed antibiotics and receive kefir during their hospital stay only
  Patients prescribed antibiotics and receive kefir for the duration of their antibiotic regimen, for a maximum of 30 days

- Monitor and record clinical findings specifically related to diarrhea, for example the incidence, duration, and recurrence of diarrhea, for patients enrolled in the study.

- Carry out statistical data analyses in order to assess the efficacy of daily kefir intake on reducing the incidence of diarrhea and CDI

Background and Significance

Clostridium difficile infections are of great concern for hospitals nationwide and *C. difficile* remains the most common nosocomial infection in North America [1]. The majority of patients with *C. difficile* infection respond to orally administered metronidazole or vancomycin, but 5-30% of patients go on to develop recurrent infection and diarrhea, despite antibiotic treatments[2]. The idea that gut microbiota influence susceptibility and recovery from disease is becoming more widely reported[3]. Disruption of normal bacterial flora in the gut during systemic antibiotic use has been attributed to the uncontrolled growth of bacteria that are otherwise suppressed such as *C.*

difficile. Even after an antibiotic regimen has concluded, restoration of the normal gut microbiota is thought to be paramount to interrupting the cycle of *C. difficile* infection. The idea of reintroducing normal microbial flora to patients with recurrent *C. difficile* infections has been the foundation upon which the use of probiotic agents, including fecal transplants, has been introduced[4]. The use of probiotics to treat antibiotic-associated diarrhea, and specifically *C. difficile* infections, has been studied and successes have been reported, and in particular by our group[4,5]. The emerging idea, however, is that probiotic consumption can be used to prevent the onset of a *C. difficile* infection, which requires further study. Meta-analyses have been conducted on previously reported data and ultimately conclude that the use of probiotics is safe and effective[6]. However, there remains a need for a focused and controlled study assessing the efficacy of kefir consumption on the incidence of *C. difficile* infections at St. Luke's Hospital in Duluth, MN, and CHI Creighton University Medical Center in Omaha, NE. The data collected from this study have the potential to change clinical practice at not only our institutions, but at any hospital or institution that has a population of patients undergoing systemic antibiotic treatment.
Risk factors for persistent *C. difficile* infection include age greater than 65 years, poor nutritional status, recent abdominal surgery, prolonged hospitalization and recent stay in the ICU[2]. The purpose of this study is to determine if administering at-risk patients a daily regimen of kefir can reduce the incidence of *C. difficile* infections. Because treatment for *C. difficile* infections is difficult and expensive, and recurrent infections are common, a way to prevent these infections is of great interest. A preventive measure that reduces the incidence of diarrhea and *C. difficile* infections will clearly improve patient health, and will have a multi-fold impact on how patients are cared for while in the hospital as well as post-discharge. There is potential for cost-saving, as well, in the form of the direct cost of antibiotic treatments and prolonged hospital stays. Additionally, the cost in time and effort to prevent the spread of these infections will be reduced, as well, all of which ultimately improve patient care.

Preliminary Studies

Many studies have been published indicating a potential for probiotics as a prophylactic against diarrhea associated with antibiotic use in humans[6]. Additionally, a recent report in a rodent model suggests that kefir consumption concomitantly with antibiotic treatment may prevent the development of *C. difficile*-associated diarrhea[7]. However, to our knowledge there have not been any studies published that have investigated the preventative effects of kefir consumption specifically related *C. difficile* infection in human patients. This protocol will serve as an initial study for a subset of at-risk patients and the data collected will provide guidance for future studies to include a more broad range of at-risk patients.

Research Design and Methods

Subjects

The objective is to identify a population of at-risk patients that are undergoing treatment at St. Luke's Hospital (Duluth, MN) and CHI Creighton University Medical Center (Omaha, NE). The study will include patients who have been prescribed a systemic antibiotic of any kind (administered by oral or parenteral route), but have not yet begun the treatment regimen. There will be three study groups:

Patients prescribed antibiotics and receive no kefir
Patients prescribed antibiotics and receive kefir during their hospital stay only
Patients prescribed antibiotics and receive kefir for the duration of their antibiotic regimen, for a maximum of 30 days

All patients will receive a journal to track their kefir intake (if in Group C) and any loose stools or diarrhea for 30 days after their hospital stay. Additionally, all patients will receive a follow-up phone 3-5 days after discharge from the hospital, and weekly thereafter for 30 days, to determine if diarrhea has occurred (total of 4 phone calls). Three attempts will be made to reach the patient for each scheduled phone call. All patients will be instructed to call their physician if symptoms of diarrhea occur. The aims of this study are to determine the incidence of antibiotic-associated diarrhea in each of the study groups as well as the presence of *C. difficile* in the stool of those patients that report having diarrhea.

Risk factors for C. difficile infection:
Age over 65 years
Prolonged stay in an intensive care unit
Antibiotic use
Hypoalbuminemia
Compromised immune system

Inclusion criteria for this study: *Patients who have*
Been admitted to the medical unit on 7 West at St. Luke's Hospital (Duluth, MN), the 4600 or 5500 floor at CHI Creighton University Medical Center (Omaha, NE)
Been prescribed a systemic antibiotic of any kind (administered by oral or parenteral route), and have not yet started taking antibiotics or have started taking antibiotics within 6 hours prior to study enrollment
Consented to be randomized and take part in the study and are adults greater than 19 years of age

Exclusion criteria for this study: *Patients who are/have*
Tube feeding
Undergoing dialysis and other renal treatment
An existing *C. difficile* infection
A recent history of *C. difficile* infection (within the last 3 months)
A recent history of antibiotic use (within the last 3 months)
Inflammatory bowel disease, Crohn's disease, or other chronic gastrointestinal syndrome
Transferred from the ICU
A history of acquired of genetic immunodeficiencies; active, acute or chronic serious infections (i.e., viral hepatitis, HIV/AIDS), or autoimmune disorders
Undergoing gastrointestinal surgery, radiation, or cytotoxic chemotherapy
Allergy to milk protein

Randomization Plan

Patients will be randomized into each of three study groups by using a computer-generated random number system. As patients are enrolled onto the study, a number will be drawn from an envelope that has previously been assigned to one of the three study groups. Patients will be enrolled into the study group that corresponds to the number drawn.

Data Collection and Accrual Methods

Patients who meet inclusion criteria and consent to be part of the study will be assigned to one of the three study groups at random:

Group A: Receive no kefir
Group B: Receive kefir during their hospital stay only
Group C: Receive kefir for the duration of their antibiotic regimen, for a maximum of 30 days

During the standard admissions interview, patients are asked about any allergies, including milk protein allergy. This type of allergy will exclude patients from participating in the study. Because kefir is 99% lactose free, lactose intolerance will not exclude individuals from participating. Per standard of care, a physical exam will be performed and a symptom-directed exam will be performed at any follow-up appointments, typically one month after discharge from the hospital.

The consent process will be carried out by Jenna Wentzlaff, PharmD., with assistance from pharmacy interns on rotation at St. Luke's Hospital in Duluth, MN. Consenting will be carried out by John M. Horne, M.D., Leopoldo Alberto Dobronski Jacome, M.D., and Javeria Syed, MD at the medical center in Omaha, NE. The consenting process will be carried out at the institution at which the patient is hospitalized. This process will be done on a continuous and at-need basis such that patients who enroll on this study are not delayed their antibiotic and/or kefir regimens.

Patients in study groups consuming kefir will receive a postcard that includes the nutrition facts for a 4-ounce serving of kefir. Lifeway brand Kefir® will be used as it is widely available and comes in several palatable flavors. The probiotic content of Lifeway Keir remains constant, regardless of flavor.

Patients in Group A will receive their prescribed antibiotics during their hospital stay and will not receive kefir. These patients will be instructed to not consume any yogurt, kefir, or other probiotic products during their hospital stay and for 30 days post-discharge.

Patients in Groups B and C will begin receiving kefir on the same day that their prescribed antibiotic regimen begins. At least 4 ounces of kefir will be administered 3 times a day to patients enrolled on this study. A 4 oz. glass of kefir will be provided with every mealtray, and nursing staff at each of the participating institutions will administer the kefir to the patients. The amount of kefir consumed by the patient will be recorded by the nursing staff and entered in the electronic medical record. Four ounces is a standard serving of yogurt and is well-tolerated by patients. This serving size will help maintain ongoing live probiotic cultures in the individuals receiving kefir.

For patients in Group B, administration of kefir by the nursing staff will continue for the duration of the patients' hospital stay only. For patients in Group C, kefir will be prescribed at the same

minimum dose as given during their hospital stay (at least 4 ounces of kefir 3 times each day, not to exceed 12 ounces per day).

All patients will be given a journal in which they can record any symptoms of a *C. difficile* infection (i.e., diarrhea). Additionally, patients in Group C will record their kefir consumption on a daily basis. All patients will receive a follow-up phone 3-5 days after discharge from the hospital, then weekly for a total of 30 days from their hospital discharge (a total of 4 phone calls). Three attempts will be made to reach the patient for each scheduled phone call. Patients will be asked if they have had any incidence of diarrhea in order for nursing staff and physicians to assess if *C. difficile* infection has occurred.

All patients will be asked to contact their physician at the hospital at which they received treatment if they experience diarrhea after their hospital stay. These patients will be offered a fecal *C. difficile* test, as per standard of care, from the laboratory within the hospital from which they received care. A clinical diagnosis is required to determine if an individual has a *C. difficile* infection. The criterion that must be met is a patient who has 3 or more watery stools in a 24 hour period. Stool collection and testing will be carried out per treatment guidelines[8].

Patients that develop a *C. difficile* infection during the study will be immediately treated per standard of care and will be considered treatment failures[8]. However, these patients will be contacted for follow-up information pertinent to the study (i.e., duration of symptoms, and efficacy of standard treatment). Follow up contacts will be made via telephone; an initial call will be made 3-5 days after discharge from the hospital and patients will be contacted weekly thereafter for 30 days (a total of 4 phone calls). Three attempts will be made to reach the patient for each scheduled phone call. During these phone calls, patients will be asked if they have any concerns or symptoms, specifically related to diarrhea.

If at any point during the hospitalization or during the follow-up timeframe a patient receiving kefir displays signs or symptoms of an invasive bacterial, yeast, or fungal infection, blood cultures and other cultures as deemed clinically appropriate (urine culture, sputum culture, wound culture, etc.) will be obtained. Signs and symptoms of an invasive infection include, but are not limited to, the following: fever, chills, elevated WBC concentration, elevated serum C-reactive protein (CRP) and/or procalcitonin levels, and hypotension.
If a suspected invasive infection occurs during the course of kefir administration, the following empiric treatment regimen will be considered:

- Intravenous administration of vancomycin plus piperacillin/tazobactam plus fluconazole
    - Intravenous daptomycin will be used for patients with a previously documented vancomycin allergy
    - An intravenous fluoroquinolone (ciprofloxacin or moxifloxacin) will be used for patients with a documented penicillin allergy
    - Intravenous micafungin will be used for patients with a documented fluconazole allergy

Initial intake screening for inclusion in the kefir research study will include an evaluation of known allergies. Antibiotic therapy will be adjusted in accordance with identification of the causative organism(s) and corresponding antibiotic susceptibilities. Treatment duration will be determined based on standards of care for the clinical situation and the causative organism.

Data Analysis

The primary outcome variables will be 1) any diarrhea post antibiotic administration and 2) any *C. difficile* infection post antibiotic administration. No further typing of isolates will be performed on samples obtained as it is beyond the scope of this study. The three treatment groups will be compared using logistic regression with covariates of study location, age, gender, antibiotic type and doses, hypoalbuminemia, and compromised immune system. Model selection based on the Akaike Information Criterion will be used to select covariates in final models. The treatment groups will also be compared without covariate adjustment. As needed, missing data will be handled using multiple imputation after assessment of missing at random considerations. Dependent and independent variables in planned analyses will be included in imputations along with any auxiliary variables thought to be potentially related to predicting the missing data. In addition, in order to include other auxiliary covariates in the imputations that are potentially related to missingness, logistic regression will be performed with the dependent variable missing/not missing. Only imputed values of independent variables will be used in the analyses. Multiple imputation will be performed with the R package mi.[9]

The data being collected from the patient diaries are to be used to monitor compliance with antibiotic use, kefir consumption, and any symptoms of C. diff infection. These data will be particularly useful in the event that there is no difference seen in C. diff infection rates among the study groups. From the self-reported patient data, compliance can be monitored and exclusion from the study may be warranted in the case of non-compliance.

Settings

Patients will be drawn from the identified population on 7 West at St. Luke's Hospital (Duluth, MN) and the 4600 or 5500 floor at CHI Creighton University Medical Center (Omaha, NE). Data will be collected during the patients' stay and will be collected from the patient journals post-discharge. All data collected will be shared among co-investigators named on this study from St. Luke's Hospital (Duluth, MN) and CHI Creighton University Medical Center (Omaha, NE).

Measurement Endpoints

Primary Endpoint: The primary endpoint will be the incidence of diarrhea among the three randomized study groups.

Co-Primary Endpoint: The co-primary endpoint will be an assessment of the safety of kefir when administered to individuals undergoing antibiotic treatment.

Secondary Endpoints: The secondary endpoint will be the incidence of C. difficile infection among the three randomized study groups. No further isolate typing will be performed on samples obtained in this study.

Data Management Plan
Data on subjects will be obtained by the nursing staff on 7 West of St. Luke's Hospital in Duluth, MN. Clinical information, as outlined above in Data Collection and Accrual Methods, will be entered into the electronic medical record as advised by the Clinical Research Nurse at each of the institutions/departments participating in this study. Additionally, data will be entered into an electronic database on an ongoing basis throughout the study in order to facilitate data analysis after the study has concluded.

Evaluation Plan
The conduct of the study will be evaluation on the basis of:

Accrual of the adequate number of patients to populate the study groups
Completion of all components of the study
Successful dissemination of the results and findings

Analysis Plan
Data from this study will be analyzed using the methods described above. Data will be submitted for statistical analysis after patient identifiers have been removed and only the assigned study number is used.

Study Limitations
A limitation of this study is patient compliance with consumption of appropriate amounts of kefir for the assigned study group. For example, it is imperative that the patients who are asked to not drink any kefir, or are asked to only drink kefir during their hospital stay but not at home until the conclusion of the study comply with their kefir regimen. Additionally, the use of kefir as the probiotic agent introduces a broad range of microbiota to the patient and the particular organism(s) that may be responsible for improving the incidence of *C. difficile* infections will not be identified.

Timeline
The duration of this study will be approximately 6 months; this should allow for an appropriate number of patients to be enrolled in the study, per current patient load at our institutions, and will allow time for follow-up interviews to be conducted. At the conclusion of the study, the primary and secondary endpoints will be analyzed in order to develop a manuscript for reporting our findings, the duration of which will take approximately 1 year.

Dissemination of Results
A manuscript describing our findings will be completed and submitted for publication. In addition, these data may be presented to local or national audiences at conferences or meetings as deemed appropriate.

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