A Randomized Clinical Trial Measuring the Influence of Kefir on Antibiotic-Associated Diarrhea

The Measuring the Influence of Kefir (MILK) Study

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Objective: To examine the role of commercially available kefir, a fermented milk similar to yogurt but containing different fermentation microbes, in preventing antibiotic-associated diarrhea (AAD). Probiotics have shown some promise in preventing AAD.

Design: A double-blinded randomized placebo-controlled allocation concealment clinical trial.

Setting: Primary care patients in the Washington, DC, metropolitan area.

Participants: A total of 125 children aged 1 to 5 years presenting to primary care physicians.

Intervention: Kefir drink or heat-killed matching placebo.

Main Outcome Measure: The primary outcome was the incidence of diarrhea during the 14-day follow-up period in children receiving antibiotics.

Results: There were no differences in the rates of diarrhea per group, with 18% in the active group and 21.9% in the placebo group (relative risk 0.82; 95% confidence interval, 0.54-1.43). Additionally, there were no differences in any secondary outcomes among the groups. However, there were some interesting interactions among initial health at enrollment, age of participants, and sex that require further study.

Conclusions: In our trial, kefir did not prevent AAD. Further independent research on the potential of kefir needs to be conducted.

Trial Registration: clinicaltrials.gov Identifier: NCT00481507


A CUTE DIARRHEA IN YOUNG children is almost always caused by infections or antibiotics. Children are often placed on antibiotics, and the rate of antibiotic-associated diarrhea (AAD) is 20% to 35%. Currently, the mainstay of treating AAD is proper hydration and, if necessary, substituting different antibiotics. Probiotics are live microorganisms that, when administered in sufficient amounts, confer a health benefit to the host. Probiotics have the potential to prevent AAD.

Often, probiotics are ingested as one would consume drugs, in powder, pill, or liquid forms designed specifically for medicinal benefit. Alternatively, probiotics are included as ingredients in fermented dairy products such as yogurt as a functional food—a food that provides health benefits beyond their nutritional value. However, simply because one probiotic is efficacious does not mean other probiotics or the same probiotic at a different dose or delivery method will be similarly effective.

The promise of using functional foods to mitigate disease and promote health is one of the major reasons so many resources are being placed in this exciting new field. Kefir is a fermented milk similar to yogurt but containing different fermentation microbes. According to the Food and Agriculture Organization/World Health Organization, kefir is defined as a “starter culture prepared from kefir grains, Lactobacillus kefiri, and species of the genera Leuconostoc, Lactococcus, and Acetobacter growing in a strong specific relationship. Kefir grains constitute both lactose-fermenting yeasts (Klyuyveromyces marxianus) and non-lactose-fermenting yeasts (Saccharomyces unisporus, Saccharomyces cerevisiae, and Saccharomyces exiguus).” It is believed that these probiotics deliver beneficial bacteria to the gut, improving gastrointestinal

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health, and may protect against AAD. We were thus interested in examining the role of commercially available kefir in preventing AAD.

### METHODS

#### STUDY DESIGN

A double-blind, randomized, placebo-controlled allocation concealment trial was conducted in Washington, DC, Northern Virginia, and the Medstar DC areas of Maryland. Participants consumed active or control drink for 10 consecutive days while being treated with antibiotics for upper respiratory tract infections. The Georgetown University institutional review board in Washington, DC, approved all aspects of the trial and participants' parents signed informed consent. An independent Data and Safety Monitoring Board met and reviewed data at predetermined stages and were alerted to adverse events. Adverse events were defined by the parent as events possibly related to the drink.

#### PARTICIPANTS

Children were enrolled from primary care offices after physicians had prescribed antibiotics for upper respiratory infections. Generally healthy children aged between 1 and 5 years were enrolled. Children were excluded from participation for taking any other medicines regularly, lactose intolerance, allergy to lime flavors, inability of parent to speak English or Spanish, active diarrhea or constipation, and chronic disease. In addition, parents had to be willing to refrain from giving their child any other cultured dairy products or probiotics during the 2 weeks of the study.

The randomization scheme was generated using permuted blocks with block size equal to 8. It was impossible for research personnel involved with participants to adjust randomization or discern what drinks participants were receiving, ensuring true allocation concealment.

#### INTERVENTION

The intervention was Probugs (Lifeway Foods, Inc, Chicago, Illinois), a drink that is commercially available in the United States. Each production was produced specifically for the study. During the 8 months of enrollment from September 9th, 2007, until April 10th, 2008, 141 messages were left on the recruitment line; 61 were rolled from these callers, or 89% of the original families unable to participate owing to not meeting inclusion criteria. One hundred twenty-five participants were enrolled from these callers, or 89% of the original families who left messages on the recruitment line; 61 were allocated into the active group and 64 into the placebo (Figure).

#### OUTCOME MEASURES

The study was designed to determine the role of commercially available kefir as a dietary management to prevent AAD. The primary outcome was a clinical determination of diarrheal incidence by blinded research personnel using parental reports during follow-ups and diary data. Secondary outcomes were chosen based on previous probiotic research showing some effect; thus, we included absences from daycare or school owing to illness, missed parental work owing to the child being ill, vomiting, stomach pain, constipation, sneeze nose, cough, earaches, fever, irritability, lethargy, and loose stools. Adverse events were collected at the regularly scheduled follow-ups or parents had a 24-hour phone number to call and report any adverse events. In discussion with the institutional review board and the Data and Safety Monitoring Board, it was agreed that adverse events were defined either by the parent or health care provider as any event that could possibly be related to the study drink. Serious adverse events were defined as any incidence of death, a life-threatening event, hospitalization, prolonged hospital stay, or an event resulting in permanent disability.

#### STATISTICAL AND POWER ANALYSIS

Setting the significance at $\alpha = 0.05$ (2-tailed), sample sizes of 62 per group were required for the study to have 80% power of statistically detecting a difference in the rates of diarrhea of 0.20 or more. This computation assumes the rate of diarrhea in the placebo group is 0.30 vs 0.10 in the kefir group. This difference was selected as the smallest difference that is clinically important and reasonable to detect.

Initially, various descriptive techniques (means, medians, standard deviations, histograms, box plots) were used to examine the characteristics of each study group. Either parametric or non-parametric statistical tests (depending on the level of measurement) were used to test the difference in the baseline variables between the groups. Multiple logistic regression was used to compare the rates of diarrhea between the groups, adjusting for various baseline covariates and interactions. Pearson and deviance residuals were calculated for checking assumptions. Plots of residuals by explanatory variables, probabilities, and order of entry into the study (from first to last) showed no outliers or patterns. For follow-up data, because subjects were repeatedly measured at baseline (day 0) and at days 6, 11 and 15, differences between the groups were tested using generalized linear modeling (for the outcome of diarrhea) and linear mixed-effects modeling (for continuous outcome variables such as 0-10 Likert health scores). In the latter case, the random slope and intercept models were fitted and parameter terms were estimated using restricted maximum likelihood estimation. Data was entered in SPSS data files, and all statistical tests were performed using SAS (SAS Inc, Carey, North Carolina). Statistical significance was set a priori at $P = 0.05$. All statistical analyses were performed using the intent-to-treat principle.

#### RESULTS

During the 8 months of enrollment from September 9th, 2007, until April 10th, 2008, 141 messages were left on the recruitment line (Figure). Sixteen participants were unable to participate owing to not meeting inclusion criteria. One hundred twenty-five participants were enrolled from these callers, or 89% of the original families who left messages on the recruitment line; 61 were allocated into the active group and 64 into the placebo (Figure).
There were no major differences regarding any of the baseline characteristics between the 2 groups (Table 1). The average ages were 2.9 years in the active group and 3.2 years in the placebo group. Most of both groups spent at least 20 hours per week in daycare or school at baseline. On a 10-point Likert scale of overall health, the placebo group rated 9.1 and the active group 8.9 (10=extremely healthy, rated by the parents) before the study started. Additionally, 18% of the interviews were conducted in Spanish (data not shown).

COMPLIANCE

Both groups averaged 6.5 drinks in the 10-day intervention period. In the placebo group, 27% of the group consumed 3 or fewer drinks; 13%, 4 to 6 drinks; and 60%, 7 or more drinks. Rates were similar in the active group, with 27% of the group consuming 3 or fewer drinks; 18%, 4 to 6 drinks; and 55%, 7 or more drinks. Neither group of parents was better at predicting what drinks their child was consuming; 64% in the active group and 67% in the placebo group believed they were receiving the kefir drink (P=.84). Five participants in the active group and 8 in the placebo group reported consuming other probiotics during the study period. Loss to follow-up was exceptionally low. Only 4 participants in each group were unable to be contacted at the final follow-up on day 15.

PRIMARY AND SECONDARY OUTCOMES

As shown in Table 2, there were no differences in the rates of diarrhea per group, with 18% in the active group and 21.9% in the placebo group (relative risk, 0.82; 95% confidence interval, 0.54-1.43). Additionally, there were no differences in any secondary outcomes among the groups.

At day 0 of the study, parents from both groups rated their child’s health similarly on a 10-point scale, 6.0 in the control and 6.1 in the active group (1=extremely unhealthy, 10=extremely healthy). Both groups had improved to 9.5 on day 15, and at no point during follow-up did either group differ statistically. There were also no differences in change in activity due to illness, missed school, or missed parental work among the groups (data not shown).
ADDITIONAL ANALYSES

Based on analysis from the multivariate modeling and initial health, age and sex group interaction effects were observed. Although these terms were not statistically significant, they revealed potential future hypotheses to be considered. These interactions could be predicted a priori and were not listed on the clinical trials site when we registered the trial. Examining the groups by participants with initial health at enrollment of 5 or less on the 10-point Likert scale, it demonstrated diarrhea in 31% of the placebo group compared with 23% of the active group. However, participants with a score greater than 5 were more similar, with 20% in the placebo group and 16% in the active group having diarrhea.

Additionally, the 77 participants aged 3 to 5 years had much lower rates of diarrhea, with the placebo group at 14% compared with 6% in the active group, while participants aged 2 years or younger had a 38% rate of diarrhea in the placebo group compared with 33% in the control group.

Most interestingly, a sex difference was observed, with boys in the placebo group having diarrhea at a rate of 32% vs 24% in the active group. This is in contrast to 12% of the girls in the placebo group and 10% in the active group.

ADVERSE EVENTS

Safety in the study was excellent, as expected for a food. In the active group, 1 parent reported emesis that they believed was related to the drink, and in the control group 1 parent reported constipation. Both participants recovered within days without any subsequent sequelae.

The kefir we studied did not show a statistically significant reduction in AAD compared with placebo based on analysis for the entire subject group. Our overall rates were consistent with previously reported AAD rates. Additionally, it does not appear that the kefir resulted in improved secondary outcomes such as runny nose and emesis. As expected, the older participants had much lower rates of diarrhea. However, the absolute differences in the older groups were rather large, with a 57% increase in diarrhea in the placebo group. Another finding that is much more difficult to explain is that boys had much higher rates of diarrhea than girls (28% vs 11%, respectively). Additionally, boys in the placebo group had a 25% increase in diarrhea compared with the boys in the active group.

Antibiotic-associated diarrhea is a serious disease that results in high rates of morbidity, decreased compliance, and increased public health costs. Although educating physicians and the lay public has led to dramatic decreases in antibiotic prescriptions, approximately 25% of all visits for children younger than 5 years still result in a prescription for antibiotics.20-23 In fact, children aged 3 to 36 months average more than 2 antibiotic prescriptions per year, with nearly 30% receiving more than 4 prescriptions per year.24 Additionally, in 1991, the outpatient costs of treating diarrhea in children younger than 3 years was calculated at $0.6 to 1 billion per year, excluding emergency department visits and hospitalizations.25 Thus, effective preventive measures for AAD are of utmost importance.

We initially were interested in this study because many physicians in our area are recommending yogurts, kefirs, and probiotic supplements to prevent AAD. However, the data for these foods and supplements are limited and are often provided by industry-conducted studies. In fact, a Cochrane review of AAD found only 10 studies that met their inclusion criteria.25 Interestingly, only the per protocol analysis showed significant results while the intent-to-treat analysis found no differences. Also, the review found a diversity of products, modalities of ingestion, and strains. Additionally, the Cochrane review commented that current research does not allow determination of age effect. Our study shows clear differences in age that need further clarification.

Our study has several important limitations that need to be noted. We did not assess stools or culture stools to evaluate the presence of pathogenic organisms. The most serious outcome of AAD is Clostridium difficile and, owing to financial constraints, we were unable to evaluate participants for the presence of C difficile. We also did not independently examine children and relied on parental reports. We chose this method for 2 reasons. We studied a functional food, not a medicinal product; parents will thus feed their children without any physician input, and we felt it was best to assess it under similar conditions. Additionally, most of our outcomes were patient-oriented, and asking parents to assess outcomes is at times preferable for these types of outcomes. Most importantly, we had extremely low rates of loss to follow-up, and thus our method of data collection led to robust valid data. However, clearly some of these assessments are subjective and could vary by evaluator. Additionally, our population was generally very healthy. In the small subgroup in which parents described lower baseline health status, there were much higher rates of diarrhea and greater effect size differences. It is possible that if our source population was not as healthy we would have had different results. Finally, we did not assess probiotic dosages throughout the study. Thus, it is possible that the dosages participants received were different, as probiotic colony-forming units decrease over time. However, no participant consumed products past the expiration date, similar to how they would drink the product if purchased in a store. We only studied 150 mL each day; it is possible a higher dosage of probiotics might have had different outcomes.

Our randomized clinical trial did not find that kefir consumed in conjunction with antibiotics resulted in lower rates of AAD. It is important to recognize that this trial studied specific strains at specific dosages and our findings cannot be extrapolated for other strains or outcomes. There are some intriguing data that we believe deserve further elucidation and may hold promise for kefir’s role in AAD prevention. We also believe that it is important that commercial products continue to be independently studied and subjected to high-quality research techniques, as many products appear to present themselves as a panacea while lacking patient-oriented outcome data.

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REFERENCE


Call for Papers

Archives of Pediatrics and Adolescent Medicine will devote their May 2010 issue to papers on the effects of life experiences occurring during the critical window of birth to age 3 years on the emotional and psychological health and development—or ill health—of children both during that age and at later ages during childhood and adolescence. Papers submitted by September 30, 2009, have the best chance of acceptance.

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