Title: Probiotic yogurt for the prevention of antibiotic-associated diarrhea in adults. A randomized double-blind placebo-controlled trial.

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ABSTRACT

Goal: To evaluate the effect of yogurt supplemented with probiotic bacteria on the prevention of antibiotic-associated diarrhea (AAD) in hospitalized patients.

Background: Diarrhea following antibiotic administration is a frequent clinical problem. The usefulness of probiotics for the prevention of AAD in the hospitalized adult population remains unclear.

Study: A randomized, double blind, placebo-controlled clinical trial was conducted in 314 hospitalized patients (mean age 76 years) who started antibiotic treatment. Patients were randomized (2:2:1) to receive a daily amount of 200 ml of placebo yogurt (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*), 200 ml of probiotic yogurt (previous plus *Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* subsp. *lactis* Bb-12 and *Lactobacillus casei* subsp. *casei* Lc-01 or no yogurt (unblinded control) within 48 hours of beginning the antibiotic therapy and up to 5 days after stopping the antibiotic. Patients were followed up with for one month to determine occurrence of diarrhea.

Results: The rate of diarrhea was 23.0% probiotic versus 17.6% placebo, absolute risk reduction -5.35%, 95% CI -15.4 to 4.7 %, p=0.30. Rate of diarrhea was similar in the unblinded external control and in the blinded study groups combined (20.9 % versus 20.2 % respectively, p=0.91). There was no difference in the duration of diarrhea, maximum number of bowel movements or prolonged admission because of diarrhea among the groups. All cause mortality did not differ between groups.

Conclusions: The combined probiotic strains LA-5, BB-12 and LC-01 do not have an effect in the prevention of AAD in hospitalized patients.

Keywords: Probiotics; Antibiotic-associated diarrhea; adults; randomized controlled trial.

Introduction

Antibiotic-associated diarrhea (AAD) is a frequent complication (5-30%) of antimicrobial therapy.[1,2] This rate may increase in hospitalized patients due to comorbidities, and may differ according to the antibiotic administered among other factors. Antibiotics most commonly associated with AAD are beta-lactams, clindamycin and fluoroquinolones. Cephalosporins, aminopenicillins and clindamycin are associated with high risk (amoxicillin-clavulanate up to 25%)[3] and fluoroquinolones with intermediate risk.[1-3] AAD is associated with patient discomfort, a longer hospital stay and sometimes requires antibiotic treatment interruption.

Disturbance of the intestinal microbiota is considered a potential cause for AAD. In addition, age-related alterations in gut physiology have an important effect on lowering the diversity, composition and functional characteristics of gut microbiota.[4,5]

Attempts to recolonize the mucosa and restore gastrointestinal microbiota equilibrium may help to reduce AAD. Probiotics are defined as live microorganisms that confer a health benefit on the host when administered in adequate amounts.[6] Specific recommendations of the World Gastroenterology Organization (WGO) for AAD indicate that there is evidence of efficacy of certain probiotic strains in adults or children who are receiving antibiotic therapy. [7] Nonetheless, recent meta-analyses, have stated that probiotic administration could be less effective in the elderly. [8] Moreover, *Saccharomyces cerevisiae* var. *boulardii* (*S. boulardii*), which stands out in the prevention of AAD due to intrinsic antibiotic resistance, has recently shown no evidence in a large cohort of adult hospitalized patients.[9] Other trials have failed to provide consistent beneficial effects.[10] The usefulness of probiotics for the prevention

of AAD in hospitalized patients remains unclear, and additional high quality trials are needed.

Lactobacillus acidophilus LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Lactobacillus casei* subsp. *casei* LC-01 are among the most widely used and potentially beneficial probiotics.[7,11,12] Changes in the microbiota of patients admitted to a hospital suggest the convenience of using high-dose and multi-strain preparations of lactobacilli and bifidobacteria to provide restorative effects of the complex gut ecosystem. Therefore, we designed a randomized placebo-controlled double blind study to assess the effect of a multispecies (*B. lactis* Bb-12, *L.acidophilus* La-5 y *L. casei* Lc-01) probiotic yogurt on the prevention of AAD in adult hospitalized patients. The placebo in this study provides a strong differential character in our trials, which allows the focus on the AAD-preventive effect of the probiotic strains.

Methods

Study design and participants

The study represents a single centre, prospective, randomized, double blind, placebo-controlled clinical trial that was conducted at Hospital Universitario Fundación Alcorcón (Madrid, Spain) from June 2005 to February 2008. Patients admitted to the hospital older than 18 years who were prescribed amoxicillin-clavulanate or levofloxacin (oral or intravenous) in their treatments and who were able to take food and drink orally, were recruited from the Internal Medicine and Pneumology wards. Exclusion criteria were pregnancy, allergy to penicillins or levofloxacin, intolerance to lactose or dairy products, diarrhea on admission or within

the preceding week, reported recurrent diarrhea, or bowel disease that could result in diarrhea, severe immunosuppression, active neoplasia, HIV infection, regular probiotic treatment before admission, or laxative use or enema within 48 hours before admission. Patients were followed up with for one month to determine occurrence of diarrhea.

The study was conducted in accordance with the principles of the Declaration of Helsinki and 'good clinical practice' guidelines. The ethics committee of Hospital Universitario Fundación Alcorcón, as well as the Spanish Ministry of Science approved the final protocol. Oral and written informed consent was obtained from all patients before inclusion in the trial. According to progressive regulations regarding non-drug related clinical trials, our protocol was retrospectively registered (Registration number ISRCTN46764354).

Randomization and blinding

After stratification by antibiotic (amoxicillin-clavulanate, levofloxacin), patients were randomly allocated by a randomized computer-generated list in blocks of 10 to receive in a 2:2:1 way 150-200 ml daily of a probiotic yogurt drink , a 150-200 ml of a placebo yogurt drink, or none (unblinded control arm). The randomization assignment was unavailable to investigators until patients had signed informed consent and enrolled in the trial. All patients were specifically instructed to avoid fermented milk or yogurt different from the ones provided in the trial. Researchers assessed participants' consumption and recorded missed or refused yogurts to evaluate compliance.

Both the probiotic and placebo yogurts were packed in identical containers. The probiotics and placebo were identical in color, smell and had a similar taste. All

doctors, nurses, clinical research staff and patients were unaware of the presence or absence of the probiotic strains within the yogurts administered to the patients until the full completion of the trial.

Procedures

The probiotic yogurt contained *L. acidophilus* La-5 (5×10^6 colony-forming units, cfu/ml), *L. casei* Lc-01 (1×10^7 cfu/ml), and *B. lactis* Bb-12 (1×10^8 cfu/ml) as probiotics, and *S. thermophilus* STY-31 (2×10^9 cfu/ml), and *L. bulgaricus* LBY-27 (5×10^6 cfu/ml) as starter strains for milk fermentation. The placebo yoghurt contained the same starter strains *S. thermophilus* STY-31 (1×10^9 cfu/ml) and *L. delbrueckii subsp. bulgaricus* LBY-27 (1×10^8 cfu/ml). Both yogurts were elaborated and kindly provided by Ganadería Priégola (Madrid, Spain). Viable counts of the species were carried out in both products to ensure strain viability throughout the study. [13]

Researchers and medical staff identified patients who had been prescribed amoxicillin-clavulanate or levofloxacin within 48 hours of the first antibiotic dose. After obtaining written informed consent, patients were randomized and prescribed the appropriate study yogurt (or none) as described above. Nursing staff dispensed the yogurts and were instructed to pour 150-200 ml daily into a cup for the patient (10^9-10^{10} cfu of probiotic strains per dose) during all the period of antibiotic course and for 5 additional days after the stop of antibiotic treatment. The appropriate amount of yogurt was provided to patients upon release from the hospital with appropriate instructions for completing the assigned treatment. Patients included in the control group (no yogurt) had an identical clinical follow up.

The following variables were registered: age, sex, living in a nursing home, previous diagnosis of diabetes mellitus, heart failure, renal insufficiency, cirrhosis,

chronic bronchitis or cognitive impairment. A study chart filled by the patients (or nurses / family when appropriate) was used to record bowel movements and feces quality, tolerance and adherence to study yogurt. Tolerance was assessed by the presence of abdominal pain, bloating, or vomiting. Adherence to the study yogurt was considered appropriate if the percentage of prescribed drinks that were consumed was higher than 80%.

Researchers checked the chart before hospital discharge for accuracy. When there was evidence of diarrhea, a stool sample was requested and analyzed for enteropathogens (*Salmonella sp, Shigella and Campylobacter*), parasites, and *Clostridium difficile* toxin. All patients received a self-stamped envelope to return the study chart at the end of the study. Additionally, all patients had a follow-up telephone call scheduled around one month after the end of antibiotic therapy.

The primary outcome was the rate of diarrhea up to one month after the end of antibiotic therapy. The WHO definition of diarrhea was used: 3 or more loose or watery stools per day for two or more days.[14] Secondary outcomes were severity of diarrhea defined as the maximum number of bowel movements per day; duration of diarrhea (days with more than two loose stools); necessity to stop antibiotic treatment to treat AAD; necessity to use intravenous fluid to treat AAD; prolonged hospital admission or readmission because of AAD; mortality; tolerance to yogurt and compliance.

Sample size and statistical analysis

The study was designed with an estimated prevalence of AAD related to amoxicillin-clavulanate of 25%.[1-3, 15] To detect a 50% reduction of AAD in the probiotic group with α=0.05 and a power of 80%, we estimated a sample size of 304, that was increased by 3% to account for potential losses during follow-up. We

performed an intention to treat analysis. All patients who had consumed at least one serving of the study yogurt were included in the analysis. All comparisons between the placebo and probiotic group were conducted in a blinded manner. Results were compared by the Fisher's exact test or chi square as appropriate for the appearance of diarrhea, mortality and categorical variables. Number of stools per day, length of diarrhea and continuous variables were compared with Student`s t test or U Mann Whitney test as appropriate. Multivariate logistic regression was used to evaluate a potential influence of relevant clinical variables on the main outcome. Additionally, comparisons were made between the 'control' group and placebo and probiotic groups combined to further detect potential important effects of the 'placebo drink'.

Results

Patients and protocol

During the study period 620 patients were assessed and 314 patients entered in the study (figure 1). Reasons for exclusion were mainly not meeting inclusion criteria (90%), in most patients because of previous antibiotic treatment for more than 2 days or other antibiotics different from amoxicillin-clavulanate or levofloxacin. Patients disliking yogurt or dairy products were also excluded. Patients were allocated to one of the 3 groups (2:2:1). There were no clinically relevant differences between the 3 groups at baseline (table 1). The most common reason for antibiotic use was respiratory infection (86.8%), including bronchitis (56.3%) and pneumonia (30.5%). Other reasons were urinary tract infection 12 (3.8%), cellulitis 16 (5.1%), multiple site infection 6 (19%) and other reasons 7 (2.2%). First antibiotic was replaced by a different one during the follow-up in 25.5% patients.

Probiotic strain viability was assessed in 22 random samples during the 4 week of expected yogurt use. *L. acidophilus* La-5 counts were reduced by a 12% at the end of the 4 week period. All the remaining strains of the probiotic and placebo yogurts maintained a similar viability during the period of use (less than 3% decrease of Log cfu/ml at 4 weeks).[13] Compliance was similar for the probiotic and placebo drinks (69,2% vs 66.2%, p=0.769). Main alleged reason for non-compliance was palatability.

Follow up and Main outcome

The main outcome was evaluated in all cases that received at least one dose of the study yogurt (100% of cases). The last observation carried forward was used for patients with incomplete follow up. Eighteen patients (5.7%) had an incomplete follow up (Figure 1): 9 (7.3%) from the probiotic group , 7 (5.6%) from the placebo group, and 2 (3%) from the control group, p=0.461. No significant differences were found for clinical variables between patients with complete and incomplete follow-up (Table 1).

Incomplete follow up was due primarily to a lack of answer to repeated telephone calls after not fulfilling charts (n=14) or incomplete clinical chart filling (n=4).. Fifteen patients (5.1%) died during the follow-up: 5 (4.4%) in the probiotic group, 5 (4.2%) in the placebo, and 5 (7.7%) in the control group respectively, but information regarding diarrhea occurrence until death was available. These patients were also included in the analysis. Thus, the intention to treat analysis of the primary outcome was carried for 247 patients (122 assigned to probiotics and 125 assigned to placebo). The occurrence of AAD was also evaluated for 67 patients in the unblinded control group.

For the primary end-point, there were no significant differences in the rate of diarrhea between patients assigned to probiotic or placebo yogurt: (23.0% versus 17.6%, absolute risk reduction -5,35% , 95% confidence interval 95% CI -15,4 to 4.7 %, p=0.30 (Table 2). As a sensitivity analysis, we performed 3 additional evaluations: 1) restricted to patients with complete follow up¸2) worst case scenario, assuming the presence of diarrhea in all cases with incomplete follow-up; and 3) best case scenario for probiotics: assuming the presence of diarrhea for all patients in the placebo group with incomplete follow-up and the absence of diarrhea in all patients belonging to the probiotic group with incomplete follow-up (Table 3). None of the comparisons were suggestive of a protective effect of probiotics on AAD.

Interestingly, the rate of diarrhea was similar in the unblinded external control to the one found in the blinded study groups (20,9% unblinded versus 20,2 % combined blinded study group, p=0,91). Similar figures were also found for secondary end-points in the unblinded control and the combined blinded study group (Table 2).

A total of 44 patients reporting diarrhea provided stools samples for microbiological cases (69%). Microbiologists were not informed about the intervention assigned to the patients. Enteropathogens evaluated were: *Salmonella* sp, *Campylobacter* and *Shigella.* No enteropathogens were isolated or parasites were found in any sample. Two samples from 2 different patients (both from the probiotic group) were positive for *C. difficile* toxin antigen test. Both patients recovered after treatment with metronidazole (1) or vancomycin (1)

Multivariate analysis

The rate of diarrhea was not associated with the type of antibiotic (either amoxicillin-clavulanate or levofloxacin), previous antibiotic use or adherence to study

drink. However, a slightly higher proportion of patients on levofloxacin were randomly assigned to placebo (20,8 vs 16.4% p=0,19). To account for potential imbalances between placebo and probiotic groups (though none of them were statistically significant) we did evaluate the effect of major clinical variables on the main outcome of the trial by multivariate logistic regression analysis. As shown in table 4, age, sex, type of antibiotic and the presence of diabetes, cancer, dementia, heart failure did not modify the effect of probiotics on the development of diarrhea.

Interestingly, yogurt intolerance was associated with about a 4 fold increase in the risk of diarrhea (OR 3,85; 95% CI 1,32 to 11.18 p=0.009), with similar effects on probiotic (OR 3,19, 95% CI 0.89 to 11.38; p= 0.06) and placebo groups (OR 5,05; 95% CI 0.67 to 37,99; p 0.08). Furthermore, by logistic regression analysis, the model including yogurt intolerance as a covariate did not modify the effect of probiotics on AAD (OR 1,152 95% CI 0,650-2,043; p 0,628).

Secondary outcomes

For the secondary outcomes, there were also no differences among groups for the severity of diarrhea, maximum number of stools per day, duration of diarrhea, necessity to stop antibiotic treatment to treat AAD, use of intravenous fluids to treat AAD prolonged or a new admission because of AAD or mortality (table 2). Among the patients included in the blinded study, 10 patients died, 5 from the probiotic group and 5 from the placebo group (p=0.573). All deaths were attributed to uncontrolled infection or underlying conditions. None of them were considered to be related to the study protocol according to the attending clinicians. One patient from the probiotic group died because of mesenteric ischemia, attributed to cardiac embolism due to atrial fibrillation. Five additional patients died in the unblinded control (7.7%, vs 4.3%

in blinded study patients p=.393). Since this group did not received study yogurt, causes of death were obviously unrelated to the study drinks.

Side effects.

Regarding side effects, a 77% of patients reported information about tolerance (fullness of abdomen, bloating /flatulence or vomiting). There were no reports of other adverse events related to the study yogurts, although tolerance to probiotic yogurt was slightly worse than placebo: 4 patients (3.4%) had intolerance in the placebo group versus 11 (9.7%) in the probiotic group, p=0.0063.)

Discussion

In this study we have found no effect of the probiotic strains LA-5, BB-12 and LC-01 to prevent AAD in hospitalized patients. Other clinical variables included in the secondary end points also failed to show any relevant difference between the probiotic and placebo groups, consistent with a lack of clinically relevant effects in our setting.

Different probiotic strains may have dissimilar effects in the prevention of ADD. Up to now, *Lactobacillus rhamnosus* GG and *Saccharomyces boulardii* are the only species that have demonstrated a protective effect on AAD in children and adult patients.[7] In contrast, *L. acidophilus* La-5 and *B. lactis* Bb-12 have not demonstrated significantly effects on AAD in adult patients.[16] In spite of the increased risk of AAD in hospitalized adult patients, few studies have evaluated the role of specific probiotic strains in this setting.

Our study was a randomized, double blind, placebo-controlled trial. The groups were well balanced and the statistical analysis was also carried out in a blinded manner. In addition, the microbiological quality of the probiotic and placebo drinks was assessed along the study period. Our study was adequately powered, a high proportion of evaluated patients was included, and had good rates of compliance and follow-up. We had complete follow-up for 296 patients (>94%). We conducted an intention to treat analysis with the last observation carried forward for patients with incomplete follow up. In a sensitivity analysis, even the best-case scenario for probiotics did not suggest a protective effect of probiotics on AAD,

Indeed, this is one of the largest clinical trials evaluating the effects of probiotics in the prevention of AAD in adults. As previously mentioned, the number of randomized trials assessing probiotics for AAD in adults is low and has limitations regarding small size of the trials, unblinded design, and poor documentation of the probiotic strains.[8] In addition, some meta-analysis have raised the issue of a publication bias in favor of the beneficial effect of probiotics.[17]The Placide trial, the largest study so far to evaluate the efficacy of a multispecies and multistrain probiotic lyophilized formulation in the elderly, described that strains *L. acidophilus* CUL60 and CUL21, *Bifidobacterium bifidum* CUL20 and *B. lactis* CUL34 were not effective in prevention of AAD.[10] In spite of this, a meta-analysis included in the Placide paper, as well as an accompanying editorial, still suggests a role for lactobacilli in the prevention of ADD[10]. However, this meta-analysis included an old preliminary work that never reached formal publication and was considered not adequate for inclusion in the Cochrane review on the topic.[18] If the meta-analysis is repeated by excluding this paper, the effect of probiotic bacteria on AAD was no longer significant [Risk difference -0.02 (95% CI -0.04, 0,01), (Supplemental Figure). The addition of our

data further challenges the contention of a protective effect of lactobacilli in the prevention of AAD.

We made a particular effort in an attempt to optimize the effect of the probiotic mixture based on that (1) the delivery as a yogurt-derived product may improve patient compliance and (2) the use of a high bacterial dose (10^9-10^{10} cfu/day), whose viability was checked for all the treatment period[13]. Besides the tested probiotic strains, our results do not suggest a clinically relevant effect of the standard yogurt on AAD, since the rates of diarrhea in the parallel randomized unblinded group assigned to no intervention were essentially identical, to both the placebo-yogurt group and the probiotic group. Therefore, neither the standard nor the probiotic-enriched yogurt seems to attenuate ADD. Our results are in contrast with several clinical trials that have evaluated the effect of lactic acid bacteria delivered in yogurt in the prevention of AAD[11,12, 19-22] Among them, four have reported a beneficial effect. However, 2 trials were not placebo-controlled blinded trials.[11, 19] Hickson et al used a milkshake as 'placebo' that could be easily differentiated from 'yogurt'. In addition, milkshake could favor diarrhea development, given the higher lactose content of milk. [20] Finally, Wenus et al [21] used a heat-killed placebo yogurt, but included just 63 patients with a short follow-up. On the other hand, Conway et al [12] the most extensive trial (414 patients), designed with placebo and control arms similar to our study, did not find any beneficial effect. However, the study was carried out in an outpatient setting, included a high proportion of children, had a short follow up and a low rate of diarrhea. Finally, Lönnermark did not find diarrhea rates differences in a randomized study with L plantarum. [22]

While we formally tested the effect of probiotics on adult patients, most patients in our trial were elder. In this regard both the population and the results are essentially in agreement with the largest trial published so far. On the other hand, our results should be applied to younger adults with caution.

In our setting, probiotic drink was not associated with important side effects. Mild abdominal intolerance was marginally superior in the probiotic yogurt as compared to the placebo. Probiotic therapy was not associated with prolonged hospital stay or readmission. Finally, there were no effects on mortality. Some reports have raised the issue of potential serious side effects associated with probiotic therapy. Indeed, increased mortality has been described in patients with acute pancreatitis randomized to receive a probiotic bacterial mixture. [23] Our patients received a similar dose of probiotic bacteria (10^9-10^{10} cfu daily) as the patients in the pancreatitis trial. However, the clinical setting, probiotic mixture and way of administration are quite different from ours. In any event, probiotics may have beneficial as well as detrimental effects and should receive identical strict and careful clinical evaluation as other therapeutic interventions. [24]

There are some limitations of our trial. First, our trial was designed to detect an important reduction (50%) of ADD. Although our results do not suggest a protective effect of probiotics, our trial was not powered to detect a milder protective effect. Second, the probiotic strains used in our trial were sensitive to the antibiotics received by the patients (unpublished results). It is conceivable that probiotic strain survival has been seriously hampered by this treatment and limited its potential effect. Indeed, we did not find differences in the *Bifidobacterium* and *Lactobacillus* counts in feces along the study among patients. [25] The extension of probiotic administration for 5 days after antibiotic withdrawal might not have been sufficient to

overcome this problem. Other probiotics, such as yeasts, may not be affected by this limitation. This reason may underlie the better outcome in the prevention of AAD when *Saccharomyces boulardi* was used as probiotic. [26,27].

Third, yogurt intolerance was associated with probiotics as well as with the presence of diarrhea. However, multivariate analysis indicated that yogurt intolerance did not modify the effect of probiotics on AAD. We believe that yogurt intolerance may be heralding the presence of diarrhea, rather than provoking it. Finally, we suggest that standard yogurt may not be useful for the prevention of AAD. However, our paper was not properly powered to address this issue.

In conclusion, our study does not support a role of the combined probiotic strains *L. acidophilus* LA-5, *B. lactis* BB-12 and *L. casei* LC-01 in the prevention of AAD in hospitalized (mostly elder) patients. The role of specific probiotic strains and ways of administration in different clinical settings merits specific evaluation.

Trial registration number (retrospectively registered, 2013): ISRCTN46764354

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Figure legends

Figure 1. CONSORT 2010 Flow Diagram

Supplemental Figure. Meta-analysis of trials of lactobacilli or bifidobacteria, or both, in the prevention of antibiotic-associated diarrhea in older patients. Reproduction of Meta-analysis from Allen et al 1 excluding one Abstract reference that did not reach full publication after 10 years. Maentel –Haenszel fixed effects analysis.

Figure

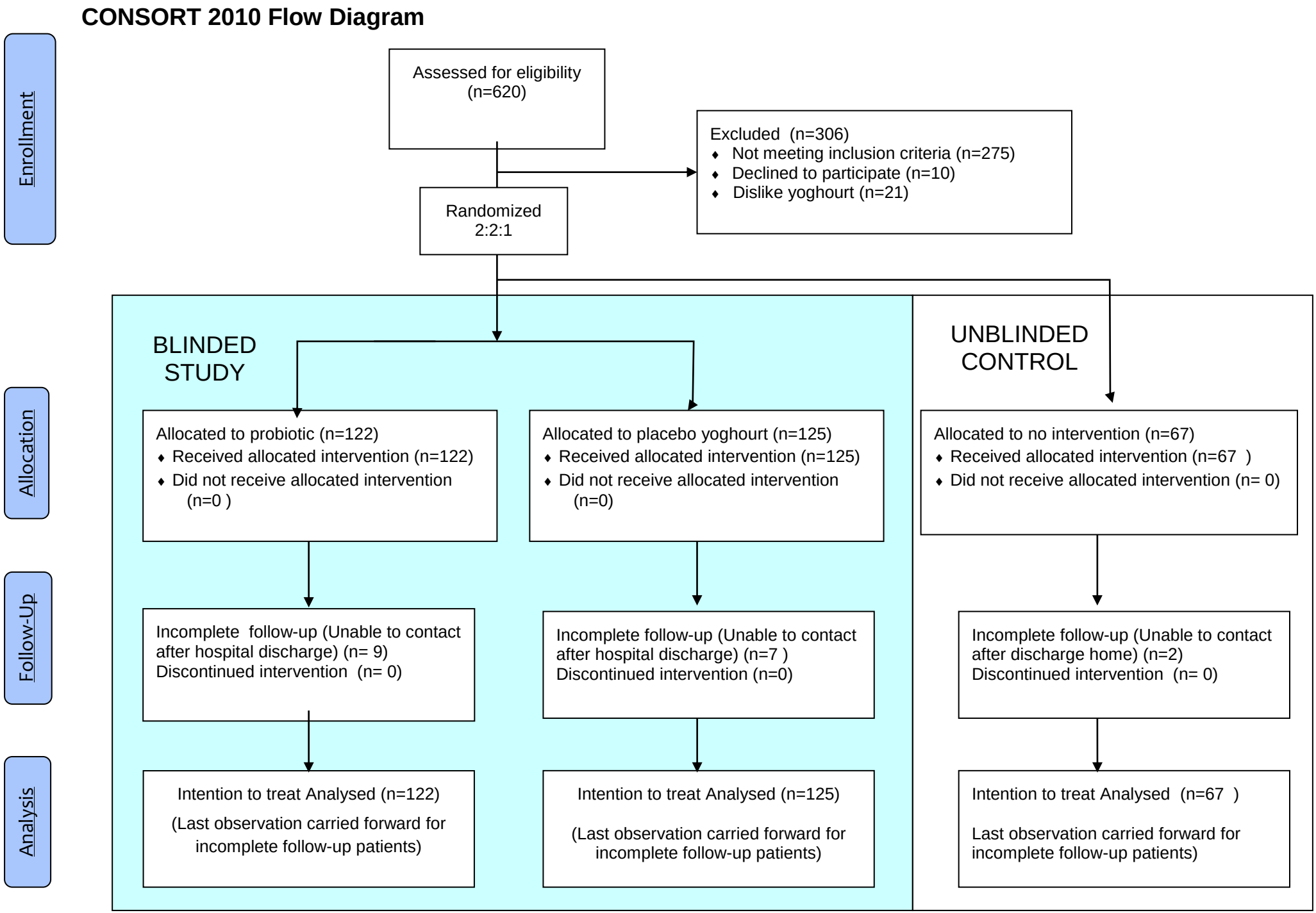

CONSORT 2010 Flow Diagram
Enrollment
Allocation
Follow-Up
Analysis
Assessed for eligibility (n=620)
Excluded (n=306)
♦ Not meeting inclusion criteria (n=275)
♦ Declined to participate (n=10)
♦ Dislike yoghourt (n=21)
Randomized 2:2:1
BLINDED STUDY
UNBLINDED CONTROL
Allocated to probiotic (n=122)
♦ Received allocated intervention (n=122)
♦ Did not receive allocated intervention (n=0)
Allocated to placebo yoghourt (n=125)
♦ Received allocated intervention (n=125)
♦ Did not receive allocated intervention (n=0)
Allocated to no intervention (n=67)
♦ Received allocated intervention (n=67)
♦ Did not receive allocated intervention (n= 0)
Incomplete follow-up (Unable to contact after hospital discharge) (n= 9)
Discontinued intervention (n= 0)
Incomplete follow-up (Unable to contact after hospital discharge) (n=7)
Discontinued intervention (n=0)
Incomplete follow-up (Unable to contact after discharge home) (n=2)
Discontinued intervention (n= 0)
Intention to treat Analysed (n=122)
(Last observation carried forward for incomplete follow-up patients)
Intention to treat Analysed (n=125)
(Last observation carried forward for incomplete follow-up patients)
Intention to treat Analysed (n=67)
Last observation carried forward for incomplete follow-up patients)

Table 1. Clinical and epidemiological variables at admission

	BLINDED GROUP			UNBLINDED GROUP
	Probiotic (n 122)	**Placebo (n 125)**	**Both (n 247)**	**None (n 67)**
Women [n (%)]	59 (48.4)	55 (44.0)	114 (46.2)	31 (46.3)
Mean age years (SD)	73.5 (16.3)	76.7 (10.6)	75.08 (14.6)	76.5 (14.6)
Levofloxacin group [n (%)]	20 (16.4)	26 (20.8)	46 (18.6)	18 (26.9)
Comorbidities [n (%)]				
Heart failure	47 (38.5)	59 (47.6)	106 (43.1)	30 (44.8)
Renal insufficiency	14 (11.5)	15 (12.1)	29 (11.8)	8 (11.9)
Cognitive impairment	14 (11.5)	17 (13.7)	31 (12.6)	7 (10.4)
Cirrhosis	1 (0.8)	1 (0.8)	2 (0.8)	0
Diabetes mellitus	25 (26.6)	30 (31.9)	55 (29.3)	14 (20.9)
Chronic bronchitis	42 (44.7)	47 (50.0)	89 (47.3)	36 (53.7)
Laboratory variables [mean (SD)]				
Mean (SD) white cell count (/l)	12,281 (5920)	11,481 (4720)	11,880 (5355)	11,372 (5581)
Platelet count (/l)	241908 (89168)	237000 (87787)	239444 (88327)	259254 (95109)
Haemoglobin (g/l)	14.1 (1.5)	12.8 (1.9)	13,5 (1.4)	12.8 (2.2)
Creatinine (/l)	1.2 (0.5)	1.2 (0.4)	1.2 (0.5)	1.2 (0.6)
ALT(/l)	26.2 (33.5)	30.0 (36.8)	28.1 (35.1)	32.7 (59.6)
Length of previous antibiotic treatment [mean (SD)]	1.8 (1.2)	1.5 (1.0)	1.7 (1.1)	1.6 (0.9)
Indication for antibiotics [n (%)]				
Respiratory tract infections /	104 (87.9)	109 (88.0)	213 (86.2)	57 (87.7)

Pneumonia				
Urinary tract infection	4 (3.3)	5 (4.0)	9 (3.6)	3 (4.3)
Cellulitis	7 (5.7)	6 (4.8)	13 (5.3)	3 (4.6)
More than one	4 (3.3)	2 (1.6)	6 (2.4)	0 (0.0)
Other	3 (2.5)	2 (1.6)	5 (2.0)	2 (3.1)
Length of intravenous antibiotic treatment (days) [mean (SD)]	4.3 (2.7)	4.1 (2.2)	4.2 (2.4)	3.9 (2.1)
Length of oral antibiotic treatment (days) [mean (SD)]	8.0 (5.1)	8.1 (5.8)	8.0 (5.4)	7.5 (3.0)

Table

Table 2. Main and Secondary Outcomes

	BLINDED GROUP				UNBLINDED GROUP	
	Probiotic (n 122)	**Placebo (n 125)**	**p***	**Both (n 247)**	**Control (n 67)**	**p#**
Main outcome: Diarrhea						
n	28	22	0.30	50	14	0.91
%	23.0	17.6		20.2	20.9	
Secondary outcomes						
Duration of diarrhea,						
days	3.2	3.7	.742	3.4	3.3	.984
SD	3.2	5.1		4.0	1.36	
Max nº stools/day,						.355
n	4.1	5.1	.189	4.5	6.5	
SD	2.2	2.6		2.4	7.3	
Stop antibiotic						
n	5	9	.399	14	4	.486
%	4.1	7.2		5.7	6.0	
Intravenous fluid						
n	2	1	.606	3	2	.171
%	1.6	0.8		1.2	2.9	
Prolonged inpatient admission						
n	3	0	.243	3	2	.086
%	2.4			1.2	3.0	
New admission						
n	0	1	.488	1	0	0.896
%		0.8		0.4		
Yogurt intolerance						
n	11	4	.063	15	NA	
%	9.0	3.2		6.1		
Mortality						
n	5	5	.573	10	5	.393
%	4.1	4.2		4.3	7.5	

*comparison between placebo and probiotic

#comparison between blinded and unblinded group

n number, SD standard deviation

NA: not applicable

Stop antibiotic: patients who have to stop antibiotic because of diarrhea
Max nº stools/day: maximum number of bowel movements per day
Intravenous fluid: need of using intravenous fluid because of diarrhea
Prolonged inpatient admission: prolonged inpatient admission because of diarrhea
New admission: new admission for any reason in the follow-up period
Yogurt intolerance: abdominal symptoms related to yogurt

Table

Table 3. Main outcome. Effect of probiotic yogurt on antibiotic associated diarrhea. Sensitivity analysis.

Analysis	Groups		% difference	difference 95% CI	p	OR	OR 95% CI
	Placebo	Probiotic					
A. Initention to treat (LOCF)	17.6	23.0	5.4	-4.7 to 15.4	0.30	1.40	0.75-2.60
B. Intention to treat (Modified)	18.6	24.8	6.6	-4-5 to 16.7	0.26	1.44	0.77-2.70
C. Worst case scenario	23.2	30.3	7.1	-3.9 to 18.0	0.21	1.44	0.82-2.54
D. Best case scenario for probiotics	23.2	23.0	-0.2	-10.3 to 10.8	1	0.99	0.55-1.78

The rates (%) of diarrhea among patients assigned to placebo or probiotic yogurt is shown, as well as the different % between groups and its 95% confidence intervals. In addition, odds ratio for the development of diarrhea and its 95% CI is shown (OR <1 favors probiotics; OR > 1 favors placebo). The analysis was conducted as follows: A) Intention to treat analysis. All randomized patients were evaluated. Last observation was carried forward for patients with incomplete follow up; B). ITT modified by excluding patients with incomplete follow up; C) Worst case scenario. All patients with incomplete follow up were considered as having diarrhea; D) Best case scenario for probiotics. assuming the presence of diarrhea for all patients in the placebo group with incomplete follow-up and the absence of diarrhea in all patients belonging to the probiotic group with incomplete follow-up. As shown, in no case scenario probiotic treatment was associated with a significant change in the rate of diarrhea as compared to placebo.

CI: confidence interval

OR: odds ratio

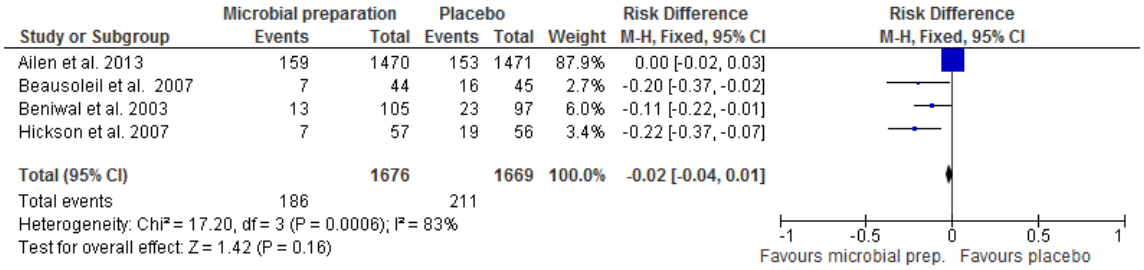
Table

Table 4. Effects of probiotics and clinical variables on the main outcome by logistic regression analysis

						95% C.I.for EXP(B)	
Univariate		B	S.E.	p	Exp(B)	Lower	Upper
	Probiotic	**0,255**	**0,284**	**0,368**	**1,291**	**0,74**	**2,251**
	Constant	-1,466	0,185	0	0,231		
Bivariate (Model 2)							
	Probiotic	**0,141**	**0,292**	**0,628**	**1,152**	**0,65**	**2,043**
	Yogurt intolerance	**1,42**	**0,529**	**0,007**	**4,139**	**1,468**	**11,67**
	Constant	-1,518	0,188	0	0,219		
Multivariate (model 3)							
	Probiotic	**0,114**	**0,3**	**0,705**	**1,12**	**0,623**	**2,016**
	Yogurt intolerance	**1,484**	**0,544**	**0,006**	**4,411**	**1,52**	**12,8**
	Antibiotic	0,083	0,38	0,827	1,087	0,516	2,288
	Age (years)	0,002	0,011	0,856	1,002	0,981	1,023
	Sex	-0,165	0,303	0,586	0,848	0,469	1,535
	Dementia	-0,069	0,466	0,883	0,934	0,375	2,326
	Diabetes	-0,647	0,401	0,106	0,524	0,239	1,148
	COPD	-0,054	0,316	0,864	0,947	0,51	1,76
	Heart Failure	-0,352	0,315	0,263	0,703	0,379	1,303
	Constant	-1,307	0,816	0,109	0,271		

Model 1 Univariate: Probiotics; Model 2. Bivariate: probiotic + yogurt intolerance; Model 3. Probiotic + yogurt intolerance + Antibiotic +Age (years) +Sex + Dementia + Diabetes + COPD (Chronic Obstructive Pulmonary Disease) + Heart Failure + Constant. All variables were forced into the models regardless of significance. S.E. Standard Error; C.I. Confidence Interval. See results section for details of the analysis.

Figure 2. Meta-analysis of trials of lactobacilli or bifidobacteria, or both, in the prevention of antibiotic-associated diarrhea in older patients. Reproduction of Meta-analysis from Allen et al 1 excluding one Abstract reference that did not reach full publication after 5 years. Maentel –Haenszel fixed effects analysis.



Risk difference -0.02 (95% CI -0.04, 0,01)