Do probiotics prevent antibiotic associated diarrhoea? Results of a multicentre randomised placebo controlled trial

Article (Accepted Version)

Do Probiotics prevent antibiotic associated diarrhoea? Results of a multicentre randomised placebo controlled trial

Authors: C Rajkumar[1], M Wilks[2], J Islam[1], K Ali[1], J Raftery[4], K.A. Davies[1], J Timeyin[3], E Cheek[5], J Cohen[1] on behalf of the investigators.

Affiliations:
1. Brighton and Sussex Medical School, Universities of Brighton & Sussex, Brighton, UK
2. Barts Health NHS Trust and Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary, University of London, London, UK
3. Brighton and Sussex University Hospitals NHS Trust, Brighton, UK
4. University of Southampton, Southampton, UK
5. University of Brighton, Brighton, UK

Correspondence:
Professor C Rajkumar
Brighton and Sussex Medical School, Audrey Emerton Building
Royal Sussex County Hospital
Eastern Road
Brighton, BN2 5BE
United Kingdom
Email: C.rajkumar@bsms.ac.uk

Abstract

Background: Antibiotic-associated diarrhoea (AAD) is a side-effect of antibiotic consumption and probiotics have shown to reduce AAD.

Methods: We conducted a multicentre, double-blind, placebo-controlled, randomised trial to evaluate the role of *L. casei* DN114001 (combined as a drink with two regular yoghurt bacterial strains) in reducing AAD and *C. difficile* infection in patients aged over 55 years. The primary outcome was the incidence of AAD during 2 weeks follow up.

Results: 1127 patients, (mean age $\pm$ SD 73.6 $\pm$ 10.5), were randomised to the active group (n=549) or placebo group (n=577). Both groups were followed up as per protocol. Patients experiencing AAD during follow-up was 19.3% (106/549) in probiotic group vs 17.9% (103/577) in placebo group (unadjusted OR 1.10, 95% CI 0.82 - 1.49, p = 0.53).

Conclusions: We did not find any significant evidence of a beneficial effect of the specific probiotic formulation in preventing AAD in this elderly population drawn from a number of different UK hospitals. However, in the UK and in many other healthcare systems there have, in recent years, been many changes in antibiotic stewardship policies, an overall decrease in incidence in *C. difficile* infection, as well as an increased awareness of infection prevention, and modifications in nursing practice. In the light of these factors, it is impossible to conclude definitively from the current trial that the study-specific probiotic formulation has no role in preventing AAD, and it is our view that further trials may be indicated, controlling for these variables.

Background

Antibiotics have revolutionised medical care. However, they have the potential to alter gut micro flora or microbiota adversely, with AAD as a possible consequence. AAD may range in severity from a mild self-limiting disease to a fulminant life-threatening illness, particularly when *C. difficile* infection (CDI) is involved [1].

In healthy individuals, the indigenous intestinal microbiota helps maintain a stable environment by competing with exogenous pathogens for nutrients, surface receptors and physical space probably through 'colonisation resistance' [2]. Commonly-used antibiotics (such as co-amoxiclav, fluorquinolones and cephalosporins) can disrupt this balance, leading to exogenous pathogen colonisation and overgrowth precipitating AAD. Antibiotics may lead to osmotic diarrhoea due to accumulation of unfermented small carbohydrate molecules in intestinal lumen [3]. Consequently, concerns were raised about older patients with multiple co-morbidities and frequent hospital admissions receiving multiple courses of antibiotics [4]. The vulnerability of the older population is further compounded by an ageing immune system (immunosenescence) and changes in intestinal microbial diversity [5, 6].

Probiotics are live micro-organisms thought to counteract antibiotic-induced disruption of the microbiota [7-9]. Three meta-analyses [10 - 12] showed that probiotics may reduce the risk of AAD. However, all three meta-analyses expressed concern over the clinical applicability of such finding, given the large heterogeneity of trial designs, in particular a lack of consistency in defining AAD, follow-up duration, blinding, patient age and the type of probiotics used.

A 2012 meta-analysis [11] of 63 RCTs involving 11,811 patients has cast doubt on the effectiveness of probiotics in preventing AAD in older patients (> 65 years). Hempel et al (2012) identified only three studies that contributed data on this subgroup, giving an overall smaller (and not statistically-significant) benefit after meta-analysis of relative risk (RR) of 0.81, 95% CI 0.40 - 1.63. This is in contrast to the more substantial (and statistically-significant) benefit (RR 0.58, 95% CI 0.50 - 0.68) for the primary analysis of data from patients of all ages. Hempel et al (2012) concluded that more trials are warranted to evaluate the potential benefits of probiotics in older patients receiving antibiotics. Our previous trial published in the BMJ using the same product as this trial showed a substantial reduction in the incidence of AAD compared to a placebo [13]. PLACIDE [14], a similar but larger UK trial in 2941 older patients (age >65 years) ran concurrently with our own study. This study did not show any benefit of probiotic preparations in preventing AAD.

In the context of lack of clear evidence to support or refute the utility of probiotic therapy in preventing AAD in older patients, we conducted a large, multicentre, double-blind, placebo-controlled, RCT to evaluate the clinical and cost effectiveness of *L. casei* DN114001 (combined as a drink with two regular yoghurt cultures, *L. bulgaricus* and *S. thermophilus*) in preventing AAD and CDI in older patients (defined as age > 55 years).

Methods

This was a multi-centre, double-blind, parallel-group RCT of a probiotic yoghurt product against a placebo, allocated on a 1:1 ratio, in patients aged >55 years.

Study Population

We initially recruited hospital inpatients aged at least 55 years, able to give written informed consent, with Barthel Index of Activities of Daily Living of >15. In a subsequent protocol amendment, after 220/1126 subjects were recruited, eligibility criteria were updated to allow subjects lacking capacity to participate via proxy assent,

subjects from residential and nursing homes, and all Barthel index scores to be included. These amendments were needed to optimize recruitment across all study centres. Recruitment of 1127 patients took place over a four-year period from October 2009 to July 2013.

Subjects were eligible if they were within 48 hours of starting antibiotics prescribed for a minimum of 72 hours. The criteria were then expanded to include hospital patients who had started antibiotics in the seven days prior to admission as long as prescription continued for 72 hours as an inpatient. Antibiotic therapy could be single or multiple antibiotics, administered orally or intravenously. Patients needed to be capable of taking oral preparations of study product or placebo.

In total, 28 centres located in NHS hospitals across England agreed to participate, with 26 recruiting at least one patient. List of exclusion criteria shown in table 1.

Consent and assent procedures

Our initial intention was to recruit patients on the basis that they were capable of providing written informed consent after reading Patient Information Sheet. After consultation with the Trial Steering Committee (TSC - appendix 1) and approval by the Research Ethics Committee (REC), an assent process was introduced to permit recruiting patients unable to provide informed consent. In such cases a family member or friend ('consultee') was asked to provide assent. Where assent had been used, but patients subsequently regained the ability to consent, they were invited to provide informed consent. If a patient declined to consent then they would no longer continue in the trial.

A favourable opinion was given by the Brighton East Research Ethics Committee, Brighton, UK. REC number: 09/H1107/10. Clinical Trial registration number: NCT01087892). The trial was adopted by the clinical research network (Surrey and Sussex) part of the National Institute for Clinical Research (NIHR) UK (IRAS No.: 1553, CPMS No.: 7528).

Interventions

Patients were randomised in a 1:1 equal allocation to drink the contents of 100ml containers of a fermented milk preparation of *L.casei* DN114001 (10^8 colony-forming units [cfu]/ml) as well as two regular yoghurt cultures *L. delbrueckii* subspecies *bulgaricus* and *S.thermophilus* (10^6 cfu/ml) *or* to consume a matched non-fermented acidified placebo provided in a similar container. Allocation was stratified by centre and by age group (55-69 years and ≥ 70 years). Quality checks on the bacterial content of the trial product were conducted using a) samples from different batches supplied by the production plant and b) samples collected at random from different centres at the end of a 2-week batch dispensed.

A formal taste test, conducted by a panel, independent of the trial personnel, scored both active and placebo products on taste, texture, smell and consistency and concluded that both products are indistinguishable.

The yoghurt drinks were prescribed twice daily whilst patients were taking antibiotics and then for the following seven days. Adherence was verified by a research nurse. For discharged patients, adherence was assessed by nurses telephoning patients at home. In patients returning to Residential or Nursing homes, appropriate staff were telephoned to obtain information.

Sample size calculation

Initial sample size was based on our meta-analysis [15].

Assumptions were:

• Prevalence of AAD of 20% in the placebo control arm

• A 40% reduction in such prevalence in the treated arm to 12% (based on an Odds Ratio [OR] of 0.55) was likely to produce a statistically-significant result.

An initial sample size of 440 in each group (total 880) would have 90% power to detect such an effect using a two-group chi-square test with a two-sided significance level at $p < 0.05$, allowing for withdrawals and inappropriate randomisation as detected previously [13]. The sample size was finalized at 1200 based on a lower

than expected AAD incidence combined with a higher than expected drop-out rate and an expected treatment effect less than 8% given the recognised decline in disease severity. The protocol revision was agreed by the Trial Steering Committee (TSC), and approved by the Sponsor, Funder and REC.

Randomisation

For each centre-age group stratum, a random allocation was carried out using random permutated blocks of length four. The randomisation allocation sequences were generated using Random Allocation Software 1.0 [16]. The randomisation sequence was concealed from the research team. Study product was allocated by an external research administrator. The research team was blinded to patient allocation. Envelopes were provided for unblinding at individual sites, but this was not used.

Primary Outcome

The primary outcome was defined as the incidence of AAD in the active and placebo groups during or for 14 days after stopping probiotic or placebo drinks (or at the last follow-up contact if this was earlier). During the study, the Bristol Stool Chart (BSC) [17] was introduced after protocol amendments to improve assessment of stool consistency. BSC was first used by study staff after recruiting 571/1126 subjects. BSC were given to patients to take home as an aide memoire. Information was subsequently recorded in a Clinical Research Folder (CRF) after telephone contact by nursing staff.

Diarrhoea was initially defined in the protocol as more than two liquid stools a day for three or more days in quantities in excess of normal for each patient (type 6-7 BSC).

The incidence of AAD in this study was lower than had been expected. This mirrored a UK trend seen at the time, as a result of hospital measures introduced to reduce *C. difficile* rates, for example improved hand-washing

techniques and changes in antibiotic stewardship. Following research team discussions, a protocol amendment was agreed to adopt a broader AAD definition as follows: loose stools, i.e. taking the shape of the receptacle or corresponding to Bristol Stool Chart types 5-7 and a stool frequency perceived as too high by the patient. This was based on diarrhoea definition considered compatible with *C. difficile* infection by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) [18]. The change in definition of diarrhoea was approved by the REC and the funder. This amendment occurred after 710/1126 subjects were recruited. However, all previously reported cases of loose stools were discussed by an End Point Committee (EPC), blinded to study product allocation, composed of independent experts and sponsor study team members. The status of all trial patients prior to this change in diarrhoea definition as the primary endpoint was reassessed by the EPC following computer-based analysis of CRF records mentioning diarrhoea of any type. The EPC decided to identify all cases before and after amendment as positive by using a new definition of diarrhoea episode, i.e. when there was a frequency of at least two liquid stools (type 5-7 on the BSC) in 24 hours.

Secondary outcomes

The secondary outcomes listed in the final protocol were:

- The incidence of *C. difficile* toxin detected by enzyme - linked immunosorbent assay (ELISA) testing from a stool sample taken at the onset of diarrhoea in active and placebo groups.
- The duration of AAD in active and placebo groups.
- The rate of recurrence of AAD in active and placebo groups to the end of the follow up period based on ESCMID definition.
- The rate of recurrence of *C. difficile* toxin-positive diarrhoea in active and placebo groups from hospital discharge to the end of follow-up period.
- Quality of life assessed by the Short Form 12 (SF-12)
- The length of hospital stay from entering the study until the end of the follow-up period in active and placebo groups.
- The clinical and cost-effectiveness of the intervention as evidenced by health economic calculations.

Antibiotics used were classified as high risk, medium risk and low risk of causing *C. difficile* infection. A list of the antibiotic classification is included in the appendix 2 [19].

Microbiological Analyses

Stool samples collected within 48 hours of randomisation and subsequent samples taken during any episodes of diarrhoea were sent for microbial analysis. Samples were cultured for Salmonella, Shigella, Campylobacter, Escherichia coli 0157 and examined for ova, cysts and parasites, *Staphylococcus aureus, Klebsiella oxytoca* and *Candida albicans* by standard methods (Health Protection Agency 2013) [20]. Norovirus was tested by polymerase chain reaction (PCR) as described by Apaza et al 2012 [21]. Faecal specimens were alcohol shocked and cultured to recover *C. difficile* as recommended by the Anaerobe Reference Laboratory, Cardiff. *C. difficile* PCR was performed using the method of Wroblewski et al 2009 [22]. Any *C. difficile* isolates were assessed for toxin production using a Quick Check *C. difficile* toxin lateral flow device card (Alere, UK) that combines an enzyme immune assay toxin assay with a glutamate dehydrogenase test.

Adverse events reporting

An adverse event (AE) was defined as any unwanted effect or abnormal laboratory result indicative of disease or organ toxicity that occurred in a study subject, whether or not related to the study product. Instances of diarrhoea not severe enough to constitute an end-point were also recorded as an AE.

AEs were categorised as mild, moderate or severe by the local Principal Investigator, according to the extent of interference with the patient's daily activity. A serious adverse event (SAE) was defined as any event considered to be life-threatening, resulting in disability or permanent injury, leading to hospital readmission or prolonged stay, or resulting in death. The research team acting on behalf of the Sponsor was faxed notification of any SAE by the centres within 24 hours of being made aware of such events. The TSC and the combined Data Monitoring and Safety Committee (DMSC) involving independent experts and co-investigators and the trial statistician, reviewed each SAE. The trial statistician also reviewed data on a monthly basis to establish if there was a statistical difference in mortality indicating a need to stop the trial for safety reasons.

Statistical Analysis

Statistical analysis was carried out on the final locked dataset. The dataset contained observations on 1126 patients. The statistical analysis plan contains details of the statistical methodology used.

Baseline characteristics were compared between treatment groups using appropriate statistics: mean and standard deviation for normally distributed measures, median and quartiles for non-normal continuous measures, and count

and percentage for categorical measures. The primary outcome, the revised definition of AAD adjudicated by the EPC, was compared between treatment groups using chi-squared test and odds ratio with 95% CI. A backwards selection logistic regression model was fitted including stratification factors (centre and age) as well as other baseline variables associated with AAD at the p=0.1 level. Due to small patient numbers and events in some centres, a random-effects logistic regression model was fitted to estimate the treatment effect adjusted for centre (as a random effect) and age.

There were 10 patients that were AAD negative and/or had a date of diarrhoea onset that did not equate to end-point criteria. These 10 subjects were not included in the survival analysis.

To compare incidence between treatment groups, a survival analysis was carried out on time to first AAD onset. AAD negative patients were censored at the earliest notification of death, withdrawal or end of follow-up. Treatment groups were compared using a log-rank test and hazard ratio estimated using a Cox proportional hazards model both unadjusted and adjusted for centre and age.

The groups were analysed as intention to treat including patients that did not meet all inclusion and exclusion criteria [23]. A per protocol analysis excluding subjects with major protocol deviations, was also undertaken. Major deviations were defined as consuming less than 50% of the product or not meeting all eligibility criteria at study entry. All statistical analyses were carried out in Stata 10.1 (StataCorp. Stata Statistical Software: Release 10. College Station, TX: StataCorp LP 2007).

Results

Patient characteristics

Over the recruitment period of four years, 1127 patients were included (Figure 1). One patient, who did not give informed consent, was excluded from all analyses (Figure 1). Thirty-one patients, who did not meet all inclusion and exclusion criteria, were included in intention to treat analyses. One hundred and twenty-eight (23%) in the active Probiotic group and 149 (26%) in the placebo group were censored for AAD because they withdrew early or died during the follow-up period (Figure 1).

Baseline characteristics by randomised group are presented in Table 2. There were larger imbalances than expected for the two stratifications factors, age group and centre, and a noticeably greater proportion of patients in

the active group who had higher risk antibiotics (14.6%) compared with the placebo group (10.0%). Inspection of the randomisation codes showed that the planned blocking strategy was impractical; the randomisation sequence was not followed with frequent gaps in the codes allocated. In effect, a simple randomisation scheme was followed for the rest of the trial.

The median and interquartile range (IQR) follow-up (randomisation to end of follow-up) in active and placebo groups was 27 (13-31) and 27 (13-30) days respectively.

Frequency of AAD

The percentage of patients experiencing AAD was 19.3% (106/549) in the active group compared to 17.9% (103/577) in the placebo group (odds ratio 1.10, 95% CI 0.82 to 1.49, P=0.53, Table 3). Adjustment for centre, age and use of antibiotics at high risk of causing *C.difficile* did not affect this finding. Two variables were associated with AAD at the p<0.1 level: albumin and potassium. Neither variable remained in the logistic model after backwards selection.

In the per-protocol population the percentage of patients experiencing AAD was 19.0% (79/416) in the active group compared to 18.0% (79/438) in the placebo group (odds ratio 1.07, 95% CI 0.75 to 1.50, P=0.72, Table 4). Adjustment for centre and age did not affect this finding (Table 4).

Survival analyses

A) Death

There were 13 deaths in the active group and 23 in the placebo group. Table 5 gives the life table analysis showing no statistical difference between the groups (95% CI 0.28 to 1.09).

B) Onset of AAD

The majority of AAD occurred in both the active and placebo groups within the first 15 days. AAD at 30-days after randomisation was 20.7% in the active group vs. 19.0% in the placebo group. There was no evidence of a difference in survivor functions comparing time to AAD in the active and placebo groups (log-rank test P=0.62).

The estimated unadjusted hazard ratio was 1.07 (95% CI 0.82 to 1.40). Adjustment for centre and age did not affect this finding (Table 4).

In the per-protocol population there was no evidence of a difference in survivor functions comparing time to AAD in the active and placebo groups (log-rank test P=0.78). The estimated unadjusted hazard ratio was 1.05 (95% CI 0.77 to 1.43). Adjustment for centre and age did not affect this finding (Table 5).

Cost analyses

The mean cost per patient was £279 higher in the active group. The active group was "dominated" (higher proportion with diarrhoea, higher cost) by the placebo group but these differences were not statistically significant.

Quality of Life (QOL)

The active group achieved a slightly higher quality of life, which when expressed in Quality-Adjusted Life Years (QALY) (0.0005, 95% CI from -0.003 to +0.004) and combined with the higher cost in the active group, (neither of which were statistically significant) would put its incremental cost per QALY at just over £0.5m far above any acceptable threshold such as that used by National Institute For Health and Care Excellence (NICE) of £30,000.

On analysing compliance to study product, we found that product consumption was similar in each group (table 6). It should be noted that all cases of AAD were analysed by the DMSC applying the third definition of AAD.

Microbiological analysis

Microbiological analysis of baseline stool specimens showed that there were eleven cases of *C.difficile* positive stools in the placebo group and eight in the active group. *S. aureus* was positive in six placebo and four active patients. *C. albicans* was positive in fifty-three placebo and forty-six active samples. *K. oxytoca* was detected in three placebo and nine active samples. *C. perfringens* was not detected in any sample. Norovirus was detected in two placebo samples and one active sample.

AAD stool samples showed *C. difficile* positive in two patients in the placebo group and one in the active group. *S. aureus* was not detected in any sample. *C. albicans* was detected in six placebo and eight active groups. *K. oxytoca* and *C. perfringens* were not detected in any samples. Norovirus was detected in one placebo and one active sample.

Discussion

The results did not show benefit of this specific probiotic drink in preventing AAD in an elderly population. The percentage of patients who experienced AAD was 19.3% (106/549) in the active group compared to 17.9% (103/577) in the placebo group (unadjusted OR 1.10, 95% confidence intervals 0.82 - 1.49, p = 0.53). The wide confidence limits for total mortality demonstrate that we have not excluded an unrelated benefit of active treatment but this would require a much larger and differently designed RCT.

The finding of no benefit needs to be interpreted in light of a dramatic reduction in CDI incidence seen immediately before the trial commenced in the UK. Between 2007 and 2009 the total number of CDI cases reported to Public Health England had declined from 16,864 to 6,407 [24]. Our findings should therefore be extrapolated only to populations where CDI incidence is low.

The main study finding differs from our earlier (albeit in a smaller trial) result of a substantial and clinically-significant reduction in the risk of AAD with the same probiotic formulation [13]. Such disparity in the overall effect may result from the smaller size of the earlier study (135 *vs*. 1126 patients) or differences in AAD and *C. difficile* associated diarrhoea (CDAD) occurrences (34% and 17% respectively in control group) or in the study design such as the definitions of a primary outcome, eligibility criteria, the antibiotics used, the length of AAD reporting period, and the diversity of clinical settings in which the current trial was conducted. The diversity of including both patients treated in general wards in small District General Hospitals, and patients treated in specialist units in large teaching hospitals, constitute a strength of this pragmatic trial, but can also be regarded as a weakness. Nursing practices, antibiotic stewardship and infection control policies were different in different centres, so we cannot exclude the influence of these factors on the study results.

Eight other studies have used the same product we used in this trial. A retrospective cohort study performed in elderly patients with proximal femur fractures showed no effect of the product ~~reported similar results~~ on CDAD [25].A recent study, 32 ICU (intensive care unit) patients and matched with contemporary controls receiving two bottles daily of the study product boluses via feeding tube, found that AAD was documented in 12.5% of the probiotic group and 31.3% in the control group. One patient in the probiotic group developed CDI compared to three in the control group [26]. The authors considered the study product to be safe and appropriate as a preventative measure for AAD and CDI in ICU patients. Two additional clinical studies in an hospitalised population showed a statistically significant effect of the same product in reducing AAD frequency, although they used a different diarrhoea definition [27]. Three other unpublished studies performed by the Imperial College

London and Brighton and Sussex University Hospitals Trust, included one pilot intervention and two observational studies, all investigating the effect of the same product in hospitalised patients (authors' unpublished observations). Only descriptive statistics were produced by the intervention trial and the first observation one, which showed both a decrease of AAD and/or post antibiotics CDAD in subject group or ward taking the active product. The second observation trial showed a reduction of CDI rates, albeit non-statistically significant, on the wards receiving the product compared with the previous year. Albeit with their own limitations, [28 -33] these seven studies showed consistent results in term of potential effect of the product in reducing AAD and/or CDAD and CDI with some conflicting results in terms of statistical significance specifically on CDAD or CDI. These differences are likely to reflect the differences in clinical setting, and heterogeneity of study populations.

The PLACIDE trial (UK NIHR funded trial) did not show any benefit of a different preparation in preventing AAD (RR 1.04, 95% CI 0.84 - 1.28). That trial differed from ours in that the intervention involved capsules containing lyophilised powder derived from four probiotic micro-organisms two strains of *Lactobacillus acidophilus* and two strains of bifidobacterium (*Bifidobacterium bifidum* and *B lactis*). It is not clear why these strains were chosen, as they have not been used in any previous trials. As the bacteria were lyophilized it is not clear what the dose of living bacteria was but nevertheless PLACIDE study would be a fair comparator to our own study [14].

PLACIDE authors updated current evidence on probiotics in preventing AAD in an older population and found limited evidence of benefits in a meta-analysis [14]. We intend to update their meta-analysis with new data from our current trial.

One possible explanation for the similar findings of both PLACIDE and our trial is the introduction of more restrictive antibiotic prescribing practices [34]. Other possibilities, raised in correspondence [35] following the publication of the PLACIDE study, include the timing of probiotic administration relative to that of antibiotics, differing opinions on statistical handling of trials such as these as well as lack of statistical power due to falling incidence rates. In addition there is variability in flora in 'live' yogurt preparations and it is not clear what the most effective combination of organisms to prevent AAD is, so it seems that more work is required. Furthermore, we have also considered the possibility that the described changes in inclusion criteria of study population, the

primary outcome, the randomisation scheme and the symptoms-reporting process after the study commenced could have influenced the study results.

The present evidence does not provide support for the use of the probiotic drink used in this study. However, further investigation is warranted to better identify the conditions, design and population characteristics for which a detection of the study product effect is possible and reproducible to address the conflicting results across the above cited studies. Nevertheless, as the incidence of *C. difficile* is now stable [36] it may be that in the future further consideration should be given to probiotic strategies if warranted. More specifically, further trials with this study product may be needed before concluding that the probiotic product is not effective in preventing AAD by putting an emphasis on controlling the confounding factors among which the infection control measures, and local antibiotic guidelines.

Acknowledgment and support

Financial support for this trial and the preparation of the interventions for the trial were provided by Danone, France.

We would like to acknowledge the input provided by Professor C.J. Bulpitt in the design of the trial, management of the trial and help with the interpretation of the data and the manuscript.

We would like to thank Professor J Wright for her contribution to the grant application, recruitment and the manuscript.

We would also like to thank Ms M Shelton for providing help with preparing the manuscript.

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Tables and figures

Table 1 – Exclusion Criteria

We excluded potential participants according to the following criteria:

- Age less than 55 years
- Currently pregnant
- On percutaneous endoscopic gastrostomy or nasogastric feed
- Evidence of diarrhoea on admission or within the preceding week
- Severe life-threatening illness
- Allergy or hypersensitivity to any component for example to milk proteins of the two preparations (probiotic or placebo).
- Surgery within the last 4 weeks or bowel-related surgery within the last 3 months
- Enrolled in another clinical study within the last 4 weeks
- Severe evolving or active pathology or infection of the gastrointestinal tract, such as inflammatory bowel disease, Crohn's disease or ulcerative colitis, diverticular disease, biliary tract disease or liver cirrhosis
- Any clinical condition affecting the pancreas, including acute and chronic pancreatitis
- Any medical condition such that patient life expectancy was predicted as less than 3 months by the admitting consultant, validated by a member of the trial team
- Taking immuno-suppressive drugs or cytotoxic drugs
- Post-transplant status
- Using prednisolone > 10 mg/day (or equivalent of dexamethasone) continuously for > 2 weeks prior to entering the trial
- Prosthetic heart valves or a history of endocarditis
- Consumption of probiotic drinks containing live organisms or over the counter probiotic preparations daily for > 7 days
- Foreign travel within the last 7 days.

Table 2: Baseline characteristics by treatment group. N (%) except where indicated. Percentages are of group total excluding missing values

		Active (N=549)	Placebo (N=577)
Gender	Male	268 (48.8)	284 (49.2)
	Female	281 (51.2)	293 (50.8)
Age	Mean age in years (SD)	73.7 (10.5)	73.5 (10.5)
	55 – 69	220 (40.1)	235 (40.8)
	70 or over	329 (59.9)	341 (59.2)
BMI	Median (IQR)	26.6 (23.1 – 1.2)	26.7 (23.0 – 1.6)
Number of co-morbidities	Median (IQR)	2 (1 – 2)	2 (1 – 2)
Barthel Index	Median (IQR)	20 (18 – 20)	20 (17 – 20)
Laxative post admission	Yes	23 (4.2)	25 (4.3)
	No	526 (95.8)	552 (95.7)
Duration of antibiotic course (days)	Median (IQR)	6 (3 – 8)	5 (3 – 8)
Number of antibiotics prescribed	Median (IQR)	2 (1 – 3)	2 (1 – 3)
High risk antibiotics		79 (14.4%)	57 (9.9%)

Table 3: Number (%) of patients experiencing AAD by treatment group with estimated odds ratios from logistic regression

	Active (N=549)	Placebo (N=577)	Odds ratio (95% CI)	P-value
Unadjusted	106 (19.3)	103 (17.9)	1.10 (0.82 to 1.49)	0.53
Adjusted*			1.14 (0.84 to 1.56)	0.39
Adjusted 2**			1.13 (0.83 to 1.54)	0.45

* Adjusted for age and centre, N=1109 as model could not be fitted in some strata
** Adjusted for age, centre and use of high-risk antibiotics, N=1109 as model could not be fitted in some strata

Table 4: Per-protocol population: Number (%) of patients experiencing AAD by treatment group with estimated odds ratios from logistic regression.

	Active (N=416)	Placebo (N=438)	Odds ratio (95% CI)	P-value
Unadjusted	79 (19.0)	79 (18.0)	1.07 (0.75 to 1.50)	0.72
Adjusted*			1.10 (0.77 to 1.58)	0.61
Random effects**			1.10 (0.76 to 1.55)	0.63

* Adjusted for age and centre, N=841 as model could not be fitted in some strata

** Logistic model treating centres as random effects, N=854

Table 5: Deaths (total person-years follow-up) with estimated hazard ratios and P-values from Cox regression

	Active (N=549)	Placebo (N=577)	Hazard ratio** (95% CI)	p-value
Unadjusted	13 (43.8)	23(44.0)	0.58 (0.29 to 1.14)	0.11
Adjusted*			0.55 (0.28 to 1.09)	0.09

* Adjusted for age and centre

** The proportional-hazards assumption of the Cox model was assessed using Schoenfeld residuals and there was no evidence that this assumption had been violated in the either the unadjusted (p=0.55) or adjusted model (p=0.40)

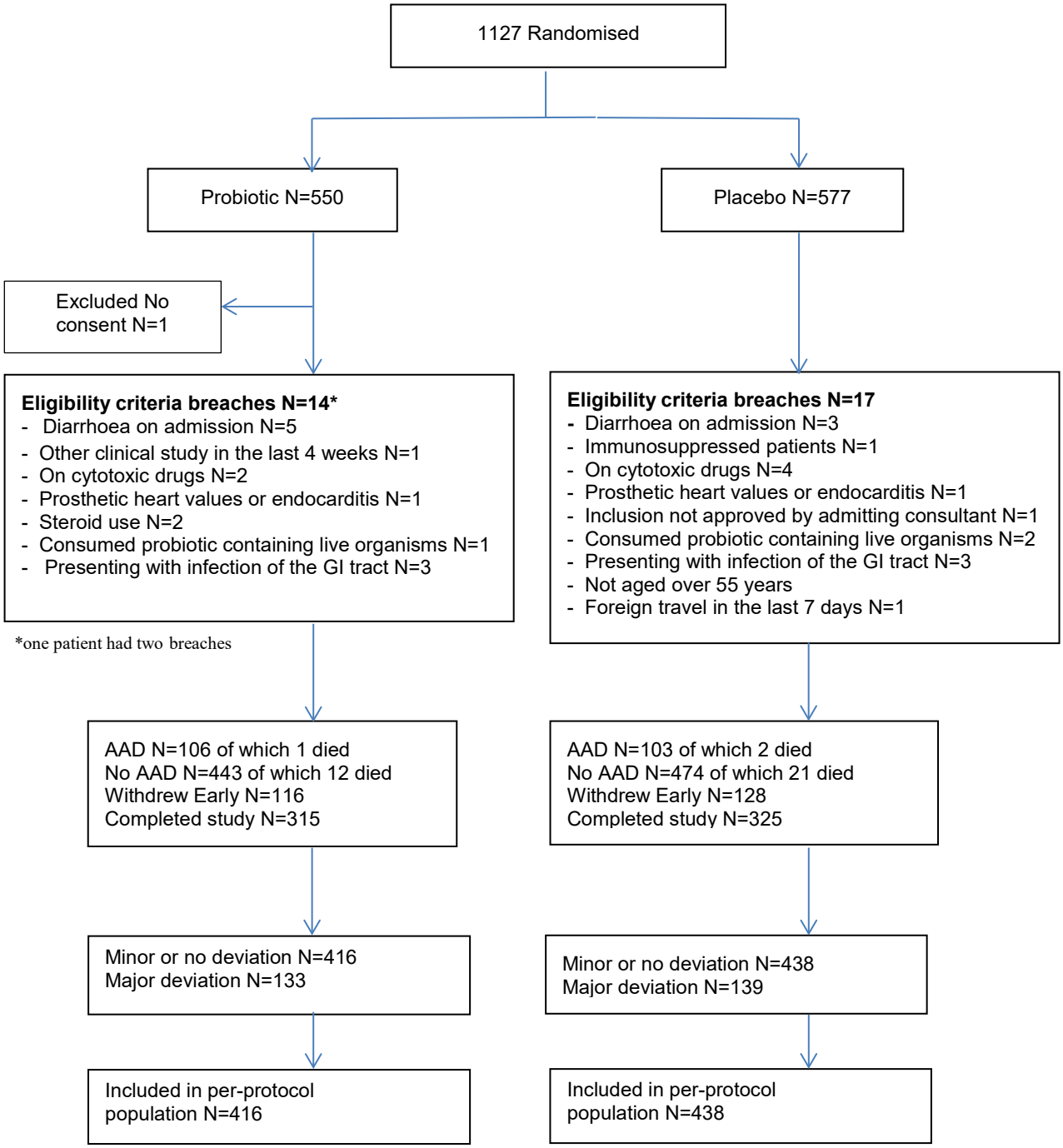
Table 6. The amount of probiotic consumed in each group

		allocation				
		Active		Placebo		
		Count	Column N %	Count	Column N %	fisher's exact
Consumed less than 50% of product	Yes	130	23.7%	133	23.1%	0.833
	No	419	76.3%	444	76.9%	
	Total	549	100.0%	577	100.0%	

		allocation				
		Active		Placebo		
		Count	Column N %	Count	Column N %	Fisher's exact
compliant	At least 80%	360	65.6%	383	66.4%	0.801
	<80%	189	34.4%	194	33.6%	
	Total	549	100.0%	577	100.0%	

		allocation				
		Active		Placebo		
		Count	Column N %	Count	Column N %	Kendall's Tau
compliance	0-49%	130	23.7%	133	23.1%	0.777
	50-79%	59	10.7%	61	10.6%	
	80%+	360	65.6%	383	66.4%	
	Total	549	100.0%	577	100.0%	

Figure 1: CONSORT Diagram



Appendix 1

Membership of Trial Steering Committee (TSC)
Professor John Potter (Chair), Professor Cameron Swift, Professor Roger Finch (Retired September 2010), Professor George Griffin (Commence April 2011)

Membership of combined Data Monitoring/Safety Committee (DMSC)
Professor Stephen Jackson (Chair) Professor Alan Sinclair (Retired June 2010) Dr Mark Wansbrough-Jones (Retired July 2013) Senior Lecturer, Dr Nigel Beckett (Commenced February 2011) (Consultant Physician/ Honorary Clinical Senior Lecturer), Elizabeth Cheek (Trial Statistician) Senior Lecturer (now retired), Professor Christopher Bulpitt (Co-Investigator)

Membership of End Point Committee: Dr Alan Ireland (Chair Consultant Gastroenterologist Consultant), Dr Mark Bayliss (Independent Member) Consultant Physician; **Co-opted Members of End Point Committee: Coordinating team:** Dr Jasmin Islam (Research Fellow), Jean Timeyin (Trial Co-ordinator), Elizabeth Cheek (Trial Statistician) Senior Lecturer (now retired), Professor Christopher Bulpitt (Co-Investigator).

List of Principal Investigators and Sites
Dr Juliet Wright MBBSMD FRCP: Brighton & Sussex University Hospital Trust, BN2 5BE
Dr Usharani NatarajanMRCOGMRCPI: East Surrey Hospital RHI 5RH
Dr Claire Nicholl BSc FRCP: Cambridge University Hospital Trust CB2 2QQ
Dr Graeme Dewhurst MB ChB FRCP: St Richards Hospital, Chichester PO 19 6SE
Dr Mahilal Fonseka MBBS MRCP: Eastbourne District General Hospital BN21 2YS
Dr David SlovickMB BS FRCP: Ealing Hospital NHS Trust UB1 3HW
Dr Peter Maskell MBBSMRCP: Maidstone and Tunbridge Wells NHS Trust ME16 9QQ
Dr SubirMukherjee MBBS MD FRCP: Queen Elizabeth Queen Mother Hospital CT9 4AN
Dr Khalid Ali MBBSMD FRCP: Princess Royal Hospital RH16 4EX
Dr Raad Nari MBBSMRCP: Ashford and St Peters Hospital KT16 0PZ
Dr Anser Qureshi MBBS FRCP: Broomfield Hospital, Chelmsford CMI 7HT
Dr David Gertner MBBSMRCP: Basildon Hospital SS16 5NL
Dr Zubair Khan MBBSMRCP: Yeovil District Hospital BA21 4AT
Dr Naval Shinh MBBSMRCP: Norfolk and Norwich University NR4 7UY
Dr Charles Bodmer MB ChB MD FRCP: Colchester Hospitals University CO4 5JL
Dr Carmen Martin-Marero MRCP MD: St Georges Hospital, London SW17 ORE
Dr Andrew Poullis BSc MBBS MD FRCP: St Georges Hospital, London SW17 ORE
Dr Richard Pollok MBBS PhD FRCP: St Georges Hospital, London SW17 ORE
Dr Aftab Ala MBBS FRCP MD: Frimley Park Hospital NHS Trust SW17 7 HS
Professor Anoop ChauhanMB ChB FRCP PhD: Portsmouth Hospitals NHS Trust PO6 3LY
Dr Mehool Patel MBBS FRCP: Lewisham Healthcare NHS Trust SE13 6LH
Dr Helen Roberts MB CHB FRCP: University Hospital Southampton SO16 6YD
Dr Simon Conroy MB CHB MRCP: University Hospitals of Leicester LE2 7LX
Dr Donald McGowan MBBS MRCP: University Hospitals of Morecambe Bay LA1 4RP
Dr Rohan Pathansali MBBS FRCP: Kings College Hospital SE5 9RS
Dr Chi Yuen Yau MB CHB MA: Stoke Mandeville Hospital HP21 8AL
Dr Evangelos Vasileiadis MBBSMRCP: Hillingdon Hospital UB8 3NN
Dr Achyut Guleri MBBSMD FRCPath: Blackpool Victoria Hospital FY3 8NR
Dr David Orr MB BCH MRCP: Lancashire Teaching Hospitals PR2 9HT
Dr David Aldulami MBBS MRCP: Worcestershire Acute Hospitals BN8 7UB

Appendix 2

Supplementary table of Antibiotics classified according to risk of *C.difficle* infection
(To be included in supplementary data)

High risk of *C.difficile* infection	**Medium risk of *C.difficile* infection**	**Low risk of *C.difficile* infection**
Cilastatin/imipenem, Ertapenem, Imipenem, Meropenem (Carbapenem)	Benzylpenicillin (penicillin G), Phenoxymethylpenicillin (penicillin V) (Penicillin - β-lactamase sensitive)	Amikacin, Gentamicin, Kanamycin, Neomycin (Aminoglycoside)
Cefaclor, Cefoxitin, Cefprozil, Cefuroxime (2nd generation cephalosporin)	Cloxacillin, Flucloxacillin, Oxacillin, Nafcillin (Penicillin - β-lactamase resistant)	Fosfomycin (Cell wall synthesis inhibitor)
Cefdinir, Cefditoren, Cefixime, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuten, Ceftizoxime, Ceftriaxone (3rd generation cephalosporin)	Amoxicillin, Amoxicillin/clavulanate, Amoxicillin/clarithromycin/lansoprazole, Ampicillin, Ampicillin/sulbactam, Piperacillin, Piperacillin/tazobactam,Ticarcillin/clavulanate (Penicillin - extended spectrum, combination)	Teicoplanin (Glycopeptide)
Cefepime (4th generation cephalosporin)	Cefadroxil, Cefalexin, Cefazolin (1st generation cephalosporin)	Ornidazole (Imidazole)
Ciprofloxacin, Levofloxacin, Moxifloxacin, Norfloxacin, Ofloxacin (Fluoroquinoline)	Azithromycin, Clarithromycin, Erythromycin, Pristinamycin, Roxithromycin, Monobactam, Aztreonam (Macrolide)	Daptomycin (Lipopeptides)
Clindamycin (Lincosamide)	Dalfopristin/quinupristin (Streptogramin)	Nitrofurantoin (Nitrofuran)
Pivampicillin, Temocillin (Penicillin - extended spectrum)		Linezolid (Oxolidinone)
		Colistin, Rifamycin, Rifampicin, Rifampin (Polymyxin)
		Sulfamethoxazole, Sulfamethoxazole/trimethoprim, Sulfasalazine, Trimethoprim (Antifolate and/or sulfonamide)
		Doxycycline, Minocycline, Tetracycline, Tigecycline (Tetracycline)