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Probiotics reduce symptoms of antibiotic use in a hospital setting: A randomized dose response study

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ABSTRACT

Probiotics are known to reduce antibiotic associated diarrhea (AAD) and Clostridium difficile associated diarrhea (CDAD) risk in a strain-specific manner. The aim of this study was to determine the dose-response effect of a four strain probiotic combination (HOWARU® Restore) on the incidence of AAD and CDAD and severity of gastrointestinal symptoms in adult in-patients requiring antibiotic therapy. Patients ($n = 503$) were randomized among three study groups: HOWARU® Restore probiotic 1.70×10^{10} CFU (high-dose, $n = 168$), HOWARU® Restore probiotic 4.17×10^{9} CFU (low-dose, $n = 168$), or placebo ($n = 167$). Subjects were stratified by gender, age, and duration of antibiotic treatment. Study products were administered daily up to 7 days after the final antibiotic dose. The primary endpoint of the study was the incidence of AAD. Secondary endpoints included incidence of CDAD, diarrhea duration, stools per day, bloody stools, fever, abdominal cramping, and bloating. A significant dose-response effect on AAD was observed with incidences of 12.5, 19.6, and 24.6% with high-dose, low-dose, and placebo, respectively ($p = 0.02$). CDAD was the same in both probiotic groups (1.8%) but different from the placebo group (4.8%; $p = 0.04$). Incidences of fever, abdominal pain, and bloating were lower with increasing probiotic dose. The number of daily liquid stools and average duration of diarrhea decreased with higher probiotic dosage. The tested four strain probiotic combination appears to lower the risk of AAD, CDAD, and gastrointestinal symptoms in a dose-dependent manner in adult in-patients.

1. Introduction

Antibiotics have provided great medical benefits and have enabled the control of numerous infectious diseases. However, the use of antibiotics may be accompanied by gastrointestinal disturbances; most notably antibiotic-associated diarrhoea (AAD) [1]. The incidence of AAD ranges between 5 and 39% and varies according to individual susceptibility, the patient environment, and the class of antibiotics administered [2,3].

While AAD can be caused by multiple pathogens and has multifactorial etiology, between 10 and 25% of all episodes of AAD, and virtually all those seen in antibiotic-induced pseudo-membranous colitis, are caused by *C. difficile* [4,5]. Incidence rates and proportion of severe cases of *C. difficile*-associated diarrhea (CDAD), and associated mortality, are on the rise [5–7].

Specific probiotic strains have been shown to have various beneficial health effects [8–11]. Recent meta-analyses concluded that probiotics produced relative risk reductions of 44–57% for AAD and 41–71% for CDAD [3,10,12]. Specific probiotics, therefore, seem to be promising as an adjunct to antibiotics to reduce the risk of AAD. Although several studies of specific probiotics against AAD exist, there is currently only one dose-response study comparing 5 and 10×10^{10} CFU; that is limited to elderly [13]. The primary objective of the present study was therefore to investigate the effect of a specific combination of probiotic strains, which has earlier been shown to stabilize the intestinal microbiota [14], on the incidence of AAD at two different doses (4.7×10^{9} and 1.70×10^{10} CFU) in a hospital setting in adults aged 30–70. The secondary objectives were to investigate their influence on the severity and duration of AAD and CDAD.

2. Methods

All research procedures were in strict accordance with a pre-defined protocol and were registered at clinicaltrials.gov

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Table 1
Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Males and females	Pregnant or breastfeeding women
Age 30–70 years	Subjects presenting with active diarrhea (3 or more liquid stools per 24-hour period).
Prescribed antibiotic therapy for a minimum of 3 days and a maximum of 14 days	Subjects with a history of daily consumption of probiotics, fermented milk and/or yogurt
Expected to remain hospitalized for a minimum of 5 days	Subjects known to have shown a previous reaction, including anaphylaxis, to any substance in composition of the study product
Subjects who have received less than 36 h of antibiotic therapy	Subjects presenting with an active, non-controlled intestinal disease such as Crohn's disease or ulcerative colitis
Informed consent obtained after verbal and written information	A previous documented *C. difficile* infection <3 months prior to study initiation
Subjects having a telephone available (mobile, work, home)	Ostomised subjects; parenteral nutrition users
Subjects having a refrigerator at home	Subjects on an immunosuppressive therapy, or any health condition causing immunosuppression (including hematological malignancies, AIDS)
	Ongoing or recent use of antibiotic therapy in the 30 days prior to the study product first administration
	Subjects with planned administration of antibiotics other than broad-spectrum penicillin, cephalosporin or clindamycin for the treatment of an infection
	Subjects with concomitant participation in another clinical trial
	Subjects who are not likely to comply with study requirements
	Subjects with self-reported lactose intolerance

(NCT01143623). The study was reviewed and approved by the Shanghai Nutrition Society and conducted in accordance with the declaration of Helsinki. The CONSORT Statement for randomized trials served as a template for reporting this clinical study [15].

3. Study design

The study was a triple-blind, randomized, placebo controlled, dose-ranging study with a 1:1:1 allocation ratio among three parallel study groups. Subjects were equally stratified by gender, age (30–49 years and 50–70 years) and duration of antibiotic treatment (3–8 days and 9–14 days).

4. Study volunteers

Subjects were recruited at Changhai Hospital (Shanghai, China) and were hospitalized for various diseases requiring antibiotic therapy. Subjects were evaluated for eligibility based on pre-defined inclusion and exclusion criteria (Table 1) and were asked to sign the informed consent form. Subjects were subsequently randomized and received both antibiotics and one of the 3 study products. Baseline characteristics are shown in Table 2.

5. Study products

The probiotic study products marketed as HOWARU® Restore, consist of equal amounts of *Lactobacillus acidophilus* NCFM® (ATCC 700396), *Lactobacillus paracasei* Lpc-37 (ATCC SD5275), *Bifidobacterium lactis* Bi-07 (ATCC SD5220), and *Bifidobacterium lactis* Bl-04 (ATCC SD5219), and were supplied in size 0, V-caps capsules (Capsugel, Peapack, NJ, USA) by Danisco USA, Inc. (Madison, Wisconsin, USA).

Table 2
Baseline subject characteristics.

Characteristics	Placebo (n = 167)	High dose (n = 168)	Low dose (n = 168)
Days on antibiotics			
3–8 days	84 (50.3%)	84 (50.0%)	84 (50.0%)
9–14 days	83 (49.7%)	84 (50.0%)	84 (50.0%)
Male gender	84 (50.3%)	84 (50.0%)	84 (50.0%)
Current smoker	56 (33.5%)	50 (29.8%)	53 (31.5%)
Age; years[a]	50.0 ± 11.0	50.5 ± 11.0	49.3 ± 11.4
Diastolic pressure; mm Hg[a]	77.5 ± 8.1	77.0 ± 6.5	76.6 ± 6.6
Systolic pressure; mm Hg[a]	127.0 ± 10.9	127.5 ± 10.5	125.9 ± 10.6
BMI; kg/m^2[a]	23.3 ± 2.5	23.3 ± 2.4	23.4 ± 2.5
Antibiotics administered			
Broad spectrum penicillin	43 (25.8%)	43 (25.6%)	47 (28.0%)
Cephalosporin	100 (59.9%)	92 (54.8%)	90 (53.6%)
Clindamycin	24 (14.4%)	33 (19.6%)	31 (18.5%)
Underlying disease			
Respiratory tract infection	75 (44.9%)	71 (42.3%)	67 (39.9%)
Urinary tract infection	53 (31.7%)	66 (39.3%)	54 (32.1%)
Prophylaxis before/after surgery	39 (23.4%)	31 (18.5%)	47 (28.0%)

[a] Mean ± SD.

The capsules contained the probiotic combination at either a low-dose: 4.17×10^9 colony forming units (CFU) or high-dose: 1.70×10^{10} CFU or placebo. The excipient used to adjust bacterial counts and to serve as placebo was micro crystalline cellulose (Tabulose Type 132, Blanver, Taboão da Serra, São Paulo, Brazil) containing 0.5% (w/w) Syloid 63FP Silicon Dioxide A (W.R. Grace & Co., Columbia, MD, USA) as flow aid. All products were stored refrigerated at the study site until time of use and were refrigerated by the participants throughout supplementation. No significant reduction in viable counts was observed during the study.

6. Intervention

The study product was administered daily, approximately 2 h after breakfast and antibiotic, for 10–21 days, depending on length of antibiotic administration (study product was consumed until 7 days after the last antibiotic, Fig. 2). The investigators followed subjects via a weekly telephone call for four weeks after finishing the antibiotic course, to inquire about concomitant medications, incidence/continuation of diarrhea, adverse events and compliance with study product. Subjects who developed diarrhea after discharge from hospital were asked to provide a stool specimen for *C. difficile* testing.

7. Outcomes

The primary outcome of the study was AAD incidence, defined as three or more liquid stools (Bristol Stool Chart Type 7) in a 24-hour period, and was confirmed by two physicians.

Secondary outcomes were CDAD incidence determined by testing stool samples from subjects with 2 or more liquid stools in a 24-hour period; the *C. difficile* test was conducted using a stool sample from the second diarrhea stool. Stool samples were tested for the presence of *C. difficile* using the Triage Micro *C. difficile* Panel (Biosite Inc. San Diego, USA). Cytotoxicity testing was performed on all specimens tested by triage that were antigen positive and toxin A negative. Detection of cytotoxin (Tox B) was performed by

cell culture. A stool sample was considered *C. difficile* positive if it was antigen positive with either toxin A and/or toxin B [16]. Duration of diarrhea was determined by the number of continuous days of diarrhea. Mean number of liquid stools was determined by the sum of liquid stools in the AAD episode, divided by the duration of diarrhea in days, the last passed stool with Bristol scale 7 was taken as the end of AAD.

While in hospital, data were recorded on case report forms (CRFs) by the clinician or research nurse; visual examination of liquid stools for the presence of blood, oral fever >38 °C, and presence of painful abdominal cramps, bloating, and constipation. Subjects who had not completed their full antibiotic treatment and/or study product during their stay in the hospital received the appropriate amount of each to complete treatment on an outpatient basis. Subjects were supplied with diary cards and instructed by the physician to record daily self-administration of the antibiotic and study product and to record any diarrhea, number of liquid stools per day, presence of blood in stools, painful abdominal cramping, soft stools, bloating, and constipation. After diary cards were returned by the subject, the research nurse transferred this information to the CRF.

8. Sample size

Sample size was calculated to detect a decreasing trend in the proportion of subjects with AAD with increasing probiotic dose: placebo (AAD incidence assumed to be 25%), low-dose probiotic (AAD incidence assumed to be 20%), and high-dose probiotic (AAD incidence assumed to be 10%). The study also had sufficient power to detect a trend in the proportion of subjects with CDAD (placebo incidence assumed to be 15%, low-dose probiotic incidence 10%, high-dose probiotic incidence 3%). Assuming 90% power and 2-sided alpha = 0.05, 145 evaluable subjects per group (435 total) were needed. Due to an anticipated 18% attrition rate, a total of 510 volunteers (170 per group) were enrolled. Sample size was calculated according to Mehta and co-workers [17].

9. Randomization

Randomization was pre-stratified by age (30–49 and 50–70 years), antibiotic treatment duration (3–8 and 9–14 days) and gender, and was implemented using a permuted block design, to ensure balanced subject allocation to the three study groups. Subjects were randomly allocated to one of the three treatment groups within their appropriate strata, using computer-generated randomization lists. Study products were distributed in bottles containing 25 capsules; the bottles were individually labeled with unique subject ID numbers and were provided to the site by the sponsor.

10. Statistical methods

Statistical analyses were performed on an intention to treat (ITT) basis. Variables in the initial descriptive and univariate analyses included baseline demographic data and subject characteristics, current smoking status, outcome variables including incidence of AAD and CDAD, and expected side effects including constipation, bloating, and soft stool (Bristol stool chart Type 5 and 6). Variables addressing AAD severity included each component of the severity indicator; presence of blood in liquid stools, fever, painful abdominal cramps, duration of diarrhea, and average number of liquid stools per day. Positivity for presence of *C. difficile* was examined.

Compliance with study treatment was evaluated using one-way analysis of variance (ANOVA) by calculating, for each subject, the percentage of intended product actually taken. Incidence of AAD and *C. difficile* were summarized as frequencies and percentages by study group, number of days on antibiotics, age, and gender.

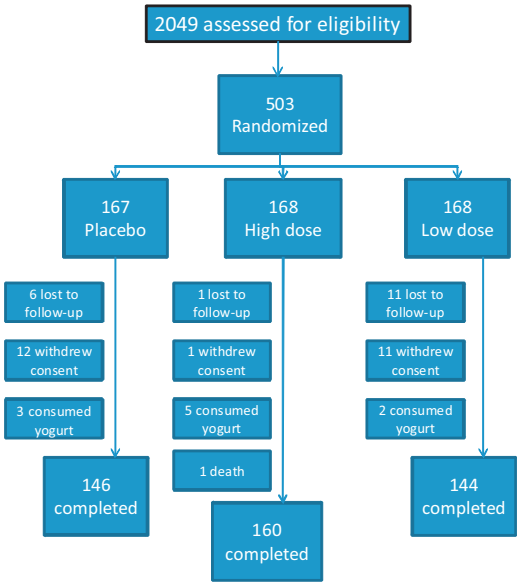

Fig. 1. CONSORT patient flow diagram. Low dose; 4.17×10^9 CFU probiotics, high dose 1.70×10^{10} CFU probiotics.

Univariate comparisons among test products were conducted using chi-square test or Fisher's exact test, as appropriate.

Multivariate comparisons of AAD and CDAD incidence by product group were made using logistic regression analyses to adjust for age, number of days on antibiotics, type of antibiotics, number of days on study product, number of hospital days, gender, body mass index (BMI), and reason for antibiotic prescription. Because age and the number of days on antibiotics are continuous but were pre-stratified into 2 categories for randomization purposes, the logistic regression model was built both ways, with the 2 variables being treated as either continuous or categorical.

A likelihood ratio model and Cochran's test of linear trend assessed dose-response effects of placebo and 2 levels of active study product on incidence of AAD and CDAD, and also to evaluate the effects of placebo and 2 dose levels of active product on side effects.

For continuous variables describing AAD severity, data were analyzed using ANOVA. A least significant difference (LSD) pairwise group comparison assessed treatment effect on diarrhea duration and average number of liquid stools, while adjusting the overall alpha level for multiple comparisons.

Statistical analyses were performed using SAS software (Version 9.1, SAS Institute, Inc., Cary, NC, USA) and BMDP software (Version 8.2, Statistical Solutions, Saugus, MA, USA).

11. Results

11.1. Recruitment and subject disposition

Between June and October 2010, 2049 subjects were assessed for eligibility. Of these, 503 were enrolled and randomized: 167 to placebo and 168 to each probiotic treatment (Fig. 1). Main reasons for non-inclusion were: lactose intolerance (26.5%), regular use of pre- and probiotic products (25.2%), not willing to participate (18.4%) and prescription of other antibiotics then defined in the inclusion/exclusion criteria (13.8%).

Subject attrition rates were significantly different between the groups ($p = 0.010$). The high-dose group (4.8%) had fewer drop outs then the low-dose (14.3%; $p = 0.0048$) and fewer drop outs then the placebo (12.6%; $p = 0.0118$), Fig. 1. Compliance was excellent (>99%) in all groups.

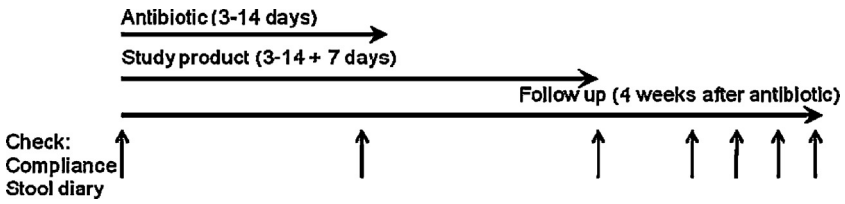

Fig. 2. Time line of study activities.

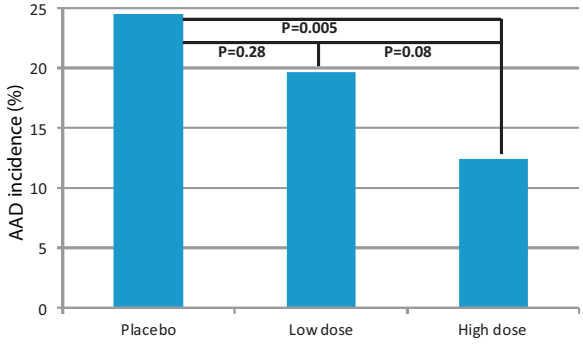

Fig. 3. Antibiotic-associated diarrhea (AAD) incidence by study group. Low dose 4.17×10^9 colony forming units (CFU), high-dose: 1.70×10^{10} CFU, placebo: microcrystalline cellulose.

12. Efficacy outcomes

12.1. Antibiotic-associated diarrhea

The highest AAD incidence was observed in the placebo group (24.6%), lowest in the high-dose group (12.5%), and intermediate in the low-dose group (19.6%), Fig. 3. Treatment effect on AAD incidence was significant ($p = 0.02$). The placebo group distinguished itself from the high-dose group ($p = 0.005$), while no significant effect was observed for placebo vs. low-dose ($p = 0.28$) or high-dose vs. low-dose ($p = 0.08$). A linear trend was identified between increasing dose of study product and decreasing incidence of AAD ($p = 0.005$).

AAD incidence rates were higher ($p = 0.004$) among older subjects then among younger subjects (12.3% vs. 6.6%) and higher ($p < 0.001$) in subjects with longer exposure to antibiotics (13.5% vs. 5.4%).

After adjustment for covariates (gender, age, and days of antibiotic use), high-dose probiotic decreased the odds of AAD incidence to 0.39 of the placebo product (95% CI: 0.21–0.72; $p = 0.003$); while the low-dose decreased to 0.72 (95% CI: 0.41–1.25) vs. Placebo, $p = 0.24$. The odds of younger subjects developing AAD was 0.46 (95%CI 0.28–0.75, $p = 0.002$) compared to older subjects. For each one-unit increase in BMI (as measured at baseline), the odds of AAD incidence (vs. non-presence) decreased 0.90 fold (95% CI: 0.81–0.99; $p = 0.04$).

12.2. Clostridium difficile-associated diarrhea

The CDAD incidence was highest in the placebo group (4.8%) and identical with high-dose and low-dose groups (1.8%) ($p = 0.04$ among groups). The comparison of placebo vs. high-dose for incidence of CDAD was statistically significant ($p = 0.02$). Although both high-dose and low-dose groups had the same incidence of CDAD the low-dose had more cases of AAD, therefore no significant difference in CDAD incidence compared to placebo was observed ($p = 0.25$).

Logistic regression adjusted for covariates identified that taking the high-dose product tended ($p = 0.098$) to decrease the odds of CDAD incidence to 0.29 of the placebo product (95% CI: 0.07–1.25). Younger subjects had only 0.15 (95% CI 0.03–0.72) of the odds of older subjects for CDAD ($p = 0.018$). Shorter exposure to antibiotics tended ($p = 0.082$) to be associated with an odds of 0.13 (95% CI 0.01–1.30) for CDAD of longer exposure.

13. Number needed to treat (NNT)

In order to prevent one case of AAD the NNT with high dose product was 8.4. To prevent one case of CDAD, the NNT with high-dose product was 33.6.

14. Diarrhea-associated symptoms

Fever rates differed significantly between the groups ($p = 0.04$, Table 3), being significantly lower in the high-dose group (1.88% $p = 0.02$) compared to placebo (8.2%) with a trend ($p = 0.09$) for fewer cases of fever in the low-dose group (3.5%). A significant linear trend was observed ($p = 0.011$) for increase in fever rate across treatment groups, which was confirmed by the likelihood ratio test for dose response effect ($p = 0.034$).

Abdominal pain differed significantly between the groups ($p < 0.001$). Fewer subjects in the high-dose group abdominal pain (2.5%; $p < 0.001$) then in the placebo group (19.2%), as well as compared to the low-dose (13.9%; $p = 0.001$). When considering diarrhea cases only, similar differences were seen; the high-dose group had significantly few cases (19.1%) of abdominal pain then placebo (71.1%; $p = 0.001$) and a trend for fewer cases compared to low-dose (61.3%; $p = 0.010$). A strong linear trend was observed ($p < 0.001$); also the likelihood ratio test indicated a dose-response effect ($p < 0.0001$).

The average number of liquid stools (Table 3) was reduced from 2.2 (SD = 1.1) in the placebo group to 1.7 (SD = 0.6) in the high-dose group ($p = 0.034$) and to 2.0 (SD = 0.9) in the low-dose group ($p = 0.04$). There was no difference in the average number of liquid stools between the two doses tested.

Diarrhea duration (Table 3) was reduced from 5.4 days (SD = 1.8) in the placebo to 2.6 days (SD = 0.93) in high-dose group ($p < 0.001$) and to 3.5 days (SD = 1.3) in the low-dose group ($p < 0.001$). Diarrhea lasted significantly shorter in the high-dose group as compared to the low-dose group ($p = 0.03$).

Bloating was significantly different between the treatment groups ($p = 0.03$, Table 3). Bloating in the high-dose group was reduced (1.3%) compared to the placebo group (7.5%; $p = 0.01$) and a linear trend indicated significance ($p = 0.008$). Also the likelihood ratio test indicated a dose response effect ($p = 0.02$).

Soft stool was reported by 89 subjects, with no difference between the treatment groups ($p = 0.17$). A linear trend ($p = 0.06$) was identified, though the likelihood ratio test did not indicate a significant dose response ($p = 0.16$).

14.1. Safety outcomes

The adverse event rate in the placebo, high-dose, and low-dose group was 7.2%, 4.2%, and 4.2%, respectively. Adverse events reported included: allergy to seafood (2), arrhythmia (2), fever (10), headache (2), left upper arm fracture (1), runny nose (4), and

Table 3
Severity of diarrhea-associated symptoms, for the whole study group and diarrhea cases only.

	Variable	Placebo	High-dose	Low-dose	*p*-value
Total population	*n*	167	168	168	Fisher's exact test
	Fever (>38 °C)	12 (8.22%)	3 (1.88%)	5 (3.47%)	0.035
	Blood in stool	3 (2.05%)	0 (0.00%)	1 (0.69%)	0.133
	Abdominal pain	28 (19.18%)	4 (2.50%)	20 (13.89%)	<0.001
	Constipation	0 (0.00%)	0 (0.00%)	0 (0.00%)	–
	Bloating	11 (7.53%)	2 (1.25%)	5 (3.47%)	0.029
	Soft stool	36 (22.60%)	23 (14.38%)	30 (18.75%)	0.168
Diarrhea cases	*n*	41 (24.6%)	21 (12.5%)	33 (19.6%)	One-way ANOVA
	Number of liquid stools (mean ± SD)	2.24 ± 1.07	1.71 ± 0.64	1.97 ± 0.86	<0.001
	Duration of diarrhea (Days, mean ± SD)	5.37 ± 1.77	2.57 ± 0.93	3.45 ± 1.28	0.041

vomiting (4). One serious adverse event was reported during the study. A 38-year-old male with a history of coronary heart disease died of a myocardial infarction. No adverse events were judged by the investigators to be related to the study product.

15. Discussion

Diarrheas have been an early target for probiotic treatment, and various meta-analyses have indicated that many probiotics reduce the incidence of AAD [3,10,12]. The present study shows a reduction in the incidence, duration, and severity of AAD and some associated symptoms; in a dose dependent manner. The study also indicates a reduction in CDAD incidence.

The overall incidence of AAD in the current study was 18.9% and in the placebo group 24.6%. This is much in line with what has been reported earlier: 2–29% [3,18], depending on the antibiotic used. The CDAD incidence was 2.8% overall and 4.8% in the placebo group; which is lower then what has been reported earlier: 10–50% [18–20]. Drop outs were least in the high-dose group; this was mainly due to more subjects being lost to follow up and withdrawing consent (Fig. 1). One could speculate that since the lower dose and placebo were less effective hence fewer volunteers were motivated to continue.

The incidence of AAD was found to be higher in older subjects; an observation that has been made before [18,19]. Also, longer exposure to antibiotics increases the AAD risk [2], as was the case in the current study, although this effect was not significant after adjustment for treatment group and age group. The observation that an increase in BMI reduces the risk for AAD is new, to our knowledge. It should, however, be noted that the subjects had a BMI in the normal range.

In the present study; the higher of the 2 doses of probiotics was able to reduce the incidence of AAD from 24.6 to 12.5%; i.e., almost half, with a NNT of 8.4. This range of reduction is similar to what has been reported in the various meta-analyses: relative risks of 0.43 [3], 0.51 [12], 0.53 [19], and 0.58 [10] and a NNT of 8. Similarly for CDAD; the high-dose was found to be associated with a significant reduction in incidence compared to placebo: 1.8% vs. 4.8% respectively, with a NNT of 33.6. This reduction is comparable to the relative risks observed by Hempel et al. [10], relative risk 0.29, and McFarland [3], relative risk 0.59.

Abdominal pain was reduced only in the high-dose group, when focusing on the diarrhea cases only, the low-dose group also showed a trend for reduced abdominal pain. This reduction in abdominal pain is interesting, as *L. acidophilus* NCFM®, one of the probiotic components in the tested preparation, has earlier been shown to increase the pain threshold in rats by inducing the expression of μ-opioid and canabinoid 2 receptors [21]. Both average number of liquid stools and average duration of diarrhea were significantly reduced by both the high and the low dose as compared to placebo.

Bloating was significantly reduced by the high dose compared to placebo. This is of interest as the combination of *L. acidophilus* NCFM® and *B. lactis* Bi-07, which are both included in the tested product, has been observed to reduce bloating in subjects functional bowel disorders [22].

The present study was designed to examine dose-response. In general, the higher dose tested performed better then the low dose for most endpoints, also those that did not reach statistical significance. This is in line with earlier reports, in which high doses have been suggested to be more efficacious in the prevention of AAD [3,23]. A recent dose-response study with a probiotic combination (*L. acidophilus* CL1285® and *Lactobacillus casei* LBC80R®) also demonstrated dose-response effects for various symptoms of AAD in elderly subjects [13]. In contrast to our study, the lower dose in this study was able to significantly reduce both AAD and CDAD. The doses used in that study were, however, considerably higher than those used in our study.

The present study is one of the largest studies with probiotics and AAD and is the only dose-response study with probiotics on AAD in adults. The results indicate that the higher tested dose of the HOWARU® Restore probiotic combination is more efficacious then the lower dose in reducing AAD symptoms, duration, and incidence in a hospital setting. Nevertheless, the results should not be interpreted that a higher dose is always superior for any probiotic strain (combination) and any health target; that remains to be determined in more detail

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Conflict of interests: None

Specific author contributions: Planning of the study: Arthur Ouwehand, Cai DongLian, Xu Weijian, Morgan Stewart, Jiayi Ni, Tad Stewart, Larry Miller; conducting study and collecting data: Cai DongLian, Xu Weijian; interpreting data: Arthur Ouwehand, Morgan Stewart, Jiayi Ni, Larry Miller; drafting manuscript: Arthur Ouwehand, Morgan Stewart, Larry Miller; approval of submitted final draft: Arthur Ouwehand, Cai DongLian, Xu Weijian, Morgan Stewart, Jiayi Ni, Tad Stewart, Larry Miller.

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Potential competing interests: Arthur Ouwehand is an employee of DuPont Nutrition & Health; DuPont Nutrition & Health manufactures and markets the probiotics tested in this study.

References

[1] Wistrom J, Norrby S, Myhre E, Erikson S, Grandström G, Lagergren L. Frequency of antibiotic-associated diarrhoea in 24462 antibiotic-treated hospitalized patients: a prospective study. J Antimicrob Chemother 2001;47:43–50.
[2] McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis 1998;16:292–307.

[3] McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. Am J Gastroenterol 2006;101:812–22.
[4] Barbut F, Petit J. Epidemiology of *Clostridium difficile* associated infections. Clin Microbiol Infect 2001;7:405–10.
[5] Morris A, Jobe B, Stoney M, Sheppard BC, Deveney CW, Deveney KE. Clostridium difficile colitis: an increasingly aggressive iatrogenic disease? Arch Surg 2002;137:1096–100.
[6] Pépin J, Valiquette L, Alary M, Forget K, Pépin K, Chouinard D. *Clostridium difficile* associated diarrhea in a regionof Quebec from 1991 to 2003: a changing pattern of disease severity. Can Med Assoc J 2004;171:466–72.
[7] Curry J. Excerpt from Pseudomembranous Colitis; 2007 http://www.emedicine.com/med/byname/Pseudomembranous-Colitis.htm
[8] FAO/WHO. Guidelines for the evaluation of probiotics in food; 2002 http://www.who.int/foodsafety/publications/fs_management/probiotics2/en/
[9] de Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S, Heller K, Schrezenmeir J. Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized, controlled trial. Vaccine 2006;24:6670–4.
[10] Hempel S, Newbery SJ, Maher AR, Wang Z, Miles JN, Shanman R. Probiotics for the prevention and treatment of antibiotic-associated diarrhoea: a systematic review and meta-anlysis. JAMA 2012;307:1959–69.
[11] Björksten B. Diverse microbial exposure–consequences for vaccine development. Vaccine 2012;30:4336–40.
[12] Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. Lancet Infect Dis 2006;6:374–82.
[13] Gao XW, Mubasher M, Fang CY, Reifer C, Miller LE. Dose-response efficacy of a proprietary probiotic formula of *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* LBC80R for antibiotic-associated diarrhea and *Clostridium difficile*-associated diarrhea prophylaxis in adult patients. Am J Gastroenterol 2010;105:1636–41.
[14] Engelbrektson A, Korzenik JR, Pittler A, Sanders ME, Klaenhammer TR, Leyer G, Kitts CL. Probiotics to minimize the disruption of faecal microbiota in healthy subjects undergoing antibiotic therapy. J Med Microbiol 2009;58:663–70.
[15] Moher D, Schulz KF, Altman D. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA 2001;285:1987–91.
[16] Massey V, Gregson DB, Chagla AH, Storey M, John MA, Hussain Z. Clinical usefulness of components of the triage immunoassay, enzyme immunoassay for toxins A and B, and cytotoxin B tissue culture assay for the diagnosis of *Clostridium difficile* diarrhea. Am J Clin Pahol 2003;119:45–9.
[17] Mehta CR, Patel NR, Senchaudhuri P. Exact power and sample size computations for the Cochran–Armitage trend test. Biometrics 1998;54:1615–21.
[18] Bartlett J. Clinical practice. Antibiotic-associated diarrhea. N Engl J Med 2002;346:334–9.
[19] Asha NJ, Tompkins D, Wilcox MH. Comparative analysis of prevalence, risk factors, and molecular epidemiology of antibiotic-associated diarrhea due to *Clostridium difficile*, *Clostridium perfringens*, and *Staphylococcus aureus*. J Clin Microbiol 2006;44:2785–91.
[20] Videlock EJ, Cremonini F. Meta-analysis: probiotics in antibiotic-associated diarrhea. Aliment Pharm Ther 2012, http://dx.doi.org/10.1111/j.1365-2036.2012.05104.x.
[21] Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C, Dubuquoy L, et al. *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. Nat Med 2007;13:35–7.
[22] Ringel Y, Ringel-Kulka T, Maier D, Carroll I, Galanko JA, Leyer G, et al. Probiotic bacteria *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. J Clin Gastroenterol 2011;45:518–25.
[23] Doron SI, Hibberd PL, Gorbach SL. Probiotics for prevention of antibiotic-associated diarrhea. J Clin Gastroenterol 2008;42:S58–63.