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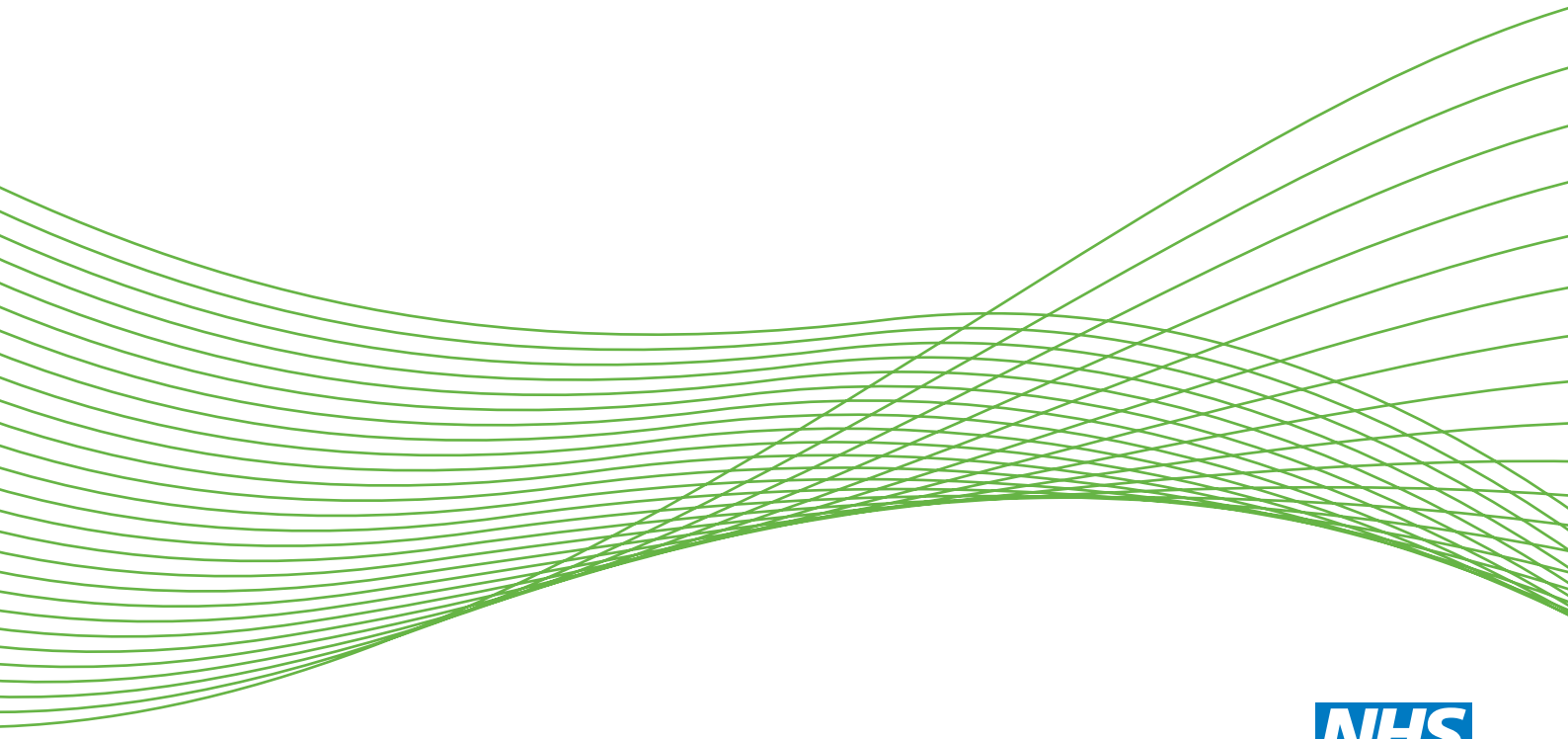

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Abstract

A high-dose preparation of lactobacilli and bifidobacteria in the prevention of antibiotic-associated and *Clostridium difficile* diarrhoea in older people admitted to hospital: a multicentre, randomised, double-blind, placebo-controlled, parallel arm trial (PLACIDE)

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Background: Antibiotic-associated diarrhoea (AAD) occurs most commonly in older people admitted to hospital and within 12 weeks of exposure to broad-spectrum antibiotics. Although usually a mild and self-limiting illness, the 15–39% of cases caused by *Clostridium difficile* infection [*C. difficile* diarrhoea (CDD)] may result in severe diarrhoea and death. Previous research has shown that probiotics, live microbial organisms that, when administered in adequate numbers, are beneficial to health, may be effective in preventing AAD and CDD.

Objectives: To determine the clinical effectiveness and cost-effectiveness of a high-dose, multistrain probiotic in the prevention of AAD and CDD in older people admitted to hospital.

Design: A multicentre, randomised, double-blind, placebo-controlled, parallel-arm trial.

Setting: Medical, surgical and elderly care inpatient wards in five NHS hospitals in the UK.

Participants: Eligible patients were aged ≥ 65 years, were exposed to one or more oral or parenteral antibiotics and were without pre-existing diarrhoeal disorders, recent CDD or at risk of probiotic adverse effects. Out of 17,420 patients screened, 2981 (17.1%) were recruited. Participants were allocated sequentially according to a computer-generated random allocation sequence; 1493 (50.1%) were allocated to the probiotic and 1488 (49.9%) to the placebo arm.

Interventions: Vegetarian capsules containing two strains of lactobacilli and two strains of bifidobacteria (a total of 6×10^{10} organisms per day) were taken daily for 21 days. The placebo was inert maltodextrin powder in identical capsules.

Main outcome measures: The occurrence of AAD within 8 weeks and CDD within 12 weeks of recruitment was determined by participant follow-up and checking hospital laboratory records by research nurses who were blind to arm allocation.

Results: Analysis based on the treatment allocated included 2941 (98.7%) participants. Potential risk factors for AAD at baseline were similar in the two study arms. Frequency of AAD (including CDD) was similar in the probiotic (159/1470, 10.8%) and placebo arms [153/1471, 10.4%; relative risk (RR) 1.04; 95% confidence interval (CI) 0.84 to 1.28; $p = 0.71$]. CDD was an uncommon cause of AAD and occurred in 12/1470 (0.8%) participants in the probiotic and 17/1471 (1.2%) in the placebo arm (RR 0.71; 95% CI 0.34 to 1.47; $p = 0.35$). Duration and severity of diarrhoea, common gastrointestinal symptoms, serious adverse events and quality of life measures were also similar in the two arms. Total health-care costs per patient did not differ significantly between the probiotic (£8020; 95% CI £7620 to £8420) and placebo (£8010; 95% CI £7600 to £8420) arms.

Conclusion: We found no evidence that probiotic administration was effective in preventing AAD. Although there was a trend towards reduced CDD in the probiotic arm, on balance, the administration of this probiotic seems unlikely to benefit older patients exposed to antibiotics. A better understanding of the pathogenesis of AAD and CDD and the strain-specific effects of probiotics is needed before further clinical trials of specific microbial preparations are undertaken. Evaluation of the effectiveness of other probiotics will be difficult where other measures, such as antibiotic stewardship, have reduced CDD rates.

Trial registration: This trial is registered as ISRCTN70017204.

Funding: This project was funded by the NIHR Health Technology Assessment programme and will be published in full in *Health Technology Assessment*; Vol. 17, No. 57. See the NIHR Journals Library website for further project information.

Contents

List of abbreviations

AAD	antibiotic-associated diarrhoea
ABMUHB	Abertawe Bro Morgannwg University Health Board
ACE	angiotensin-converting enzyme
CDD	*Clostridium difficile* diarrhoea
CDDFT	County Durham and Darlington Foundation Trust
CDI	*Clostridium difficile* infection
CEAC	cost-effectiveness acceptability curve
cfu	colony-forming unit
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CTA	clinical trial authorisation
EQ-5D	European Quality of Life-5 Dimensions
GP	general practitioner
HIV	human immunodeficiency virus
HRQL	health-related quality of life
ICER	incremental cost-effectiveness ratio
IMP	investigational medicinal product
IQR	interquartile range
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	intention to treat
MCS	mental component summary
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Agency
NCIMB	National Collection of Industrial, Food and Marine Bacteria
NGT	nasogastric tube
NIHR	National Institute for Health Research
OR	odds ratio
PCS	physical component summary
PLACIDE	probiotic lactobacilli and bifidobacteria in antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in the elderly
PP	per protocol
PPI	proton pump inhibitor
PT	preferred term
QALY	quality-adjusted life-year
QoL	quality of life
RCT	randomised controlled trial
REC	Research Ethics Committee
RR	relative risk
SAE	serious adverse event
SF-12 v2	generic Short Form questionnaire-12 items version 2
SOC	system organ class
VAS	visual analogue scale
WCC	white blood cell count

Scientific summary

Background

Antibiotic-associated diarrhoea (AAD) is diarrhoea that occurs in association with antibiotic treatment without any other cause. It complicates between 5% and 39% of antibiotic courses and occurs within 12 weeks of exposure to antibiotics. Major risk factors include admission to hospital, age ≥ 65 years and exposure to broad-spectrum antibiotics. Antibiotics cause diarrhoea through several mechanisms, including disruption of the gut microbial flora, which impairs colonisation resistance and changes gut physiology, and direct effects on the gut mucosa. The major pathogen associated with AAD is *Clostridium difficile*, which accounts for 15–39% of AAD cases. AAD is usually a mild, self-limiting illness but *C. difficile* diarrhoea (CDD) may result in severe illness with pseudomembranous colitis, toxic megacolon and often death. The frequency and severity of CDD have increased in recent years as a result of the emergence of the hypervirulent 027 strain. In the UK, CDD increases health-care costs by some £4000 per patient affected.

Probiotics are live microbial organisms which, when administered in adequate numbers, are beneficial to health. Probiotics may prevent or ameliorate AAD through several mechanisms, including antipathogen effects, such as secretion of bacteriocins and competition for nutrients and binding sites, and enhancement of the immunological barrier function and integrity of the gut mucosa. Probiotics have rarely led to adverse events even when administered to vulnerable populations, such as preterm infants and people with severe illness.

Although systematic reviews have provided some evidence that probiotics may be effective in preventing AAD, the reviews included trials of many different probiotics (including single strains of bacteria, the yeast *Saccharomyces boulardii* and mixtures of organisms), different administration regimens (including mode of delivery, number of organisms and probiotics combined with prebiotics), and patient populations who were diverse in age and exposure to antibiotics. Thus, the statistical heterogeneity in the results of meta-analysis probably arose from the marked clinical heterogeneity between studies. In addition, follow-up did not always cover the period of risk for AAD and research design and reporting were often poor.

Objectives

The primary objectives were to determine the clinical effectiveness and cost-effectiveness of a high-dose, multistrain probiotic in the prevention of AAD and CDD in older people (aged ≥ 65 years) admitted to hospital. The study was designed to inform whether or not the probiotic should be administered as part of routine health care to older people receiving antibiotics in secondary health care. Therefore, we aimed to undertake a pragmatic study that included participants who would be representative of older people admitted to hospitals in the industrialised world, with causes of diarrhoea identified, and patients managed, according to usual hospital practice. Secondary objectives were to assess the effect of the probiotic on the severity and duration of AAD, the acceptability and serious adverse events (SAEs) of the probiotic preparation and its effect on quality of life (QoL).

Methods

We undertook a multicentre, randomised, double-blind, placebo-controlled, parallel-arm trial.

Setting

We recruited inpatients from five hospitals in two NHS regions in the UK. We recruited from several clinical specialties including those known to have high rates of AAD, such as nephrology and care of the elderly.

Population

Inclusion criteria

- People aged at least 65 years and admitted to hospital.
- People exposed to one or more oral or parenteral antibiotics within the last 7 days or about to start antibiotic treatment.
- Consultant approval to invite the patient to join the study.

Exclusion criteria

People were excluded if they:

- already had diarrhoea, which was defined as three or more watery or loose stools (Bristol Stool Form Scale types 5–7) in the preceding 24 hours
- were sufficiently immunocompromised to require isolation and/or barrier nursing
- had severe illness requiring high-dependency or intensive care
- had a prosthetic heart valve
- had suffered from CDD in the previous 3 months
- had inflammatory bowel disease that had required specific treatment in the previous 12 months
- had suspected acute pancreatitis, which was defined as abdominal pain with serum amylase or lipase greater than three times the institutional upper limit of normal
- had a known abnormality or disease of mesenteric vessels or coeliac axis that may compromise gut blood supply
- had a jejunal tube in situ or were receiving jejunal feeds
- had a previous adverse reaction to probiotics, or
- were unwilling to discontinue their existing use of probiotics.

Participants were allocated sequentially to the probiotic or placebo arm according to a computer-generated random allocation sequence using blocks of variable sizes and stratified by centre.

Intervention

Informed by the findings of meta-analysis, we selected a high-dose, multistrain preparation of lactobacilli and bifidobacteria. The probiotic preparation consisted of a vegetarian capsule containing 6×10^{10} live bacteria as lyophilised powder, two strains of *Lactobacillus acidophilus* [CUL60/National Collection of Industrial, Food and Marine Bacteria (NCIMB) 30157 and CUL21/NCIMB 30156] and two strains of bifidobacteria (*Bifidobacterium bifidum* CUL20/NCIMB 30153 and *Bifidobacterium lactis* CUL34/NCIMB 30172). The placebo capsules were identical in appearance and contained inert maltodextrin powder. The dose was one capsule per day with food, taken between antibiotic doses where possible, for 21 days.

Outcomes

The main outcome measures were the occurrence of AAD within 8 weeks and CDD within 12 weeks of recruitment. Secondary outcomes were severity and duration of AAD, gastrointestinal symptoms, occurrence of pseudomembranous colitis, need for colectomy, duration of hospital stay, QoL, SAEs or death, acceptability of the probiotic and its viability at point of administration, and cost-effectiveness.

Outcomes were assessed by research nurses who were blind to participant allocation. Research nurses followed up participants daily during hospital stay and then weekly by telephone call until 8 weeks post recruitment. We also reviewed laboratory records to identify stools positive for *C. difficile* up to 12 weeks after recruitment. Diarrhoea was defined as three or more watery or loose stools (Bristol Stool Form Scale

types 5–7) in 24 hours. Diarrhoea stools were tested for intestinal pathogens and *C. difficile* according to routine laboratory practice. QoL was assessed by generic Short Form questionnaire-12 items version 2 (SF-12 v2) and European Quality of Life-5 Dimensions (EQ-5D) questionnaires administered at recruitment and at 4 and 8 weeks. Cost-effectiveness was evaluated from the perspective of the UK NHS focusing on the resources used by each participant. Differences between the two arms in costs and outcomes were used in a cost–consequences analysis with cost per quality-adjusted life-year (QALY) gained as the primary outcome.

Results

Patient recruitment was undertaken between 1 December 2008 and 28 February 2012. Out of 17,420 patients screened, 2981 (17.1%) were recruited. The main causes of non-participation were patients' unwillingness to join the trial (52.1%) and presence of exclusion criteria (18.4%). We allocated 1493 participants at random to the probiotic arm and 1488 to the placebo arm. Analysis by treatment allocated covered 2941 (98.7%) participants. The median age of participants was 77.1 years and comorbid illnesses were common; 54.6% participants suffered from hypertension, 24.1% from chronic obstructive pulmonary disease (COPD) and 22.9% from diabetes mellitus. Demographic factors and potential risk factors for AAD at the baseline were similar in the two study arms except for a sex imbalance (52.9% of participants were male in the probiotic arm and 46.2% were male in the placebo arm).

Antibiotic exposure varied between the centres but was similar in the two study arms. The most common indication for antibiotics was the treatment of respiratory, thoracic and mediastinal disorders (34.9% of participants). Antibiotics were prescribed for prophylaxis, mainly for surgical and medical procedures, in 24.3% participants. The most commonly prescribed antibiotics were penicillins (71.8% of participants were prescribed a penicillin and 56.1% of all participants were prescribed a broad-spectrum penicillin) and cephalosporins (24.3% of participants were prescribed cephalosporins). Most participants (78.8%) were exposed to antibiotics from two or more different classes and 62.8% received antibiotics for ≥ 7 days. However, 9.1% received only a single dose of antibiotic.

Non-antibiotic drug treatment was common, and similar, in the two study arms. Overall, 48.1% of participants were receiving an antihypertensive, 40.7% aspirin, 39.4% a proton pump inhibitor (PPI) and 29.7% an angiotensin-converting enzyme (ACE) inhibitor, with many participants receiving a combination of drugs.

Compliance was similar in the two study arms. Nearly all participants (99.6%) took at least one dose of the trial interventions and 52.5% completed the full course of 21 days.

The frequency of AAD (including CDD) was similar in the probiotic (159/1470, 10.8%) and placebo arms [153/1471, 10.4%; relative risk (RR) 1.04; 95% confidence interval (CI) 0.84 to 1.28; $p = 0.72$]. Most episodes of AAD (55.4%) were managed in hospital and stool samples were collected and tested for diarrhoeal pathogens in 58.6% of cases. The frequency and duration of gastrointestinal symptoms during AAD were similar in the two study arms. CDD was an uncommon cause of AAD and occurred in 12/1470 (0.8%) participants in the probiotic and 17/1471 (1.2%) in the placebo arm (RR 0.71; 95% CI 0.34 to 1.47; $p = 0.35$).

In patients with CDD, bloating was less common in the placebo arm (17.6%) than in the probiotic arm (58.3%; risk difference 40.7%; 95% CI 7.4% to 74.0%) but other gastrointestinal symptoms, clinical findings and investigations, and classification of severity of CDD were similar in the two study arms. During follow-up, no patient was identified as having pseudomembranous colitis, toxic megacolon or life-threatening CDD or as having died from CDD.

The frequency of AAD and CDD was similar across the study centres. In covariate analysis, AAD occurred more commonly with longer duration of antibiotic treatment [≤ 8 days compared with > 8 days; odds ratio (OR) 0.48; 95% CI 0.36 to 0.62], antacid therapy (no antacid therapy compared with some antacid therapy; OR 0.74; 95% CI 0.58 to 0.95) and longer duration of hospital stay (< 7 days compared with ≥ 7 days; OR 0.74; 95% CI 0.55 to 0.99). CDD was also more common with longer duration of antibiotic therapy (≤ 8 days compared with > 8 days; OR 0.13; 95% CI 0.03 to 0.55). However, during covariate analysis, taking account of risk factors and the number of days that participants took the trial interventions did not materially alter probiotic effect for either AAD or CDD. Sex was not a risk factor for AAD and did not modulate probiotic effect.

For all participants, the frequency of common gastrointestinal symptoms was similar in the two study arms except for flatus occurring during administration of the trial interventions, which was marginally less common in the placebo arm (10.2%) than in the probiotic arm (12.5%; risk difference 2.3%; 95% CI 0.0% to 4.6%). Duration of hospital stay was similar in the probiotic arm ($n = 1452$; median 4 days, range 1–11 days) and placebo arm ($n = 1447$; median 4 days, range 1–11 days; $p = 0.87$).

The frequency of SAEs and the proportion of participants experiencing at least one SAE were similar in both arms. Across both arms, a SAE resulted in death in 143 participants (4.9%), was life-threatening in 10 participants (0.3%), resulted in prolonged hospitalisation in 447 participants (15.2%), resulted in persistent or significant disability or incapacity in four participants (0.1%) and was considered another significant medical event in 11 participants (0.4%). SAEs took the form of gastrointestinal disorders in 79 (2.7%) participants and infections and infestations in 43 (1.5%).

Perhaps not surprisingly, QoL tended to improve in both arms during follow-up. The change from baseline in EQ-5D index values, visual analogue scale (VAS) and SF-12 v2 scores was similar across both arms except that the change in VAS was less in the probiotic arm than in the placebo arm at 8 weeks (−1.76; 95% CI −3.32 to −0.19). However, this difference of < 2 on the 100-point VAS scale was unlikely to be clinically significant.

Sixty-seven unused trial capsules returned by participants were analysed in an independent laboratory. The 33 capsules allocated as placebo according to the randomisation sequence were inert (contained no viable bacteria) and all 34 capsules allocated as probiotic according to the randomisation sequence had $\geq 1.62 \times 10^{10}$ viable bacteria.

Total health-care cost per patient was remarkably similar in the probiotic arm (£8020; 95% CI £7620 to £8420) and placebo arm (£8010; 95% CI £7600 to £8420). The probiotic was not cost-effective, with an incremental cost-effectiveness ratio (ICER) of £189,662 per QALY. Across both arms, the average duration of hospital stay was 5.58 days (95% CI 2.78 to 8.39 days) longer for patients with AAD than for those without and there was an associated average increase in health-care cost of £4530 (95% CI £3440 to £5620).

Conclusions

We did not find adequate evidence to suggest that probiotic administration was effective in preventing AAD. Although there was a trend towards reduced CDD in the probiotic arm, the administration of this probiotic seems unlikely to benefit older patients exposed to antibiotics. Alternative probiotic preparations may be effective in the prevention of AAD. However, future clinical trials should be guided by a better understanding of the mechanisms underlying AAD and the strain specific effects of probiotics. Furthermore, a probiotic is less likely to be effective where other measures have reduced CDD rates.

Implications for health care

- The high-dose, multistrain preparation of lactobacilli and bifidobacteria evaluated in our study is unlikely to benefit unselected older inpatients exposed to antibiotics.
- The effectiveness of our preparation in preventing CDD was unclear. However, even if it is effective, the falling prevalence of CDD needs more patients to take the probiotic to prevent a single case.
- Clinical judgement regarding the benefits and risks of novel interventions to prevent AAD needs to take account of its reduced frequency as a result of other preventative measures, such as antibiotic stewardship.
- The administration of additional medications to vulnerable older people, many of whom are already taking multiple medications, may not be well tolerated in practice.
- The probiotic preparation was not associated with SAEs in our study. However, surveillance for potentially uncommon adverse events is required in future studies.

Implications for research

- A better understanding of the multiple mechanisms underlying AAD and CDD, and how these vary in specific populations and the strain-specific effects of probiotics, is needed before further clinical trials of specific probiotic preparations are undertaken.
- More research to identify populations at increased risk of AAD and CDD is needed to facilitate the future evaluation of probiotic interventions.
- The design of studies to evaluate the efficacy of alternative probiotics in the prevention of CDD needs to consider the effect of other measures that have reduced the frequency of *C. difficile* infection (CDI) in some health-care institutions.
- Further research into the effect of probiotics on patient QoL will be necessary to better determine patient benefit and cost-effectiveness.

Trial registration

This trial is registered as ISRCTN70017204.

Funding

This project was funded by the NIHR Health Technology Assessment programme and will be published in full in *Health Technology Assessment*; Vol. 17, No. 57. See the NIHR Journals Library website for further project information.

Chapter 1 Introduction

Background

Antibiotic-associated diarrhoea (AAD), defined as diarrhoea in association with antibiotic treatment and without an alternative cause, occurs in 5–39% people within 12 weeks of exposure to antibiotics.[1,2] The predominant mechanism underlying AAD is disruption of the commensal gut flora. This impairs colonisation resistance and facilitates the emergence of a range of gut pathogens.[2,3] The major diarrhoeal pathogen associated with antibiotic treatment is *Clostridium difficile*, which accounts for 15–39% of AAD cases.[4] Altered commensal flora may also result in diarrhoea through changes in the metabolism of carbohydrates, short-chain fatty acids and bile acids, and some antibiotics cause diarrhoea through direct effects such as increased gut motility.[2,5]

Clostridium difficile is an anaerobic bacterium that produces resistant spores that persist long term in the environment. Transmission is faecal–oral and 4–21% patients may acquire the infection during admission to hospital through contact with colonised patients, contaminated fomites and the hands of health-care staff. *C. difficile* diarrhoea (CDD) occurs in both endemic and outbreak scenarios.[6] Since 2003, an increase in the frequency and severity of CDD in North America and Europe has been attributable to the emergence of the hypervirulent 027 strain, which may produce higher amounts of toxin.[7–9]

Antibiotic-associated diarrhoea occurs typically in older people admitted to hospital.[1,2] ADD complicates treatment with many antibiotic classes but especially broad-spectrum penicillins, cephalosporins, clindamycin and long-duration antibiotic treatments.[10] Additional risk factors include prolonged hospital stay, previous hospital admission, previous gastrointestinal surgery and use of a nasogastric tube (NGT).[3] In a retrospective study of European hospitals, risk factors for CDD included age $\geq$ 65 years, severe comorbidity and recent treatment with cephalosporins and aminopenillin-beta (β)-lactamase inhibitor combinations.[11] In a prospective study of people aged > 18 years admitted to Canadian hospitals, age, exposure to antibiotics, treatment with proton pump inhibitors (PPIs) and previous hospital admission within the last 2 months predicted CDD.[12]

The severity of AAD varies greatly. Although usually a mild, self-limiting illness, it is associated with prolonged hospital stay and increased health-care costs. *C. difficile* infection may remain asymptomatic, but intestinal pathology results from secretion of toxins A and B causing increased mucosal fluid secretion and inflammation. Symptoms range from mild, self-limiting diarrhoea to severe diarrhoea complicated by pseudomembranous colitis, toxic megacolon and death.[6,8]

Estimates of the financial burden of *C. difficile* infection (CDI) to the health-care service have varied between $2454 and $16,464 for every health-care-acquired case in the USA,[13–15] £4107 in the UK[16] and €7147 in Germany.[17] The annual cost of health care for CDD in the USA has been estimated to be $3B.[18,19] Nosocomial CDI increases hospital stay by between 7 and 26 days,[16,17,20] and prolonged hospital admission was identified as the main cost driver in most studies.[13,15,16] Furthermore, an increase in length of hospital stay due to more severe disease in recent years has resulted in a rise of the incremental cost of CDI.[21]

Treatment

Uncomplicated AAD usually responds to withdrawal of the offending antibiotics. CDD usually responds to treatment with metronidazole or vancomycin, but 20–25% patients go on to suffer from a recurrent form of the disease. Novel modes of treatment include probiotics, immunoglobulin infusion and faecal transplant from healthy donors.[3,6]

Prevention of antibiotic-associated and *Clostridium difficile* diarrhoea

The frequency and severity of CDD in hospitals in the industrialised world have led to comprehensive strategies for prevention. These include decontamination of the environment, hand washing and isolation of patients with diarrhoea.[6] Antibiotic stewardship programmes have also been effective in reducing infection rates.[6,22,23]

Probiotics

Probiotics are defined as live microbial organisms which, when administered in adequate numbers, are beneficial to health.[24] Although clinical trials are undertaken to determine whether or not a microbial preparation has a health benefit in a specific population, the term 'probiotic' is commonly used to refer to the preparation being evaluated and it is used in this sense here. Bacteria used as probiotics are among the organisms 'generally regarded as safe' by the Food and Agriculture Organization of the United Nations.[25] Live bacteria from several genera and the yeast *Saccharomyces boulardii* have been administered to vulnerable groups such as preterm infants and people with human immunodeficiency virus (HIV) infection without adverse effects.[26] Adverse events occurring in clinical trials evaluating the prevention of AAD have not been ascribed to probiotic intake.[26] Administration of lactic acid bacteria has been associated in rare cases with septicaemia in immunocompromised people and endocarditis in people with artificial heart valves.[27] Despite the apparent low risk of adverse events, careful assessment of safety in clinical trials is recommended.[26]

Rationale

Specific probiotic strains have been identified *in vitro* and *in vivo* to possess several mechanisms that may prevent or ameliorate AAD and CDD through enhanced barrier function of the gut epithelium.[28] First, potential pathogens are killed or inhibited by the secretion of antimicrobial peptides and probiotics compete for attachment sites on intestinal mucus and epithelium. Acidification of the gut contents through the production of lactic acid also inhibits pathogen growth. Second, mucosal immunity is enhanced through increased secretory immunoglobulin A production and stimulation of antimicrobial peptide secretion by host cells. Finally, through direct effects on the epithelium, probiotics increase the secretion of mucus, enhance tight junction integrity and decrease epithelial cell apoptosis.

At the time that probiotic lactobacilli and bifidobacteria in antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in the elderly (PLACIDE) was developed, meta-analyses had suggested that probiotics may be effective in the prevention of AAD. McFarland pooled data from 25 randomised controlled trials (RCTs), which included a total of 2810 adults and children.[29] There was significant heterogeneity ($\chi^2 = 82.5$; $p < 0.001$) in a fixed-effects model. In random-effects analysis, the relative risk (RR) for AAD was 0.43 [95% confidence interval (CI) 0.31 to 0.58] in participants receiving a probiotic. This meta-analysis included all of the trials included in previous systematic reviews, which had broadly similar findings.[30–33] Although meta-analysis has provided some evidence that probiotics may be effective in the prevention of AAD, the marked differences between trials in the microorganisms evaluated (single strains and mixtures of bacteria, the yeast *S. boulardii*), administration regimens (mode of delivery, number of organisms, probiotics combined with prebiotics), patient populations (age and exposure to antibiotics) and period of follow-up for AAD probably underlie the statistical heterogeneity in the results and weaken the evidence for probiotic effectiveness.

Much less evidence from clinical trials was available for probiotics in the prevention of CDD. A pilot study in elderly hospitalised patients reported that 30 out of 138 (21.7%) patients developed diarrhoea and 5 out of 69 (7.2%) in the placebo group compared with 2 out of 69 (2.9%) who had received a

combination of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* tested positive for *C. difficile* toxin. Stool culture suggested that the main effect of the probiotic was neutralisation of toxin rather than prevention of colonisation.[34] Thomas *et al.* had assessed *Lactobacillus rhamnosus* GG for the prevention of AAD in 267 adults who were monitored for an average of 21 days, but the number of patients from whom stool samples had been collected and in which *C. difficile* was detected was too small to assess probiotic effect.[35] In randomised trials of 193[36] and 180 adult patients,[37] the occurrence of CDD was similar in those receiving *S. boulardii* and those receiving the placebo. Although there was trial evidence for probiotics in the treatment of established CDD or the prevention of recurrence,[29] we are not aware of any other studies that had assessed probiotics in the prevention of CDD in adults.

We are not aware of any studies that have assessed the effect of probiotic on quality of life (QoL) in patients at risk of AAD.

Selection of the probiotic preparation

Although several mechanisms whereby probiotic organisms enhance gut barrier function have been identified,[28] it remains unclear which of these are most relevant to the prevention or amelioration of AAD and to what extent these mechanisms are common to many different probiotic organisms or are strain specific. Therefore, the scientific evidence to inform the selection of a specific probiotic intervention for the prevention of AAD is limited. The meta-analyses included trials that had evaluated many different bacterial preparations and the yeast *S. boulardii*.[29] The bacterial interventions included single strains, mixtures of different organisms and mixtures of probiotics with prebiotics. Dosages (number of organisms) and modes of administration also varied markedly between studies. Factors associated with greater efficacy in preventing AAD in meta-analysis included the use of *S. boulardii* or *L. rhamnosus* GG, mixtures of probiotics and preparations with high numbers of organisms.[29] Efficacy was similar for bacterial preparations [five trials conducted on 384 adults and children; odds ratio (OR) 0.34; 95% CI 0.19 to 0.61] and yeast preparations (four trials conducted on 830 participants; OR 0.39; 95% CI 0.25 to 0.62).[31]

In an attempt to maximise gut colonisation and, therefore, colonisation resistance, we used a multistrain preparation of *Lactobacillus* sp. and *Bifidobacterium* sp., bacterial species that had been evaluated extensively in clinical trials, with a high number of viable bacteria (total 6×10^{10} organisms per day). We intended to undertake a pragmatic trial to assess the clinical effectiveness and cost-effectiveness of the probiotic preparation in older people receiving antibiotics in secondary health-care settings representative of those in industrialised counties and with the causes of diarrhoea determined by routine laboratory practice.

Chapter 2 Methods

Trial design

Probiotic lactobacilli and bifidobacteria in antibiotic-associated diarrhoea and *Clostridium difficile* in the elderly was a prospective, multicentre, pragmatic, two-armed, double-blind, randomised, placebo-controlled trial with equal randomisation.

Approvals obtained

The Research Ethics Committee (REC) for Wales approved the study on 27 November 2008 (number 08/MRE09/18).

A clinical trial authorisation (CTA) was granted for the live bacterial preparation by the Medicines and Healthcare products Regulatory Agency (MHRA) on 6 October 2008 (CTA number AAD-CDD-001). The details of the REC for Wales, local RECs, competent authorities and research and development department approvals are provided in *Appendix 1*. The trial was assigned the International Standard Randomised Controlled Trial Number (ISRCTN) ISRCTN70017204 and EudraCT number 2007–002876–32.

Trial sites

Inpatients were recruited from medical and surgical wards of secondary care hospitals from two NHS regions: the Abertawe Bro Morgannwg University Health Board (ABMUHB) in South West Wales (Singleton, Morriston and Princess of Wales hospitals), and the County Durham and Darlington Foundation Trust (CDDFT; the University Hospital of North Durham and Darlington Memorial Hospital). Details of the trial sites are included in *Appendix 2*.

Participant inclusion criteria

People aged ≥ 65 years who were admitted to hospital and had been exposed to one or more antibiotics within the preceding 7 days, or were about to start antibiotic treatment, were eligible to join the study. Approval to invite the patient to participate in the study was secured from the patient's consultant.

Participant exclusion criteria

People were excluded if they:

- already had diarrhoea, which was defined as three or more watery or loose stools (Bristol Stool Form Scale types 5–7)[38] in the preceding 24 hours
- were sufficiently immunocompromised to require isolation and/or barrier nursing
- had a severe illness requiring high-dependency or intensive care
- had a prosthetic heart valve
- had suffered from CDD in the previous 3 months
- had inflammatory bowel disease that had required specific treatment in the previous 12 months
- had suspected acute pancreatitis, which was defined as abdominal pain with serum amylase or lipase greater than three times the institutional upper limit of normal

- had a known abnormality or disease of mesenteric vessels or coeliac axis that may compromise gut blood supply
- had a jejunal tube in situ or were receiving jejunal feeds
- had a previous adverse reaction to probiotics
- were unwilling to discontinue their existing use of probiotics.

Investigational medicinal products

The active preparation consisted of a vegetarian capsule containing 6×10^{10} live bacteria as lyophilised powder comprising two strains of *L. acidophilus* [CUL60, National Collection of Industrial, Food and Marine Bacteria (NCIMB) 30157 and CUL21, NCIMB 30156; 3×10^{10} colony-forming units (cfu)] and two strains of *Bifidobacterium* (*B. bifidum* CUL20, NCIMB 30153 and *Bifidobacterium lactis* CUL34, NCIMB 30172; 3×10^{10} cfu). Obsidian Research Ltd, Port Talbot, UK, prepared the investigational medicinal products (IMPs) according to the randomisation schedule. The organisms were available commercially through BioCare® Ltd (Lakeside, Birmingham, UK) and Pharmax (Bellevue, WA, USA). The placebo capsules were identical to the probiotic capsules but contained inert maltodextrin powder. The dose was one capsule per day with food and, where possible, between antibiotic doses, for 21 days.

Each participant was provided with a bottle labelled with his or her unique study number and containing 21 capsules. The IMPs were given to participants on the day of recruitment and administration during the hospital stay was supervised by the research nurses in ABMUHB and by the ward nurses in CDDFT. To prevent cross-contamination, strict hygiene procedures were followed, for example where capsules were opened, and the contents were mixed with fluids for administration to participants with difficulty swallowing. Although the live bacteria had a shelf life of 2 months at room temperature, participants were instructed to keep the capsules in the refrigerator following discharge from the hospital and encouraged to complete the 21-day course.

In vitro antibiotic testing demonstrated that the lactobacilli and bifidobacteria were sensitive to broad-spectrum antibiotics. All four strains were sensitive to ceftriaxone, chloramphenicol, erythromycin, linezolid and tetracycline.

After recruitment of the first 50 participants, a research nurse perceived a slight difference in the colour of the powder contained in the probiotic and placebo capsules, which were transparent. Therefore, the IMPs were resupplied in opaque capsules according to the original random allocation sequence. No unmasking of participant allocation occurred.

To ensure the quality of the IMPs, unused capsules were collected from participants on an opportunistic basis (e.g. from participants who withdrew before completing the 21-day course). A laboratory independent of the research team performed quantitative bacterial culture. The findings were sent to the independent statistician to check against the randomisation sequence and for the total count of viable organisms.

Objectives

Primary objectives: to determine the clinical effectiveness and cost-effectiveness of a live preparation of two strains of lactobacilli and two strains of bifidobacteria in the prevention of AAD and CDD in people aged ≥ 65 years who were exposed to oral or intravenous antibiotics and who were representative of patients admitted to secondary care NHS facilities in the UK.

Secondary objectives: to assess the effect of the probiotic on the duration and severity of AAD, the acceptability of the probiotic preparation, serious adverse events (SAEs) of the probiotic preparation and its effect on QoL.

Outcomes

Primary outcomes: the occurrence of AAD within 8 weeks and CDD within 12 weeks of recruitment.

Secondary outcomes: the severity and duration of AAD; the severity and duration of CDD and incidence of recurrence within the follow-up period; the number of days with abdominal pain, bloating, flatus and nausea; the incidence of pseudomembranous colitis, the need for colectomy; well-being and QoL; duration of hospital stay; frequency of SAEs; the acceptability of the live bacterial preparation (to identify if the participants had any difficulty taking the bacterial preparation vs. the placebo); the viability of the bacterial preparation at point of administration and death.

Sample size

Based on a review of previous clinical trials, we estimated that AAD would occur in 20% and CDD in 4% of participants in the placebo arm. The detection of a 50% reduction in CDD to a frequency of 2% in the active arm with 80% power at the 5% significance level required 2478 subjects (1239 in each arm). This number of participants would provide a power of > 99% to detect a 50% reduction in the risk of AAD (to 10% frequency) and a power of 90% to detect a 25% risk reduction in AAD (to 15% frequency) in the active arm at the 5% significance level. We planned to recruit 2974 participants to allow for 10% drop-outs and 10% loss to follow-up due to deaths unrelated to diarrhoea.

Randomisation

The independent statistician prepared a random allocation sequence using blocks of variable sizes and stratified by hospital using SAS® PROC PLAN, version 9.1 (SAS Institute Inc., Cary, NC, USA). The sequence allocated participants in a 1 : 1 ratio to the two arms of the study.

Blinding

The allocation sequence was not available to any member of the research team until databases had been completed and locked. In view of the established safety record of lactobacilli and bifidobacteria[27] and the sensitivity of the organisms used in this study to broad-spectrum antibiotics, there was no provision for the emergency unblinding of participants. Therefore, copies of the random allocation sequence were not held at the recruitment sites.

Recruitment

The research nurses received training in 'Good Clinical Practice' and were fully conversant with all aspects of the trial. Specific training was given in assessment of patient eligibility, recruitment and obtaining informed consent, the trial protocol, reporting of adverse events and collecting information. Presentations were made to clinical, nursing and pharmacy staff to ensure their familiarity with the purpose and conduct of the trial. Permission to approach patients and invite them to join the study was obtained from hospital consultants.

Research nurses visited wards daily to identify eligible patients and provide them with verbal and written information (see *Appendix 3*). Patients were revisited either later the same day or the next day after they had had the opportunity to discuss with relatives/carers and health-care personnel whether or not they wished to join the study. For people unable to provide consent, information was provided to their relatives/carers and assent sought (see *Appendix 3*). Although reasons for not joining the study were

requested, patients and their relatives/carers were free to decline to participate without giving a reason. Patients in whom consent or assent was obtained were allocated the next unique study number in the random sequence for that site and the research nurses provided them with the corresponding trial preparation.

Baseline assessment

Information recorded at recruitment included basic demographic characteristics, use of cigarettes and alcohol, source of admission, principal diagnosis or reason for admission, comorbid illnesses, duration of hospital stay prior to recruitment, non-antibiotic drug treatment, indication for antibiotic treatment and antibiotics prescribed (see *Appendix 4*).

Participant follow-up

Participants were visited daily by the research staff during hospital stay. Changes to antibiotic treatment, gastrointestinal symptoms (including the presence of diarrhoea), compliance, difficulties taking the IMPs and occurrence of SAEs were recorded onto standard forms (see *Appendix 4*). The same information was requested weekly, after discharge from hospital, via a telephone questionnaire and continued for 8 weeks post recruitment. In addition, participants were encouraged to contact a named member of the research staff to report potential SAEs at any time during follow-up. Review of laboratory data regarding stool assays was continued until 12 weeks after recruitment.

Trial completion

Participants had completed the trial when they:

- had completed follow-up
- had withdrawn and declined collection of further follow-up data
- were lost to follow-up
- had died.

A chart showing participant flow through the study is included as *Appendix 5*.

Measurement of primary outcomes

Diarrhoea was defined as three or more loose stools (stool consistency 5–7 on the Bristol Stool Form Scale)[38] in a 24-hour period. Diarrhoea was also diagnosed in participants with frequent stools that they described as loose but who were unable to describe stool consistency using the Bristol Stool Form Scale. The presence of diarrhoea according to these criteria was confirmed by the research nurses during admission with either the patient, their carers or a member of the medical staff. After discharge, this was confirmed during telephone interview.

Stool samples for analysis were collected only during episodes of diarrhoea, including diarrhoea that occurred after discharge from hospital. Stools were analysed for diarrhoeal pathogens according to routine NHS laboratory practice. Analyses included bacterial culture for *Salmonella* spp., *Shigella* spp., *Campylobacter* and *Escherichia coli* 0157 and wet film for ova, cysts and parasites. Detection of viruses depended on the clinical context (e.g. suspected norovirus outbreak). In ABMUHB, *C. difficile* toxins were detected by an in-house tissue culture assay with confirmation by enzyme immunoassay Premier™ Toxins A&B (Meridian Bioscience, Inc., Cincinnati, OH, USA). In CDDFT, toxins were detected by the VIDAS®

C. difficile A&B (bioMérieux SA, Marcy l'Etoile, France). To improve the detection of CDD from June 2010, CDDFT also introduced detection of the *C. difficile* antigen glutamate dehydrogenase C. DIFF QUIK CHEK® (TECHLAB® Inc., Blacksburg, VA, USA) to be used in conjunction with the toxin assay. Further stools samples were requested if a cause of diarrhoea was not identified and especially if there was clinical suspicion of CDD. Stools positive for *C. difficile* toxin were cultured and the isolates sent to a central reference laboratory for ribotyping.

Antibiotic-associated diarrhoea was defined as occurring in association with antibiotic therapy but without diarrhoeal pathogens detected on routine laboratory analysis or an alternative explanation (e.g. laxative treatment). Among patients with AAD, CDD was diagnosed in those with stools testing positive for *C. difficile* toxins.

Measurement of secondary outcomes

Information regarding the severity and duration of AAD and CDD, number of stools per day and stool consistency, incidence of recurrence of CDD within the follow-up period, the number of days with abdominal pain, bloating, flatus and nausea, duration of hospital stay and acceptability of the live bacterial preparation was collected by the research nurses during follow-up (see *Appendix 4*). CDD was investigated and managed according to the current hospital practice and clinical and laboratory information from clinical records was recorded (see *Appendix 4*). Information regarding the occurrence of pseudomembranous colitis, the need for colectomy and death was also collected from the patient's case records. The information from follow-up and patient case records was used to classify the severity of CDD according to UK national guidelines (see *Appendix 6*).[39] The severity of CDD was classified independently by two assessors, WH and SA, and differences resolved by discussion.

Quality of life was assessed by the generic Short Form questionnaire-12 items version 2 (SF-12 v2) administered by research nurses at baseline and at 4 and 8 weeks. SF-12 v2 QoL subscales (social function, role function–emotional, role function–physical, general health, mental health, bodily pain, vitality, physical functioning), physical component summary (PCS) and mental component summary (MCS) scores were calculated and quality assured according to the QualityMetric Incorporated guidance[40] with imputation of missing scores using the SF-12 v2 Missing Data Estimator software where possible.[40] SF-12 v2 subdomain, PCS and MCS scores were allocated a value of 0 for the lowest/worse score and 100 for the highest/best score.

Serious adverse events were identified and reported according to standard guidelines.[41] Suspected unexpected serious adverse reactions were to be reported immediately to the independent safety monitor and, if appropriate, the ethics committee in accordance with local and national requirements, which were identified as:

- any manifestation of infection (e.g. abscess, bacterial endocarditis, bacteraemia) in which lactobacilli or bifidobacteria were isolated from pathological specimens
- the development of pancreatitis, which was defined as abdominal pain with serum amylase or lipase concentration equal to or greater than three times the institutional upper limit of normal
- the development of multiorgan failure
- the development of bowel ischaemia.

Additional data collected

Information was collected to identify subgroups of participants who may be at increased risk of AAD and CDD. This included potential risk factors at baseline, antibiotic treatment and duration of hospital stay.

Data management

All data were collected on standardised forms that were checked for missing values by the trial manager. Routine laboratory records were accessed to record results of stool analyses. Data were entered into Microsoft Access® (Microsoft Corporation, Redmond, WA, USA) and included range checks and double entry. Databases were compared using Microsoft Excel® (Microsoft Corporation, Redmond, WA, USA) to identify data entry errors.

Antibiotics were classified according to *British National Formulary* categories (see *Appendix 7*).[42] Indications for antibiotic treatment were classified according to the system organ class (SOC) terminology of the Medical Dictionary for Regulatory Activities (MedDRA).[43] Participants were allocated to each SOC for either suspected or proven infection of that organ or system. Antibiotic treatment for suspected infection where no system or organ was identified was classified as 'suspected sepsis but site unclear'. SAEs were coded according to the most appropriate preferred terms (PTs) of the MedDRA.[43]

After data cleaning, databases were locked and forwarded to the trial statistician for analysis. Initial analysis according to the randomisation sequence identified the two arms of the study as only 'A' or 'B'. The identity of the two arms was only revealed after the Data Monitoring and Ethics Committee had reviewed these data and approved the analysis.

Statistical analysis

Demographic and baseline data were summarised by recruitment hospital and treatment group. Continuous variables were summarised using number of observations, median and interquartile range (IQR) and categorical variables by the number and percentage of events.

Analysis of primary and secondary end points was performed in a modified intention-to-treat (ITT) population that excluded a small number of subjects who withdrew shortly after randomisation and did not have follow-up data. The chi-squared test or Fisher's exact test was used to compare proportions. Risk difference and RR together with the 95% CIs were calculated using a generalised linear model that included treatment as a single predictor. Similarly, CIs for the ORs of AAD and CDD were estimated from logistic regression models. Secondary continuous outcomes with no repeated measurements were summarised using number of observations, median and IQR. The *t*-test or Mann–Whitney method were used to compare continuous variables. Duration data were summarised by median and IQR and compared using the Mann–Whitney method. No transformation was used for any variables.

Analysis of primary end points was also performed by logistic regression model adjusting for the following prespecified baseline characteristics and potential risk factors for AAD that may be likely to affect the occurrence of the primary end point:

- centre
- age
- sex
- antibiotic class
- duration of antibiotic treatment
- antacid therapy (including PPI treatment)
- NGT in situ
- previous gastrointestinal surgery
- recent previous hospital admission
- duration of hospital stay.

We intended to include all 10 prespecified variables in the logistic model but some were not entered because their effects were inestimable. A per-protocol (PP) population excluded participants who did not receive any IMP doses or in whom compliance was unclear. Additional analyses also assessed probiotic effect on primary outcomes in participants who took all 21 doses, 14 or more doses, or seven or more doses of the IMP. Analysis methods for the PP population were similar to those described for the modified ITT population. All analyses were performed on both the modified ITT and PP populations.

SAS version 9.2 was used for data analyses.

Quality of life analysis

The main analysis compared the change from baseline in SF-12 v2 PCS and MCS composite scores at 4 weeks in the two study arms. We also compared SF-12 v2 PCS and MCS composite scores at 8 weeks and scores for individual SF-12 v2 subdomains (social function, role function–emotional, role function–physical, general health, mental health, bodily pain, vitality and physical functioning) at 4 and 8 weeks. Composite scores were compared using mixed model analysis using the SF-12 v2 score at baseline as a covariate, treatment, visit, interaction between the treatment and visit as fixed effects, and subject as a random effect. During the trial, some subjects dropped out, resulting in some incomplete observations. These incomplete observations were not computed but were assumed to be missing at random in the mixed model analysis. The treatment difference, together with the 95% CI at each visit, was derived from the mixed model.

Economic analysis

The cost-effectiveness evaluation was undertaken from the perspective of the UK NHS. Costs were assigned to the resources utilised by each participant. These consisted of the bacterial preparation, staff time involved in administering the preparation, treatment relating to potential adverse events, the assessment of cases of diarrhoea (stool collection and culture/toxin assay, endoscopy) and diarrhoea management costs (laundry, antibiotics, increased hospital stay and comorbidities). Unit costs (cost year 2011) were derived from published information[44–46] and through discussions with relevant clinical and finance department staff. Missing data were addressed using the imputation-based method for QoL data and censored data relating to costs using the weighted cost method with known cost histories.[47] In view of the short timescale of the project, there was no discounting of the costs or benefits. Costs and benefits would have been discounted at the conventional rate of 3.5% if the time scale of the follow-up had exceeded 1 year.

Cost differences between the two arms of the study were determined and used in conjunction with differences in outcomes in undertaking a cost–consequences analysis, with cost per case of AAD averted as the primary outcome measure. We planned to undertake subgroup analyses to determine the relative cost-effectiveness of preventative strategies in different risk groups as indicated in the covariate analysis. In addition, a cost–utility analysis considered the differences in costs between the two study arms and differences in quality-adjusted life-years (QALYs) derived from European Quality of Life-5 Dimensions (EQ-5D) responses.

Resource use and costs

Health-care contacts

The number of health-care contacts, duration of initial hospital stay, days spent in care facilities and number and duration of readmissions were recorded routinely within the trial. If the discharge date was not known, the end of follow-up was assumed to be the discharge date. A weighted unit cost of £334.17

was applied for every day a participant spent in hospital (*NHS reference costs 2011*; extrapolated).[44] For the base-case analysis, all other health-care contacts were treated as general practitioner (GP) visits and published care home costs were applied on a daily rate basis.[45]

Antibiotics

Antibiotic use was collected throughout the 8-week follow-up period and costed using published sources.[46] Staff time was assumed to be 5 minutes for the administration of oral antibiotics and 20 minutes for intravenous or intramuscular antibiotics. As 66% of doses during the study period were delivered orally and 34% intravenously, staff cost per dose was weighted to 10.1 minutes per dose and costed at £16.33 per dose.[45] Missing data on antibiotic dose were replaced by the most common value. If no data were available on number of doses per day or duration of antibiotic course, it was assumed that the patient was receiving a full course of the recorded antibiotic.

Intervention implementation

For the cost-effectiveness analysis, it was assumed that every patient would receive a course of 21 oral capsules containing the probiotic formulation at a retail cost of £10 and that capsules that were not taken by the participant would go to waste (as a high proportion of participants finished their course at home after hospital discharge). While the patient was in hospital, staff time of 5 minutes was allocated for administration of each dose. The number of days in hospital (and thus number of nurse contacts) was calculated individually for each patient according to his or her intervention start and hospital discharge dates. Nursing time was allocated even if the patient declined the intervention, as time would have been used for patient interaction and assessment of the situation. No staff time was allocated after the patient withdrew or died. Nursing time was costed at £8.08 per 5 minutes.[45] The cost of placebo formulation and staff cost for administration in the control group were excluded from the cost-effectiveness analysis as 'routine use' was considered.

Episodes of diarrhoea

Costs regarding health-care resource use, antibiotics and increase in length of hospital stay associated with diarrhoea were collected routinely during the trial. Other costs, such as diagnostics, clinical review, cleaning, laundry and isolation, were sourced from outside the trial setting. Resource use and costs of laboratory tests for *C. difficile* detection were obtained using a microcosting approach based on internal standard operating procedures and discussions with key laboratory staff and purchasing officers. Costs of other diagnostic tests (*Salmonella*, *Shigella*, *Campylobacter*, *E. coli* 0157 and norovirus) were taken from the literature.[48] Published reference costs[44] were used to estimate the costs of diagnostic (endoscopy, abdominal computerised tomography and radiography) and therapeutic (colectomy) procedures, which were then weighted to reflect the probability of the event occurring in a population suffering from diarrhoea based on recent publications.[49,50] Costs of patient assessment, including for review of antibiotic treatment and nutritional requirements, and infection control measures were based on discussions with infection control nurses, clinicians and microbiologists. The medical team in the base case was assumed to consist of the treating clinician (costed as registrar), a gastroenterologist, a microbiologist, an infection control nurse, a ward nurse and a pharmacist. Staff time was estimated to be 45 minutes for the registrar and 15 minutes for the other professionals and unit costs were obtained from the literature.[45] Cleaning after patient discharge from the hospital or relocation after a diarrhoea episode was costed based on discussions with key members of infection control staff and includes domestic staff time, specialist cleaning agents (hypochlorite; TUFFIE 5®, Vernacare UK, Bolton, UK) and special cleaning equipment as well as laundry, steam cleaning and use of an autoclave. Costs were obtained from hospital human resource and purchasing departments, wholesalers, published literature[51] and microcosting. All costs were allocated once per patient diarrhoea episode.

Daily costs of diarrhoeal disease included daily cleaning and bed and ward closures. Costing of daily cleaning included domestic staff time, specialist cleaning agents and special cleaning equipment. Costs were obtained from human resources and purchasing departments as well as wholesalers. A lost bed-day

due to closures was costed at £334.17 by published reference costs[44] and weighted for specialties (e.g. whether or not the bed is in intensive care) and activity.[44] Data on frequency of ward closures due to CDD and number of positive cases identified per year were obtained from discussions with key staff and Public Health Wales reports.[52] It was assumed that 1 in 115 cases resulted in an outbreak and subsequent ward closure. Based on an average ward size of 28 beds in Singleton and Morriston hospitals, ward closures could cost up to £9356.76 per day and occur in 0.87% of positive cases. Thus, a weighted cost of £81.40 was applied to each diarrhoea case per day to account for potential ward closures.

The cost per stool included disposables such as gloves and aprons, laundry and staff time for patient check-ups, spot cleaning and changing of beds. Costing of spot cleaning included nursing time, specialist cleaning agents and special cleaning equipment. Data on number of stools per day, duration of diarrhoea episode in days and whether or not the episode was managed in hospital were collected routinely as part of the trial. These data were used to calculate the cost of a diarrhoea episode individually for each patient by applying the one-off episode costs (microbiology, review, procedures, end cleaning) and adding daily and per stool costs according to duration and stool frequency. No cost was applied to participants whose diarrhoea was managed entirely at home. Episodes managed in care homes were treated as hospital episodes.

Cost-effectiveness analyses

Patient responses from EQ-5D questionnaires at baseline and after 4 and 8 weeks were translated into utility scores using a scoring algorithm. We planned to use health and cost outcomes to calculate the cost of probiotics per QALY gained in a cost–utility analysis, to obtain the cost per case of diarrhoea averted in a cost-effectiveness analysis and to present and compare outcomes in tabular form in a cost–consequences analysis. The results of cost-effectiveness analyses were expressed as incremental cost-effectiveness ratios (ICERs) and calculated by dividing the cost difference between the two alternatives being compared by the difference in the effect/benefit. In cost–utility analysis, the effect was expressed in QALYs. The cost per QALY was calculated by dividing the difference in costs by the difference in QALYs for each comparison.

Generally, the National Institute for Health and Care Excellence considers an intervention cost-effective if one of the following applies.[53]

- The intervention is less costly and more clinically effective than all other relevant alternatives. In this case, no ICER is calculated as the strategy in question dominates the alternatives.
- The intervention has an ICER of < £20,000 per QALY compared with the next best alternative. This means that an investment of up to £20,000 in order to achieve an additional QALY is considered cost-effective.

Sensitivity analysis

Sensitivity analyses investigated the robustness of the results to changes in estimated costs and outcomes and probabilistic sensitivity analysis used bootstrap resampling to determine the probability that preventative strategies were within certain thresholds. We planned to assess the budgetary impact from a NHS perspective of adopting a policy of administering the bacterial preparation to prevent or ameliorate AAD and CDD in the target population.

During univariate sensitivity analysis, all ICERs were recalculated after changing the value of a range of parameters individually to estimate the robustness of the ICER (*Table 1*). Prolonged inpatient stay is the main cost driver when considering the cost of diarrhoea.[13,15,16] Other potentially important cost differences between the probiotic and placebo arms included staff time, which is naturally subject to variation, and diarrhoea-associated costs such as cleaning, laundry, microbiology, assessment, diagnostic and therapeutic procedures. As these costs were thought to make up a large proportion of the total health-care costs, the impact of changes to parameters contained within these costs was evaluated.

TABLE 1 Parameter changes for univariate sensitivity analysis

Parameter	Minimum	Maximum	Change from base case
Costing of diarrhoea (£)			
Microbiology ABMUHB	30.17	109.88	50% discount on consumables; two samples tested per patient
Microbiology CDDFT	27.51	90.56	50% discount on consumables; two samples tested per patient
Ward and bed closures	0.00	497.61	No cost effect; double the amount of wards closed per year
Clinical assessment and review	103.01	259.67	Reduced staff time, no gastroenterologist; increased staff time
Cleaning, laundry and disposables	57.15	85.73	± 20%
Other costs (£)			
Cost of one hospital inpatient day	267.34	401.00	± 20%
Other parameters			
Staff time/dose probiotics (minutes)	2.5	10	Half; double

Probabilistic sensitivity analysis with changes to the values of all chosen parameters (usually within the 95% CI or a reasonable, defined range) calculated the probability that the intervention was cost-effective when all uncertainty associated with the individual parameters was considered. Results of the probabilistic sensitivity analysis were expressed as per cent probability that the intervention was cost-effective. Cost-effectiveness acceptability curves (CEACs) were generated to depict the probability of the intervention being cost-effective at different willingness-to-pay thresholds.

Chapter 3 Protocol changes

Inclusion and exclusion criteria

In practice, on assessment for eligibility, some patients were severely ill and not expected to survive for the intended period of follow-up; therefore, they were not approached regarding joining the study. Similarly, participants who were nil by mouth at initial assessment were not approached.

Follow-up

We had intended that diarrhoea outcomes would be assessed during antibiotic treatment and for 8 weeks after stopping antibiotics. However, prolonged follow-up for participants on long courses of antibiotics was not feasible. In practice, daily follow-up during hospital stay or weekly follow-up after discharge from hospital was continued to 8 weeks after recruitment. Review of laboratory data regarding stool assays was continued until 12 weeks after recruitment.

Assessment of quality of life

We considered modifying tools validated to measure QoL in treatment-induced diarrhoea in people with HIV[54] and older patients with faecal incontinence.[55] However, we considered that completion of additional questionnaires would be too onerous for elderly inpatients and these were not pursued.

Chapter 4 Results

A total of 17,420 in-patients aged ≥ 65 years and who had been exposed to one or more antibiotics were assessed for eligibility (*Figure 1*). Exclusion criteria were present in 3202 (18.4%) patients, 9068 (52.1%) declined to participate, 2130 (12.2%) were too unwell to join the study and 39 (0.2%) were nil by mouth. We recruited 2981 (17.1%) patients, at randomisation 1493 (50.1%) were allocated to the probiotic and 1488 (49.9%) to the placebo arm.

In total, 2941 (98.7%) were included in the analysis according to treatment allocated; 23 in the probiotic arm and 17 in the placebo arm were excluded. The identity of the IMP was unknown in seven participants (six allocated to the probiotic and one to the placebo arm) due to an error in IMP labelling at one hospital site. No outcome data were available in 23 patients who were lost to follow-up. In each arm of the study, these included six patients who declined further participation shortly after randomisation without giving a reason and contact was lost with four patients from each arm. Exclusion criteria were present at recruitment in three patients and the details of antibiotic treatment could not be determined in one patient in the probiotic arm. Six participants were recruited to the study for a second time and all were allocated to the placebo arm. Possible carry-over effects from their first involvement in the study could not be excluded and; therefore, data from their second involvement were excluded.

Participant characteristics according to intervention arm

Consent to participate in the trial was provided directly by 1398 patients (95.1%) in the probiotic arm and 1392 patients (94.6%) in the placebo arm. For patients unable to give consent themselves, assent was usually provided by a family member: daughter [in 24 cases (1.6%) in the probiotic arm and 34 cases (2.3%) in the placebo arm], wife [in 19 cases (1.3%) in the probiotic arm and 13 cases (0.9%) in the placebo arm], or son [in 15 cases (1.0%) in the probiotic arm and 18 cases (1.2%) in the placebo arm].

Baseline demographic and patient characteristics were similar in the two intervention arms except for a greater proportion of males than females in the probiotic arm and vice versa in the placebo arm (*Table 2*). The frailty of the study population is reflected in the median age of 77.1 years and common occurrence of comorbid illnesses: 54.6% of participants suffered from hypertension, 24.1% from chronic obstructive pulmonary disease (COPD) and 22.9% from diabetes. Participant age ranged from 65.0 to 107.5 years in the probiotic arm and from 65.0 to 104.4 years in the placebo arm. More participants were recruited during the winter than in the summer months. The majority of patients were admitted to hospital from home and approximately one-third had been admitted to hospital within the previous 8 weeks. Very few people of non-white ethnic origin were recruited. Cigarette smoking was uncommon, but approximately one in three participants drank alcohol. Recent consumption of foods containing live bacteria was uncommon among all participants and occurred with a similar frequency in both study arms.

Participant characteristics according to centre

Overall, 1873 (63.7%) inpatients were recruited in hospitals in ABMUHB (Singleton, Morriston and Princess of Wales) and 1068 (36.3%) in CDDFT (Durham and Darlington). In ABMUHB, recruitment began with a pilot study of 50 patients in Morriston Hospital on 1 December 2008 to evaluate the recruitment methods and data collection forms. Methods were found to be reliable and these patients were included in the final analysis. Recruitment continued until 28 February 2012 and a total of 1479 patients (50.3% of total) were recruited (see *Table 2*). Recruitment in Singleton Hospital began on 9 June 2009 but was terminated on 9 February 2011, after 203 (6.9%) patients had been recruited, because of falling numbers of eligible patients due to service reconfiguration. To maintain patient numbers, recruitment was undertaken at Princess of Wales Hospital from 5 May 2011 to 10 January 2012 and 191 (6.5%) patients were recruited. The start of recruitment was delayed in CDDFT for operational reasons. Darlington Memorial Hospital

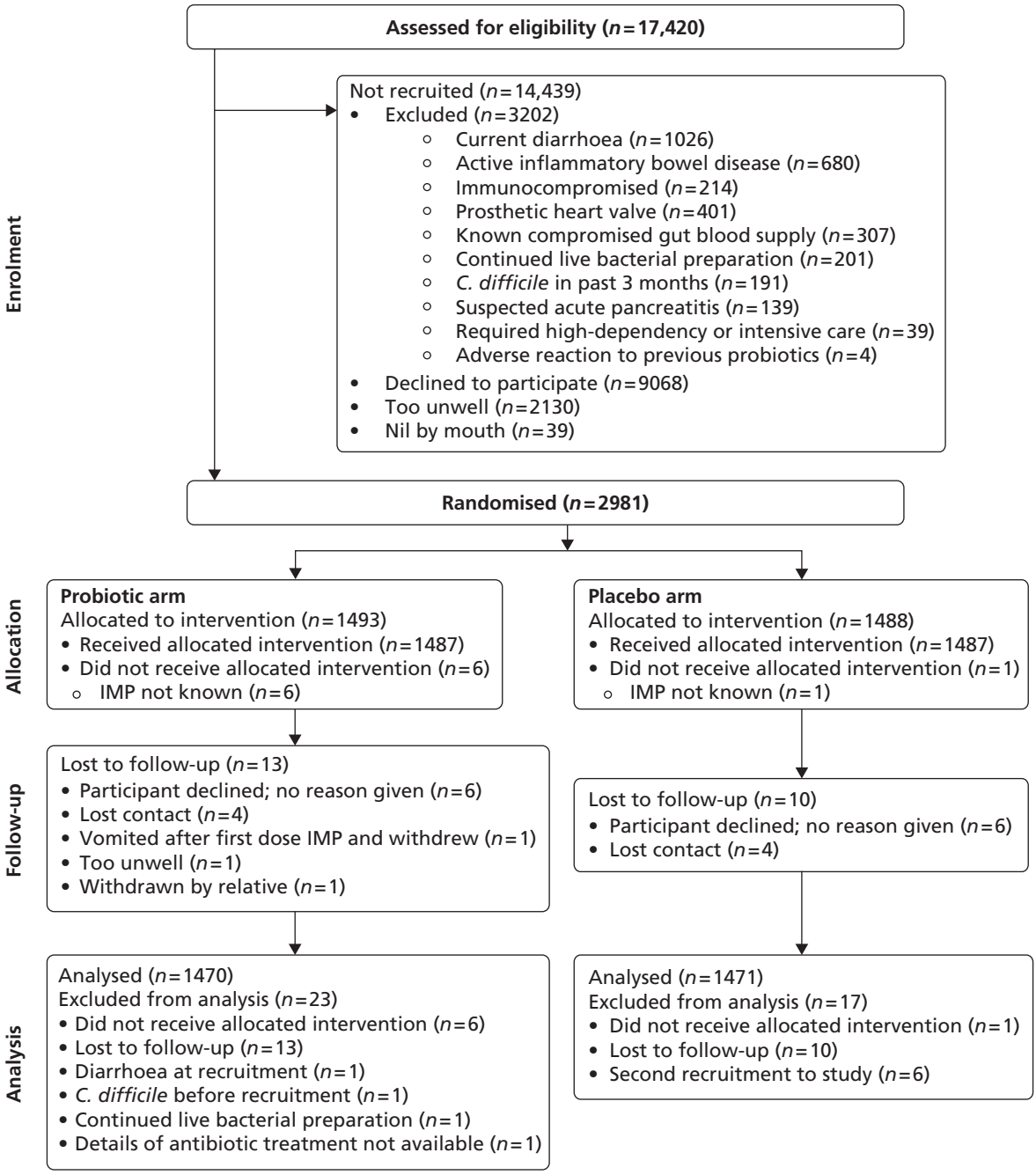

FIGURE 1 Trial profile.

recruited 521 (17.7%) patients from 12 October 2009 to 27 February 2012 and University Hospital of North Durham recruited 547 (18.6%) patients from 17 November 2011 to 28 February 2012 (see *Table 2*).

Baseline participant characteristics were generally similar across the hospital sites (*Table 3*) with some exceptions. The greater proportion of males in the probiotic arm and females in the placebo arm occurred in all centres except for Singleton Hospital, where there were more females than males in the probiotic arm (data not shown). Participants recruited at Singleton Hospital were more likely to be female and tended to be older than participants from other hospitals. The period during which recruitment in each centre occurred was reflected in the lower proportion of patient recruitment during the winter months in Princess of Wales than in other hospitals. The frequency of COPD and hospital admission in the previous 8 weeks were both more common in hospitals in CDDFT than in ABMUHB.

TABLE 2 Demographic variables and participant characteristics according to intervention arm

Characteristic	Probiotic[a] (*n* = 1470)	Placebo[a] (*n* = 1471)	Total (*n* = 2941)
Median age in years (IQR)	77.2 (70.8–83.6)	77.0 (71.3–83.5)	77.1 (71.0–83.5)
Male, *n/N* (%)	777/1470 (52.9)	679/1471 (46.2)	1456/2941 (49.5)
Ethnicity, *n/N* (%) white	1459/1461 (99.9)	1461/1464 (99.8)	2920/2925 (99.8)
Recruited during winter (October–March), *n/N* (%)	845/1470 (57.5)	845/1471 (57.4)	1690/2941 (57.5)
Where admitted from	**n = *1469***	**n = *1468***	**n = *2937***
• Home (%)	1345 (91.6)	1334 (90.9)	2679 (91.2)
• Residential care (%)	58 (3.9)	67 (4.6)	125 (4.3)
• Other hospital (%)	37 (2.5)	39 (2.7)	76 (2.6)
• Other (%)	29 (2.0)	28 (1.9)	57 (1.9)
Hospital			
• Singleton (%)	102 (6.9)	101 (6.9)	203 (6.9)
• Morriston (%)	742 (50.5)	737 (50.1)	1479 (50.3)
• Princess of Wales (%)	94 (6.4)	97 (6.6)	191 (6.5)
• Durham (%)	269 (18.3)	278 (18.9)	547 (18.6)
• Darlington (%)	263 (17.9)	258 (17.5)	521 (17.7)
Cigarette smoker, *n/N* (%)	140/1470 (9.5)	120/1471 (8.2)	260/2941 (8.8)
Drinks alcohol, *n/N* (%)	459/1470 (31.2)	482/1471 (32.8)	941/2941 (32.0)
Comorbid illnesses			
• Hypertension (%)	779/1455 (53.5)	812/1457 (55.7)	1591/2912 (54.6)
• COPD (%)	350/1459 (24.0)	354/1462 (24.2)	704/2921 (24.1)
• Diabetes (%)	357/1465 (24.4)	314/1468 (21.4)	671/2933 (22.9)
• Asthma (%)	237/1462 (16.2)	232/1465 (15.8)	469/2927 (16.0)
• Renal disease (%)	127/1455 (8.7)	139/1461 (9.5)	266/2916 (9.1)
• Dementia or Alzheimer's (%)	61/1449 (4.2)	80/1459 (5.5)	141/2908 (4.8)
• Irritable bowel syndrome (%)	28/1465 (1.9)	26/1465 (1.8)	54/2930 (1.8)
• Other (%)	978/1452 (67.4)	1010/1458 (69.3)	1988/2910 (68.3)
Previous gastrointestinal surgery, *n/N* (%)	203/1448 (14.0)	212/1449 (14.6)	415/2897 (14.3)
NGT in situ, *n/N* (%)	5/1469 (0.3)	2/1464 (0.1)	7/2933 (0.2)
Hospital admission in last 8 weeks, *n/N* (%)	488/1470 (33.2)	448/1471 (30.5)	936/2941 (31.8)
Median number of hospital admissions in last 8 weeks (IQR)	1465 0.0 (0.0–10)	1467 0.0 (0.0–1.0)	2932 0.0 (0.0–1.0)
Live bacteria consumed in last 7 days, *n/N* (%)	72/1470 (4.9)	45/1471 (3.1)	117/2941 (4.0)

a Denominator varies according to availability of information.

TABLE 3 Demographic variables and participants characteristics according to centre

	ABMUHB[a]			CDDFT[a]	
Variable	Singleton	Morriston	Princess of Wales	Durham	Darlington
Number participants recruited	*n* = 203	*n* = 1479	*n* = 191	*n* = 547	*n* = 521
Median age in years (IQR)	79.9 (74.1–86.3)	76.8 (70.6–83.4)	76.0 (70.4–82.7)	77.7 (71.3–84.2)	76.4 (70.8–82.1)
Male, *n* (%)	85 (41.9)	755 (51.0)	93 (48.7)	271 (49.5)	252 (48.4)
Ethnicity, *n/N* (%) white	203/203 (100.0)	1467/1469 (99.9)	188/188 (100.0)	543/544 (99.8)	519/521 (99.6)
Recruited during winter (October–March) *n/N* (%)	130/203 (64.0)	819/1479 (55.4)	66/191 (34.6)	368/547 (67.3)	307/521 (58.9)
Where admitted from					
• Home (%)	184 (90.6)	1312 (88.8)	184 (96.3)	506 (93.0)	493 (94.6)
• Residential care (%)	14 (6.9)	61 (4.1)	3 (1.6)	28 (5.1)	19 (3.6)
• Other hospital (%)	2 (1.0)	64 (4.3)	1 (0.5)	4 (0.7)	5 (1.0)
• Other (%)	3 (1.5)	41 (2.8)	3 (1.6)	6 (1.1)	4 (0.8)
Cigarette smoker, *n/N* (%)	16/203 (7.9)	122/1479 (8.2)	18/191 (9.4)	66/547 (12.1)	38/521 (7.3)
Drinks alcohol, *n/N* (%)	66/203 (32.5)	487/1479 (32.9)	43/191 (22.5)	160/547 (29.3)	185/521 (35.5)
Comorbid illnesses					
• Hypertension, *n/N* (%)	88/198 (44.4)	827/1467 (56.4)	106/186 (57.0)	261/545 (47.9)	309/516 (59.9)
• COPD, *n/N* (%)	54/201 (26.9)	216/1468 (14.7)	53/189 (28.0)	216/544 (39.7)	165/519 (31.8)
• Diabetes, *n/N* (%)	48/202 (23.8)	336/1477 (22.7)	40/191 (20.9)	138/543 (25.4)	109/520 (21.0)
• Asthma, *n/N* (%)	43/202 (21.3)	194/1473 (13.2)	26/191 (13.6)	96/542 (17.7)	110/519 (21.2)
• Renal disease, *n/N* (%)	20/202 (9.9)	99/1467 (6.7)	13/188 (6.9)	68/540 (12.6)	66/519 (12.7)
• Dementia or Alzheimer's disease, *n/N* (%)	17/202 (8.4)	73/1458 (5.0)	3/191 (1.6)	32/539 (5.9)	16/518 (3.1)
• Irritable bowel syndrome, *n/N* (%)	3/202 (1.5)	22/1475 (1.5)	5/191 (2.6)	13/542 (2.4)	11/520 (2.1)
• Other, *n/N* (%)	91/199 (45.7)	929/1470 (63.2)	132/187 (70.6)	420/542 (77.5)	416/512 (81.3)
Previous gastrointestinal surgery, *n/N* (%)	21/194 (10.8)	201/1460 (13.8)	18/188 (9.6)	81/537 (15.1)	94/518 (18.1)
NGT in situ, *n/N* (%)	1/203 (0.5)	1/1477 (0.1)	1/191 (0.5)	2/541 (0.4)	2/521 (0.4)
Hospital admission in last 8 weeks, *n/N* (%)	50/203 (24.6)	311/1479 (21.0)	30/191 (15.7)	304/547 (55.6)	241/521 (46.3)
Number of hospital admissions in last 8 weeks, *n*, median (IQR)	201, 0.0 (0.0–0.0)	1473, 0.0 (0.0–0.0)	191, 0.0 (0.0–0.0)	546, 1.0 (0.0–1.0)	521, 0.0 (0.0–1.0)
Live bacteria consumed in last 7 days, *n/N* (%)	7/203 (3.4)	65/1479 (4.4)	6/191 (3.1)	12/547 (2.2)	27/521 (5.2)

a Denominator varies according to availability of information.

Indications for initial antibiotic treatment

Indications for antibiotic treatment classified according to the MedDRA SOC[43] were similar in the two study arms (*Table 4*). The most common indication was 'respiratory, thoracic and mediastinal disorders'. Antibiotic treatment for suspected sepsis where the site was unclear was given to a small proportion of patients. About one in four patients in each arm of the study received antibiotics for prophylaxis rather than the treatment of infection and nearly all of these were for surgical and medical procedures.

In keeping with differences in the frequency of COPD according to centre, a greater proportion of the patients in hospitals in CDDFT than ABMUHB were treated for 'respiratory, thoracic and mediastinal disorders' (*Table 5*).

Antibiotic exposure

All of the participants were receiving one or more antibiotics when they started the IMPs. The date the participant began taking the antibiotics before recruitment was known in 1448 participants in the probiotic and 1443 in the placebo arm. The median (IQR) period of exposure to antibiotics before starting the IMP was 3.0 days (2.0–6.0 days) in both study arms ($p = 0.38$).

During the period 7 days before, and 8 weeks following, recruitment, the most commonly used antibiotic class was the penicillins, with over half of all participants receiving a broad-spectrum penicillin.

TABLE 4 Indications for initial antibiotic treatment according to MedDRA SOC[43] and intervention arm

Indication for initial antibiotic treatment	Probiotic ($n = 1470$) (%)	Placebo ($n = 1471$) (%)	Total ($n = 2941$) (%)
• Blood and lymphatic system disorders	2 (0.1)	0 (0.0)	2 (0.1)
• Cardiovascular disorders	5 (0.3)	1 (0.1)	6 (0.2)
• Ear and labyrinth disorders	4 (0.3)	1 (0.1)	5 (0.2)
• Eye disorders	0 (0.0)	1 (0.1)	1 (0.0)
• Gastrointestinal disorders	14 (1.0)	8 (0.5)	22 (0.7)
• Hepatobiliary disorders	32 (2.2)	23 (1.6)	55 (1.9)
• Infections and infestations	2 (0.1)	3 (0.2)	5 (0.2)
• Injury, poisoning and procedural complications[a]	67 (4.6)	56 (3.8)	123 (4.2)
• Musculoskeletal and connective tissue disorders[a]	18 (1.2)	17 (1.2)	35 (1.2)
• Nervous system disorders	3 (0.2)	3 (0.2)	6 (0.2)
• Renal and urinary disorders[a]	265 (18.0)	278 (18.9)	543 (18.5)
• Reproductive system and breast disorders	2 (0.1)	3 (0.2)	5 (0.2)
• Respiratory, thoracic and mediastinal disorders[a]	501 (34.1)	525 (35.7)	1026 (34.9)
• Skin and subcutaneous tissue disorders	166 (11.3)	147 (10.0)	313 (10.6)
• Surgical and medical procedures[a]	338 (23.0)	363 (24.7)	701 (23.8)
• Vascular disorder	2 (0.1)	3 (0.2)	5 (0.2)
Suspected sepsis but site unclear	49 (3.3)	39 (2.7)	88 (3.0)

a The indication for antibiotics in some patients in these categories was prophylaxis rather than treatment [in total, 345 (23.6%) in the probiotic and 370 (25.1%) in the placebo arm]. With the exception of seven patients in each arm, prophylaxis was given for surgical and medical procedures.

TABLE 5 Indications for initial antibiotic treatment according to MedDRA SOC and centre

	ABMUHB			CDDFT	
Indication for initial antibiotic treatment	Singleton (*n* = 203) (%)	Morriston (*n* = 1479) (%)	Princess of Wales (*n* = 191) (%)	Durham (*n* = 547) (%)	Darlington (*n* = 521) (%)
Blood and lymphatic system disorders	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.2)
Cardiovascular disorders	0 (0.0)	3 (0.2)	1 (0.5)	0 (0.0)	2 (0.4)
Ear and labyrinth disorders	0 (0.0)	1 (0.1)	1 (0.5)	2 (0.4)	1 (0.2)
Eye disorders	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)
Gastrointestinal disorders	0 (0.0)	17 (1.1)	1 (0.5)	2 (0.4)	2 (0.4)
Hepatobiliary disorders	7 (3.4)	24 (1.6)	5 (2.6)	13 (2.4)	6 (1.2)
Infections and infestations	1 (0.5)	2 (0.1)	1 (0.5)	0 (0.0)	1 (0.2)
Injury, poisoning and procedural complications	2 (1.0)	100 (6.8)	6 (3.1)	5 (0.9)	10 (1.9)
Musculoskeletal and connective tissue disorders	0 (0.0)	18 (1.2)	2 (1.0)	5 (0.9)	10 (1.9)
Nervous system disorders	0 (0.0)	4 (0.3)	0 (0.0)	1 (0.2)	1 (0.2)
Renal and urinary disorders	56 (27.6)	284 (19.2)	37 (19.4)	105 (19.2)	61 (11.7)
Reproductive system and breast disorders	1 (0.5)	0 (0.0)	1 (0.5)	1 (0.2)	2 (0.4)
Respiratory, thoracic and mediastinal disorders	90 (44.3)	331 (22.4)	64 (33.5)	313 (57.2)	228 (43.8)
Skin and subcutaneous tissue disorders	25 (12.3)	162 (11.0)	21 (11.0)	52 (9.5)	53 (10.2)
Surgical and medical procedures	9 (4.4)	488 (33.0)	49 (25.7)	28 (5.1)	127 (24.4)
Vascular disorder	0 (0.0)	3 (0.2)	1 (0.5)	0 (0.0)	1 (0.2)
Suspected sepsis but site unclear	12 (5.9)	42 (2.8)	1 (0.5)	18 (3.3)	15 (2.9)

About one in four participants were exposed to a cephalosporin. Antibiotic exposure was similar in the two study arms (*Table 6*).

Antibiotic exposure varied according to centre (see *Appendix 9*, *Table 31*). In hospitals in CDDFT, exposure to broad-spectrum penicillins was greater than in hospitals in ABMUHB (67.8–70.6% vs. 47.3–57.1%, respectively), but exposure to cephalosporins was lower (1.9–3.3% vs. 13.6–29.1%, respectively), as was exposure to quinolones (6.9–7.5% vs. 8.4–21.2%, respectively).

Fewer than 1 in 10 participants received only a single dose of an antibiotic and most received antibiotics for ≥ 7, with one-third treated for at least 14 days (*Tables 7* and *8*). The majority of participants were exposed to antibiotics from two or more classes. Exposure to combination therapy and duration of antibiotic therapy was similar in the two study arms.

TABLE 6 Antibiotic exposure according to intervention arm. The number and percentage of participants who received therapy with the antibiotic during the period 7 days before recruitment to the end of follow-up at 8 weeks

Antibiotic (classes and individual drugs)	Probiotic (*n* = 1470) (%)	Placebo (*n* = 1471) (%)	Total (*n* = 2941) (%)
Penicillins	***1052 (71.6)***	***1061 (72.1)***	***2113 (71.8)***
Benzylpenicillin	115 (7.8)	99 (6.7)	214 (7.3)
Penicillinase-resistant penicillin – flucloxacillin	322 (21.9)	310 (21.1)	632 (21.5)
Broad-spectrum penicillins	822 (55.9)	829 (56.4)	1651 (56.1)
• Amoxicillin	310 (21.1)	323 (22.0)	633 (21.5)
• Ampicillin	2 (0.1)	1 (0.1)	3 (0.1)
• Co-amoxiclav	612 (41.6)	623 (42.4)	1235 (42.1)
Anti-pseudomonas penicillins	127 (8.6)	118 (8.0)	245 (8.3)
• Piperacillin	3 (0.2)	0 (0.0)	3 (0.1)
• Piperacillin + tazobactam	125 (8.5)	118 (8.0)	243 (8.3)
Cephalosporins	***359 (24.4)***	***356 (24.2)***	***715 (24.3)***
First generation	77 (5.2)	74 (5.0)	151 (5.1)
• Cefalexin	77 (5.2)	73 (5.0)	150 (5.1)
• Cefradine	0 (0.0)	1 (0.1)	1 (0.0)
Second generation	290 (19.7)	304 (20.7)	594 (20.2)
• Cefuroxime	27 (1.8)	24 (1.6)	51 (1.7)
• Cefaclor	1 (0.1)	0 (0.0)	1 (0.0)
• Cefixime	284 (19.3)	293 (19.9)	577 (19.6)
Third generation	11 (0.7)	10 (0.7)	21 (0.7)
• Cefotaxime	1 (0.1)	2 (0.1)	3 (0.1)
• Ceftazidime	7 (0.5)	7 (0.5)	14 (0.5)
• Ceftriaxone	3 (0.2)	2 (0.1)	5 (0.2)
Other antibiotics			
Carbapenems and other β-lactams	33 (2.2)	29 (2.0)	62 (2.1)
• Ertapenem	0 (0.0)	1 (0.1)	1 (0.0)
• Imipenem	2 (0.1)	3 (0.2)	5 (0.2)
• Meropenem	31 (2.1)	26 (1.8)	57 (1.9)
Tetracyclines	211 (14.4)	222 (15.1)	433 (14.7)
• Demeclocycline	0 (0.0)	2 (0.1)	2 (0.1)
• Doxycyline	199 (13.5)	213 (14.5)	412 (14.0)
• Lymecycline	0 (0.0)	1 (0.1)	1 (0.0)
• Oxytetracycline	10 (0.7)	4 (0.3)	14 (0.5)
• Tetracycline	2 (0.1)	4 (0.3)	6 (0.2)
Aminoglycosides	182 (12.4)	196 (13.3)	378 (12.9)
• Gentamicin	182 (12.4)	195 (13.3)	377 (12.8)
• Tobramycin	0 (0.0)	1 (0.1)	1 (0.0)

continued

TABLE 6 Antibiotic exposure according to intervention arm. The number and percentage of participants who received therapy with the antibiotic during the period 7 days before recruitment to the end of follow-up at 8 weeks (*continued*)

Antibiotic (classes and individual drugs)	Probiotic (n = 1470) (%)	Placebo (n = 1471) (%)	Total (n = 2941) (%)
Macrolides	249 (16.9)	251 (17.1)	500 (17.0)
• Azithromycin	13 (0.9)	11 (0.7)	24 (0.8)
• Clarithromycin	203 (13.8)	210 (14.3)	413 (14.0)
• Erythromycin	43 (2.9)	41 (2.8)	84 (2.9)
Clindamycin	18 (1.2)	14 (1.0)	32 (1.1)
Sulphonamides and trimethoprim	228 (15.5)	242 (16.5)	470 (16.0)
• Cotrimoxazole	0 (0.0)	6 (0.4)	6 (0.2)
• Trimethoprim	228 (15.5)	236 (16.0)	464 (15.8)
Metronidazole	171 (11.6)	142 (9.7)	313 (10.6)
Quinolones	185 (12.6)	180 (12.2)	365 (12.4)
• Ciprofloxacin	171 (11.6)	157 (10.7)	328 (11.2)
• Levofloxacin	14 (1.0)	21 (1.4)	35 (1.2)
• Moxifloxacin	2 (0.1)	5 (0.3)	7 (0.2)
• Norfloxacin	0 (0.0)	1 (0.1)	1 (0.0)
Glycopeptides	103 (7.0)	75 (5.1)	178 (6.1)
• Teicoplanin	82 (5.6)	61 (4.1)	143 (4.9)
• Vancomycin	27 (1.8)	20 (1.4)	47 (1.6)
Anti-tuberculous antibiotics	26 (1.8)	20 (1.4)	46 (1.6)
• Ethambutol	1 (0.1)	2 (0.1)	3 (0.1)
• Rifampicin	26 (1.8)	20 (1.4)	46 (1.6)
• Streptomycin	1 (0.1)	0 (0.0)	1 (0.0)
Others	38 (2.6)	53 (3.6)	91 (3.1)
• Daptomycin	1 (0.1)	0 (0.0)	1 (0.0)
• Linezolid	3 (0.2)	0 (0.0)	3 (0.1)
• Nitrofurantoin	26 (1.8)	45 (3.1)	71 (2.4)
• Sodium fusidate	9 (0.6)	8 (0.5)	17 (0.6)

Non-antibiotic drug treatment

Use of drugs other than antibiotics was common, with many participants receiving antihypertensive therapy, aspirin, PPIs or angiotensin-converting enzyme (ACE) inhibitor therapy. Non-antibiotic drug treatment was similar in the two study arms (*Table 9*).

Primary outcomes

Antibiotic-associated diarrhoea (including CDD) occurred with a similar frequency in the probiotic arm (159 participants, 10.8%) and placebo arm (153 participants, 10.4%; RR 1.04; 95% CI 0.84 to 1.28; $p = 0.71$; *Table 10*). This included 12 participants with frequent stools that they described as looser than normal but who were unable to describe stool consistency using the Bristol Stool Form Scale.[38]

TABLE 7 Combination of antibiotic therapies according to intervention arm

Combination antibiotic therapy[a]	Probiotic (*n* = 1470)	Placebo (*n* = 1471)	Total (*n* = 2941)
Number (%) participants who received an antibiotic from			
One class only	310 (21.1)	310 (21.1)	620 (21.1)
Two classes	407 (27.7)	397 (27.0)	804 (27.3)
Three or more classes	753 (51.2)	764 (51.9)	1517 (51.6)

a Antibiotics were classified as follows: penicillins, cephalosporins, carbapenems and other β-lactams, tetracyclines, aminoglycosides, macrolides, clindamycin, sulphonamides and trimethoprim, metronidazole, quinolones, glycopeptides, antituberculous antibiotics and others.

TABLE 8 Duration of antibiotic therapy according to intervention arm

Duration of antibiotic therapy	Probiotic[a] (*n* = 1406)	Placebo[a] (*n* = 1398)	Total (*n* = 2804)
Number (%) participants who received			
Single dose	133 (9.5)	123 (8.8)	256 (9.1)
1–6 days' treatment	389 (27.7)	398 (28.5)	787 (28.1)
7–13 days' treatment	402 (28.6)	426 (30.5)	828 (29.5)
≥ 14 days' treatment	482 (34.3)	451 (32.3)	933 (33.3)

a This is not the full sample size owing to availability of information.

TABLE 9 Non-antibiotic drug treatment according to intervention arm

Drugs	Probiotic,[a] *n/N* (%)	Placebo,[a] *n/N* (%)	Total, *n/N* (%)
Antacid therapies			
PPI	582/1459 (39.9)	567/1460 (38.8)	1149/2919 (39.4)
H_2 blocker	96/1449 (6.6)	74/1454 (5.1)	170/2903 (5.9)
Antacid	30/1457 (2.1)	34/1460 (2.3)	64/2917 (2.2)
Other drugs			
ACE inhibitor	425/1449 (29.3)	436/1453 (30.0)	861/2902 (29.7)
Antihypertensive	679/1450 (46.8)	716/1453 (49.3)	1395/2903 (48.1)
Aspirin	597/1458 (40.9)	589/1458 (40.4)	1186/2916 (40.7)
Oral hypoglycaemic agent	208/1460 (14.2)	188/1460 (12.9)	396/2920 (13.6)
Non-steroidal anti-inflammatory drug	158/1450 (10.9)	135/1454 (9.3)	293/2904 (10.1)
Insulin	96/1459 (6.6)	78/1460 (5.3)	174/2919 (6.0)
Feed containing probiotic	8/1459 (0.5)	9/1457 (0.6)	17/2916 (0.6)

a Denominator varies according to availability of information.

TABLE 10 Primary outcomes according to intervention arm

Outcome	Probiotic *n/N* (%)	Placebo *n/N* (%)	RR (95% CI) [*p*-value]	OR (95% CI) [*p*-value]	Risk difference (95% CI) [*p*-value]
AAD[b]	159/1470 (10.8)	153/1471 (10.4)	1.04 (0.84 to 1.28) [0.72]	1.04 (0.83 to 1.32) [0.72]	0.42 (–1.81 to 2.64) [0.72]
CDD	12/1470 (0.8)	17/1471 (1.2)	0.71 (0.34 to 1.47) [0.35]	0.70 (0.34 to 1.48) [0.35]	–0.34 (–1.05 to 0.37) [0.35]

Clostridium difficile diarrhoea was uncommon and occurred in 12 (0.8%) participants in the probiotic arm and 17 (1.2%) participants in the placebo arm (RR 0.71; 95% CI 0.34 to 1.47; $p = 0.35$; see *Table 10*). Based on this effect size and the low prevalence of CDD, the number needed to treat to prevent one case is 295. This would be reduced to 95 for an effect size at the lower limit of the 95% CI (a threefold reduction in CDD in the probiotic arm). The corresponding number needed to harm (the upper 95% CI) is 267.

Secondary outcomes

Clostridium difficile was isolated from stools in two participants with mild loose stools (not meeting the study criteria for diarrhoea) in the probiotic arm. One participant in each arm had an episode of CDD after an initial episode of AAD that was not associated with CDI; the participant allocated to the placebo arm required surgery for CDD and the participant allocated to the probiotic arm had gallstones and died during the episode of CDD. One patient with known carcinoma of the head of the pancreas with a biliary stent in situ died during an episode of CDD that occurred after withdrawal from the trial.

The adjusted treatment effect on occurrence of AAD from covariate analysis was similar to the unadjusted effect after controlling for nine prespecified covariates. Covariate analysis identified that the occurrence of AAD could be predicted by the duration of antibiotic treatment, antacid therapy and duration of hospital stay (*Table 11*).

The frequency of AAD was similar in each centre: Morriston 162/1479 (11.0%), Singleton 20/203 (9.9%), Princess of Wales 21/191 (11.0%), Durham 56/547 (10.2%) and Darlington 53/521 (10.2%; $p = 0.97$). Subgroup analyses showed that the distribution of cases of AAD according to prespecified potential risk factors for AAD, including those identified as risk factors in covariate analysis, was similar in the two intervention arms and there was no evidence of a statistically significant interaction between prespecified potential risk factors for AAD and intervention arm (*Table 12*).

Most episodes of AAD (73.7%) occurred within 4 weeks of recruitment. On average, episodes of AAD lasted for 2 days with four stools in 24 hours and of consistency seven on the Bristol Stool Form Scale (*Table 13*). The most commonly associated symptoms were urgency, abdominal pain and nocturnal diarrhoea. The latter tended to occur more frequently in the placebo than the probiotic group ($p = 0.051$) and other characteristics of the diarrhoea episodes were similar in the two study arms (see *Table 13*). Most episodes of AAD were managed in hospital and stool samples were collected and tested for diarrhoeal pathogens in 58.6% of all cases. For many episodes of AAD, the short duration and occurrence after discharge from hospital complicated the collection of a stool specimen for testing for pathogens.

As with AAD, the adjusted treatment effect for CDD was similar to the unadjusted estimate (*Table 14*). Covariate analysis showed that duration of antibiotic treatment was associated with CDD.

The frequency of CDD was similar in each centre: Morriston 21/1479 (1.4%), Singleton 2/203 (1.0%), Princess of Wales 0/191 (0.0%), Durham 3/547 (0.5%) and Darlington 3/521 (0.6%; $p = 0.15$).

TABLE 11 Adjusted treatment effect and potential risk factors for AAD: covariate analysis

Variable[a]	Comparison	OR (95% CI)
Intervention arm	Probiotic vs. placebo	1.00 (0.78 to 1.27)
Centre	Singleton vs. Darlington	0.69 (0.38 to 1.25)
	Morriston vs. Darlington	0.84 (0.58 to 1.21)
	Princess of Wales vs. Darlington	1.08 (0.62 to 1.88)
	Durham vs. Darlington	0.93 (0.62 to 1.41)
Age	≤ 77 years vs. > 77 years	1.05 (0.81 to 1.35)
Sex	Male vs. female	1.07 (0.83 to 1.37)
Antibiotic class	Penicillins vs. other	0.93 (0.70 to 1.22)
	Cephalosporins vs. other	1.41 (0.97 to 2.04)
Duration of antibiotic therapy	≤ 8 days vs. > 8 days	0.48 (0.36 to 0.62)
Any antacid therapy	No vs. yes	0.74 (0.58 to 0.95)
Previous gastrointestinal surgery	No vs. yes	0.95 (0.67 to 1.33)
Recent previous hospital admission	No vs. yes	1.05 (0.80 to 1.38)
Duration of hospital stay	< 7 days vs. ≥ 7 days	0.74 (0.55 to 0.99)

a The variable NGT in situ was excluded from the analysis because of a failure of convergence in the logistic regression model.

In subgroup analysis, there was a statistically significant interaction between intervention arm and age ($p = 0.0015$; *Table 15*). In patients aged > 77 years, the frequency of CDD was significantly lower in the probiotic arm than in the placebo arm. In contrast, the frequency of CDD was similar in the two intervention arms for patients aged ≤ 77 years. In addition, the interaction between treatment group and duration of antibiotic treatment was of borderline statistical significance ($p = 0.054$). There was no evidence of a significant interaction between the intervention arm and other prespecified potential risk factors for CDD (*Table 15*).

The timing of onset of CDD was similar to that of AAD, with 75.8% cases occurring within 4 weeks of recruitment. On average, the duration of CDD was 6.5 days and duration was similar in the two study arms (*Table 16*). Bloating was less common in the placebo arm than in the probiotic arm (risk difference 40.7%; 95% CI 7.4% to 74.0%) and median stool frequency tended to be lower in the placebo arm than in the probiotic arm (see *Table 16*). Otherwise, gastrointestinal symptoms, clinical findings and investigations and classification of severity were similar in the two study arms. During follow-up, no patient was identified as having peritonitis, ileus, toxic megacolon or life-threatening CDD or as having died from CDD. The majority of patients in both study arms were managed in hospital.

Seven (0.5%) participants in the probiotic arm and 10 (0.7%) participants in the placebo arm had diarrhoea due to other causes (RR 0.70; 95% CI 0.27 to 1.84). In the probiotic arm, six had norovirus diarrhoea and one was diagnosed with non-specific colitis. In the placebo arm, six had norovirus diarrhoea, one had diarrhoea after taking laxatives, two patients attributed diarrhoea to drinking a large volume of fruit juice and one had melaena associated with abnormal clotting.

Overall, 2927/2940 (99.6%) participants took at least one dose of the IMP, with a similar proportion in the probiotic (1462/1469, 99.5%) and placebo arms (1465/1471, 99.6%; $p = 0.78$; compliance unknown for one participant in the probiotic arm). The median number of days that participants were observed or

TABLE 12 Subgroup analyses of AAD by prespecified risk factors

Category	Probiotic,[a] *n/N* (%)	Placebo,[a] *n/N* (%)	RR (95% CI)	*p*-value for interaction
Centre				
Singleton	11/102 (10.8)	9/101 (8.9)	1.21 (0.52 to 2.79)	0.28
Morriston	81/742 (10.9)	81/737 (11.0)	0.99 (0.74 to 1.33)	
Princess of Wales	15/94 (16.0)	6/97 (6.2)	2.58 (1.05 to 6.37)	
Durham	26/269 (9.7)	30/278 (10.8)	0.90 (0.54 to 1.47)	
Darlington	26/263 (9.9)	27/258 (10.5)	0.94 (0.57 to 1.57)	
Age				
≤ 77 years	86/730 (11.8)	70/732 (9.6)	1.23 (0.91 to 1.66)	0.15
> 77 years	73/740 (9.9)	83/739 (11.2)	0.88 (0.65 to 1.18)	
Sex				
Male	91/777 (11.7)	68/679 (10.0)	1.17 (0.87 to 1.57)	0.25
Female	68/693 (9.8)	85/792 (10.7)	0.91 (0.68 to 1.24)	
Antibiotic class				
Penicillins	78/761 (10.2)	77/777 (9.9)	1.03 (0.77 to 1.39)	0.87
Cephalosporins	31/227 (13.7)	26/220 (11.8)	1.16 (0.71 to 1.88)	
Other	50/482 (10.4)	50/474 (10.5)	0.98 (0.68 to 1.43)	
Duration of antibiotic treatment				
≤ 8 days	48/694 (6.9)	52/709 (7.3)	0.94 (0.65 to 1.38)	0.66
> 8 days	107/712 (15.0)	99/689 (14.4)	1.05 (0.81 to 1.35)	
PPI treatment				
No	86/877 (9.8)	79/893 (8.8)	1.11 (0.83 to 1.48)	0.55
Yes	72/582 (12.4)	72/567 (12.7)	0.97 (0.72 to 1.32)	
Any antacid therapy (including PPI treatment)				
No	77/802 (9.6)	74/834 (8.9)	1.08 (0.80 to 1.47)	0.73
Yes	81/657 (12.3)	77/627 (12.3)	1.00 (0.75 to 1.34)	
NGT in situ				
No	159/1464 (10.9)	153/1462 (10.5)	1.04 (0.84 to 1.28)	–
Yes	0/5 (0.0)	0/2 (0.0)		
Previous gastrointestinal surgery				
No	132/1245 (10.6)	129/1237 (10.4)	1.02 (0.81 to 1.28)	0.71
Yes	25/203 (12.3)	23/212 (10.8)	1.14 (0.67 to 1.93)	
Recent previous hospital admission				
No	107/982 (10.9)	105/1023 (10.3)	1.06 (0.82 to 1.37)	0.85
Yes	52/483 (10.8)	47/444 (10.6)	1.02 (0.70 to 1.48)	
Duration of hospital stay				
< 7 days	39/524 (7.4)	40/498 (8.0)	0.93 (0.61 to 1.42)	0.54
≥ 7 days	117/928 (12.6)	111/949 (11.7)	1.08 (0.85 to 1.37)	

a Denominator varies according to availability of information.

TABLE 13 Severity of AAD and frequency of associated symptoms according to intervention arm

Outcome	Probiotic[a]	Placebo[a]	Total	*p*-value
Duration (days), *n*, median (IQR)	135, 2.0 (1.0–4.0)	125, 3.0 (1.0–6.0)	260, 2.0 (1.0–5.0)	0.11
Number stools per 24 hours, *n*, median (IQR)	158, 4.0 (3.0–5.0)	152, 4.0 (3.0–5.0)	310, 4.0 (3.0–5.0)	0.69
Stool consistency, *n*, median (IQR)	152, 7.0 (6.0–7.0)	145, 7.0 (6.0–7.0)	297, 7.0 (6.0–7.0)	0.85
Nausea, *n/N* (%)	35/154 (22.7)	37/145 (25.5)	72/299 (24.1)	0.57
Vomiting, *n/N* (%)	20/155 (12.9)	16/149 (10.7)	36/304 (11.8)	0.56
Bloating, *n/N* (%)	32/154 (20.8)	31/146 (21.2)	63/300 (21.0)	0.92
Flatus, *n/N* (%)	41/153 (26.8)	45/146 (30.8)	86/299 (28.8)	0.44
Abdominal pain, *n/N* (%)	55/154 (35.7)	65/147 (44.2)	120/301 (39.9)	0.13
Tenesmus, *n/N* (%)	8/154 (5.2)	7/145 (4.8)	15/299 (5.0)	0.88
Fever, *n/N* (%)	6/152 (3.9)	4/143 (2.8)	10/295 (3.4)	0.59
Faecal incontinence, *n/N* (%)	27/151 (17.9)	31/147 (21.1)	58/298 (19.5)	0.48
Nocturnal diarrhoea, *n/N* (%)	44/151 (29.1)	59/148 (39.9)	103/299 (34.4)	0.051
Urgency, *n/N* (%)	78/151 (51.7)	85/146 (58.2)	163/297 (54.9)	0.26
Blood in stool, *n/N* (%)	3/135 (2.2)	3/134 (2.2)	6/269 (2.2)	0.99
Mucus in stool, *n/N* (%)	7/132 (5.3)	12/131 (9.2)	19/263 (7.2)	0.23
Managed in hospital, *n/N* (%)	93/157 (59.2)	75/146 (51.4)	168/303 (55.4)	0.17
Stool sample tested, *n/N* (%)	93/158 (58.9)	88/151 (58.3)	181/309 (58.6)	0.92

a Denominator varies according to availability of information.

TABLE 14 Adjusted treatment effect and potential risk factors for CDD: covariate analysis

Variable[a]	Comparison	OR (95% CI)
Intervention arm	Probiotic vs. placebo	0.65 (0.29 to 1.47)
Centre	Singleton vs. Darlington	0.52 (0.04 to 6.08)
	Morriston vs. Darlington	1.56 (0.34 to 7.20)
	Princess of Wales vs. Darlington	0.00 (0.00 to incalculable)
	Durham vs. Darlington	0.75 (0.10 to 5.46)
Age	≤ 77 vs. > 77 years	0.95 (0.42 to 2.18)
Sex	Male vs. female	1.12 (0.49 to 2.58)
Type of antibiotics	Penicillins vs. other	0.43 (0.15 to 1.21)
	Cephalosporins vs. other	1.80 (0.69 to 4.67)
Duration of antibiotic therapy	≤ 8 days vs. > 8 days	0.13 (0.03 to 0.56)
Any antacid therapy (including PPI treatment)	No vs. yes	0.49 (0.21 to 1.12)
Previous gastrointestinal surgery	No vs. yes	1.41 (0.41 to 4.88)
Recent previous hospital admission	No vs. yes	0.97 (0.40 to 2.34)
Duration of hospital stay	< 7 days vs. ≥ 7 days	0.00 (0.00 to incalculable)

a The variable NGT in situ was excluded from the analysis because of a failure of convergence in the logistic regression model.

TABLE 15 Subgroup analyses of CDD by prespecified risk factors

Category	Probiotic,[a] *n*/*N* (%)	Placebo,[a] *n*/*N* (%)	RR (95% CI)	*p*-value for interaction
Centre				
Singleton	1/102 (1.0)	1/101 (1.0)	0.99 (0.06 to 15.62)	0.91
Morriston	8/742 (1.1)	13/737 (1.8)	0.61 (0.25 to 1.47)	
Princess of Wales	0/94 (0.0)	0/97 (0.0)	–	
Durham	1/269 (0.4)	2/278 (0.7)	0.52 (0.05 to 5.67)	
Darlington	2/263 (0.8)	1/258 (0.4)	1.96 (0.18 to 21.50)	
Age				
≤ 77 years	9/730 (1.2)	3/732 (0.4)	3.01 (0.82 to 11.07)	0.0015
> 77 years	3/740 (0.4)	14/739 (1.9)	0.21 (0.06 to 0.74)	
Sex				
Male	8/777 (1.0)	7/679 (1.0)	1.00 (0.36 to 2.74)	0.31
Female	4/693 (0.6)	10/792 (1.3)	0.46 (0.14 to 1.45)	
Type of antibiotic				
Penicillins	3/761 (0.4)	6/777 (0.8)	0.51 (0.13 to 2.03)	0.86
Cephalosporins	4/227 (1.8)	5/220 (2.3)	0.78 (0.21 to 2.85)	
Other	5/482 (1.0)	6/474 (1.3)	0.82 (0.25 to 2.67)	
Duration of antibiotic treatment				
≤ 8 days	2/694 (0.3)	0/709 (0.0)	Incalculable	0.054
> 8 days	10/712 (1.4)	16/689 (2.3)	0.60 (0.28 to 1.32)	
PPI treatment				
No	6/877 (0.7)	7/893 (0.8)	0.87 (0.29 to 2.59)	0.70
Yes	6/582 (1.0)	9/567 (1.6)	0.65 (0.23 to 1.81)	
Any antacid therapy (including PPI treatment)				
No	5/802 (0.6)	7/834 (0.8)	0.74 (0.24 to 2.33)	0.10
Yes	7/657 (1.1)	9/627 (1.4)	0.74 (0.28 to 1.98)	
NGT in situ				
No	12/1464 (0.8)	17/1462 (1.2)	0.70 (0.34 to 1.47)	1.00
Yes	0/5 (0.0)	0/2 (0.0)	–	
Previous gastrointestinal surgery				
No	10/1245 (0.8)	15/1237 (1.2)	0.66 (0.30 to 1.47)	0.85
Yes	1/203 (0.5)	2/212 (0.9)	0.52 (0.05 to 5.71)	
Recent previous hospital admission				
No	7/982 (0.7)	13/1023 (1.3)	0.56 (0.22 to 1.40)	0.38
Yes	5/483 (1.0)	4/444 (0.9)	1.15 (0.31 to 4.25)	
Duration of hospital stay				
< 7 days	0/524 (0.0)	0/498 (0.0)	–	1.00
≥ 7 days	11/928 (1.2)	16/949 (1.7)	0.70 (0.33 to 1.51)	

a Denominator varies according to availability of information.

TABLE 16 Severity of CDD, frequency of associated symptoms and investigations according to intervention arm

Outcome	Probiotic[a]	Placebo[a]	Total	*p*-value
Duration (days), *n*, median (IQR)	11, 5.0 (3.0–8.0)	11, 9.0 (6.0–13.0)	22, 6.5 (3.0–12.0)	0.16
Number stools per 24 hours, *n*, median (IQR)	12, 5.0 (3.0–6.0)	17, 3.0 (3.0–4.0)	29, 4.0 (3.0–5.0)	0.057
Stool consistency, *n/N*, median (IQR)	12, 7.0 (6.0–7.0)	17, 7.0 (6.0–7.0)	29, 7.0 (6.0–7.0)	0.62
Nausea, *n/N* (%)	3/12 (25.0)	6/17 (35.3)	9/29 (31.0)	0.56
Vomiting, *n/N* (%)	1/12 (8.3)	2/17 (11.8)	3/29 (10.3)	0.77
Bloating, *n/N* (%)	7/12 (58.3)	3/17 (17.6)	10/29 (34.5)	0.023
Flatus, *n/N* (%)	7/12 (58.3)	6/17 (35.3)	13/29 (44.8)	0.22
Abdominal pain, *n/N* (%)	8/12 (66.7)	10/17 (58.8)	18/29 (62.1)	0.67
Tenesmus, *n/N* (%)	2/12 (16.7)	1/17 (5.9)	3/29 (10.3)	0.35
Fever, *n/N* (%)	2/12 (16.7)	1/17 (5.9)	3/29 (10.3)	0.35
Faecal incontinence, *n/N* (%)	3/12 (25.0)	5/16 (31.3)	8/28 (28.6)	0.72
Nocturnal diarrhoea, *n/N* (%)	7/12 (58.3)	11/17 (64.7)	18/29 (62.1)	0.73
Urgency, *n/N* (%)	8/12 (66.7)	8/17 (47.1)	16/29 (55.2)	0.30
Blood in stool, *n/N* (%)	0/11 (0.0)	0/16 (0.0)	0/27 (0.0)	–
Mucus in stool, *n/N* (%)	0/11 (0.0)	4/15 (26.7)	4/26 (15.4)	0.063
Managed in hospital, *n/N* (%)	9/12 (75.0)	14/17 (82.4)	23/29 (79.3)	0.63
Findings on examination and clinical investigations				
Fever (temperature ≥ 38.5 °C), *n/N* (%)	1/8 (12.5)	0/11 (0.0)	1/19 (5.3)	0.23
Abdominal distension, *n/N* (%)	1/8 (12.5)	4/11 (36.4)	5/19 (26.3)	0.24
Abdominal tenderness, *n/N* (%)	1/7 (14.3)	3/11 (27.3)	4/18 (22.2)	0.52
WCC (× 10^9/l), *n*, median (IQR)	12, 8.5 (7.9–14.6)	14, 11.7 (7.6–16.7)	26, 9.6 (7.8–16.7)	0.92
Creatinine, *n*, median (IQR)	9, 122 (64.0–207.0)	13, 108 (58.0–133.0)	22, 109 (58.0–155.0)	0.43
Sigmoidoscopy or colonoscopy performed, *n/N* (%)	0/11 (0.0)	1/15 (6.7)	1/26 (3.8)	0.38
Severity classification,[b] n (%)				
1 – mild	7 (63.6)	7 (50.0)	14 (56.0)	0.79
2 – moderate	1 (9.1)	2 (14.3)	3 (12.0)	
3 – severe	3 (27.3)	5 (35.7)	8 (32.0)	

WCC, white blood cell count.
a Denominator varies according to availability of information.
b See *Appendix 6*.

reported taking the IMP in the first 3 weeks was similar in the probiotic [*n* = 1469, 21 days (IQR 14–21 days)] and placebo arms [*n* = 1471, 21 days (IQR 14–21 days); *p* = 0.55; *Figure 2*]. The full 21-day course was completed by 52.5% of participants. Overall, 1076/2934 (36.7%) participants reported that they disliked taking the IMP, and this proportion was similar in the probiotic (529/1466, 36.1%) and placebo arms (547/1468, 37.3%; *p* = 0.51). Taking account of compliance in covariate analysis did not materially alter the risk of AAD (OR 1.02; 95% CI 0.80 to 1.30) or CDD (OR 0.66; 95% CI 0.30 to 1.47).

Unused IMPs were collected opportunistically at three time points during the study, from participants who had withdrawn or died, for assessing correct identity according to active versus placebo and number of viable organisms in the probiotic preparation. Thirty-four probiotic capsules were tested and all contained $\geq 1.62 \times 10^{10}$ viable bacteria. All of the 33 placebo capsules tested were sterile.

During the first 3 weeks while participants were taking the IMPs, the duration of hospital stay was similar in the probiotic [*n* = 1469, median 6 days (IQR 2–13 days)] and placebo arms [*n* = 1470, median 6 days (IQR 2–13 days); *p* = 0.65]. The most commonly reported gastrointestinal symptoms were nausea (14.9%), abdominal pain (13.4%) and diarrhoea (any loose stools reported by the participants; 12.3%; *Table 17*). The frequency of gastrointestinal symptoms was similar in the two study arms with the exception of flatus, which was marginally less common in the placebo than the probiotic arm (risk difference 2.3%; 95% CI 0.0% to 4.6%). Futhermore, although very few participants had a NGT in situ, this was significantly more common in the probiotic than placebo arm. With these two exceptions, the duration that symptoms were present was also similar in the two study arms ($p > 0.17$ for all comparisons).

There were no statistically significant differences in either the frequency or duration of gastrointestinal symptoms or other morbidity according to study arm during weeks 4–8 of the study (data not shown). Overall, average duration of hospital stay was known in 2899 participants and was similar in the probiotic [*n* = 1452, median 4 days (IQR 1–11 days)] and placebo arms [*n* = 1447, median 4 days (IQR 1–11 days); *p* = 0.87; *Figure 3*].

Eighteen participants were excluded from PP analysis. Seven patients in the probiotic and six in the placebo arm declined to take any of the IMPs. Investigation of the IMP labelling error that occurred at one centre resulted in the IMPs being withdrawn before completion of the 21-day course in one participant in the probiotic arm and four in the placebo arm. Among these participants excluded from PP analysis, none developed CDD, but one allocated to the probiotic arm developed AAD. Analysis of primary outcomes in the PP population did not materially alter the assessment of the efficacy of the intervention (data not shown). In addition, the risk of developing AAD or CDD was as similar among those participants who took all 21 IMP doses, 14 or more doses or seven or more doses as it was in all participants (data not shown).

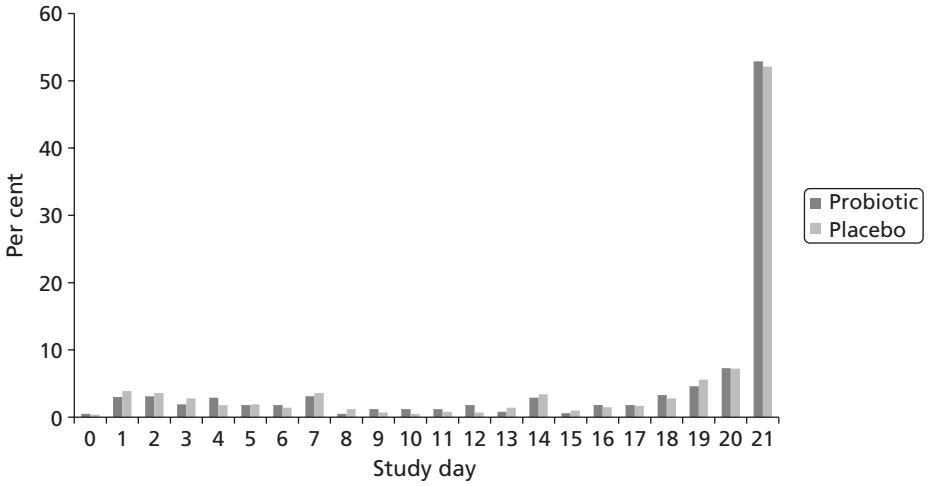

FIGURE 2 Total number of days participants took the IMPs according to intervention arm.

TABLE 17 Frequency of gastrointestinal symptoms and other morbidity in the first 3 weeks according to intervention arm

Variable	Probiotic,[a] *n/N* (%)	Placebo,[a] *n/N* (%)	Total, *n/N* (%)	*p*-value
Diarrhoea	189/1460 (12.9)	172/1464 (11.7)	361/2924 (12.3)	0.33
Nocturnal diarrhoea	55/1459 (3.8)	51/1464 (3.5)	106/2923 (3.6)	0.68
Faecal incontinence	46/1460 (3.2)	53/1463 (3.6)	99/2923 (3.4)	0.48
Tenesmus	22/1458 (1.5)	22/1464 (1.5)	44/2922 (1.5)	0.99
Abdominal pain	200/1458 (13.7)	193/1464 (13.2)	393/2922 (13.4)	0.67
Nausea	228/1458 (15.6)	207/1462 (14.2)	435/2920 (14.9)	0.26
Vomiting	124/1459 (8.5)	110/1463 (7.5)	234/2922 (8.0)	0.33
Bloating	155/1457 (10.6)	143/1464 (9.8)	298/2921 (10.2)	0.44
Flatus	183/1459 (12.5)	149/1462 (10.2)	332/2921 (11.4)	0.045
NGT in situ	8/1460 (0.5)	1/1463 (0.1)	9/2923 (0.3)	0.019
Other morbidity	442/1462 (30.2)	463/1468 (31.5)	905/2930 (30.9)	0.44
Sought consultation for new health problem	238/1469 (16.2)	257/1471 (17.5)	495/2940 (16.8)	0.36

a Denominator varies according to availability of information.

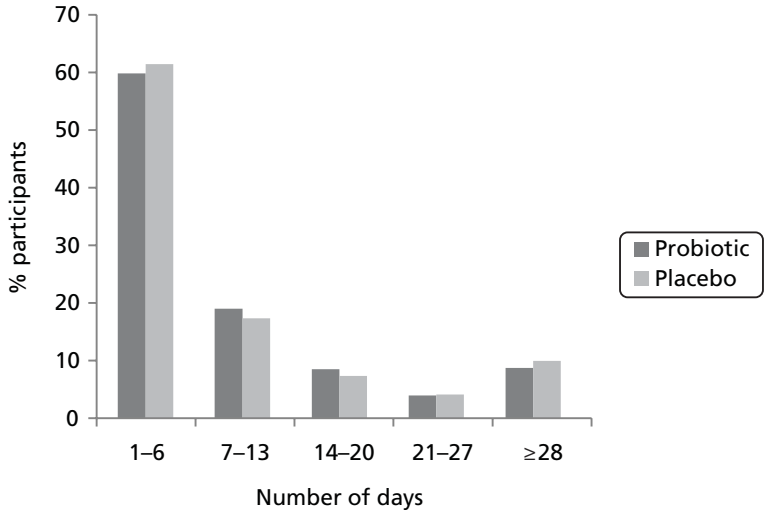

FIGURE 3 Duration of hospital stay according to intervention arm.

Serious adverse events

Serious adverse events were common in the study population with 578 (19.7%) participants experiencing one or more SAE (*Table 18*). The most common MedDRA SOC classifications for SAEs were respiratory, thoracic and mediastinal disorders, gastrointestinal disorders, and cardiac disorders.

Serious adverse events classified as gastrointestinal disorders occurred in 79 (2.7%) participants with a similar frequency in both study arms (see *Table 18*). With SAEs classified according to MedDRA PTs[43] (see *Appendix 9*, *Table 32*), gastrointestinal haemorrhage occurred in 15 participants in the probiotic arm (specified as upper gastrointestinal haemorrhage in four participants and lower in five participants) and 11 participants in the placebo arm (specified as upper gastrointestinal haemorrhage in one participant and

TABLE 18 Serious adverse events classified according to MedDRA SOC[a,b] and intervention arm

SOC	Probiotic (*n* = 1470) (%)	Placebo (*n* = 1471) (%)	Total (*n* = 2941) (%)
Blood and lymphatic system disorders	7 (0.5)	5 (0.3)	12 (0.4)
Cardiac disorders	42 (2.9)	28 (1.9)	70 (2.4)
Ear and labyrinth disorders	1 (0.1)	0 (0.0)	1 (0.0)
Eye disorders	0 (0.0)	1 (0.1)	1 (0.0)
Gastrointestinal disorders	44 (3.0)	35 (2.4)	79 (2.7)
General disorders and administration site conditions	14 (1.0)	9 (0.6)	23 (0.8)
Immune system disorders	0 (0.0)	4 (0.3)	4 (0.1)
Infections and infestations	20 (1.4)	23 (1.6)	43 (1.5)
Injury, poisoning and procedural complications	20 (1.4)	21 (1.4)	41 (1.4)
Investigations	0 (0.0)	1 (0.1)	1 (0.0)
Metabolism and nutrition disorders	3 (0.2)	9 (0.6)	12 (0.4)
Musculoskeletal and connective tissue disorders	4 (0.3)	4 (0.3)	8 (0.3)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	11 (0.7)	11 (0.7)	22 (0.7)
Nervous system disorders	13 (0.9)	15 (1.0)	28 (1.0)
Psychiatric disorders	3 (0.2)	0 (0.0)	3 (0.1)
Renal and urinary disorders	22 (1.5)	25 (1.7)	47 (1.6)
Respiratory, thoracic and mediastinal disorders	83 (5.6)	87 (5.9)	170 (5.8)
Skin and subcutaneous tissue disorders	13 (0.9)	4 (0.3)	17 (0.6)
Social circumstances	4 (0.3)	1 (0.1)	5 (0.2)
Surgical and medical procedures	9 (0.6)	11 (0.7)	20 (0.7)
Vascular disorder	8 (0.5)	7 (0.5)	15 (0.5)
Unclassified[c]	5 (0.3)	4 (0.3)	9 (0.3)
Any SAE	294 (20.0)	284 (19.3)	578 (19.7)

a Diagnoses for SAEs were based on all available clinical evidence. SAEs were classified according to an organ system wherever possible. For example, 'cellulitis' was classified as 'skin and subcutaneous tissue disorders' in preference to 'infections and infestations'.
b SAEs were coded according to the most appropriate SOC of the MedDRA.[42]
c Where there were insufficient details to allow reliable classification (e.g. 'chest pain'), the SAE was unclassified.

lower in seven participants). Peptic ulcer occurred in four participants in the probiotic arm (specified as duodenal ulcer in one participant and perforated peptic ulcer in two participants) and one participant in the placebo arm experienced a perforated duodenal ulcer. Abdominal pain occurred in four participants in the probiotic arm and three in the placebo arm. Gastroenteritis occurred in three participants in the probiotic arm and two in the placebo arm. Constipation occurred in one participant in the probiotic arm and two in the placebo arm. Peritonitis, volvulus and dysentery each occurred in one participant in the probiotic arm and appendix abscess, colostomy performed, diarrhoea, liver abscess and pancreatitis each occurred in one participant in the placebo arm. There was no occurrence of intestinal ischaemia.

The frequency of SAEs that were, or may have been, due to bacterial infection occurred with similar frequency in each arm (see *Appendix 9*, *Table 32*). Pneumonia occurred in 52 participants in the probiotic

arm (specified as caused by *Pseudomonas* sp. in two of these participants) and 53 in the placebo arm (specified as caused by *Pseudomonas* sp. in three of these participants). Abscesses occurred in one participant in the probiotic arm (specifically, a groin abscess) and four participants in the placebo arm (specifically groin, mediastinal, liver and psoas abscesses). Urinary tract infection occurred in 15 participants in the probiotic arm and 12 in the placebo arm, wound infection or cellulitis occurred in 16 participants in the probiotic arm and nine in the placebo arm, infected implant site occurred in two participants in the probiotic arm and one in the placebo arm and infected haematoma occurred in one participant in the probiotic arm. Sepsis occurred in 10 participants in the probiotic and 12 in the placebo arm and organ failure in one participant in each study arm.

The most frequent SAEs classified according to MedDRA PTs[43] were pneumonia (3.3%), obstructive pulmonary disorder (1.6%) and falls (1.1%). Overall, 143 (4.9%) participants experienced a SAE that resulted in death, 10 (0.3%) SAEs that were considered to be life-threatening, 447 (15.2%) SAEs that prolonged hospitalisation, four (0.1%) SAEs that resulted in persistent or significant disability or incapacity and 11 (0.4%) experienced other SAEs that were considered to be significant medical events (see *Appendix 9*, *Table 32*). The proportion of patients in each SAE severity category, the frequency of individual SAEs within each category and the proportion of participants experiencing one or more SAE were similar in the two study arms. (see *Appendix 9*, *Table 32*).

Following the occurrence of a SAE, the patient's clinical team withdrew the IMP from 14 (0.5%) participants and discontinued them temporarily in 90 (3.1%) participants, with a similar proportion in each study arm (see *Appendix 9*, *Table 33*). A common reason for discontinuing the IMPs was to reduce the number of medications for the patient rather than any concern regarding the safety of the bacterial organisms.

Quality of life analysis

European Quality of Life-5 Dimensions

There was a tendency for the EQ-5D visual analogue scale (VAS) and index values to increase over time from baseline to 4 weeks and then 8 weeks, indicating an improvement in health status over time within each of the study arms. Median scores were similar in the two study arms (*Table 19*).

The change from baseline EQ-5D VAS to that at 4 weeks was similar in both study arms but at 8 weeks there was a statistically significant difference between the two arms. However, this was a change of less than 2 points on the 100-point scale and, therefore, is unlikely to represent a clinically important change in health status (*Table 20*).

TABLE 19 Summary statistics of EQ-5D VAS and index values by intervention arm and visit

Visit	Probiotic,[a] *n*, median (IQR)	Placebo,[a] *n*, median (IQR)	Total, *n*, median (IQR)
VAS			
Baseline	1432, 50.0 (40.0–70.0)	1435, 50.0 (40.0–70.0)	2867, 50.0 (40.0–70.0)
4 weeks	1160, 60.0 (50.0–75.0)	1197, 60.0 (50.0–75.0)	2357, 60.0 (50.0–75.0)
8 weeks	1133, 60.0 (50.0–78.0)	1151, 65.0 (50.0–80.0)	2284, 61.0 (50.0–80.0)
Index values			
Baseline	1457, 0.62 (0.22–0.74)	1461, 0.62 (0.24–0.74)	2918, 0.62 (0.22–0.74)
4 weeks	1180, 0.69 (0.52–0.81)	1219, 0.69 (0.52–0.81)	2399, 0.69 (0.52–0.81)
8 weeks	1153, 0.71 (0.59–0.81)	1178, 0.69 (0.59–0.82)	2331, 0.69 (0.59–0.81)

a The number of participants in each arm decreased over time because of loss due to follow-up.

TABLE 20 Mixed model analysis of EQ-5D change from baseline in VAS health status scores and index score

Comparison	Difference (95% CI)	*p*-value
VAS		
Probiotic vs. placebo at 4 weeks	−0.44 (−1.98 to 1.11)	0.58
Probiotic vs. placebo at 8 weeks	−1.76 (−3.32 to −0.19)	0.028
Index values		
Probiotic vs. placebo at 4 weeks	0.00 (−0.02 to 0.03)	0.74
Probiotic vs. placebo at 8 weeks	0.01 (−0.01 to 0.03)	0.45

Generic Short Form questionnaire-12 items version 2

As with the EQ-5D, there was a tendency for SF-12 v2, MCS, PCS and subdomain scores, with the exception of vitality, to increase over time, also indicating that the level of health increased in both study arms (*Table 21*).

Analysis of changes from baseline in SF-12 v2 summary and subdomain scores at 4 weeks and 8 weeks by treatment allocated showed no statistically significant differences between the two study arms (*Table 22*).

Economic analysis

Resource use and costs

Health-care contacts

Average duration of initial hospital stay was 0.03 days longer in the probiotic arm than in the placebo arm (*Table 23*). Overall, during the 8-week follow-up period, 18.3% patients were readmitted to hospital with a similar frequency in each study arm; however, patients in the probiotic arm remained in hospital for 0.62 days less than those in the placebo arm during each readmission. In the probiotic arm, 38.4% of patients reported other health-care contacts for a new problem compared with 40.9% in the placebo arm (costed as GP visits) and spent an additional 0.11 days, on average, in care facilities. None of these differences was statistically significant.

The mean cost of health-care contacts per patient was similar in the two trial arms (*Table 24*).

Antibiotics

The mean cost of antibiotics was £105.38 in the probiotic arm and £90.94 in the placebo arm. Staff costs for administration of antibiotics was £759.71 in the probiotic and £738.34 in the placebo arm. Overall antibiotics cost per patient was £35.80 less in the placebo arm than in the probiotic arm, but the difference was not statistically significant (*Table 25*).

Intervention implementation

The mean nursing time required to administer the probiotic course was 39 minutes at a cost of £63.02. Including the retail cost of the formulation and accounting for duration of hospital stay, the mean implementation cost of the probiotic was £73.02 (range £10.00–179.68; *Table 25*). No adverse events requiring additional health-care contacts were observed.

Episodes of diarrhoea

A summary of costs associated with gastroenteritis while patients are in hospital and collected outside the trial can be found in *Table 26*. When all causes of diarrhoea were included but the costs of antibiotics, other health-care contacts and increased duration of hospital stay were excluded, an episode of diarrhoea cost £402.63 more in the placebo arm than in the probiotic arm. When only AAD was considered, the

TABLE 21 Generic Short Form questionnaire-12 items version 2, MCS, PCS and subdomain scores by intervention arm and visit

Subdomains and component summaries	Probiotic,[a] *n*, median (IQR)	Placebo,[a] *n*, median (IQR)	Total, *n*, median (IQR)
Physical function			
Baseline	1458, 25.0 (0.0–50.0)	1461, 25.0 (0.0–50.0)	2919, 25.0 (0.0–50.0)
4 weeks	1179, 25.0 (0.0–50.0)	1214, 25.0 (0.0–50.0)	2393, 25.0 (0.0–50.0)
8 weeks	1150, 50.0 (0.0–50.0)	1176, 50.0 (0.0–50.0)	2326, 50.0 (0.0–50.0)
Role physical			
Baseline	1458, 37.5 (25.0–62.5)	1461, 37.5 (25.0–62.5)	2919, 37.5 (25.0–62.5)
4 weeks	1179, 50.0 (25.0–75.0)	1214, 37.5 (25.0–75.0)	2393, 42.5 (25.0–75.0)
8 weeks	1150, 50.0 (25.0–75.0)	1176, 50.0 (25.0–75.0)	2326, 50.0 (25.0–75.0)
Bodily pain			
Baseline	1458, 50.0 (25.0–100.0)	1461, 50.0 (25.0–100.0)	2919, 50.0 (25.0–100.0)
4 weeks	1179, 75.0 (50.0–100.0)	1214, 75.0 (50.0–100.0)	2393, 75.0 (50.0–100.0)
8 weeks	1150, 75.0 (50.0–100.0)	1176, 75.0 (50.0–100.0)	2326, 75.0 (50.0–100.0)
General health			
Baseline	1458, 25.0 (25.0–60.0)	1461, 25.0 (25.0–60.0)	2919, 25.0 (25.0–60.0)
4 weeks	1179, 60.0 (60.0–85.0)	1214, 60.0 (60.0–85.0)	2393, 60.0 (60.0–85.0)
8 weeks	1150, 60.0 (25.0–60.0)	1176, 60.0 (25.0–67.5)	2326, 60.0 (25.0–60.0)
Vitality			
Baseline	1458, 25.0 (0.0 to 50.0)	1461, 25.0 (0.0 to 50.0)	2919, 25.0 (0.0 to 50.0)
4 weeks	1179, 25.0 (0.0 to 50.0)	1214, 25.0 (0.0 to 50.0)	2393, 25.0 (0.0 to 50.0)
8 weeks	1150, 25.0 (25.0 to 50.0)	1176, 25.0 (25.0 to 50.0)	2326, 25.0 (25.0 to 50.0)
Social function			
Baseline	1458, 50.0 (25.0 to 75.0)	1461, 50.0 (0.0 to 100.0)	2919, 50.0 (0.0 to 100.0)
4 weeks	1179, 50.0 (25.0 to 75.0)	1214, 50.0 (25.0 to 75.0)	2393, 50.0 (25.0 to 75.0)
8 weeks	1150, 75.0 (25.0 to 100.0)	1176, 75.0 (25.0 to 100.0)	2326, 75.0 (25.0 to 100.0)
Role emotional			
Baseline	1458, 70.5 (37.5 to 100.0)	1461, 62.5 (37.5 to 100.0)	2919, 62.5 (37.5 to 100.0)
4 weeks	1179, 75.0 (50.0 to 100.0)	1214, 75.0 (50.0 to 100.0)	2393, 75.0 (50.0 to 100.0)
8 weeks	1150, 75.0 (50.0 to 100.0)	1176, 75.0 (50.0 to 100.0)	2326, 75.0 (50.0 to 100.0)
Mental health			
Baseline	1458, 62.5 (50.0 to 87.5)	1461, 62.5 (50.0 to 87.5)	2919, 62.5 (50.0 to 87.5)
4 weeks	1179, 75.0 (50.0 to 87.5)	1214, 75.0 (50.0 to 87.5)	2393, 75.0 (50.0 to 87.5)
8 weeks	1150, 75.0 (60.0 to 87.5)	1176, 75.0 (50.0 to 87.5)	2326, 75.0 (55.9 to 87.5)
MCS			
Baseline	1458, 33.3 (26.2 to 41.9)	1461, 33.32 (25.8 to 41.9)	2919, 33.32 (25.9 to 41.9)
4 weeks	1179, 37.50 (30.9 to 43.8)	1214, 37.63 (30.5 to 43.6)	2393, 37.51 (30.7 to 43.7)
8 weeks	1150, 38.12 (28.3 to 45.4)	1176, 38.35 (29.3 to 45.5)	2326, 38.25 (28.9 to 45.4)

continued

TABLE 21 Generic Short Form questionnaire-12 items version 2, MCS, PCS and subdomain scores by intervention arm and visit (*continued*)

Subdomains and component summaries	Probiotic,[a] *n*, median (IQR)	Placebo,[a] *n*, median (IQR)	Total, *n*, median (IQR)
PCS score			
Baseline	1458, 45.8 (36.0 to 54.4)	1461, 45.4 (36.2 to 54.4)	2919, 45.5 (36.2 to 54.4)
4 weeks	1179, 48.0 (38.2 to 55.3)	1214, 47.94 (37.9 to 55.4)	2393, 47.98 (38.1 to 55.4)
8 weeks	1150, 51.0 (41.0 to 57.0)	1176, 50.64 (40.2 to 56.8)	2326, 50.86 (40.6 to 56.8)

a The number of participants in each arm decreased over time because of loss due to follow-up.

TABLE 22 Mixed model analysis of SF-12 v2 change from baseline in MCS, PCS and subdomain scores

Comparison	Difference (95% CI)	*p*-value
Physical function		
Probiotic vs. placebo at 4 weeks	0.61 (–1.95 to 3.17)	0.64
Probiotic vs. placebo at 8 weeks	–0.28 (–2.87 to 2.31)	0.83
Role physical		
Probiotic vs. placebo at 4 weeks	1.44 (–1.04 to 3.92)	0.26
Probiotic vs. placebo at 8 weeks	0.91 (–1.61 to 3.42)	0.48
Bodily pain		
Probiotic vs. placebo at 4 weeks	0.63 (–1.52 to 2.77)	0.57
Probiotic vs. placebo at 8 weeks	0.49 (–1.68 to 2.66)	0.66
General health		
Probiotic vs. placebo at 4 weeks	–0.88 (–3.05 to 1.30)	0.43
Probiotic vs. placebo at 8 weeks	–1.29 (–3.50 to 0.92)	0.25
Vitality		
Probiotic vs. placebo at 4 weeks	–1.26 (–3.21 to 0.68)	0.20
Probiotic vs. placebo at 8 weeks	–1.49 (–3.46 to 0.48)	0.14
Social function		
Probiotic vs. placebo at 4 weeks	0.19 (–2.58 to 2.97)	0.89
Probiotic vs. placebo at 8 weeks	–0.55 (–3.36 to 2.26)	0.70
Role emotional		
Probiotic vs. placebo at 4 weeks	0.88 (–1.54 to 3.29)	0.48
Probiotic vs. placebo at 8 weeks	1.77 (–0.68 to 4.22)	0.16
Mental health		
Probiotic vs. placebo at 4 weeks	0.55 (–1.16 to 2.26)	0.53
Probiotic vs. placebo at 8 weeks	1.33 (–0.40 to 3.06)	0.13
MCS		
Probiotic vs. placebo at 4 weeks	0.29 (–0.63 to 1.20)	0.54
Probiotic vs. placebo at 8 weeks	–0.51 (–1.44 to 0.41)	0.28
PCS score		
Probiotic vs. placebo at 4 weeks	0.13 (–0.76 to 1.03)	0.77
Probiotic vs. placebo at 8 weeks	0.51 (–0.39 to 1.41)	0.27

TABLE 23 Health-care contacts per patient by intervention arm

Health-care contact	Probiotic (*n* = 1470)	Placebo (*n* = 1471)	Total (*n* = 2941)
Mean duration of initial hospital stay in days (95% CI)	17.38 (16.36 to 18.39)	17.35 (16.31 to 18.39)	17.36 (16.63 to 18.09)
Number of readmissions (%)	260 (17.7)	279 (19.0)	539 (18.3)
Mean duration of readmission inpatient stay in days (95% CI)	10.99 (9.78 to 12.19)	11.61 (10.38 to 12.84)	11.31 (10.45 to 12.17)
Mean number of other health-care contacts per patient	0.69	0.73	0.71
Mean number of days in care home per patient (95% CI)	3.73 (3.17 to 4.28)	3.62 (3.08 to 4.15)	3.67 (3.29 to 4.06)

TABLE 24 Mean cost of health-care contacts per patient by intervention arm: base case

Health-care contact	Probiotic (95% CI)	Placebo (95% CI)	*p*-value
Mean cost of initial hospital stay (£)	5806.60 (5467.94 to 6145.27)	5797.66 (5450.63 to 6144.68)	0.97
Mean cost of readmissions (£)	3672.01 (3268.90 to 4075.12)	3879.50 (3468.30 to 4290.70)	0.48
Mean cost of other health-care contacts (£)	64.47 (57.50 to 71.44)	63.85 (59.45 to 68.25)	0.88
Mean cost of care home (£)	2680.96 (2464.09 to 2897.82)	2505.79 (2298.39 to 2713.18)	0.25

TABLE 25 Mean cost of antibiotics, probiotics and episodes of diarrhoea per patient by intervention arm: base case

Health event	Probiotic (95% CI)	Placebo (95% CI)	*p*-value
Mean cost of antibiotics including staff time (£)	865.09 (816.65 to 913.54)	829.29 (780.38 to 878.19)	0.31
Mean cost of probiotic including staff time (£)	73.02 (70.20 to 75.83)	–	–
Episode of diarrhoea (all causes included) (£)	1817.20 (1519.88 to 2114.52)	2219.83 (1725.61 to 2714.06)	0.17
Mean cost of an episode of AAD (£)	1742.15 (1438.39 to 2045.92)	2220.38 (1696.52 to 2744.23)	0.12
Mean total cost (£)	8020.11 (7622.31 to 8417.90)	8011.37 (7600.53 to 8422.22)	0.98

differential cost was £478.23 more in the placebo arm than in the probiotic arm (see *Table 25*). However, these differences were not statistically significant.

Independent of study arm, the mean duration of hospital stay was 22.31 days for patients with AAD versus 16.73 days for non-diarrhoea patients. This difference of 5.58 days (95% CI 2.78 to 8.39 days) accrued, on average, £4531.36 (95% CI £3439.80 to £5622.92) more health-care costs ($p = 0.01$). In addition to increased length of hospital stay, the main cost drivers for this difference were additional costs of £1976.66 (95% CI £1677.24 to £2276.09) attributed to assessment and management of diarrhoea episodes including microbiology, staff time, diagnostics, cleaning, laundry and infection control measures.

Total health-care cost

Total health-care cost was £8.74 greater in the probiotic arm than in the placebo arm (see *Table 25*). According to our analysis, the main cost drivers that make up a high proportion of the total health-care

TABLE 26 Cost components of diarrhoea episodes

Cost component	Base case (£)	Range (£)
Per patient episode		
Microbiology at ABMUHB hospitals	54.94	30.17–109.88
Microbiology at CDDFT hospitals	45.28	27.51–90.56
Diagnostic and therapeutic procedures	69.65	49.25–101.25
Clinical assessment and review	194.76	103.01–259.67
End cleaning	20.76	16.62–24.91
Per day		
Bed and ward closures	415.57	0–497.61
Daily cleaning	9.54	7.63–11.45
Per stool		
Disposables and staff time	8.38	6.70–10.06
Spot cleaning and changing	32.85	26.28–39.42

costs were the duration of the initial hospital stay and readmissions, staff time for antibiotic and probiotic administration and diarrhoea-associated costs (including microbiology, clinical review and assessment, diagnostic and therapeutic procedures, disposables, cleaning, laundry and infection control procedures).

Utility and quality-adjusted life-years

Mean EQ-5D index values at the baseline were 0.51 for both the probiotic and placebo arms, and this value increased over time. At 4 weeks, scores for both trial arms were 0.60, and at 8 weeks this had further increased to 0.64 in the probiotic and 0.63 in the placebo arm. The slightly better 8-week follow-up outcome for the patients who were administered the probiotic (average utility difference of 0.01) was not statistically significant. Extrapolated to 1 year, the total QALY gain in the probiotic group was 0.0004 as no further QALY gain was to be expected after 8 weeks and any further changes in QoL would probably be due to general recovery (*Table 27*).

TABLE 27 Cost–utility of probiotic in comparison to placebo: base case

Outcome	Difference: probiotic minus placebo	*p*-value
Incremental utility – 8 weeks (95% CI)	0.01 (–0.01 to 0.03)	0.45
Incremental QALY – 1 year (95% CI)	0.0004 (–0.0006 to 0.0014)	–
Incremental total health-care cost (95% CI)	£8.74 (–£4.32 to £21.78)	0.98
ICER (1 year)	£22,701 per QALY	
Probability cost-effective at £20,000	0.48	
Probability cost-effective at £30,000	0.54	
Probiotic implementation cost (95% CI)	£73.02 (£70.20 to £75.83)	
ICER (1 year)	£189,662 per QALY	
Probability cost-effective at £20,000	< 0.01	
Probability cost-effective at £30,000	0.02	

Cost-effectiveness and uncertainty

Base-case analysis showed only a small total health-care cost difference between the probiotic and placebo arms (see *Table 25*). This was mainly due to the relatively small implementation cost of the probiotic and the marginal cost savings for diarrhoea episodes in the probiotic arm. The cost difference resulted in an ICER of £22,701 per QALY at 1 year with a probability of the intervention being cost-effective at a £20,000 willingness-to-pay threshold of 48%. The CEAC depicts the probability of the intervention being cost-effective at different willingness-to-pay thresholds. (*Figure 4*).

If the implementation costs of the probiotics only are taken into account, without consideration of any downstream effects, the ICER increases to £189,662 per QALY at 1 year with a probability of cost-effectiveness at £30,000 of 2% (*Figure 5*). Thus, based on a £30,000 willingness-to-pay threshold and implementation costs, probiotics are not cost-effective.

Results of the cost–consequences analysis are reported in *Table 28*. As overall differences in costs and clinical outcomes between the two arms were small, the clinical effectiveness and cost-effectiveness of probiotics in the prevention of AAD in this study can be considered limited. Even though probiotics

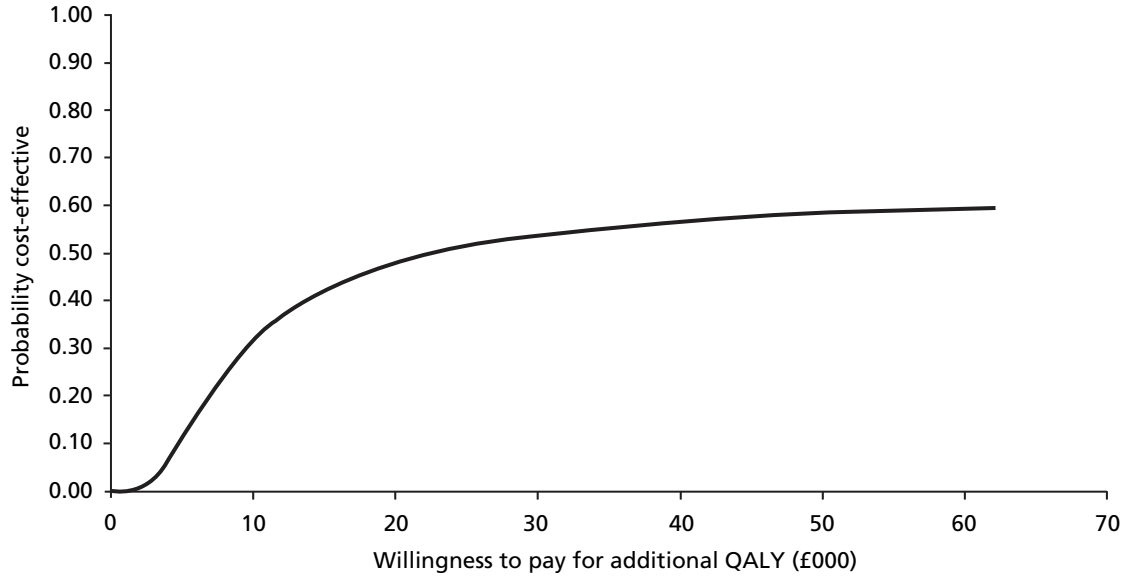

FIGURE 4 Cost-effectiveness acceptability curve, base case analysis: total health-care cost.

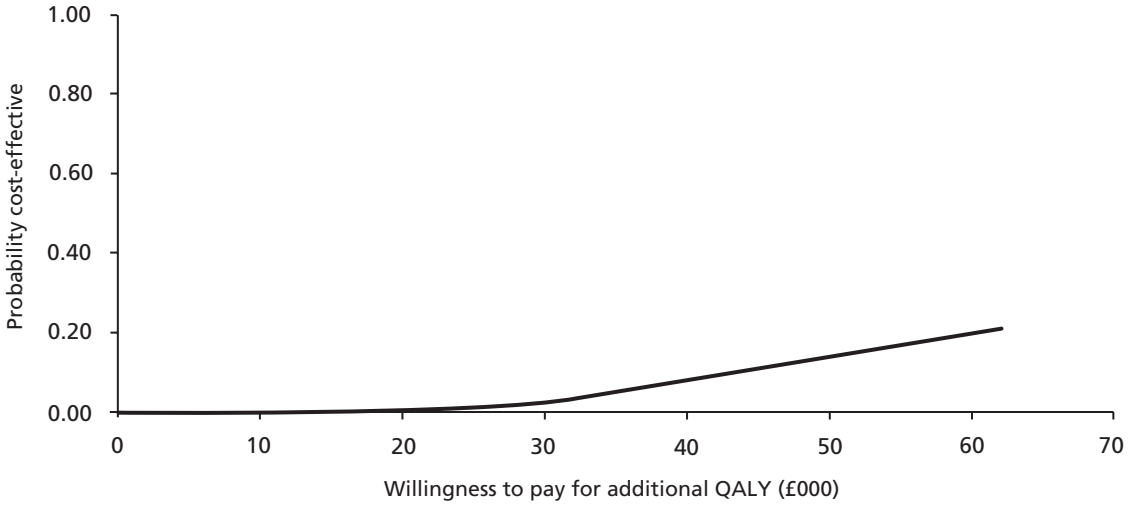

FIGURE 5 Cost-effectiveness acceptability curve, base case analysis: probiotics implementation cost.

TABLE 28 Clinical effectiveness and cost-effectiveness outcomes: cost–consequences analysis[a]

Impact variable	Probiotic	Placebo	Difference	*p*-value
Costs impact				
Implementation cost per patient (£)	73.02 (70.20 to 75.83)	0.00 (0.00 to 0.00)	73.02 (70.20 to 75.83)	0.01
Total health-care cost per patient (£)	8020.11 (7622.31 to 8417.90)	8011.37 (7600.53 to 8422.22)	8.74 (–4.32 to 21.78)	0.98
Cost of AAD episode (£)	1742.15 (1438.39 to 2045.92)	2220.38 (1696.52 to 2744.23)	–478.23 (–1192.34 to 235.89)	0.12
QoL impact				
EQ-5D scores at baseline	0.51 (SD 0.341)	0.51 (SD 0.332)	0.00	–
EQ-5D changes baseline – 4 weeks	0.08 (SD 0.343)	0.08 (SD 0.334)	0.00 (–0.03 to 0.03)	0.74
EQ-5D changes baseline – 8 weeks	0.12 (SD 0.348)	0.11 (SD 0.352)	0.01 (–0.01 to 0.03)	0.45
Health impact				
Number of AAD cases per arm	159/1470 (10.8%)	153/1471 (10.4%)	6 (0.4%)	0.72
Number of CDD cases per arm	12/1470 (0.8%)	17/1471 (1.2%)	–5 (–0.4%)	0.35

SD, standard deviation.
a Values in brackets represent 95% CI unless indicated otherwise.

appeared cost-effective in the cost–utility analysis based on total health-care costs, no significant budgetary impact can be anticipated. This is due to the small differences in total cost between the probiotic and placebo arms and the lack of statistical significance in the primary outcomes. Subgroup analysis was not undertaken, as the covariate analysis did not identify any specific population that clearly benefited from receiving the probiotic. Cost per case of diarrhoea averted was not analysed as the study did not demonstrate a difference in diarrhoea frequency between the two groups.

Sensitivity analysis

Changes in the parameters included in the microcosting of a diarrhoea episode and changes in the cost of an inpatient day (the average cost per day amounted by a patient while in hospital) did not result in significant changes to the difference in overall cost between the probiotic and the placebo arms (*Table 29*). Furthermore, a decrease or increase in staff time for probiotic administration did not significantly change the cost-effectiveness results (*Table 30*). Considering probiotic implementation costs only, a reduction in staff time by 50% resulted in an ICER of £107,818 per QALY and a probability of cost-effectiveness at £30,000 of 16%, whereas doubling of staff time increased the ICER to £353,402 per QALY and was associated with a probability of cost-effectiveness at £30,000 of < 1%. The CEACs for these results can be found in *Figures 6* and *7*.

Summary of cost-effectiveness results

- Cost and duration of hospital stays, cost of diarrhoea, cost of antibiotics and total health-care cost per patient were very similar between the probiotic and the placebo arms. No statistically significant cost differences were found between the two study arms.
- Incremental total health-care cost of participants who suffered from AAD was £4531.36 (95% CI £3439.80 to £5622.92), which was significantly higher than for non-diarrhoea patients, independent of study arm. This was mainly due to increased length of hospital stay and additional diarrhoea-associated costs.

TABLE 29 Sensitivity analysis: changes in mean health-care cost per patient following parameter change within defined ranges

Parameter changed	Mean health-care cost per patient		Difference	*p*-value
	Probiotic (95% CI)	Placebo (95% CI)		
Costing of diarrhoea (£)				
All diarrhoea costs lower value	7871.70 (7483.57 to 8259.83)	7843.83 (7445.22 to 8242.44)	27.87	0.92
All diarrhoea costs upper value	8095.37 (7694.56 to 8496.19)	8111.94 (7691.43 to 8532.46)	−16.57	0.96
Other costs (£)				
Hospital inpatient day lower value	6716.00 (6389.38 to 7042.61)	6699.56 (6359.39 to 7039.73)	16.14	0.95
Hospital inpatient day upper value	9298.27 (8833.97 to 9762.58)	9312.79 (8831.04 to 9794.54)	−14.52	0.97

TABLE 30 Sensitivity analysis: changes of ICER based on probiotic implementation cost following parameter change within defined ranges

Probiotic implementation cost (£)	Cost per patient (95% CI)	ICER	Probability cost-effective
Mean staff time per dose lower value	41.51 (40.11 to 42.92)	107,818 per QALY	0.046
Mean staff time per dose upper value	136.06 (130.44 to 141.68)	353,402 per QALY	0.00

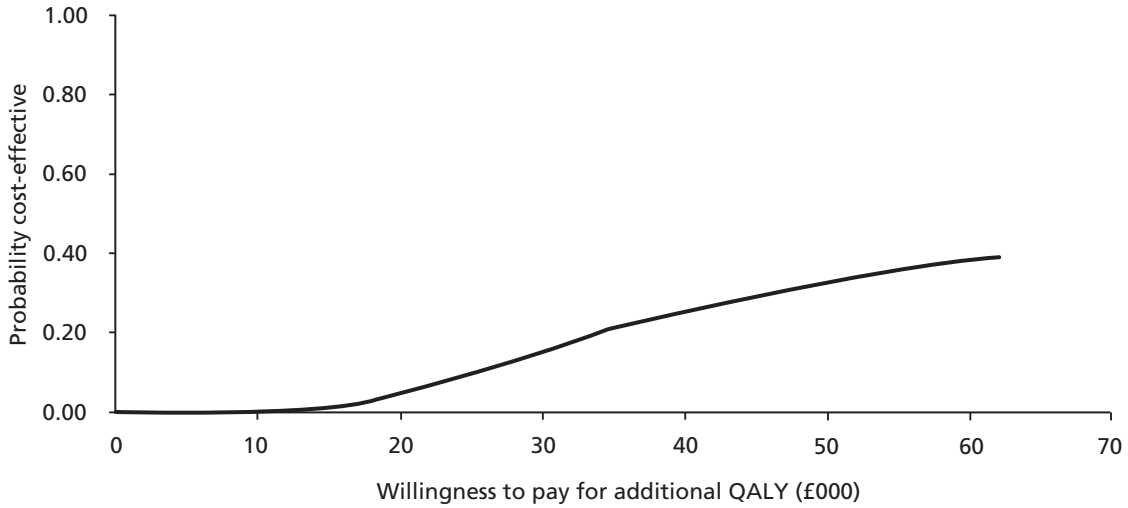

FIGURE 6 Cost-effectiveness acceptability curve, sensitivity analysis: probiotic implementation cost when staff cost halved.

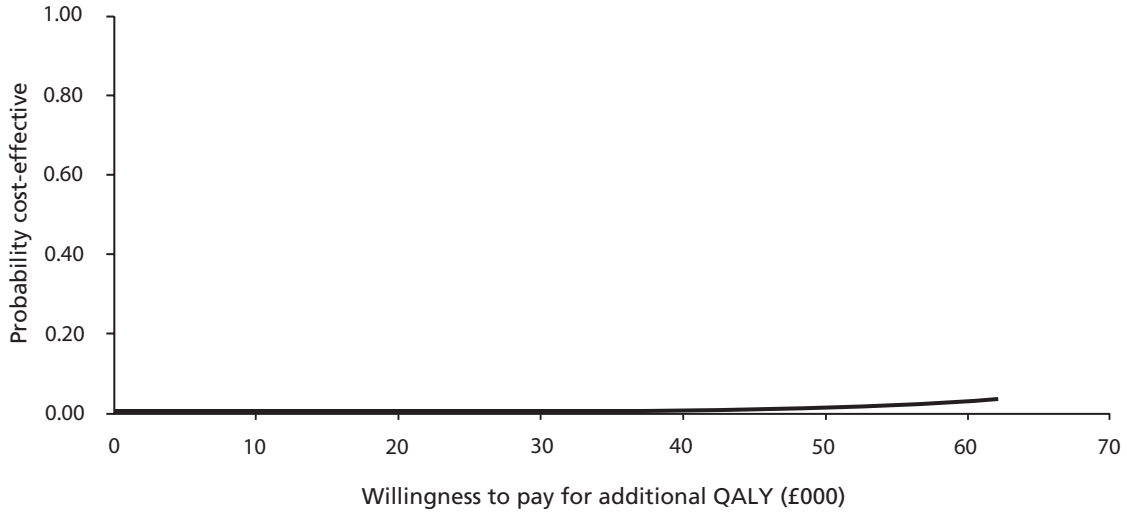

FIGURE 7 Cost-effectiveness acceptability curve, sensitivity analysis: probiotic implementation cost when staff cost doubled.

- Duration of hospital stay (initial stay and readmissions), staff time for antibiotic administration and diarrhoea-associated costs were identified as main components of the total health-care costs across both study arms.
- Between baseline and 8 weeks, mean QoL increase was 0.01 QALY higher in the probiotic than in the placebo arm; however, this difference was not statistically significant.
- The ICER associated with probiotic use at 1 year was estimated at £22,701 per QALY gained when total health-care costs were considered and £189,662 per QALY gained considering probiotic implementation costs only. However, the similarity in total cost, number of diarrhoea cases and patient QoL between the two trial arms limits the relevance of the ICERs.
- One-way sensitivity analyses did not show any significant effect on difference in total health-care costs between the trial arms and the overall conclusion of the cost-effectiveness assessment.

Chapter 5 Discussion

Here we report the results of a RCT that evaluated the clinical effectiveness and cost-effectiveness of a high-dose, multistrain bacterial preparation in the prevention or amelioration of AAD and CDD. PLACIDE was designed and undertaken in response to an increased frequency and severity of CDD in hospitals in industrialised countries[8,9] and a meta-analysis[29] that highlighted the need for more high-quality evidence regarding the role of probiotics. We aimed to undertake a pragmatic trial of older people receiving antibiotics in secondary health-care settings representative of those in industrialised countries and with the causes of diarrhoea determined by routine laboratory practice. This section summarises the key findings, compares our results with published studies, considers the strengths and limitations of PLACIDE and discusses the clinical and research implications of our findings.

Key findings

In an analysis according to treatment allocated, we did not find adequate evidence that a preparation of four strains of live lactobacilli and bifidobacteria was clinically effective in preventing or ameliorating AAD in 2941 inpatients aged between 65.0 and 107.5 years. Overall, AAD occurred in 312 (10.6%) participants, 159 (10.8%) participants in the probiotic arm and 153 (10.4%) participants in the placebo arm (RR 1.04; 95% CI 0.84 to 1.28; $p = 0.72$). CDD was an uncommon cause of AAD, occurring in 29 (1.0%) participants. CDD occurred less frequently in the probiotic (12 participants; 0.82%) arm than in the placebo arm (17 participants; 1.2%). However, this difference was not statistically significant (RR 0.71; 95% CI 0.34 to 1.47; $p = 0.35$). Analysis adjusting for potential risk factors did not significantly change the findings [OR for AAD 1.0 (95% CI 0.78 to 1.27); OR for CDD 0.65 (95% CI 0.29 to 1.47)]. Secondary outcome measures including the severity of diarrhoea, frequency and duration of gastrointestinal symptoms, duration of hospital stay, and QoL were also generally similar in the two study arms. Although an episode of AAD increased total health-care costs by £4531.36 (95% CI £3439.80 to £5622.92) independent of study arm, this was not reflected in a difference in total health-care cost between the probiotic and placebo arms. Cost-effectiveness analysis based on probiotic implementation costs showed that the probiotic was not cost-effective with an ICER of £189,662 per QALY. This result was robust to changes in the key parameters.

Comparison with other studies/reviews

Antibiotic-associated diarrhoea

Probiotic efficacy in our study (RR 1.04; 95% CI 0.84 to 1.28) is in marked contrast with meta-analyses that reported findings in adults (≥ 18 years of age),[29,56–59] as well as a recent review of 63 randomised trials (11,811 participants of all ages and mostly outpatients), in which the pooled estimate showed a statistically significant effect of probiotics (random-effects model; RR 0.58; 95% CI 0.50 to 0.68).[60] We are not aware of further trial findings published since this recent review.

Although meta-analyses have reported similar estimates of probiotic efficacy, authors have emphasised caution in the interpretation of pooled results because of marked clinical heterogeneity between studies. In the recent review, marked heterogeneity in the pooled result ($I^2 = 54\%$) was not explained by subgroup analyses according to differences between trials in the probiotic preparation used, antibiotic treatment and patient characteristics.[60] Review authors have also highlighted deficiencies in the evidence base, especially the limited number of well-conducted trials and variations in the quality control of probiotic preparations.[61] Despite the addition of many recent studies, the authors of the latest review commented that research methods and reporting were generally poor, most studies were underpowered, probiotic strains were not clearly identified, only half of the studies defined the diarrhoea outcome and the follow-up period was

limited or not specified.[60] A recent Cochrane review of probiotics in the prevention of AAD in children also highlighted the low quality of clinical trial evidence.[62]

Causes of AAD may vary according to age and other patient characteristics. For example, the effects of antibiotics may vary according to the characteristics of the pretreatment enteric flora. The composition of the enteric flora varies between individuals,[63] to a greater degree in the elderly than in younger people,[64] and is influenced by chronic disease, frailty, diet, residence and care setting.[64] Most clinical trials recruited people of all ages. Although age was not significantly associated with probiotic efficacy in metaregression analysis in the recent review, interestingly, in three trials that recruited only patients aged > 65 years, there was no clear beneficial effect of probiotics (pooled RR 0.81; 95% CI 0.40 to 1.63).[60] Age and other host factors may modulate probiotic effects and need to be considered when evaluating the effect of interventions. Recommendations that probiotics should be used routinely for the prevention of AAD[65] seem premature and more evidence is needed to inform the selection of a specific probiotic preparation for a well-defined population group.[60]

Clostridium difficile *diarrhoea*

Probiotic efficacy in our study (RR 0.71; 95% CI 0.34 to 1.47) is consistent with pooled estimates from meta-analyses,[60,66] including that of a recent meta-analysis of 20 trials including 3818 children and adults (random-effects model; RR 0.34; 95% CI 0.24 to 0.49).[67] Although this meta-analysis included trials that evaluated many different probiotics, including the yeast *S. boulardii*, and research methods and reporting were poor in many studies, there was consistency in results across studies and probiotics remained clinically effective in worse case assumptions for missing outcome data.

Interestingly, our findings suggested that the probiotic may have been clinically effective in preventing CDD in the older patients (aged > 77 years). However, we are not aware of similar findings in other reviews or randomised trials. Given the large number of analyses in our study, the apparent difference in probiotic effect according to age may have occurred by chance.

Strengths and weaknesses of probiotic lactobacilli and bifidobacteria in antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in the elderly

Research setting and population evaluated

We aimed to maximise the generalisability of our findings by limiting the participant exclusion criteria to conditions associated with diarrhoea and potential risk factors for probiotic adverse effects.[26,68,69] However, this still resulted in the exclusion of about one in five patients assessed for eligibility, and a further 1 in 10 were considered too unwell to join the study. In addition, > 50% of patients receiving antibiotics declined to participate. Reluctance to take additional medications was commonly stated as a reason for declining to join the trial. Among those recruited, use of numerous medications was common, and over one in three stated that they disliked taking the IMP as an additional medication. These practical difficulties need to be considered when developing novel interventions for the older population at risk of AAD.

Despite the relatively low conversion rate among patients who were assessed for their eligibility to participate in the trial (17.1%), we included patients from a range of medical and surgical wards in five hospitals in two NHS regions and, as far as we are aware, recruited a greater number of participants than other similar trials.[60,67] Therefore, we consider that our findings are generalisable to older patients managed in secondary care facilities in settings similar to the UK.

Overall, participant characteristics, including common comorbid illnesses and potential risk factors for AAD were similar at baseline, indicating that randomisation had been successful. The imbalance in sex between the two arms is likely to have arisen by chance and would not appear to have influenced the evaluation of probiotic efficacy; sex was not a risk factor for AAD and there was no evidence of an interaction between

sex and probiotic effect. The higher frequency of COPD in the CDDFT hospitals probably reflects exposure to mining dust and asbestos in the north-east of England related to the ship building and mining industries in the second half of the 20th century.

Treatment with PPIs has been identified as an important risk factor for CDD,[12,70] which led to recommendations for hospital antacid policies to restrict their use.[71] Antacid and PPI use were common among our participants (39.4%); both AAD and CDD were more frequent among patients receiving antacid medications, although this finding was statistically significant only for AAD. However, we found no evidence of an interaction between antacid treatment and probiotic effect and, therefore, no indication that probiotics should be targeted to this specific patient population.

Bacterial preparation

Several bacterial strains share antipathogen mechanisms such as the production of lactic acid;[72] however, other mechanisms that may prevent AAD may be strain specific. Probiotic mixtures appear to better maintain a beneficial gut microbiota than single strains, although this may be due to a greater number of organisms rather than strains.[73] Evidence for a range of health outcomes, mainly from animal studies, also suggests that multispecies mixtures may be more effective than either multistrain or monostrain probiotics.[74] In meta-analysis, subgroup analyses suggested that the use of *S. boulardii*, *L. rhamnosus* GG, probiotic mixtures and a high number of organisms were associated with greater efficacy in preventing AAD.[29] These findings informed the selection of the probiotic preparation used in our study, which consisted of a high number (6×10^{10}) of four strains of bacteria from two species, *Lactobacillus* and *Bifidobacterium*, that are most commonly evaluated in trials of the prevention of AAD.[60]

In meta-analysis of CDD, subgroup analysis did not show significant changes in probiotic effect according to probiotic strain or dose. However, there was a trend towards increased clinical effectiveness of mixtures compared with single probiotic species.[67] Although not a commensal organism of the human gastrointestinal tract, the yeast *S. boulardii* may also be clinically effective in preventing AAD in adults [10 trials, 1866 adults, pooled RR of 0.47 (95% CI. 0.35 to 0.63) with homogeneity between trials in random-effects analysis].[75]

Several authors have highlighted deficiencies in previous studies in the identification of probiotic strains and confirmation of the viability of organisms following storage and at the point of delivery. The organisms in this study are deposited in an established repository (the NCIMB, UK). Confirmation of viability, and also identity according to the random allocation sequence, was undertaken in capsules collected from the point of use.

Overall, compliance with the IMPs was good, with over half of the participants reporting completion of the full 21-day course. The similarity in compliance in the two study arms and the lack of interaction between compliance and probiotic effect in covariate analysis suggests that the absence of probiotic effect was not due to poor compliance.

Frequency of Clostridium difficile *diarrhoea*

A major limitation of our study was the uncommon occurrence of CDD, and this prevented a reliable assessment of the efficacy of the probiotic. This occurred despite recruiting patients from general medicine, elderly care and nephrology wards that are known to have the highest CDD rates.[52] In line with Department of Health recommendations,[39] all of the hospitals in this study actively implemented measures to prevent and control CDI during the period of the trial. These included antibiotic stewardship (especially reduced cephalosporin and broad-spectrum antibiotic use),[22,23] enhanced cleaning, use of chlorine-based cleaning agents, enhanced hand hygiene and improved recognition and isolation of patients with *C. difficile*.

The low frequency is in keeping with the marked fall in CDD in hospitals in the UK during the period of recruitment to this study. Mandatory reporting for patients aged > 65 years admitted to hospitals in Wales

with CDD identified 2744 cases from July 2008 to June 2009 and 1295 from April 2011 to March 2012 (a fall of 52.8%).[52] In ABMUHB, the annual number of cases fell from 344 to 229 (a fall of 33.4%) during the equivalent time periods. In patients aged > 65 years admitted by NHS Trusts in England, the total number of CDD cases fell from 20,191 in April 2009 to March 2010 to 13,836 in April 2011 to March 2012 (a fall of 31.5%).[76] The number of reported cases in CDDFT was 228 and 123, respectively (a fall of 46.1%).

Stool samples were not available to test for *C. difficile* toxins in 41.4% of the participants with diarrhoea in our study. As a result, some cases of CDD will not have been identified, and this weakens our estimate of probiotic efficacy. In practice, collection of stool samples was often difficult due to diarrhoea episodes of short duration (median duration of 2 days) and many occurring after hospital discharge. Where reported, the frequency of missing assessment for CDD has generally been lower in smaller trials (5–45%)[66] than in our pragmatic study. In view of the low sensitivity of enzyme-linked immunosorbent assay-based toxin assays in the diagnosis of CDD,[77–79] we undertook additional laboratory analyses in stored stool samples from participants with diarrhoea to try to increase the detection rate. These results will be reported in a separate publication.

Although CDI has been associated with single doses of cephalosporins,[80] the limited duration of exposure to antibiotics in some patients may have also reduced CDD frequency in our study. Although most participants in our study were exposed to antibiotics from two or more classes and 12.4% were exposed to fluoroquinolones, 9.1% received only a single antibiotic dose and a further 28.1% received treatment for ≤ 6 days. A recent retrospective review of patients aged ≥ 18 years identified the number of antibiotics prescribed, increasing cumulative dose, duration of treatment and exposure to fluoroquinolones as increasing the risk of CDD.[81] We also found that longer duration of exposure (≥ 8 days) was associated with an increased frequency of both AAD and CDD. However, the practical value of longer exposure to antibiotics as a risk factor for CDD may be limited as duration of treatment is often unpredictable when commencing antibiotic therapy. Importantly, to prevent a single case of CDD, the number of patients needed to treat with any novel preventative strategy will increase as other measures reduce the frequency of CDD. This needs to be considered in the clinical management of individual patients and also in the design of trials to assess probiotic effectiveness for this indication.

Most antibiotic classes have been associated with AAD with increased risk with broad-spectrum antibiotics such as aminopenicillins, coamoxiclav (amoxicillin and clavulanic acid), cephalosporins and clindamycin.[82] Despite differences in antibiotic prescribing, rates of AAD and CDD were similar across the centres. Antibiotic exposure was similar in the two study arms and exposure to the two main antibiotic classes, penicillins and cephalosporins, did not modulate probiotic effect. We plan to undertake more detailed analysis to identify specific antibiotic treatment regimens that may increase the risk of AAD.

Serious adverse events

The daily follow-up during hospital stay and weekly telephone calls after discharge provided opportunities to identify SAEs. SAEs were expected to occur commonly in this vulnerable population and one or more SAE occurred in about one in five participants. Based on mortality data for the 3 months after hospital admission for patients aged ≥ 65 years in England over the period 2004–7, we had expected an overall mortality rate of 9.1% (Professor David Ford, Swansea University, March 2009, personal communication). The lower mortality rate in our study (4.9%) probably results from the exclusion of severely unwell patients. The frequency and severity of all SAEs, and those that resulted in the temporary or permanent withdrawal of the IMPs, were broadly similar in the two study arms. No SAE was attributed to the participant's involvement in the study. The probiotic used in this study was not associated with adverse events in a previous study of 52 adults with irritable bowel syndrome.[83] Our findings are consistent with recent systematic reviews of probiotic safety in humans,[84] including a recent meta-analysis of 208 RCTs where mainly single strains or mixtures of *Lactobacillus* sp. and *Bifidobacterium* sp. administered to medium-risk and critically ill patients were not associated with increased adverse events, including gastrointestinal disorders and infections.[26]

Quality of life

The application of SF-12 v2, a generic tool to evaluate health-related quality of life (HRQL), to specific gastrointestinal disorders is unclear. Erminia *et al.* were unable to detect differences in SF-12 v2 scores between patients with and without lactose intolerance.[85] A systematic review that included four studies of people with irritable bowel syndrome reported that despite improvements in symptoms in the probiotic group compared with controls, little improvement was seen in HRQL.[86] However, Koloski *et al*.[87] reported poor HRQL in patients with functional gastrointestinal disorders compared with healthy controls. We found that, overall, both SF-12 v2 and EQ-5D scores improved during follow-up, which was consistent with improved HRQL as patients responded to treatment. However, administration of the probiotic did not result in a significant improvement in HRQL. We are not aware of any studies that have examined the HRQL effects of probiotics on diarrhoea.

Health economic analysis

In our study, the probiotic was not effective in preventing AAD and the total health-care costs were very similar in both arms, indicating that the probiotic had virtually no budgetary impact. Furthermore, based on a non-significant difference in QoL as assessed by EQ-5D index values with very similar data ranges in both arms, the cost–utility analysis revealed an ICER of £189,662 per QALY gained, consistent with an absence of cost-effectiveness of probiotics in regards to patient QoL. The main limitation of the cost-effectiveness analysis was the relatively low number of diarrhoea cases (especially CDD), which limited the potential to detect cost differences between the study arms.

Although the need for rigorous economic evaluation of preventative therapies for AAD has been recognised,[88,89] to our knowledge no formal cost-effectiveness evaluations of probiotics have been undertaken in appropriately powered RCTs. In contrast with our findings, Kamdeu Fansi *et al*.[90] undertook a cost–consequences analysis based on a decision tree model with a short-term horizon (3 weeks follow-up) and concluded that substantial cost savings could be achieved by the routine use of probiotics due to a significant reduction in AAD incidence and total health-care cost per patient. Their model was based on a single trial of 225 participants divided into three trial arms (two different *Lactobacillus* formulations compared with no prophylactic intervention), patient QoL was not considered and costs included in the total cost were restricted to those of microbiological testing, the probiotic preparation, antibiotics and hospital stay (which was based on data from previously published studies and assumptions). In a pilot study, probiotics were cost-effective based on the relatively low implementation cost and a lower frequency of AAD in the placebo arm.[91] However, the sample size was small ($n = 23$) and no formal cost-effectiveness analysis was undertaken. Therefore, both of these cost-effectiveness estimates have severe limitations.

In our study, AAD resulted in an average increased duration of hospital stay of 5.58 days and an average incremental total cost of £4531.36. These findings correspond well with those of previous studies, in which CDD resulted in increased length of hospital stay of 3–21 days[13,16,17,20,92,93] and was associated with additional total health-care costs of between £3101 and £6195 converted to pounds sterling, correct in 2011.[13,14,16,17] In our study, no SAEs that required additional health-care resources were attributed to the probiotics, which is in line with reports from the published literature.[94,95]

Despite only moderate evidence of clinical effectiveness, some authorities have recommended that the use of probiotics is justified for the prevention of AAD[96] and CDD.[67] The modest effect of probiotics has also led to the recommendation that they should be considered a supplement rather than a replacement for conventional therapy.[97] In our study, the similar diarrhoea and QoL outcomes in the two study arms and lack of cost-effectiveness of the probiotic preparation lead us to recommend that further research is needed before a specific probiotic preparation can be recommended for a specific population group.

Clinical implications

- The high-dose, multistrain preparation of lactobacilli and bifidobacteria evaluated in our study is unlikely to benefit unselected older inpatients exposed to antibiotics.
- Clinical judgement regarding the benefits and risks of novel interventions to prevent AAD needs to take account of the impact of other preventative measures, such as antibiotic stewardship, on disease frequency.
- The administration of additional medications to vulnerable older people, many of whom are already taking multiple medications, may not be well tolerated in practice.
- The clinical effectiveness of our preparation in preventing CDD was unclear. However, even if it is effective, the falling prevalence of CDD needs more patients to take the probiotic to prevent a single case.
- The probiotic preparation was not associated with SAEs in our study. However, surveillance for potentially uncommon adverse events is required in future studies.

Research implications

- A better understanding of the multiple potential mechanisms underlying AAD and CDD, how these may vary in specific populations and the strain-specific effects of probiotics is needed before further clinical trials of specific probiotic preparations are undertaken. Further research to identify populations at increased risk of AAD and CDD is needed to facilitate the future evaluation of probiotic interventions.
- The design of studies to evaluate the efficacy of alternative probiotics in the prevention of CDD needs to consider the effect of other measures that have reduced the frequency of CDI in some health-care institutions.
- Further research into the effect of probiotics on patient QoL will be necessary to better determine patient benefit and cost-effectiveness.

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Professor Stephen J Allen wrote the original protocol, was the chief investigator, oversaw the study, wrote the initial draft of the main report and contributed to the final report.

Ms Kathie Wareham was a co-applicant and refined the protocol, was the trial manager and contributed to the final report.

Dr Duolao Wang was a co-applicant and refined the protocol, wrote the statistical analysis plan and undertook the statistical analysis and contributed to the final report.

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Appendix 1 Regulatory approvals

Competent authority approval, MHRA. Approval granted on 8 October 2008.

Medical REC for Wales, Sixth Floor Churchill House, 17 Churchill Way, Cardiff, CF10 2TW. Approval granted on 27 November 2008.

Singleton, Morriston and Princess of Wales hospitals; Research and Development Department, ABMUHB (change of name from Abertawe Bro Morgannwg NHS Trust on 1 October 2009), Swansea, SA6 6NL. Approval granted on 11 September 2008.

County Durham and Tees Valley 2 REC. Approval granted on 17 June 2008.

Research and Development Department, Darlington Memorial Hospital, Hollyhurst Road, Darlington, DL3 6HX. Approval granted on 5 December 2008.

Appendix 2 Details of wards involved in the recruitment of patients by centre

Specialty	Morriston Hospital (*n* = 23)	Singleton Hospital (*n* = 8)	Princess of Wales Hospital (*n* = 13)	Darlington Memorial Hospital (*n* = 12)	University Hospital of North Durham (*n* = 11)
Medical specialties					
General medicine	✓	✓ × 2	–	–	✓
Medical admissions	✓	–	✓	✓	✓
Respiratory/general medicine	✓	–	✓	✓	✓
Elderly care/general medicine	–	✓	✓ × 2	✓	✓
Stroke/general medicine	–	✓	–	✓	✓
Elderly care/stroke medicine	✓	–	✓	–	✓
Cardiology/general medicine	✓	✓	✓	✓	✓
Cardiology	✓	–	–	–	–
Haematology/general medicine	–	–	–	✓	✓
Hepatology/gastroenterology/general medicine	–	–	–	✓	–
Gastroenterology/general medicine	✓	–	✓	–	–
Renal medicine	✓	–	–	–	–
Neurology/general medicine	✓	–	–	–	–
Total number of medical wards	9	5	7	7	8
Surgical specialties					
Surgical admissions	✓	–	–	–	–
Male genitourinary	✓	–	–	–	✓
Female genitourinary and gynaecology	✓	✓	✓	–	✓
Orthopaedic	✓ × 3	–	✓ × 2	✓ × 2	✓
General surgery	✓	✓ × 2	✓ × 2	✓ × 2	–
Colorectal surgery	✓	–	–	–	–
Ear, nose and throat/general surgery	–	–	✓	✓	–
Neurosurgery	✓	–	–	–	–
Burns	✓	–	–	–	–
Burns and plastic surgery	✓ × 2	–	–	–	–
Maxillary facial surgery/plastic surgery	✓	–	–	–	–
Cardiac surgery	✓	–	–	–	–
Total number of surgical wards	14	3	6	5	3

Appendix 3 Patient information sheet, consent form, advocate information sheet and advocate assent form

Patient information sheet

The PLACIDE study

Probiotic **L**actic acid bacteria and **A**ntibiotic-associated and ***C****. **di**fficile* **d**iarrhoea in the **E**lderly

Patient information sheet

Full title of the study*:* A multicentre, randomised, placebo controlled trial of lactic acid bacteria in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in patients aged 65 years and over admitted to hospital and receiving antibiotics.

Part 1

You are invited to participate in a clinical study. Before agreeing to participate in this study it is important that you read and understand why the research is being done and what will happen during it. Please take time to read this information carefully. Take time to ask as many questions as you want. The study personnel will explain any word or information you do not clearly understand. You may to talk to others about the study if you wish.

Part 1 tells you the purpose of the research and explains what will happen to you if take part.

Part 2 gives you more information about the conduct of the study if you are still interested after reading Part 1.

1. What is the purpose of the study?

We want to find out whether or not giving a probiotic food supplement prevents the diarrhoea that often affects people taking antibiotics. Probiotics are the safe, live, 'friendly' bacteria that are found in live yoghurts that you can buy in supermarkets.

Many people admitted to hospital require treatment with antibiotics. Antibiotics change the 'healthy' bacteria that live in the gut and this results in diarrhoea in about 1 in 5 people. Diarrhoea is distressing for patients and may also delay recovery from illness and prolong the hospital admission.

Occasionally, antibiotics result in an overgrowth of a potentially dangerous bacterium called '*C. difficile*'. This is also known as 'C. diff' and is talked about a lot in the press. This can cause a severe and life-threatening diarrhoeal illness that may require additional medical or surgical treatment. The clinicians working in the hospitals involved in this study perceive *C. difficile* diarrhoea as a very important problem. This underlies our interest to search for new ways to prevent this problem.

2. Why have I been chosen?

You have been approached because you fulfil the entry criteria for the study. These criteria are:

- aged 65 years or more and have been admitted to hospital
- already taking antibiotics or are about to start antibiotic treatment
- do not have diarrhoea at the moment
- have not had an adverse reaction to a probiotic food supplement in the past
- do not have an impaired immune system, an artificial heart valve or active inflammatory bowel disease.

The study is being conducted in Wales and in Durham and we hope to recruit around 2500 patients.

3. Do I have to take part?

It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw consent from the study at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

4. What will happen to me if I take part?

The doctors will treat the illness as usual, including treatment with antibiotics. If you develop diarrhoea, the nurses and doctors will take stool samples to investigate the cause and treat it in the usual way.

We will ask you to take a small amount of powder by mouth **once daily for 21 days**. You will have an equal chance of receiving either the probiotic food supplement (the active intervention) or an inactive substance (the placebo). We will also ask you to answer questions about any symptoms you may have (diarrhoea, abdominal pain, bloating, flatus, nausea) and also general questions that assess the impact of your illness on your quality of life.

We will keep in-touch with patients to check for these symptoms for 8 weeks after you have finished the course of antibiotics. Our research nurses will visit you in hospital or telephone or visit you at home if discharged from hospital.

5. Expenses and payments?

Joining the trial will not result in any expenses for you. None of the participants will receive any payment.

6. What do I have to do?

Once you have consented to join the trial, you will be asked to give information to our research nurses about any symptoms you have, including diarrhoea, and also answer some questions that assess how your quality of life is affected. You should take the trial intervention as directed together with other medications as prescribed by the doctor. Please note that it is important that the participant avoids taking any probiotic preparations (other than the trial intervention) or live yogurts during the study. The research nurse can give you more guidance on what products to avoid if needed. Please inform the study nurse if any of your medications change during the study period.

7. What is the drug that is being tested?

We are testing a probiotic food supplement and this is not a drug. The supplement consists of lactobacilli and bifidobacteria that are just like the bacteria that live in the bowels of healthy people. The bacteria are *Lactobacillus acidophilus* (2 strains: CUL60 and CUL21), *Bifidobacterium bifidum* (CUL20) and *Bifidobacterium lactis* (CUL34).

People in the placebo group will receive an inactive, inert powder called maltodextrin.

8. What are the alternative procedures or treatments?

There are no other ways for people to protect themselves against antibiotic-associated and *C. difficile* diarrhoea. The hospitals are trying their best to reduce these problems for all patients by improving hygiene and also being careful with using antibiotics.

9. What are the side effects of treatment?

Probiotics are very safe and we do not anticipate any side effects. There are a small number of case reports where probiotics may themselves have caused infections in people with markedly impaired immune systems or an artificial heart valve. We will not include such people in our study.

10. What are the other possible disadvantages and risks of taking part?
We are not aware of any disadvantages or risks of joining our study.

11. What are the possible benefits of taking part?
All patients in the study, including those in the placebo group, will benefit from regular follow-up for diarrhoea and other symptoms. This may improve the recognition of problems and overall care for all participants.

Participants who receive the active intervention may have a reduced risk of developing antibiotic-associated and *C. difficile* diarrhoea or may develop milder disease. If the food supplement proves to be successful against *C. difficile* diarrhoea, fewer cases will reduce the risk of other patients acquiring this infection.

12. What happens when the research stops?
Patients will leave the trial at the end of the 8 weeks follow-up. If you require any further treatment in respect of diarrhoea this will be under the care of the GP or hospital doctor.

We plan to publicise the findings of the study widely in the medical literature and local press. Depending on the results, the NHS may recommend probiotic food supplements as part of routine care for older people receiving antibiotics in hospitals.

13. What if there is a problem?
We do not expect any problems related to the study because probiotics are very safe. If you, or your doctor feels that the trial intervention is causing you to have an adverse effect then it will be stopped and any necessary treatment instigated.

14. Will my taking part in the study be kept confidential?
Yes. The information about you will only be known to members of the research team. All information about you will be held using a unique research number so that you cannot be identified in any results, publications or publicity related to the study.

15. Contact details?
Please note the name and phone number of your research nurse. You can contact this person at any time should you have any questions about the study.

Nurse …… Telephone number……

If you (your representative) have any questions as to your rights as a research subject you may contact:

Study team…… Telephone number……

If you have further questions about this study or your participation, or if during your participation you experience a study related injury, or any side effect should occur between the nurse visits/telephone calls, please feel free to contact the nurse for further information and or for action to be taken.

This completes Part 1 of the Information Sheet

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

16. What if relevant new information becomes available?
Sometimes during the course of a study, new information becomes available about the intervention being studied. If this happens, the doctor will tell you about it and discuss whether or not you wish to continue

in the study. If you wish to withdraw from the study, the study doctor will make arrangements for your continued care. If you decide to continue in the study, you may be asked to sign an updated consent form containing the new information. Both the researchers and an independent committee of experts will be looking-out for any new information. If the study is stopped for any reason you will be told why and your continuing care will be arranged.

17. What will happen if I don't want to carry on with the study?

You are free to withdraw consent from the study without giving a reason at any time. This will not affect the medical care that you receive in any way.

18. What if there is a problem?

Complaints: If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure (or private institution). Details can be obtained from the hospital.

Harm: If you are harmed, due to participating in the study and or this is due to someone's negligence then you may have grounds for a legal action for compensation against the Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

19. Will my taking part in the study be kept confidential?

Yes. The information about you will only be known to members of the research team. No individuals will be identified in any results, publications or publicity related to the study.

We will ask your permission to inform your General Practitioner about your participation in the study.

20. What will happen to any samples given?

If you develop diarrhoea, your doctors or nurses will collect a stool sample in the usual way. This will be tested in the NHS laboratories to identify the cause of the diarrhoea. For quality control purposes, further testing to identify the strain of bacteria will be done in some of the stools samples that test positive for *C. difficile*.

21. Will any genetic tests be done?

Genetic tests may be done on the bacteria in stool samples but not on any of the participants. The genetic results will be coded so that the patient is not identifiable.

22. What will happen to the results of the research study?

We will inform the NHS Research and Development Department and publish the findings of the study in the medical literature and widely in the local press. Depending on the results, the NHS may recommend probiotic food supplements as part of routine care for older people receiving antibiotics in hospitals. You will not be identified in any reports or publications.

23. Who is organising and funding this research?

The project is organised and carried-out by the research teams in Swansea University and NHS Trust and County Durham and Darlington Foundation Trust. The NHS Research and Development Health Technology Association are providing funding.

24. Who has reviewed the study?

The NHS Research and Development Health Technology Association has reviewed the study. It has also been given a favourable ethical opinion for conduct in the NHS by the South Wales Ethics Committee. The Medicines and Health Regulatory Authority have approved the probiotic food supplement.

Thank you for taking the time to read this patient information sheet.

Patient consent form

PATIENT CONSENT FORM

The PLACIDE study
Probiotic **L**actic acid bacteria and **A**ntibiotic-associated and **C** d**i**ff **D**iarrhoea in the **E**lderly

Name of Researcher: Dr S Allen

Please initial boxes

1. I confirm that I have read and understand the patient information sheet version 4 dated 29 April 2008 for the above study had have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that I am being invited to take part in a research study. I have not received any investigational drugs within the last four weeks and I am not taking part in any other research study at this time. I understand the risks and benefits, and I freely give my informed consent to participate in the research study described in this form, under the conditions stated in it. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical or legal rights being affected. ☐

4. I understand that relevant sections of my medical notes and data collected during the study may be examined by responsible individuals from the funding body or national, international regulatory authorities, or the NHS Trust where it is relevant to my taking part in the research. I give permission for these individuals to have access to my records. ☐

5. I give consent for my GP to be contacted and informed that I am participating in this study. ☐

6. I agree to take part in the above study. ☐

Name of patient	Date	Signature
Name of person taking consent	Date	Signature

(Copies: 1 for patient/1 hospital notes/1 research file)

Version 3
Date: 29 April 2008

Patient advocate information sheet

The PLACIDE study

Probiotic **L**actic acid bacteria and **A**ntibiotic-associated and ***C****. **di**fficile***d**iarrhoea in the **E**lderly

Patient representatives information sheet

Note: for persons being asked to consider inclusion of a patient who is unable them self to give informed consent (e.g. relative/friend/spouse) it is important that you consider all aspects of the study on their behalf and act in their best interest in respect of inclusion in the study.

Full title of the study*:* A multicentre, randomised, placebo controlled trial of lactic acid bacteria in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in patients aged 65 years and over admitted to hospital and receiving antibiotics.

Part 1

The patient you represent is invited to participate in a clinical study. Before agreeing to them participate in this study it is important that you read and understand why the research is being done and what will happen during it. Please take time to read this information carefully. Take time to ask as many questions as you want. The study personnel will explain any word or information you do not clearly understand. You may to talk to others about the study if you wish.

Part 1 tells you the purpose of the research and explains what will happen to the patient you represent if they take part.

Part 2 gives you more information about the conduct of the study if you are still interested after reading Part 1.

1. What is the purpose of the study?

We want to find out whether or not giving a probiotic food supplement prevents the diarrhoea that often affects people taking antibiotics. Probiotics are the safe, live, 'friendly' bacteria that are found in live yoghurts that you can buy in supermarkets.

Many people admitted to hospital require treatment with antibiotics. Antibiotics change the 'healthy' bacteria that live in the gut and this results in diarrhoea in about 1 in 5 people. Diarrhoea is distressing for patients and may also delay recovery from illness and prolong the hospital admission.

Occasionally, antibiotics result in an overgrowth of a potentially dangerous bacterium called '*C. difficile*'. This is also known as 'C. diff' and is talked about a lot in the press. This can cause a severe and life-threatening diarrhoeal illness that may require additional medical or surgical treatment. The clinicians working in the hospitals involved in this study perceive *C. difficile* diarrhoea as a very important problem. This underlies our interest to search for new ways to prevent this problem.

2. Why has the patient I represent been chosen?

The patient has been approached because he/she fulfils the entry criteria for the study. These criteria are:

- aged 65 years or more and have been admitted to hospital
- already taking antibiotics or are about to start antibiotic treatment
- do not have diarrhoea at the moment
- have not had an adverse reaction to a probiotic food supplement in the past
- do not have an impaired immune system, an artificial heart valve or active inflammatory bowel disease.

The study is being conducted in Wales and in Durham and we hope to recruit around 2,500 patients.

3. Does the patient have to take part?

It is up to you to decide whether or not the patient you represent can participate. If you do, you will be given this information sheet to keep and be asked to sign an assent form giving consent on behalf of the patient. As the patient's representative you are still free to withdraw assent from the study at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care the patient receives.

4. What will happen to the patient I represent if they take part?

The doctors will treat the illness as usual, including treatment with antibiotics. If the patient represent develops diarrhoea, the nurses and doctors will take stool samples to investigate the cause and treat it in the usual way.

We will ask the patient to take a small amount of powder by mouth **once daily for 21 days**. He/she will have an equal chance of receiving either the probiotic food supplement (the active intervention) or an inactive substance (the placebo). We will also ask you or the patient to answer questions about any symptoms they may have (diarrhoea, abdominal pain, bloating, flatus, nausea) and also general questions that assess the impact of their illness on their quality of life.

We will keep in-touch with patients to check for these symptoms for 8 weeks after the patient has finished the course of antibiotics. Our research nurses will visit the patient in hospital or telephone or visit you him/her at home if discharged from hospital.

5. Expenses and payments?

Joining the trial will not result in any expenses for the patient. None of the participants will receive any payment.

6. What do I have to do?

Once you have given assented on behalf of the patient to join the trial, we will ask the patient or you on his/her behalf to give information to our research nurses about any symptoms the patient has, including diarrhoea, and also answer some questions that assess how their quality of life is affected. The patient should take the trial intervention as directed together with other medications as prescribed by the doctor. Please note that it is important that the participant avoids taking any probiotic preparations (other than the trial intervention) or live yogurts during the study. The research nurse can give you more guidance on what products to avoid if needed. Please inform the study nurse if any of the patient's medications change during the study period. While in hospital the research nurse will be able to confirm all medicines that are prescribed.

7. What is the drug that is being tested?

We are testing a probiotic food supplement and this is not a drug. The supplement consists of lactobacilli and bifidobacteria, which are just like the bacteria that live in the bowels of healthy people. The bacteria are *Lactobacillus acidophilus* (2 strains: CUL60 and CUL21), *Bifidobacterium bifidum* (CUL20) and *Bifidobacterium lactis* (CUL34).

People in the placebo group will receive an inactive, inert powder called maltodextrin.

8. What are the alternative procedures or treatments?

There are no other ways for people to protect themselves against antibiotic-associated and *C. difficile* diarrhoea. The hospitals are trying their best to reduce these problems for all patients by improving hygiene and also being careful with using antibiotics.

9. What are the side effects of treatment?

Probiotics are very safe and we do not anticipate any side effects. There are a small number of case reports where probiotics may themselves have caused infections in people with markedly impaired immune systems or an artificial heart valve. We will not include such people in our study.

10. What are the other possible disadvantages and risks of taking part?

We are not aware of any disadvantages or risks of joining our study.

11. What are the possible benefits of taking part?

All patients in the study, including those in the placebo group, will benefit from regular follow-up for diarrhoea and other symptoms. This may improve the recognition of problems and overall care for all participants.

Participants who receive the active intervention may have a reduced risk of developing antibiotic-associated and *C. difficile* diarrhoea or may develop milder disease. If the food supplement proves to be successful against *C. difficile* diarrhoea, fewer cases will reduce the risk of other patients acquiring this infection.

12. What happens when the research stops?

Patients will leave the trial at the end of the 8 weeks follow-up. If the patient you represent requires any further treatment in respect of diarrhoea this will be under the care of the GP or hospital doctor.

We plan to publicise the findings of the study widely in the medical literature and local press. Depending on the results, the NHS may recommend probiotic food supplements as part of routine care for older people receiving antibiotics in hospitals.

13. What if there is a problem?

We do not expect any problems related to the study because probiotics are very safe. If the patient you represent, or your doctor feels that the trial intervention is causing you to have an adverse effect then it will be stopped and any necessary treatment instigated.

14. Will my taking part in the study be kept confidential?

Yes. The information about the patient you represent will only be known to members of the research team. All information about him/her will be held using a unique research number so that they cannot be identified in any results, publications or publicity related to the study.

15. Contact details?

Please note the name and phone number of the patient's research nurse. You can contact this person at any time should you have any questions about the study.

Nurse …… Telephone number……

If you have any questions as to the rights of a research subject you may contact:

Study team…… Telephone number……

If you have further questions about this study or the patient's participation, or if during you're their participation they experience a study related injury, or any side effect should occur between the nurse visits/telephone calls, please feel free to contact the nurse for further information and or for action to be taken.

This completes Part 1 of the Information Sheet

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

16. What if relevant new information becomes available?

Sometimes during the course of a study, new information becomes available about the intervention being studied. If this happens, the doctor will tell you about it and discuss whether or not you wish the patient to continue in the study. If you wish them to be withdrawn from the study, the study doctor will make arrangements for their continued care. If you allow them to continue in the study, you may be asked to sign an updated assent form containing the new information. Both the researchers and an independent committee of experts will be looking-out for any new information. If the study is stopped for any reason you will be told why and the patient's continuing care will be arranged.

17. What will happen if I/the patient you represent don't/doesn't want to carry on with the study?

You are free to withdraw assent for the study without giving a reason at any time. This will not affect the medical care that the patient receives in any way.

18. What if there is a problem?

Complaints: If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS complaints procedure (or private institution). Details can be obtained from the hospital.

Harm: If the patient you represent is harmed, due to participating in the study and or this is due to someone's negligence then you may have grounds for a legal action for compensation against the Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

19. Will my taking part in the study be kept confidential?

Yes. The information about the patient will only be known to members of the research team. No individuals will be identified in any results, publications or publicity related to the study.

We will ask your permission to inform the patient's General Practitioner about (their) participation in the study.

20. What will happen to any samples given?

If the patient develops diarrhoea, the doctors or nurses will collect a stool sample in the usual way. This will be tested in the NHS laboratories to identify the cause of the diarrhoea. For quality control purposes, further testing to identify the strain of bacteria will be done in some of the stools samples that test positive for *C. difficile*.

21. Will any genetic tests be done?

Genetic tests may be done on the bacteria in stool samples but not on any of the participants. The genetic results will be coded so that the patient is not identifiable.

22. What will happen to the results of the research study?

We will inform the NHS Research and Development Department and publish the findings of the study in the medical literature and widely in the local press. Depending on the results, the NHS may recommend probiotic food supplements as part of routine care for older people receiving antibiotics in hospitals. The patient you represent will not be identified in any reports or publications.

23. Who is organising and funding this research?

The project is organised and carried-out by the research teams in Swansea University and NHS Trust and County Durham and Darlington Foundation Trust. The NHS Research and Development Health Technology Association are providing funding.

24. Who has reviewed the study?

The NHS Research and Development Health Technology Association has reviewed the study. It has also been given a favourable ethical opinion for conduct in the NHS by the South Wales Ethics Committee. The Medicines and Health Regulatory Authority have approved the probiotic food supplement.

Thank you for taking the time to read this patient information sheet.

Patient advocate assent form

PATIENT ASSENT FORM
(completion by patient representative)
The PLACIDE study
Probiotic **L**actic acid bacteria and **A**ntibiotic-associated and **C** diff **D**iarrhoea in the **E**lderly

Name of Researcher: Dr S Allen

Please initial boxes

1. I confirm that I have read and understand the patient information sheet version 4 dated 29 April 2008 for the above study had have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my relative, friend, spouse is being invited to take part in a research study. To the best of my knowledge they have not received any investigational drugs within the last four weeks and they are not taking part in any other research study at this time. I understand the risks and benefits, and I freely give my informed assent for my relative, friend, spouse to participate in the research study described in this form, under the conditions stated in it. ☐

3. I understand that their participation is voluntary and that I can withdraw this assent at any time, without giving any reason and without their medical or legal rights being affected. ☐

4. I understand that relevant sections of my relatives/friend/spouse's medical notes and data collected during the study may be examined by responsible individuals from the funding body or national, international regulatory authorities, or the NHS Trust where it is relevant to their taking part in the research. I give permission for these individuals to have access to such records. ☐

5. I give consent for their GP to be contacted and informed that they are participating in this study. ☐

6. I agree to my relative/friend/spouse taking part in the above study. ☐

_____ _____ _____
Name of person giving assent Date Signature

Relationship to patient.

_____ _____ _____
Name of person taking consent Date Signature

(Copies 1 for patient/assenter/1 hospital notes/1 research file)

Version 3
Date: 29 April 2008

Appendix 4 Data collection forms

Case report form

PLACIDE study *Research number:* | | | | | *Patient initials:* |

PLACIDE study Case Report Form (CRF)

A multicentre, randomised, placebo controlled trial of lactic acid bacteria and bifidobacteria in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in patients aged 65 years and over admitted to hospital and receiving antibiotics.

Front sheet: Check id details on each page and completeness of all data. Detach pages 1 and 2 and file in register before CRF goes to data entry clerk.

Study number issued at recruitment **:** **PLACIDE**

While in the study the participant will be identified by this study number

Is the patient eligible? check inclusion / exclusion criteria (page 2) Y/N

Patient details

Name

Hospital number Age

Address and post code

Telephone:

Home:	Work:
Mobile:	E mail:

Details of next of kin

Name

Relationship: Spouse-1; Son/daughter-2; Relative-3; Carer-4; Other-5

Address and post code

Telephone:

GP details

Name

Address and post Code

Telephone:

Date completed / / signed 1

PLACIDE study *Research number:* ☐☐☐☐ *Patient initials:* ☐

Inclusion criteria*: code as Y / N

- Age ≥ 65 years ☐
- One or more doses of antibiotic within last 7 days or starting antibiotic today ☐
- Consultant approval for invitation to join study ☐

Exclude if "No" to any of these questions

Exclusion criteria*: code as Y / N

- Diarrhoea now[1] ☐
- Immunocompromised[2] ☐
- Active inflammatory bowel disease[3] ☐
- Prosthetic heart valve ☐
- Suspected acute pancreatitis[4] ☐
- Requires high dependency or intensive care[5] ☐
- Known compromised gut blood supply[6] ☐
- Naso-jejunal feeding tube *in situ* ☐
- Adverse reaction to previous probiotics ☐
- Continues on other live bacterial food preparations ☐
- *C. difficile* in past 3 months ☐

Exclude if "Yes" to any of these

1. 3 or more watery or loose stools (Bristol Stool Chart 5 - 7) in a 24 hour period
2. Compromised immunity sufficient to require isolation and barrier nursing (e.g. disseminated cancer / chemotherapy, AIDS, known immunodeficiency disease)
3. Required specific treatment in past 12 months
4. Abdominal pain + serum amylase/lipase >3 ULN
5. Not admission just for observation (e.g. post cardiac surgery)
6. Disease, stenosis or thrombosis of mesenteric vessels or coeliac axis

***If in doubt, discuss with Project Manager or Research Clinician *before* recruitment**

Date completed ☐☐ / ☐☐ / ☐☐ signed ☐ 2

PLACIDE study *Research number:* ☐☐☐☐ *Patient initials:* ☐☐

Data entry clerks: Enter the following data into the PLACIDE CRF database

Date person recruited into PLACIDE study ☐☐ / ☐☐ / ☐☐

If assent, relationship to patient: _____(see previous)

Demography

Gender: Male-1; Female-2 ☐

DOB ☐☐ / ☐☐ / ☐☐

Race: White-1; Black-2; Asian-3; Chinese-4; Other-5 and specify: ☐

Weight (kg) ☐☐☐ . ☐

Average number cigarettes / day ☐☐

Average number units alcohol / week* ☐☐

*Approx units: 1 pint beer – 2; 1 standard glass wine – 2; small measure spirits – 1.

This admission

Date of hospital admission ☐☐ / ☐☐ / ☐☐

Initial diagnosis / reason for admission

Admitted from home-1, residential care-2, other hospital-3, other -4 ☐

If other, details: ……..

Previous gastrointestinal surgery? Y / N ☐

If Yes – specify operation(s) done and year performed:

Co- morbidity (Y / N or 9 if not known):

Hypertension ☐ Asthma ☐ Diabetes ☐

COPD ☐ Renal Disease ☐ Irritable bowel syndrome ☐

Dementia or Alzheimer's disease ☐ Other co-morbidity* ☐

*If Yes - specify:

Date completed ☐☐ / ☐☐ / ☐☐ signed 3

PLACIDE study *Research number:* ☐☐☐☐ *Patient initials:* ☐

Other information

Current stool frequency / week ☐☐ stool consistency (Bristol chart) ☐

No. hospital admissions in last 8 weeks ☐ NGT *in situ* : Y / N ☐

Live bacteria consumed in last 2 weeks* ☐

* includes live yogurts (e.g. Actimil, Yakult), probiotics bought from health food shops, over internet

STUDY intervention

Day 1 is the first day that the person *had the opportunity* to take the study intervention and is either the same day as the recruitment day or the next day.

Day 1 date ☐☐ / ☐☐ / ☐☐

Start time of study intervention (nearest hour; 24 hour clock)* ☐☐

*record 99 if not known; 88 if not taken

End of study summary : Complete at the end of follow-up

Final classification of participant ☐

No diarrhoea-1; AAD-2; *C. difficile* diarrhoea-3; other cause of diarrhoea-4; died-5; withdrawn-6.

Outcome date (death, withdrawal, end of FU): ☐☐ ☐☐ ☐☐

Date of hospital discharge: ☐☐ / ☐☐ / ☐☐

Discharge and outcome dates same if participant dies or is withdrawn in hospital

If withdrawn: ☐

High dependency or ITU care-1; pancreatitis-2; bowel ischaemia-3; other-4 and details:

Notes:

Please check that all data is accurate and complete. Then submit form to Data Manager for checking prior to data entry

Date completed ☐☐ / ☐☐ / ☐☐ signed ☐ 4

PLACIDE study *Research number:* ☐☐☐☐ *Patient initials:* ☐

Medication record: Antibiotics

Record details of any antibiotics (including single doses) that the person has taken in the 8 weeks before recruitment until the end of follow-up.

- Record as much information as possible (e.g. "middle November" = 15/11/xx)
- Route: Oral/NGT = 1; IV/IM = 2; other = 3
- Record dates as DD/MM/YY; record 88/88/88 if on-going at end of follow-up; 99/99/99 if not known
- Check that the information is complete at the end of follow-up

Antibiotic 1: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic 2: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic 3: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic 4: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic 5: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic 6: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Date completed ☐☐ / ☐☐ / ☐☐ signed ☐ 5

PLACIDE study *Research number:* ☐☐☐☐ *Patient initials:* ☐

Antibiotic 7: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic 8: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic 9: ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Medication record: other drugs being taken at recruitment

Enter Y, N or 9 for don't know

- Proton Pump inhibitor[1] ☐
- Antacids ☐
- Anti-hypertensives ☐
- Oral hypoglycaemic agents ☐
- Insulin ☐
- H_2 blockers[2] ☐
- ACE inhibitors ☐
- Aspirin (used most days) ☐
- NSAIDS (used most days) ☐
- *Commercial feed + prebiotic ☐

*If Y, name:

1. Examples are: lanzoprazole, omeperazole.
2. Examples are: ranitidine, cimetidine, zantac.

Date completed ☐☐ / ☐☐ / ☐☐ signed ☐ 6

Daily follow-up log

PLACIDE study *Research number:* | | | | | *Patient initials:* |

Daily follow-up log for participants during hospital admission

- Complete daily and ask about the preceding 24 hours
- Day # 01 is the first day that the person had the opportunity to take the trial intervention (TI)
- At the end of each week, transfer **total** to the weekly FU form. If too many days "don't know", enter "9" on weekly sheet
- If new episode of diarrhoea, also complete a diarrhoea record sheet
- Document any changes to medication on medication record

Date							
Responder							
Location							

Code symptoms / TI as Y, N or 9 – don't know:								**Total Y**
Diarrhoea								
Noct diarr								
Faec incont								
Tenesmus								
Abdo pain								
Nausea								
Vomiting								
Bloating								
Flatus								
NGT								
Other (a)								

TI taken								
Problem with TI (b)								
Initials								

a) Details of other symptoms / problems:

b) Details of any problems taking trial intervention:

Notes:

File this sheet in the patient log (**not** for data entry)

signed 1

Weekly follow-up log

PLACIDE study *Research number:* ☐☐☐☐ *Patient initials:* ☐

Weekly follow-up after recruitment

- Complete on the same day each week until 8 weeks after stopping antibiotics to a maximum of 12 weeks. Ask about the preceding week
- Transfer data from daily log sheet for participants admitted to hospital
- If new episode of diarrhoea, also complete a diarrhoea record sheet

Week number: ☐☐ Date: ☐☐ / ☐☐ / ☐☐

Usual responder: Participant-1; Other-2 and give details: ☐

In the last week, number of days:

In hospital: ☐ At home / community: ☐ In other care facility: ☐

No. days that the person has experienced the following in the past week:
("0" for no symptom; "9" for don't know)

[a]Diarrhoea ☐	[a]Nocturnal diarrhoea ☐	Faecal incontinence ☐
Tenesmus ☐	Abdominal pain ☐	Nausea ☐
Vomiting ☐	Bloating ☐	Flatus ☐
	NGT in situ ☐	Other[b] ☐

[a]If ≥ 3 loose/watery stools/24 hours, complete **diarrhoea record sheet**

[b]If new problem or other symptom – specify:

Record any AEs on AE log

No. visits to GP / health facility for a new problem in the last week ☐

No. days trial intervention taken in last week (0 if after 21 days) ☐

Any problems with taking intervention? Y, N or 9 if not applicable ☐

If Yes – specify (e.g. caused nausea):

Note: Document any changes to medication on medication record

signed ☐ 1

Additional antibiotics form

PLACIDE study *Research number:* ☐☐☐☐ *Patient initials:* ☐

Use this sheet to record the details of additional antibiotics that the person has taken in the 8 weeks before recruitment until the end of follow-up. Label them beginning at "antibiotic 10".

Antibiotic…. ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic…. ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic…. ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic…. ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic…. ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic…. ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

Antibiotic…. ☐ Route ☐ Dose (mg) ☐ No. doses/day ☐

Indication: ☐

Start date ☐☐ / ☐☐ / ☐☐ Stop date: ☐☐ / ☐☐ / ☐☐

signed ☐ 1

Severe adverse events form

PLACIDE study *Research number:* | | | | | *Patient initials:* |

vers 4: 17th March 2009

Patient's age: | Clinical diagnosis |

Start date IMP | | / | | / | |

Date of onset of SAE | | | | | |

Date of end of SAE | | / | | / | |

Describe SAE – what has happened?

What is the most likely cause of this event (e.g. UTI, post-op complication)?

ICD10 code | | | . | |

Did the SAE require intervention? Yes -1; No -2 |

If Yes, describe intervention:

Study preparation discontinued? Yes -1; No -2 |

Final outcome regarding SAE |
(Full recovery -1; on-going disability/impairment -2; died -3)

Severity criteria (enter either Y or N – complete all boxes)

Life threatening		Significant disability	
Hospitalisation/ prolongation		Congenital anomaly/birth defect	
Important Medical Event		Death	

Form completed by (initials): |

Pass form to Trial Manager to complete the following:

Reported to: MHRA | MREC | R&D |

Date added to SAE database: | | / | | / | |

Diarrhoea sheet

PLACIDE study *Research number:* ☐☐☐☐ *Patient initials:* ☐

Complete a sheet for each new episode of diarrhoea occurring during participation in trial

- Diarrhoea defined as ≥ 3 loose stools (Bristol type 5, 6 or 7) in 24 hours
- New episode if occurs after at least 3 days of normal bowel habit for person

Where did the diarrhoea start? (hospital-1; home-2; other health facility-3): ☐

Date diarrhoea started: ☐☐ / ☐☐ / ☐☐

Date diarrhoea finished (88 if on-going at end of follow-up) ☐☐ / ☐☐ / ☐☐

No. stools/24 hrs (at worse): ☐☐ Stool consistency (at worse; Bristol type): ☐

Did the following symptoms occur? (Y, N or "9" for don't know)

Nausea	☐	Vomiting	☐	Bloating	☐	Flatus	☐
Abdominal pain	☐	Tenesmus	☐	Fever	☐	Faecal incontinence	☐
Nocturnal diarrhoea	☐	Urgency	☐	Blood in stools	☐	Mucus in stools	☐

Stool sample sent to microbiology lab? Y / N ☐

Date stool sample sent to lab: ☐☐ / ☐☐ / ☐☐

Infectious cause identified? (None-1; bacteria-2; virus-3; parasite-4; *C. difficile*-5) ☐

If pathogen isolated: details:

Management: (fluids only-1; anti-diarrhoeals-2; antibiotics-3; surgery-4; other-5) ☐

Where was the episode managed? (hospital-1; home-2; other health facility-3) ☐

What was the clinical diagnosis? ☐

AAD-1; *C. difficile*-2; bacterial-3; viral-4; parasite-5; other-6 and specify:

If *C. difficile*, classify severity as detailed overleaf ☐

Outcome of diarrhoea (patient survived-1; died-2) ☐

Notes (e.g. additional investigations performed; date of death):

signed ☐ 1

Classification of severity of *Clostridium difficile* diarrhoea

PLACIDE trial

Classification of patients with confirmed *C. difficile* diarrhoea (CDAD) during follow-up

Record the following information from the lab records and clinical notes for patients who developed CDAD. Where there are several reports for an individual patient, record the *most severe findings* (e.g. highest temperature, highest WCC, lowest BP) that occurred during the period that the patient had diarrhoea.

Participant initials: [ ] PLACIDE ID number: [ ]

	Date of test or examination DD/MM/YY	Worst result
Blood tests		
Total white cell count (WCC or WBC)		x 10^9/L
Creatinine		µmol/L
Findings on clinical examination		
Temperature		°C
Abdomen:		
Distended?		Y / N / not recorded
Tender to palpation?		Y / N / not recorded
Peritonitis (hard abdomen; rebound tenderness)		Y / N / not recorded
Ileus (silent abdomen; absence of bowel sounds)		Y / N / not recorded
Toxic megacolon?		Y / N / not recorded

Radiology

AXR or CT scan done? Y / N / not recorded

If Yes; enter date and report:

Other investigations

Flexible sigmoidoscopy or colonoscopy done? Y / N / not recorded

If Yes; enter date and report:

How was the CDAD treated?

Metronidazole Y / N / not recorded

Vancomycin Y / N / not recorded

Surgery (colostomy, colectomy etc.)? Y / N / not recorded

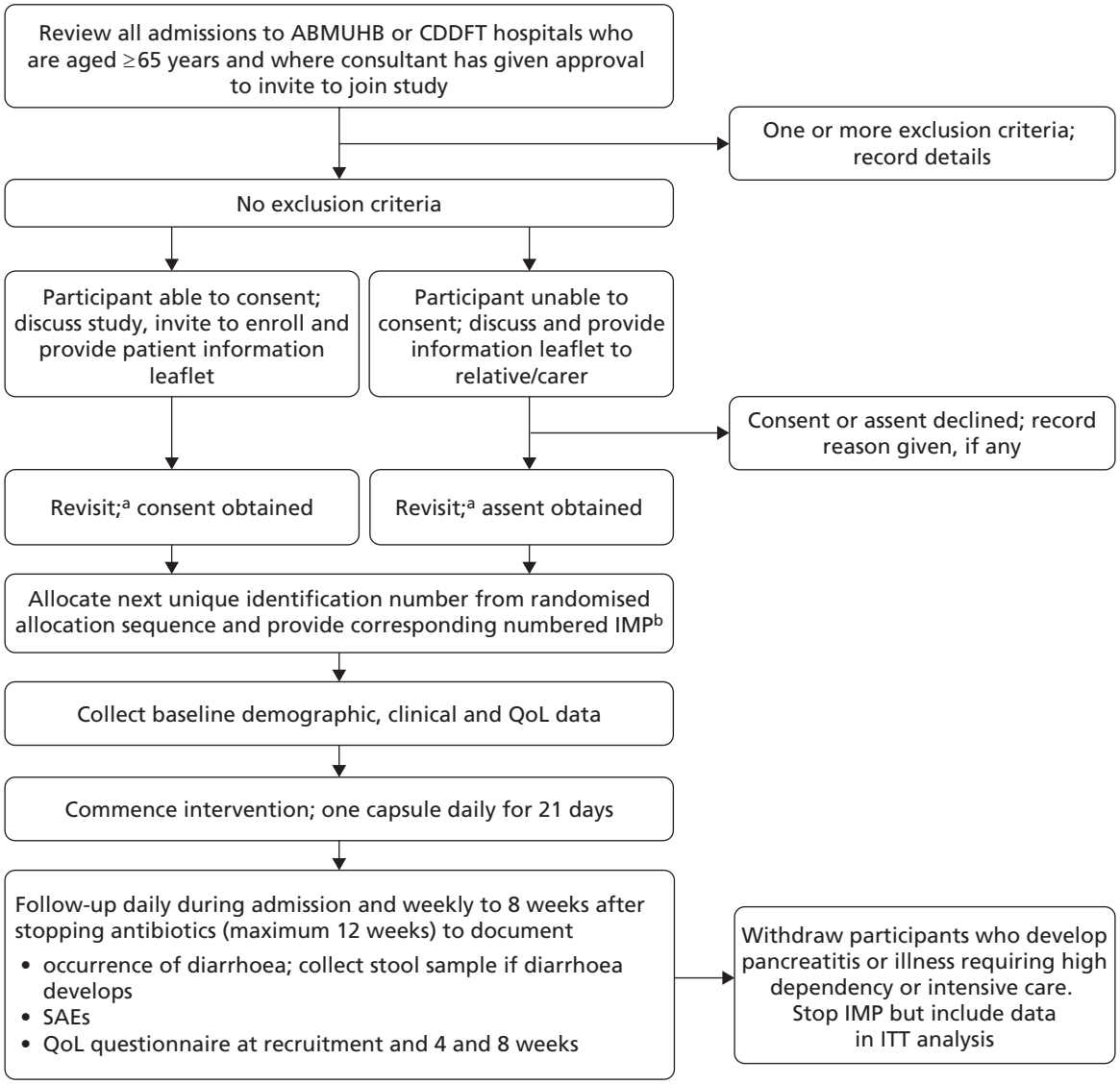
If yes – please give details:

Other Y / N / not recorded

If yes – please give details:

Please sign and date:

Appendix 5 Participant flow chart



Notes

(a) The patient or next of kin is approached for consent in the afternoon if verbal and written information about the trial is provided in the morning, or the following day if provided in the afternoon; and (b) either 21 capsules of probiotic or placebo.

Appendix 6 Criteria for severity of *Clostridium difficile* infection

Based on information from case records, the severity of episodes of *C. difficile* diarrhoea was classified according to the following guidelines:[39]

Mild

- normal white blood cell count (WCC)
- stool frequency less than three per day and
- stool consistency type 5–7 on Bristol Stool Form Scale.[38,39]

Moderate

- WCC raised but $< 15 \times 10^9$/l and
- stool frequency three to five per day.[39]

Severe

- WCC raised but $> 15 \times 10^9$/l or
- an acute rising serum creatinine (> 50% increase above baseline) or
- temperature > 38.5 °C or
- evidence of severe colitis (based on clinical examination or imaging).[39]

Life-threatening

- hypotension or
- partial or complete ileus or
- toxic megacolon or
- radiological evidence of severe disease.[39]

Appendix 7 Classification of antibiotics (according to *British National Formulary* 2012)

Antibiotic class	Drug names
Penicillins	
• Benzylpenicillin	Benzylpenicillin
• Penicillinase-resistant penicillin	Flucloxacillin
• Broad-spectrum penicillins	Amoxicillin, ampicillin, co-amoxiclav
• Antipseudomonas penicillins	Piperacillin, piperacillin plus tazobactam, ticaricillin
Cephalosporins	
• First generation	Cefalexin, cefradine, cefadroxil
• Second generation	Cefuroxime, cefaclor, cefixime
• Third generation	Cefotaxime, ceftazidime, ceftriaxone
Carbapenems and other β-lactams	Ertapenem, imipenem, meropenem, aztreonam
Tetracyclines	Tetracycline, demeclocycline, doxycycline, lymecycline, minocylcine, oxytetracycline
Aminoglycosides	Gentamicin, amikacin, tobramycin, neomycin
Macrolides	Erythromycin, azithromycin, clarithromycin
Clindamycin	Clindamycin
Sulphonamides and trimethoprim	Co-trimoxazole, trimethoprim
Metronidazole	Metronidazole
Quinolones	Nalidixic acid, ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, moxifloxacin
Glycopeptides	Vancomycin, teicoplanin
TB drugs	Ethambutol, rifampicin, streptomycin
Others	Chloramphenicol, daptomycin, sodium fusidate, linezolid, nitrofurantoin

Appendix 8 Protocol

PLACIDE: Probiotics and the prevention of AAD and CDD in older people: 06/39/02
Swansea University and NHS Trust and CDDFT, UK; 6 October 2008
Version 5

1. PROJECT TITLE: A multicentre, randomised, placebo controlled trial of lactic acid bacteria and bifidobacteria in the prevention of antibiotic-associated diarrhoea (AAD) and *Clostridium difficile* diarrhoea (CDD) in patients aged 65 years and over admitted to hospital and receiving antibiotics (06/39/02)

2. HOW THE PROJECT HAS CHANGED SINCE THE OUTLINE PROPOSAL WAS FIRST SUBMITTED

The following changes to the proposal were made in response to review of the recently published literature and recommendations from the first DMEC meeting held on 22/09/2008:

- Reference to the probiotic bacteria has been modified to "… lactic acid bacteria *and bifidobacteria* …" to more accurately identify the organisms. The description "optimal" has been removed.

- The former Swansea NHS Trust has merged to form the Abertawe Bro Morgannwg (ABM) University NHS Trust, Trust Headquarters, One Talbot Gateway, Baglan Energy Park, Baglan, Port Talbot, SA12 7BR. Tel: (01656) 752752 Fax: (01639) 687675/687676.

- We have extended the exclusion criteria following the publication of the PROPATRIA trial.[1] This RCT evaluated a high dose (10^{10} live bacteria / day) multi-strain (4 lactobacilli and 2 bifidobacteria) probiotic preparation in a total of 296 adult patients with predicted severe acute pancreatitis. Trial interventions were given via naso-jejunal (NJ) tubes together with a fibre-enriched feed (Nutrison Multi Fibre; Nutricia).

  No differences between the two intervention groups was found for the primary outcome (a composite outcome of infectious complications). No probiotic infections were identified. However, mortality was significantly higher in the probiotic than placebo group (24/152 [16%] vs. 9/144 [6%] respectively). All patients who died had multi-organ failure; all 9 cases of bowel ischaemia occurred in the probiotic group and 8 of these patients died. A higher frequency of organ failure before or during the first day of administration of the interventions in the probiotic (13.2%) versus the placebo group (4.9%) may have contributed to the increased mortality.

  The authors and accompanying commentary[2] hypothesise that live bacteria may result in bowel ischaemia in patients with impaired splanchnic circulation. Also, there may be an interaction between the probiotic preparation and the NJ feed.

  These findings in this specific patient population need to be considered alongside the numerous studies that have not identified adverse effects of probiotics (as detailed in the "safety" section of the proposal). However, in light of these potentially concerning findings and to pursue a conservative approach, we suggest 3 modifications to the PLACIDE study:

- Extend exclusion criteria. Patients with the following conditions will be not be recruited to the trial:

  a. Acute pancreatitis (defined as abdominal pain with serum amylase or lipase concentration ≥3 times the institutional upper limit of normal)
  b. Jejunal tube in-situ and/or jejunal feeding (as documented in the clinical / nursing records)
  c. Likely impaired splanchnic perfusion: any past or current abnormality or disease affecting the mesenteric arteries (as documented in the clinical records)
  d. Severe illness requiring care in either a high dependency or intensive care unit (but not planned admission to these facilities for observation only – e.g. after cardiac surgery)

- Withdrawal criteria: patients who require care in either a high dependency or intensive care unit (but not planned admission to these facilities for observation only – e.g. after cardiac surgery) will be withdrawn from the study and the trial intervention discontinued. However, these participants would be included in the intention-to-treat analysis. Patients who develop acute pancreatitis will also be excluded

- The independent statisticians will undertake an unblinded, interim analysis for important safety outcomes including the first 500 participants with complete data and report to the DMEC. Outcomes will include all SUSARs and all serious adverse events (see below) these will be handled in accordance with the EU directive 2001/20/EC.

  We expect that these modifications will result in the exclusion and withdrawal of only a small number of participants. However, the interim analysis will also assess recruitment according to targets and, as detailed in the proposal, additional recruitment sites will be invited to join the study if needed.

  References

  1. Besselink MGH *et al.* Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 2008; 371:651-59.

  2. Sand J, Nordback I. Probiotics in severe acute pancreatitis. Lancet 2008; 371:634-5.

- The maximum follow-up period will be 3 months from the date of recruitment

- Elderly people may have difficulty in describing stool consistency. Use of the validated Bristol stool chart is likely to be helpful.

- Because of labeling requirements, rather than stick sachets, the investigational medical products will be presented in a labeled bottle, each bottle containing 21 vegetarian capsules of either the probiotic food supplement or the inert placebo.

- The lactic acid bacteria and bifidobacteria have now been deposited in the National Collection of Industrial, Food and Marine Bacteria (NCIMB) and the appropriate identification numbers added to the proposal

- The two outcomes used to generate the sample size have been identified as co-primary outcomes. Other endpoints have been listed as secondary outcomes.

- Safety monitoring. The DMEC discussed at length the most appropriate methods for safety monitoring. Definitions for SUSARs were identified and procedures for the reporting of SUSARs and review of all SAEs determined. Arrangements for an interim analysis to assess safety outcomes were also discussed.

- Participant unblinding. It was agreed that immediate participant unblinding was not necessary as this would not inform clinical management. Unblinding could be undertaken by Dr Duolao as and when necessary in respect of SUSARS and adverse events.

- Although expected soon, final MHRA approval for the study has not been granted. Therefore, no changes have been made to the project timetable at this stage.

3. PLANNED INVESTIGATION

- **Research objectives**

Primary objectives: to determine the effectiveness and cost-effectiveness of an formulation of lactic acid bacteria and bifidobacteria in preventing or ameliorating AAD and CDD in people aged 65 years and over who are representative of patients admitted to secondary care NHS facilities in the UK and are exposed to oral or intravenous antibiotics.

Secondary objectives: to assess the acceptability and adverse effects of the probiotic preparation and the effect of the intervention on quality of life.

- **Existing research**

AAD is diarrhoea occurring in association with antibiotic treatment without an alternative cause (Bartlett JG; 2002). It occurs typically 2-8 weeks after exposure to antibiotics. The frequency of AAD varies markedly between studies according to risk factors such as exposure to broad-spectrum antibiotics, nosocomial infections and host factors such as age, health status, gender (McFarland LV 1998; Bartlett 2002). The following table shows the frequency of AAD in the placebo group of probiotic intervention studies undertaken in adults.

Reference	**Participants**	**No. (%) diarrhoea in placebo group**	
Studies of lactic acid bacteria			
Thomas 2001	18-93 years	40/134	(29.9)
Armuzzi 2001	mean 40 ±2 yrs; Rx *H. pylori*	8/30	(26.7)
Cremonini 2002	18-61 years; Rx *H. pylori*	6/21	(28.6)
Gotz 1979	adults	6/43	(14.0)
Wunderlich 1989	adults	6/22	(27.3)
Orrhage 1994	adults; Rx clindamycin	7/10	(70.0)
Beniwal 2003	adults	23/97	(23.7)
Studies of S. boulardii			
Surawicz 1989	Adults	14/64	(21.9)
McFarland 1995	18-86 years; Rx β lactam	14/96	(14.6)
Lewis 1998	>65 years	5/36	(13.9)
Can 2006	25-50 years	7/80	(8.8)
Overall		**136/633 (21.5%)**	

The major mechanism whereby antibiotics result in diarrhoea is through disruption of the commensal gut flora. This results in changes in carbohydrate, short chain fatty acid and bile acid metabolism and impairs colonization resistance which allows the emergence of a variety of gut pathogens. Some antibiotics also increase gut motility and may have direct effects on the gut mucosa (Bartlett JG, 2002). Although AAD is usually of moderate severity and self-limiting, it is a considerable nuisance to patients, prolongs hospital stay and increases healthcare costs.

C. difficile is an anaerobic bacterium which produces heat- and drying-resistant spores that persist long-term in the environment and make environmental control difficult. Transmission is faecal-oral and in health facilities occurs through contact with colonized patients, contaminated fomites and the hands of health care staff. Acquisition during admission is common (4-21%) and occurs in both endemic and outbreak scenarios (Barbut 2001; Poutanen 2004; Berrington 2004). Since 2003, CDD has become more frequent and associated with more severe illness in North America and Europe attributable to the emergence of a new strain which may produce higher amounts of toxin (Warny 2005; Bartlett JG, 2006). Outbreaks in the UK attract adverse media attention (Independent Newspaper, August 25th, 2005; Hospital Doctor, September 21st, 2006).

Most people who acquire the organism remain asymptomatic. However symptomatic disease has been reported to occur in 3.4-8.4/1000 admissions and to account for 10% of cases of nosocomial diarrhoea (Poutanen 2004). Severity of illness ranges from mild diarrhoea with abdominal discomfort through to pseudomembranous colitis complicated by toxic megacolon that may require colectomy and result in high case fatality. Disease mechanisms of increased mucosal fluid secretion and inflammation are due to exotoxins and both toxins A and B result in disease. The infection usually responds to treatment with metronidazole or vancomycin but 20-25% cases go on to get recurrent disease. The cost to the health services of CDD has been estimated to be £4000/case.

Exposure to antibiotics is the major risk factor for CDD and is associated with >90% cases. CDD accounts for about 25% of cases of AAD and occurs more commonly in health care facilities than in the community. CDD can occur with any antibiotic but the risk is greater with broad spectrum antibiotics (e.g. cephalosporins and β-lactamase resistant penicillins), clindamycin, antibiotic combinations and long treatment courses. CDD may occur from the first day of starting treatment or within 6 weeks or more after treatment. Other well documented risk factors include extremes of age, severity of underlying illness, use of proton-pump inhibitors, gastro-intestinal surgery and naso-gastric catheters.

Probiotics are defined as live microbial organisms which, when administered in adequate numbers, are beneficial to health (Joint FAO/WHO Expert Consultation, 2001). Probiotics are food supplements and are classified by the Food Standards Agency as “generally regarded as safe”. In general, probiotics do not cause adverse effects and have been used in people with a wide variety of different illnesses including many studies in preterm infants and also people with HIV infection. However, lactic acid bacteria have been reported to cause septicaemia in immunocompromised patients and endocarditis in people with artificial heart valves (Hammerman 2006).

In research studies, many different probiotics with varying numbers of organisms and modes of administration have been tested. There is little scientific rationale for selecting a particular strain and dosage of organisms for specific health outcomes. However, a strategy that is likely to maximize gut colonization and, thereby, colonization resistance is to use a combination of different organisms with large viable numbers of each strain.

In view of the central role of colonization resistance in preventing AAD, several double-blind, randomized, placebo-controlled trials of probiotics in the prevention of AAD and CDD in adults have been undertaken. There have also been several systematic reviews and meta-analyses conducted recently.

Antibiotic-associated diarrhoea.

A systematic review assessed studies of *Lactobacillus GG* in the prevention of AAD (Hawrelak 2005) and meta-analyses pooled data from studies of probiotics in the

prevention of AAD (McFarland 2006; Sazawal 2006; Cremonini 2002; D'Souza 2002) and of *S. boulardii* in the prevention of ADD (Szajewska 2005).

In a comprehensive meta-analysis, McFarland (2006) pooled data from 25 RCTs (total of 2,810 adults and children) and reported a reduced relative risk of AAD in participants receiving a probiotic (0.43; 95% CI 0.31 – 0.58). A wide range of probiotics were tested in these studies including single strains (including *S. boulardii)*, probiotic mixtures and probiotic and prebiotic mixtures. Dosages (number of organisms) varied markedly between studies. In sub-group analyses, factors associated with greater efficacy in preventing AAD were use of *S. boulardii* or *L. rhamnosus GG*, mixtures of probiotics and preparations with high numbers of organisms. Reported adverse events in these studies were mild but occurred with *S. boulardii* (constipation, increased thirst) and *L. rhamnosus* GG (bloating, gas). This meta-analysis included all of the studies included in reviews undertaken by other researchers.

Sazawal (2006) assessed probiotics in the prevention of acute diarrhoea. In 19 studies of AAD in adults and children which tested a variety of probiotics, the frequency of diarrhoea was reduced in the probiotic group by 0.52 (95% CI 0.35-0.65).

D-Souza (2002) included studies of *S. boulardii*, Lactic acid bacteria and a strain of enterococcus. Three trials used a probiotic combination and two were done in children. In the pooled analysis including data from 9 trials, the odds ratio (OR) in favor of the probiotic preparation over placebo in the occurrence of diarrhoea was 0.37 (95% CI 0.26 to 0.53). Importantly, the efficacy appeared to be similar for the bacterial (5 trials/384 participants; OR 0.34, 0.19 to 0.61) and yeast preparations (4 trials/830 participants; OR 0.39, 0.25 to 0.62).

Cremonini (2002) included trials in which either *Lactobacillus* or *Saccharomyces* spp. had been tested. They identified 7 randomized, placebo-controlled studies where participants had been followed-up for a minimum of 2 weeks. Overall, the relative risk of diarrhoea in the probiotic compared to the placebo group was 0.40 (95% CI 0.27 to 0.57).

Szajewska (2005) pooled data from 5 RCTs of *S. boulardii* (1076 participants including 269 children) and reported a reduction of diarrhoea in the probiotic group by 0.43 (95% CI 0.23-0.78). Although no adverse effects were reported in these studies, the authors noted reports of fungaemia occurring in people receiving *S. boulardii*.

C. difficile diarrhoea

Members of our research group (Plummer *et al*, 2004) assessed the effect of a combination of *L. acidophilus* and *B. bifidum* on CDD in a pilot study in elderly patients receiving antibiotics. Stools were cultured for *C. difficile* as well as tested for toxins A and B. Overall, 30/138 (22%) patients developed diarrhoea with 5/69 in the placebo group and 2/69 in the probiotic group testing positive for *C. difficile* toxin. In

this small study, the main effect of the intervention appeared to be neutralisation of the toxin rather than prevention of colonization with *C. difficile*.

We are not aware of any other studies that have assessed probiotics in the prevention of CDD in adults. The meta-analysis by McFarland (2006) included 5 studies, in addition to our study, but all of these were treatment trials of patients with established or recurrent CDD. Kotowska (2005) reported that *S. boulardii* reduced the risk of CDD in children by 0.3 (95% CI 0.1-0.14).

In summary, a variety of probiotics with different administration regimens appear to reduce the risk of AAD by around 50%. There is insufficient data to assess the effectiveness of probiotics in the prevention of CDD.

- **Research methods**

We will undertake a randomized, placebo-controlled, double blind trial in 5 secondary care hospitals in 2 NHS regions. All clinical, laboratory and research methods will be uniform across the centres involved in the study. To ensure that our participants are generally representative of older patients admitted to NHS hospitals throughout the UK, we will recruit from all wards admitting adult patients in Singleton and Morriston Hospitals (total 1450 beds), ABM University NHS Trust and all Medical and Care of the Elderly wards at the University Hospital of North Durham, Bishop Auckland General Hospital and Darlington Memorial Hospital, County Durham & Darlington Foundation Trust (CDDFT; 598 beds). We aim to recruit people with a wide range and severity of illnesses to ensure that our findings are directly applicable to the general hospital population. In 2005/6 (12 months), 26,692 people aged ≥65 years were admitted in Swansea and 21,676 in CDDFT 30-37% of patients received antibiotics.

Planned inclusion criteria:

- People aged ≥65 years admitted to hospital without diarrhoea and who have been exposed to one or more antibiotics within the last 7 days or are about to start antibiotic treatment

Planned exclusion criteria:

- People with known immunosuppressive disorder, prosthetic heart valve or active inflammatory bowel disease (the latter defined as requiring specific treatment in the past 12 months)
- Acute pancreatitis (defined as abdominal pain with serum amylase or lipase concentration ≥3 times the institutional upper limit of normal)
- Jejunal tube in-situ and/or jejunal feeding (as documented in the clinical / nursing records)
- Likely impaired splanchnic perfusion: any past or current abnormality or disease affecting the mesenteric arteries (as documented in the clinical records)
- Severe illness requiring care in either a high dependency or intensive care unit (but not planned admission to these facilities for observation only – e.g. after cardiac surgery)

- People with a previous history of adverse reactions to probiotics
- Informed consent not granted by patient or their carer(s)

Withdrawal criteria:

- patients who require care in either a high dependency or intensive care unit (but not planned admission to these facilities for observation only – e.g. after cardiac surgery)
- patients who develop acute pancreatitis (defined as abdominal pain with serum amylase or lipase concentration ≥3 times the institutional upper limit of normal)

The trial intervention would be discontinued for participants who are unable tolerate it and compliance to that time recorded. Their data would be included in the analysis on an intention to treat basis. Patients would continue in follow-up if they were happy to continue in the study.

Recruitment (see participant flow chart; appendix 1)

Dedicated research nurses will visit all wards twice daily, including weekends, to:

- record the total number of admissions
- record the working diagnosis/diagnoses or reason for admission in those aged ≥65 years
- apply the inclusion / exclusion criteria (as above)
- invite eligible patients to participate

The aims and methods of the study will be discussed and an approved information sheet provided. Sufficient time will be given for the participant to consider and discuss with relatives and health care personnel whether or not they wish to participate in the study.

Participants admitted to hospital in the mornings will be revisited later that day and those admitted after midday will be revisited the next morning. The research nurse will take signed, informed consent according to ICH/GCP guidelines. The consent form will be held in the investigator file, with copies filed in the hospital notes and given to the participant. A sticker will be placed on the hospital notes to signify that the patient has joined the study and the GP informed by letter. The reasons for declining to participate, if given, will be recorded.

Demographic and baseline clinical data will be recorded including the type and dose of antibiotics, duration of treatment in those already receiving antibiotics, other risk factors for CDD and episodes of CDD within the last 3 months. Participants will be required to stop any regular usage of probiotic preparations for the duration of the trial.

Generation and concealment of a simple random allocation sequence and participant allocation

A stratified randomisation by centre using blocks of variable sizes will be used to allocate subjects to either placebo or probiotic on a 1:1 basis with an aim to ensure similar numbers of patients in all centres. The randomisation codes will be produced by Dr Duolao Wang at London School of Hygiene and Tropical Medicine, using SAS PROC PLAN Version 9.1. Subjects fulfilling the eligibility criteria will be assigned a randomization code (subject numbers with an unique 11 digit identifier) starting and ending as follows:

- Centre 1, PLACIDE1001 ---- 1800
- Centre 2, PLACIDE2001 ---- 2800
- Centre 3, PLACIDE3001 ---- 3800
- Centre 4, PLACIDE4001 ---- 4800
- Centre 5, PLACIDE5001 ---- 5800

The random allocation sequence will be deposited with the DMEC who will check its reliability. It will not be available to any members of the research team.

Cultech Ltd. will prepare packs of the appropriate trial intervention (probiotic or placebo) labeled with each unique number in the series according to the random sequence. Each hospital will be supplied with a consecutive series of 100 study numbers and corresponding packs and re-supplied as recruitment progresses. Participants will be enrolled strictly sequentially in each centre. The research nurse will allocate each participant to the next unique study number in the sequence and provide the participant with the corresponding trial preparation. If a patient discontinues from the study, the patient number will not be reused, and the patient will not be permitted to re-enter the study.

Administration of intervention and follow-up to determine study outcomes

The participant will be instructed to take the first dose of the trial preparation (probiotic or placebo) on the day of recruitment. Project nurses will review participants daily during admission to identify the onset and determine the duration of diarrhoea and ask about gastrointestinal symptoms (abdominal pain, bloating, flatus, nausea), acceptability and adverse effects of the interventions. All participants will be followed-up for 8 weeks after completing antibiotic treatment. The maximum follow-up period will be 3 months from the date of recruitment Participants will also be asked to complete a quality of life questionnaire at baseline, 3 days post intervention, on hospital discharge and at the end of follow-up. We expect that most participants will have been discharged before completion of the 8 week follow-up. After discharge, follow-up will be weekly by telephone call, postal questionnaire or home visit as appropriate. Participants will be provided with a card with contact details and will have ready access to research staff by telephone throughout the study to notify the onset of diarrhoea or any other adverse events.

Diarrhoea is defined as the occurrence of 3 or more loose stools (loose stools will be identified with the help of the Bristol stool chart: Types 6 and 7) in a 24 hour period. All participants who develop diarrhoea during the study period will be asked to provide a stool sample (collected during a home visit if required). The cause of

diarrhoea will be determined by NHS laboratories according to their usual practice. Stools will be analysed for diarrhoeal pathogens (bacterial culture for Salmonella sp, Shigella sp, Campylobacter, E. Coli 0157; wet film for ova, cysts and parasites) and for *C. difficile* toxins A and B using the Biostat EIA test. If a cause of the diarrhoea is not identified, a further stool sample will be collected and tested 2 days later.

Diagnosis of the cause of diarrhoea will be based on stool analysis. AAD is defined as diarrhoea without pathogens detected on routine laboratory analysis and negative for *C. difficile* toxin. CDD is defined as diarrhoea with stools positive for *C. difficile* A or B toxin. For quality control purposes, *C. difficile* culture and confirmation by immunoassay will be undertaken in 1 in 5 *C. difficile* toxin positive stool samples collected in Swansea.

Participants who develop severe diarrhoea will be investigated and managed according to the current practice of their clinicians who will have access to the Cochrane review of antibiotic treatment of CDD (Bricker *et al* 2005). Investigations other than stool analyses are not part of the research protocol and will not be advised or undertaken solely for the purposes of this project. Information from clinical records regarding investigations and management as undertaken by the usual clinicians caring for the patients (e.g. findings at sigmoidoscopy, colectomy) will be recorded in the participant log.

- **Planned interventions**

Participants will be allocated randomly on a 1:1 basis to receive either:

a. Live bacteria of human origin: 2 strains of *Lactobacillus acidophilus* (CUL60, National Collection of Industrial, Food and Marine Bacteria [NCIMB] 30157 and CUL21, NCIMB 30156), *Bifidobacterium bifidum* (CUL20, NCIMB 30153), *Bifidobacterium lactis* (CUL34, NCIMB 30172). Prepared as 5g lyophilised powder in a capsule containing 6 x 10^{10} organisms/capsule.
b. Identical formulation of inert placebo: maltodextrin 5g

Dosage: 1 capsule/day taken with food for 21 days.

These probiotics are known to survive passage through the upper gut, adhere to intestinal mucosa and have excellent viability at the point of administration.

The rationale for the selection of these organisms is based on our previous work with probiotics in the prevention of CDD (Plummer *et al* 2004) and we have recent evidence that one of the organisms (*L. acidophilus*) neutralises *C. difficile* toxin in an epithelial cell assay *in vitro* (SPUR, Welsh Development Agency Research Grant; submitted April 2004). These probiotic preparations are already commercially available through BioCare UK and Pharmax, USA. We consider that it is important to select human commensal organisms for probiotic interventions in at-risk patients to reduce the possibility of adverse effects such as systemic infection by the probiotic strains. Therefore, we decided not to test organisms that are not part of the normal human commensal flora such as *S. boulardii*.

To ensure the quality of the probiotics, identity will be checked by established molecular techniques and viability by quantitative bacterial culture in a representative sample of the study preparations retrieved from wards on a regular basis throughout the study. This will be done by a laboratory independent of the research team to maintain masking of the allocation sequence. If any deterioration or deviation in the product is detected, including a reduction of >10% in the number of viable organisms of each strain, fresh supplies of trial preparations will be provided and testing repeated.

Outcome measures

- Diarrhoea is defined as 3 or more stools in a 24 hour period. Loose stools will be identified with the help of the Bristol stool chart (Types 6 and 7).
- Severe diarrhoea is defined as diarrhoea that requires treatment (oral or intravenous rehydration therapy for clinical dehydration and/or antibiotics) or investigation beyond stool culture (blood culture for suspected septicaemia, sigmoidoscopy for suspected PMC)
- AAD is defined as diarrhoea occurring in association with antibiotic therapy without an alternative explanation
- CDD is defined as diarrhoea not attributable to another cause and with stools positive for either *C. difficile* toxin A or B as detected by the Biostat EIA
- Pseudomembraneous colitis (PMC) is diagnosed by finding characteristic features at endoscopy and/or mucosal histology

The effect of the probiotic on the following outcomes will be determined:

Primary outcomes

During antibiotic treatment and within 8 weeks of stopping antibiotics:

a. The occurrence of AAD
b. The occurrence of CCD

Secondary outcomes

a. severity and duration of AAD
b. abdominal symptoms (abdominal pain, bloating, flatus, nausea)
c. severity and duration of CDD and incidence of recurrence within the study period
d. incidence of PMC, need for colectomy, death
e. well-being and quality of life
f. duration of hospital stay
g. adverse effects
h. acceptability of the probiotic preparation
i. viability of the probiotic at point of administration
j. risk factors for ADD, CDD and severe disease (PMC, colectomy, death)

Although not part of the main brief, an important issue is whether prevention strategies should be provided to all patients or just those at high risk of severe *C. difficile* infection. We will assess clinical outcomes according to proposed risk factors for severe CDD: age, duration of admission, severity of illness, previous episodes of CDD, specific antibiotics and usage of proton-pump inhibitors (Barbut 2001).

Estimate of likely recruitment rate

We have obtained a favourable response from senior clinicians and the Chief Nursing Officer in each of the participating hospitals to allow us to approach patients. Short

presentations will be arranged for medical and nursing staff to invite them to agree to their patients joining the study. We will announce the study in local media to raise awareness and aid recruitment.

As detailed above, and based on data from 2005/6, we expect to admit about 14,000 patients per year to the study hospitals aged ≥65 years and exposed to antibiotics. Conservatively, we expect to be able to recruit between 1:9 and 1:10 of these patients – 124 patients/month.

This estimate is supported by a limited 2-week pilot study of our recruitment process in 23 wards in Morriston Hospital during September 2005 (Elderly Care, Medical, Gastroenterology, Renal, Cardiology, General Surgery, Urology, Trauma, Burns and Orthopaedic wards). Research nurses visited the wards daily and identified a total 253 admissions aged ≥65 years. The nurses excluded 166 patients (no current or planned exposure to antibiotics – 152 patients; already had diarrhoea – 12 (inc. 1 *C. difficile*); active inflammatory bowel disease -1; previous adverse reaction to probiotic reported -1).

Eighty-seven (34.4%) patients were eligible to participate in the pilot study but 8 of these were excluded because they were either confused or not available (in theatre, undergoing investigations). Further attempts to recruit these patients would be made in an on-going study by follow-up visits and/or seeking assent from relatives. Therefore, the design of the study was then explained to 79 patients (31.2% of total admissions) and 58 (73.4%) patients stated that they would have agreed to participate in this study.

As detailed in our proposal, there were a total of 963 cases of CDD in 2005/6 in the hospitals involved in this study.

As a safeguard, we will monitor closely the number of participants reaching study endpoints in each hospital every 3 months from the beginning of the study so that we can take remedial action if needed. In Swansea, we have already undertaken research in hospitals in neighbouring Trusts and are confident that we could include additional hospitals in our study if required.

- **Ethical arrangements**

The project will be submitted to the Central Office for Research Ethics Committees (COREC) for allocation for review by an Ethics Committee (EC). The PI will report promptly all changes to the study, all unanticipated problems involving risks to participants or others and any protocol deviations which are necessary to eliminate immediate hazards to patients. Serious adverse events will be reported to the EC in accordance with national and local requirements. The Investigator will not make any changes to the study or its conduct without EC approval, except to eliminate a danger. The investigator will submit annual progress reports and a final report to the EC following the study completion or in the event of a premature termination of the study. For essential amendments after the study has started, participants will be informed and invited to sign a revised consent form should they wish to continue in the study.

- **Risks and anticipated benefits for trial participants and society, including how the benefits justify the risks**

Risk of no benefit to participants

The 50% of the participants allocated to the control arm will not derive any direct benefit from the trial intervention.

Risks of adverse effects

- Probiotics are members of the normal gut commensals and were classified in 2002 as "generally regarded as safe" by the US Food and Drug Administration (notice GRN 000049). The European Society of Paediatric Gastroenterology, Hepatitis and Nutrition Committee on Nutrition concluded that probiotics can be considered safe but surveillance for side effects is needed (ESPGHAN 2004; von Wright 2005). A recent review of the safety of lactic acid bacteria found only anecdotal reports of systemic infection that had occurred in people with severe disability, immune deficiency or prosthetic heart valves. In these few cases, it was difficult to differentiate infection caused by administered probiotics from that caused by the endogenous flora. In prospective studies, probiotics have been administered without adverse effects to vulnerable groups such as children and adults with HIV infection and preterm infants (Hammerman 2006; Schlegel 1998). We will exclude patients at high risk of probiotic infections from our study as detailed above.

- In the case of suspected sepsis developing after starting the trial intervention, laboratory staff will be alerted that the patient is enrolled in the study by a sticker attached to the laboratory request forms. They will undertake bacterial culture for the probiotic organisms as well as common bacterial pathogens. Although highly unlikely, any infection attributed to the probiotic organisms would be treated according to their pre-determined antibiotic sensitivity.

- *Participant unblinding.* Arrangements for the immediate unblinding of participant allocation are not necessary as this would not inform clinical management. If required by the DMEC, Safety Monitor or participant's clinician, unblinding could be undertaken by Dr Duolao as and when necessary.

- Research staff will have ready access to senior physicians on a 24 hour basis to discuss adverse events and safety issues as needed.

Anticipated benefits

- Regular follow-up of all participants (including those in the placebo group) for diarrhoea and any adverse events may increase the recognition of morbidity and therefore, improve overall care and outcome.

- Those who receive the active intervention may have a reduced risk of developing AAD and CDD or may develop milder disease.

- If the intervention proves to be successful against CDD, fewer cases will reduce the risk of nosocomial diarrhoea amongst other admissions.

- This large study will also provide further information about frequency and risk factors for AAD and CDD which may allow high risk groups to be better identified.

Informing potential trial participants of possible benefits and known risks of the intervention (or of no intervention or a placebo)

As part of the informed consent process, research staff will strive to ensure that all participants (and their relatives or carers where appropriate) understand that they have a 50% chance of being allocated to the placebo arm of the study and would, in that case, derive no direct benefit from the intervention. They will also explain that the probiotic preparation may not prove to be effective in preventing or ameliorating diarrhoea. Each participant will have frequent contact with a named research nurse who will be available by telephone throughout the study to answer any questions that may arise.

- *Informed consent from participants wherever possible*

Potential participants will be given a verbal and written explanation of the study by one of the study team who is experienced in taking informed consent. Participants will be encouraged to ask questions and every attempt will be made to ensure that they understand the study including that they can withdraw from the study at any time without giving a reason and without it affecting their medical care in any way. They will be given sufficient time to discuss the study with others. Once a participant has decided to enter the study, they will be asked to sign a consent form. Participants will be aware that all information will be anonymous to ensure complete confidentiality and that individual participants will not be identified in any reports or publications.

Proposed action where fully informed consent is not possible (e.g. emergency settings)

Every effort will be made to communicate details of the study to the participant but, in this older population, assent will be required in many cases where the patient is unable to give full, informed consent. Assent will be sought from next of kin, other relative or carer in line with Article 5 of the EU Directive 2001/20/EC (Clinical Trials on incapacitated adults not able to give informed legal consent). The information sheet will be sent to the relative or carer and they will be given the opportunity to ask questions of a member of the research team. We will appoint a senior clinician in each NHS Trust who is independent of the research team to act as an advisor for participants and relatives regarding their involvement in the study if they wish. Participants and relatives will also be encouraged to discuss the study with their General Practitioner. If a participant is later deemed to be able to give informed consent, then this will be sought.

Relatives / carers will be informed that they can withdraw assent at anytime without it affecting the patient's care. The participant would be withdrawn from the study if he/she declines on two consecutive occasions to take the trial preparation.

Retention of relevant trial documentation

Data containing participant's identification details will be retained for 10 years from the termination of the study. This will allow the linking of an individual participant's data with their other health records (e.g. GP record, other hospital records). Beyond this period, all participant identification details (e.g. name, contact details, hospital number) will be removed. This anonymised data set will be retained indefinitely.

Action to comply with EU Directive 2001/20/EC

MHRA are assisting us in the completion of the necessary submission for a Clinical Trial Authorisation for this study.

- **Sample size**

Conservatively, we expect ADD to occur in 20% and CDD in 4% of participants in the placebo group. To detect a 50% reduction in the frequency CDD in the probiotic group (i.e. 2% frequency) with 80% power at the 5% significance level, we will require 2,478 subjects (1,239 in each group; 1:1 allocation). At the 5% significance level, this number of participants would provide a power of >99% to detect a 50% reduction in ADD (i.e. 10% frequency) and a power of 90% to detect a 25% reduction in ADD (i.e. 15% frequency) in the probiotic group. To allow for 10% drop-outs and 10% loss to follow-up due to deaths unrelated to diarrhoea, we will recruit 2,974 participants.
On this basis, we expect 50 cases of CDD in the control group and 25 in the probiotic group over 2 years. Since we observed 963 cases of CDD in one year (2005/6) in the hospitals involved in this study, we would have to recruit less than 1 in 20 cases into our study to reach our recruitment target. We are likely to recruit more cases of CDD than required which would increase the power for all CDD comparisons.

- **Statistical analysis**

Primary outcomes will be analysed with standard methods for a multicentred RCT. Confidence intervals for the odds ratios for ADD and CDD will be estimated from regression models that include the relevant covariates (such as age, gender, specific antibiotic, centre). A similar approach will be taken for the outcomes of severity and PMC. Careful inspection of interaction terms will identify sub-group effects, and these will be interpreted in light of power relative to main effects and supporting evidence of mechanism (Brookes 2001). All analyses will be performed using the R statistical environment (Ihaka & Gentleman, 1996, *J Comp Graph Stat*, 5).

Quality of life (QoL)

There are few tools that are validated for measuring QoL in older people and none specifically targeted at treatment-induced diarrhoea. We will modify existing tools which have been validated to measure QoL in treatment-induced diarrhoea in people with HIV (Thielman 2002) and older patients with faecal incontinence (Rockwood 2000). We will also use the generic measures EQ-5D and the York SF12 (Iglesias 2001) to understand the broader health impact related to treatment-induced diarrhoea and facilitate cost-effectiveness analysis.

Health economic analysis

The health economic evaluation will be undertaken from the perspective of the NHS. Resources utilised by each participant will be logged using appropriate recoding forms and collected as part of the on-going data collection process. The resources utilised will consist of the number and cost of the probiotics, the costs of staff time involved in administering the probiotics, costs of treatments relating to adverse events, costs incurred in the assessment of cases of diarrhoea (stool collection and culture/toxin assay, endoscopy) and costs resulting from dealing with and treatment relating to cases of diarrhoea, such as laundry, antibiotics, increased hospital stay and co-morbidities. Data relating to unit costs will be collected through discussions with relevant clinicians and finance department staff, while published information will also be utilised.

Cost differences between the probiotic and placebo group will be determined and used in conjunction with differences in outcomes between groups in undertaking a cost-consequences analysis, with cost per case averted as the primary outcome measure for the economic evaluation, but with other outcomes considered. Sub-group analyses will also be conducted to determine the relative cost-effectiveness of preventive strategies in different risk groups. In addition, a cost-utility analysis will be undertaken based on the differences in costs between the two groups and differences in QALYs derived from the EQ-5D responses during the course of the investigation.

Given the timescale of the project there will be no discounting of the costs or benefits. Sensitivity analyses will investigate the robustness of the results to changes in estimated costs and outcomes and probabilistic sensitivity analysis will use bootstrap resampling to determine the probability that preventive strategies are within certain thresholds.

The budgetary impact (again from a NHS perspective) of adopting a policy of administering a probiotic preparation containing 4 strains of live bacteria to prevent or ameliorate AAD and CDD in people aged 65 years and over who are admitted to secondary care NHS facilities and receive oral or intravenous antibiotics will also be assessed as part of the health economic evaluation.

- **Research Governance –** see organogram; appendix 2

Trial Steering Committee (TSC)

The chair and members of the TSC will be appointed formally by the HTA. The proposed Chair is Professor Stephen Bain, Director of R&D, ABM University NHS Trust. Patients admitted under Professor Bain's care would be eligible to participate in the study, but he would have no other involvement in the trial. Membership would also include a service user representative, two other independent members, Dr. Steve Allen (PI), Ms. Kathie Wareham (Project Manager). Observers from the HTA and the trial sponsor (Swansea University) will be invited to all meetings and will also be able to convene additional meetings.

An initial TSC meeting before the trial start will be arranged by the PI to review and agree the trial protocol and establish a DMEC (see below). In advance of subsequent meetings, evidence regarding progress with recruitment based on eligible population, adherence to protocol, loss to follow-up and AEs will be provided. The TSC will also be required to review any new information regarding CDD, AAD and probiotics that may be relevant to the local trial.

Safety

Safety reporting will follow the requirements as described in The Medicines for Human Use (clinical Trials) Regulation 2004: SI 2004/1031.and the EU Directive 2001/20/EC.

Adverse events
All serious adverse events (SAE's) will be reported immediately to the sponsor except for those which are described in the protocol/addendum as not needing immediate reporting. The immediate reports will be followed promptly by detailed, written reports. The reports will follow the guidelines of 4.11.of the ICH Guidelines for Good Clinical Practice.

Suspected unexpected serious adverse reactions (SUSARs)
All relevant information about suspected unexpected serious adverse reactions (SUSAR) which occur during the course of the study and are fatal or life-threatening will be reported immediately/as soon as possible to the MHRA, the competent authority and the relevant Ethics Committee. This will be done within 7 days of first being aware of the reaction. Additional information would be forwarded as soon as possible and within eight days of filing the initial report. In respect of a SUSAR which is not fatal or life threatening it will be reported as soon as possible but not later than 15 days after the Sponsor is first aware of its occurrence.

SUSAR reporting
CIOMS 1 form will be used to inform the MHRA and it will include all relevant information including the EudraCT number, CTA number protocol number and Study name. Reports may be faxed, emailed or sent as electronic documents on disk.

Safety Monitor

A large number of Serious Adverse Events (SAEs) are likely in the course of this study of elderly people and a realistic approach is necessary in AE reporting bearing

in mind the excellent safety record of probiotics. SAEs will be defined according to GCP guidelines and assessed by the local research clinicians involved in the project as to their attribution. See appendix 3

In respect of what constituted a SUSAR, it was agreed that this would include the following but not limited to these serious adverse events:

- bacterial infection caused by a probiotic organism (i.e. lactobacillus or bifidobacteria)
- the development of bowel ischaemia not present at recruitment (any past or current abnormality or disease affecting the mesenteric arteries is an exclusion criterion).
- the development of pancreatitis (defined as abdominal pain with serum amylase or lipase concentration ≥3 times the institutional upper limit of normal; pancreatitis present on admission is an exclusion criterion)

These SUSARs will be reported immediately to the Independent Safety Monitor to consider their attribution to the participant's participation in the trial and also to the Ethical Committee/MHRA/ regulatory bodies in accordance with local and national requirements.

A dedicated EXCEL database will record all SAE's and SUSARs and this would be available at any time to the Safety Monitor and the DMEC.

The research team will send the Independent Statistician details of all SAEs every 3 months. The statistician will allocate these to the two intervention groups (but labeled as only "A" or "B") and discuss the findings with the Safety Monitor. These reports will be reviewed at DMEC meetings. The identity of groups "A" and "B" will be provided by the Independent Statistician immediately should either the Safety Monitor or the DMEC have any concerns regarding participant safety

In addition, the Independent Statistician will undertake an unblinded, interim analysis for important safety outcomes including the first 500 participants with complete data and report to the DMEC. Outcomes will include all SUSARs and all serious adverse events.

Data Monitoring and Ethics Committee (DMEC)

The chair and members of the DMEC will be appointed formally by the HTA. Proposed membership includes an independent Chair (Professor JG Williams, Consultant Gastroenterologist, Neath Port Talbot Hospital and Director of Welsh Office Research and Development Programme), Dr. Duolao Wang, Medical Statistics Unit, Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine and 1-2 additional independent members. Patients admitted under the care of Professor Williams would be eligible to participate in the study, but he would have no other involvement in the trial. Dr. Wang will generate and hold the random allocation sequence for the trial but is otherwise independent of the study.

Regular meetings will be organized with the PI in association with the DMEC chair. Prior to each meeting, the trial statistician will prepare a report of trial progress.

The DMEC would review data from other related studies and advise as to how this might reflect on the local study. The DMEC would also advise regarding the needs for extended funding should this be requested by either the funding body or the TSC. The DMEC will report to the subsequent TSC meeting.

Trial Management Committee

This group will be based in Swansea and include the PI, the Project Manager, the CDDFT Site Co-ordinator and CDDFT hospital site leads. It will meet frequently prior to commencing the study and at least monthly as the study progresses. It will focus on the day-to-day operation of the trial including mechanisms for the prompt reporting of adverse events.

After initial face-to-face meetings, use of teleconference facilities will help to reduce travel costs.

4. Project timetable and milestones

Key milestones:

- May - June 2008: submission to MREC; staff recruitment and training; pilot testing of patient recruitment, data collection and stool collection and analysis; development of database; preparation of trial interventions; writing Standard Operating Procedures; local meetings with NHS staff; establish TSC, DMEC and local trial management committee.
- July 2008 – June 2010: participant recruitment and laboratory analyses (target 117 participants/month for 24 months)
- July – September 2010: complete participant follow-up
- October 2010 - March 2011: clinical and cost-effectiveness data analysis; report writing, presentation of results at national and international meetings and preparation of publications
- March 2011: completion of study

5. Expertise

We consider that we have a highly experienced and committed team of investigators with strengths in each of the major areas of the study. All of the investigators have contributed to the design of the study, will be closely involved in the trial on a day-to-day basis and will also be involved in data interpretation and the writing of scientific publications. Trial management will be centered in the Clinical Research Unit (CRU) based at Morriston Hospital, Swansea.

Dr. Stephen Allen is a Reader and Honorary Consultant Paediatric Gastroenterologist. He has completed a Cochrane systematic review in probiotics in acute diarrhoea. He has extensive experience of clinical research mainly in economically-poor countries and has led research teams in both hospital

and community settings. During a 4 year period based at the MRC Laboratories, The Gambia, he was a member of the Nutrition Research Group and the Scientific Co-ordinating Committee which met monthly to review new research proposals. He is a member of the UK Medicines for Children Research Network Clinical Studies Group for General Paediatrics. As PI of this study, he will provide overall supervision of the conduct of the study, including assessing progress against milestones, supervising data management and financial control. He will report on progress and adverse events to the TSC and take the lead in the writing of trial reports and publications.

Ms. Caroline Bradley has worked as a Clinical Pharmacist within the secondary care environment for over 10 years. She has a special interest in the use of antibiotics and leads on antibiotic policy and use for County Durham and Darlington Acute Hospitals NHS Trust (CDDAH). CDDAH is an acute Trust providing healthcare across County Durham and Darlington and surrounding areas from three main acute hospitals, at Durham, Darlington and Bishop Auckland alongside other community hospitals. The Trust serves a population of 550,000 people across County Durham and Darlington, and offer services to many patients outside this area.

Recent achievements in antibiotic management are producing and managing the policy for the use of antibiotics across in the Trust, advising and monitoring the use of antibiotics with particular attention to MRSA and *C. difficile* rates. Results include reducing the average duration of IV antibiotic use, halving the rate of IV macrolide use in the Directorate of Medicine and the introduction of automatic stop orders to limit the duration of antibiotic treatment. She will oversee the management of the trial in CDDAH and be the main point of contact with the Swansea research team.

Dr. Anjan Dhar is a Consultant Gastroenterologist in the Directorate of Medicine & Elderly Care at Bishop Auckland General Hospital. He obtained D. M. in Gastroenterology in 1994 at the Postgraduate Institute of Medical Education and Research, Chandigarh, India and won a Commonwealth Fellowship in Gastroenterology, Association of Commonwealth Universities, undertaken with Professor Derek Jewell, Radcliffe Infirmary, University of Oxford, UK between 1998 and 2000. He gained extensive experience in gastroenterology from working in leading clinical and research centres including Middlesex Hospital, University College London Hospitals, The Royal Hospital, Muscat, Oman, the All India Institute of Medical Sciences, New Delhi, and Postgraduate Institute of Medical Education and Research, Chandigarh India. His research has focused on *Helicobacter pylori*, peptic ulcer and inflammatory bowel disease. He will provide supervision of clinical recruitment for the trial in Bishop Auckland General Hospital and also provide expert advice regarding clinical management of patients with ADD and CDD.

Professor Dietrich Mack is Professor of Medical Microbiology and Infectious Diseases and Honorary Consultant Microbiologist. He has extensive research experience of techniques for susceptibility determination in multiresistant nosocomial organisms like ESBL-containing enterobacteria, VRE, and staphylococci and exploring their epidemiology as well as the molecular pathogenesis of biomaterial-related staphylococcal infections. He will supervise all of the laboratory analyses undertaken in the trial including quality assurance for *C. difficile* culture and toxin assays.

Dr. Sue Plummer is the Technical Director of Cultech Ltd., Swansea, a leading manufacturer of specialist nutritional products for the healthcare industry. She leads the development of the human nutritional supplement sector and has a special interest in probiotics. She will ensure a reliable supply of the trial preparations for this study allocated according to a random sequence. She will also supervise quality control of the trial preparations and give expert guidance on new developments in the field of probiotics.

Dr. Wyn Harris is a Consultant Geriatrician with extensive clinical experience of the diagnosis and management of *C. difficile* infection in the elderly. He has completed an audit of antibiotic use in an effort to reduce the incidence of CDD and implemented prescribing guidelines. He will be primarily responsible for the welfare of trial participants in Swansea and be available for expert clinical guidance. He is an active member of the Welsh Branch of the British Geriatrics Society and this will assist in disseminating the results of the study to inform clinical practice.

Dr. Wai Yee Cheung is a Senior Lecturer in Health Services Research with expertise in the development and validation of patient-focused outcome measures. She has led development of condition-specific and systemic quality of life measures for use in many multi-centre studies, including trials funded by the HTA Programme. She will oversee the development and application of quality of life measures in the current study.

Dr. Mike Gravenor is a Reader in Epidemiology and Statistics. His research centres on the application of statistical and mathematical models to practical problems in epidemiology and the link between good data collection and sophisticated analysis techniques. He will supervise data collection and storage and perform the statistical analysis.

Professor Ceri Phillips is a health economist and Head of the Institute for Health Research at the School of Health Science, Swansea University. He has extensive experience of health economic evaluation in many projects, including HTA projects, and will oversee the detailed economic evaluation in this study.

Ms. Kathie Wareham is the Director of the Clinical Research Unit (CRU), ABM University NHS Trust. She has 25 years experience in clinical research, having spent 10 years setting-up and running phase I clinical trials unit at Smith, Kline & French (now Glaxo SmithKline). For the past 15 years, she has been responsible for setting up a research network in Swansea within and outside of the Trust. She was a member of two phase I Ethics Review Committees for 20 years and was recently an external examiner for an MSc in Clinical Research at John Moores University, Liverpool. She has successfully managed projects funded by the Welsh Office of Research and Development, Welsh Assembly Government and these have resulted in publications in leading journals. She will supervise the overall running of the trial both in Swansea and CDDAH.

The CRU has been operational for 16 years with continual growth. A purpose built facility was established in 2000 and now undertakes most of the clinical research projects in ABM University NHS Trust. It has an alliance with The School of Medicine at Swansea University. The unit has been commissioned by a number of blue chip pharmaceutical companies. It undertakes proof of concept studies, phases IIa, IIb and III across a number of disciplines. All staff are trained and updated in Good Clinical Practice guidelines. The Unit sets up unique, password-protected computer databases which are archived regularly off site in a secure location.

Dr. Helga Brown is Consultant Physician and Honorary Clinical Lecturer at University Hospital of North Durham (UHND). Most of her inpatients (850 per annum) are frail elderly. She also provides regular assessment of inpatients in the Orthopaedic and Psychiatry of Old Age departments. Her work brings her into direct contact with the patients most at risk of developing ADD and CDD. In response to an alarming rise in the incidence of CDD in UHND in 2006, Dr Brown undertook an audit of risk factors and revised the hospital guidelines for the management of CDD, in association with colleagues in microbiology. She will be the clinical lead for the study at UHND and be responsible for advising on the clinical assessment and care of trial participants.

Dr. Alwyn Foden is a Consultant Physician with an interest in Respiratory Medicine at Darlington Memorial Hospital, Co Durham. He has extensive experience of clinical trials. He has a special interest in conditions that lead to infections, especially resistant ones, and has experience in trials of anti-infective agents. He has recently completed a formal course on Good Clinical Practice. He is the Acute Care Trust representative on the Darlington Respiratory Team of the Primary Care Trust. In this study, he will be w the clinical lead for the study at Darlington Memorial Hospital and be responsible for advising on the clinical assessment and care of trial participants.

6. Service users

Mr. John Pollock has kindly agreed to represent consumers on the Steering Group. He is a retired businessman and Rotarian. He has been involved as research participant for a number of years and is fully conversant with the procedures for clinical research and issues regarding lay interpretation of consent. He fully understands the needs of participants and how best these can be met. Another service

user representative based in CDDAH will be also be invited to join the TSC. We propose to pay the service user representatives for their time spent attending meetings as well as re-imbursement of travel expenses.

7. Justification of support required

Project manager (50% FTE; 1 post in Swansea): Ms. Kathy Wareham, Director of the CRU, will undertake this post. She will take the lead for ensuring close liaison between Swansea and CDDAH. She will draft all Standard Operating Procedures for the conduct of the study. She will assess progress against milestones and take action if targets are not being met. In Swansea, she will be responsible for training the research nurses and motivating the team and will liaise with hospital clinicians and senior nurses throughout the project to maintain their support.

Study Co-ordinators (50% FTE; 1 post in each NHS region): These posts will be pivotal in ensuring efficient working practices and good lines of communication and will report directly to the project manager. They will supervise data collection from the hospital sites and following discharge, data entry, maintenance of participant files, take minutes at local meetings and ensure that interim and final reports are drafted, circulated and finalised by the project manager and PI. They will liaise with laboratory staff in respect of stool samples. The post holders will help to produce a three-monthly newsletter, which will be circulated to hospital medical and nursing staff. They will also provide back-up for patient recruitment during periods of staff leave and sickness.

Administrator (25% FTE; 1 post in Swansea): This post will provide essential administrative and secretarial support to the project teams in both NHS regions.

Research nurses (100% FTE; 3 posts in each NHS region): Six posts are required to ensure flexibility to cover all hospital wards twice daily including at weekends and complete follow-up with adequate coverage for holidays and sickness. They will liaise with ward staff in the identification of eligible participants, recognition of diarrhoea and collection of stool samples. Regular updates and a supportive working relationship with all hospital staff will ensure maximal cooperation. Research nurses will recruit participants, encourage participants to take the trial interventions daily and collect clinical outcome data. Participants will be allocated a named research nurse throughout their involvement, including after discharge.

Research Assistant – Cost-effectiveness analysis (50% FTE; 1 post in Swansea): The research assistant will conduct a detailed and comprehensive assessment involving extensive data collection and analysis in both NHS regions.

Statistician / Data Manager (30% FTE; 1 post in Swansea): This person will help with design of data collection forms, build the databases for the clinical and laboratory data and ensure that reliable data is entered into the database as the study progresses. He/she will also be responsible for the initial data analyses supported by the project statistician.

Laboratory assistant (100% FTE; 1 post in Swansea): This post is required to support NHS staff for the prompt and careful handling and analysis of a large number of stool samples from the participants. The number of stool samples for analysis will increase significantly as a result of the research project.

Data clerk (50% FTE; 1 post in each NHS region): Data entry for both clinical and laboratory data will occur at each site with exchange of records between the data clerks for double data entry and checking for errors.

Consumables: Consumables are limited to the trial preparations, minor laboratory equipment and 4 computers for data entry and maintenance of other trial documentation. The computers should be of sufficient specification to generate quality graphics for reports and to write back-up data CDs. Other consumables include stationery items, paper and stamps.

Travel: We have carefully considered the need for research staff to make regular visits between the two NHS regions to ensure that all study procedures are uniform. In addition, travel expenses will be incurred by research nurses following-up participants in their homes. A nominal fee and travel costs will be paid to the independent members of the TSC for attending meetings.

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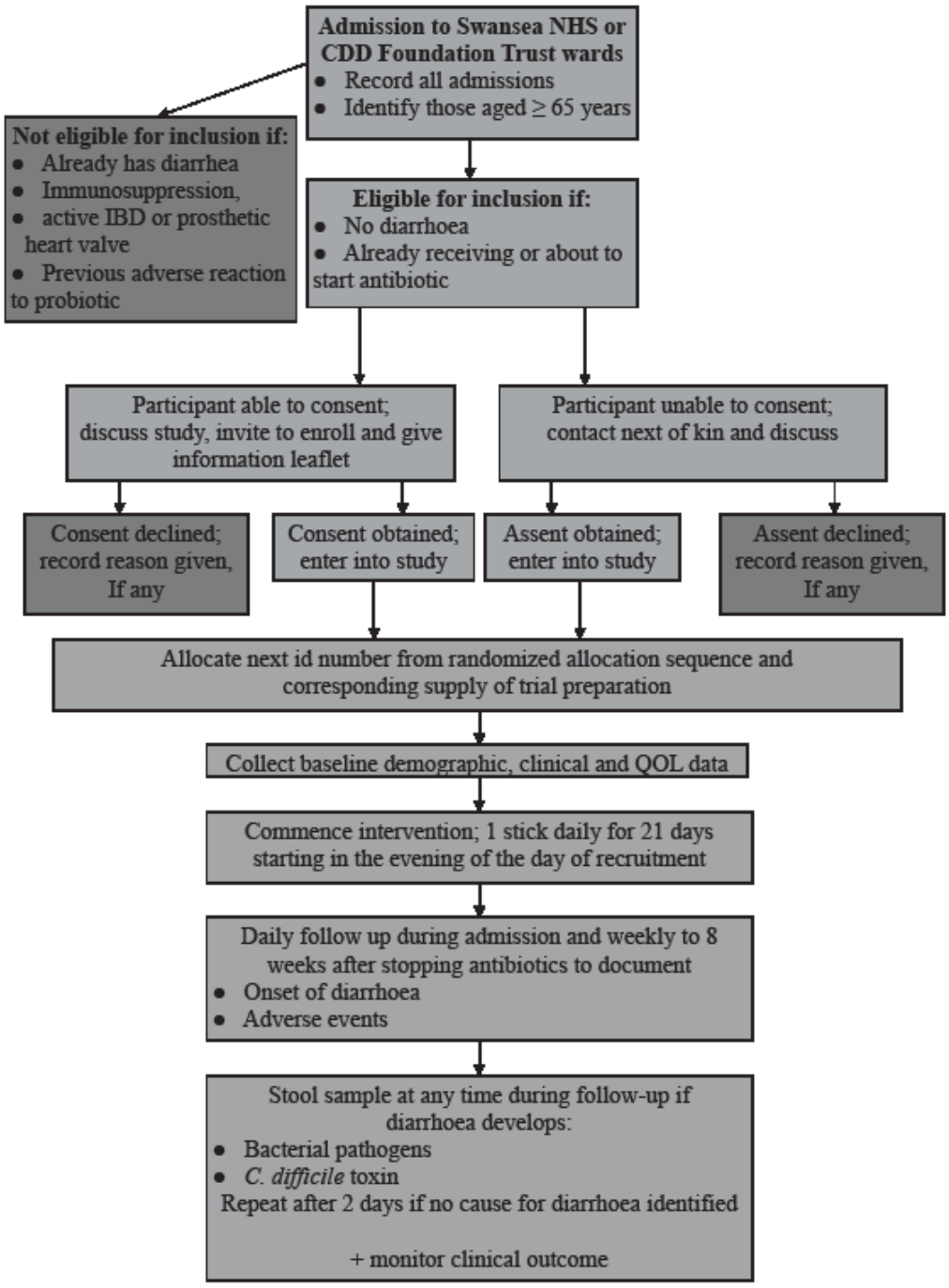
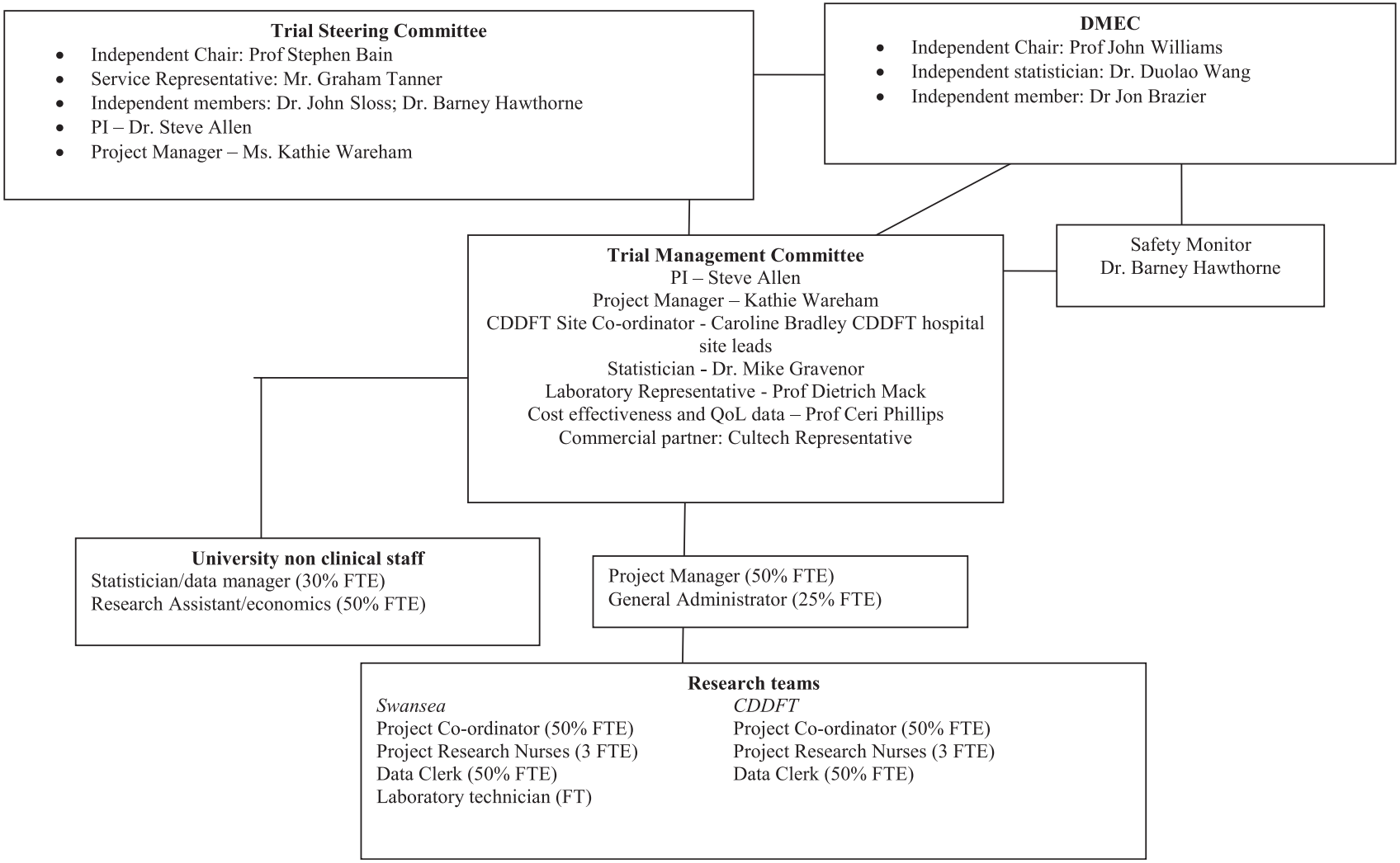
Appendix 1: Participant flow chart



Appendix 2


Trial Steering Committee
Independent Chair: Prof Stephen Bain
Service Representative: Mr. Graham Tanner
Independent members: Dr. John Sloss; Dr. Barney Hawthorne
PI – Dr. Steve Allen
Project Manager – Ms. Kathie Wareham
DMEC
Independent Chair: Prof John Williams
Independent statistician: Dr. Duolao Wang
Independent member: Dr Jon Brazier
Trial Management Committee
PI – Steve Allen
Project Manager – Kathie Wareham
CDDFT Site Co-ordinator - Caroline Bradley CDDFT hospital site leads
Statistician - Dr. Mike Gravenor
Laboratory Representative - Prof Dietrich Mack
Cost effectiveness and QoL data – Prof Ceri Phillips
Commercial partner: Cultech Representative
Safety Monitor
Dr. Barney Hawthorne
University non clinical staff
Statistician/data manager (30% FTE)
Research Assistant/economics (50% FTE)
Project Manager (50% FTE)
General Administrator (25% FTE)
Research teams
Swansea
Project Co-ordinator (50% FTE)
Project Research Nurses (3 FTE)
Data Clerk (50% FTE)
Laboratory technician (FT)
CDDFT
Project Co-ordinator (50% FTE)
Project Research Nurses (3 FTE)
Data Clerk (50% FTE)

Appendix 3 : Adverse events summary overview for guidance

All serious adverse events (SAE's) should be reported immediately to the sponsor except for those which are described in the protocol/addendum as not needing immediate reporting. The immediate reports should be followed promptly by detailed, written reports. The reports will follow the guidelines of 4.11.of the ICH Guidelines for Good Clinical Practice and the EU Directive 2001/20/EC.

Adverse Events categories for determining relation to study medication

Description	Related			Unrelated
	Probable	Possible	Remote	
Clearly due to extraneous causes	-	-	-	+
Reasonable temporal association with drug administration	+	+	-	-
May be produced by patient clinical state etc	-	+	+	+
Known response pattern to suspected drug	+	+	-	-
Disappears or decreases on cessation or reduction in dose	+	-	-	-
Reappears on rechallenge	+	-	-	-

Unrelated:
This category is applicable to those adverse events which are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment etc) and do not meet the criteria for drug relationship listed under remote, possible or probable.

Related

Probable (must have first three)
This category applies to those adverse events that are considered, with a high degree of certainty, to be related to the test drug. An adverse event may be considered probable if:

1 It follows a reasonable temporal sequence from administration of the drug
2 It cannot be reasonably explained by the know characteristics of the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3 It disappears or decreases on cessation or reduction in dose.
4 It follows a known pattern of response to the suspected drug
5 It reappears upon rechallenge

Possible (must have first two)
This category applies to those adverse events in which the connection with the study drug administration appears unlikely, but cannot be ruled out with certainty. An adverse event may be considered possible if or when:It follows a reasonable temporal sequence from administration of the drug

1 It may have been produced by the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject
2 It follows a known pattern of response to the suspected drug

Remote (must have first two)
In general, this category is applicable to an adverse event which meets the following

criteria:

1 It does not follow a reasonable temporal sequence from drug administration
2 It may readily have been produced by the subject's clinical state, environment or toxic factors, or other modes of therapy administered to the subject.
3 It does not follow a known pattern of response to the suspected drug
4 It does not reappear or worsen when the drug is readministered

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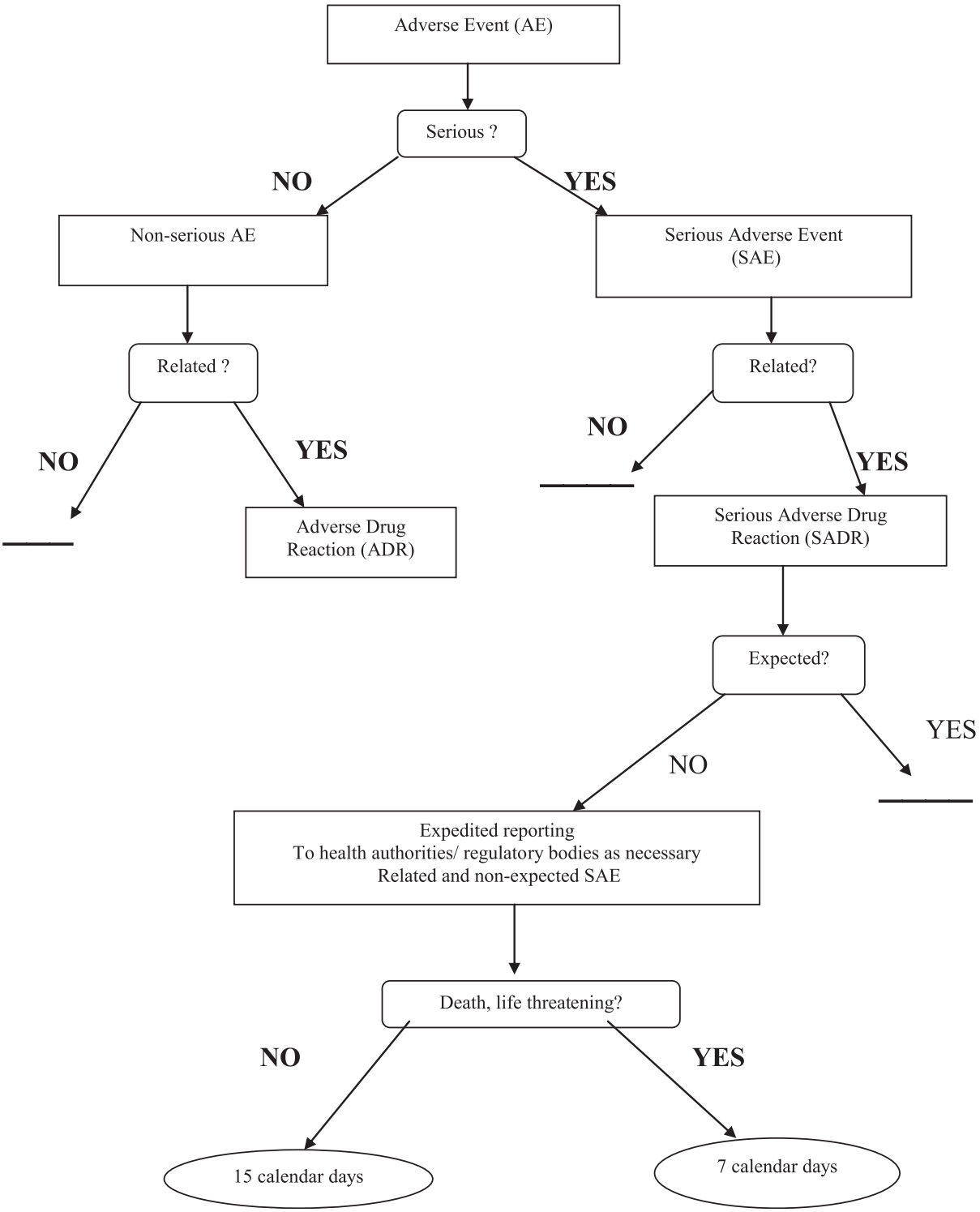
Flow Chart of the management of Adverse Events



Appendix 2b (SOP for staff managing Adverse events/SUSARs)

Notification of a serious adverse event

As the study is recruiting patients who are 65 years and older with mixed pathology and disease progression (with no upper age limit) there are expected to be a number of adverse events including death.

With the above expectation it has been decided that the following list (although not conclusive) will be used as a guideline for reporting "sudden unexpected severe adverse reactions" **(SUSARs**) and will be reported to DMEC and other regulatory bodies (MHRA) as required following the guidelines in the EU Directive 2001/20/EC.

1 Bacterial infection caused by a probiotic organism. This would be any manifestation of infection (abscess, bacterial endocarditis, bacteraemia etc.) where a lactobacillus or bifidobacteria is isolated in pathological specimens by the microbiology laboratories.

2 The development of multi- organ failure not present at recruitment (vasopressor administration for circulatory support and multi- organ failure are exclusion criteria)

3 The development of bowel ischaemia not present at recruitment (any past or current abnormality or disease affecting the mesenteric arteries is an exclusion criterion).

These SUSARs will be reported immediately to the Independent Safety Monitor to consider their attribution to the participant's participation in the trial and also to the Ethical Committee in accordance with local and national requirements.

For other serious adverse events, a summary will be provided to the Safety Monitor every 3 months and to the Chair of the DMEC every 6 months.

Procedure
The person who is first aware of the SAE/ SUSAR must notify the project manager / study co-ordinator immediately.

The investigator to be informed and to assist in completing the relevant documents. Where possible the investigator should clarify if the event was related to the trial intervention and assess the severity of the event.

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Study co-ordinator (Swansea)
Claire Fagan
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Morriston Hospital ABM NHS Trust Swansea

Appendix 9 Information regarding exposure to antibiotics according to centre and severe adverse events in the two intervention arms classified according to MedDRA preferred term and actions taken regarding the trial interventions

TABLE 31 Antibiotic exposure according to centre. The number and percentage of participants who received therapy with the antibiotic during the period 7 days before recruitment to the end of follow-up at 8 weeks

	ABMUHB			CDDFT	
Antibiotic (classes and drugs)	Singleton, *n* (%)	Morriston, *n* (%)	Princess of Wales, *n* (%)	Durham, *n* (%)	Darlington, *n* (%)
Penicillins	138 (68.0)	968 (65.4)	147 (77.0)	436 (79.7)	424 (81.4)
Benzylpenicillin	18 (8.9)	130 (8.8)	23 (12.0)	20 (3.7)	23 (4.4)
Penicillinase-resistant penicillin – flucloxacillin	29 (14.3)	372 (25.2)	68 (35.6)	83 (15.2)	80 (15.4)
Broad-spectrum penicillins	116 (57.1)	700 (47.3)	96 (50.3)	371 (67.8)	368 (70.6)
• Amoxicillin	32 (15.8)	224 (15.1)	43 (22.5)	209 (38.2)	125 (24.0)
• Ampicillin	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	2 (0.4)
• Co-amoxiclav	97 (47.8)	559 (37.8)	68 (35.6)	227 (41.5)	284 (54.5)
Antipseudomonas penicillins	18 (8.9)	126 (8.5)	24 (12.6)	52 (9.5)	25 (4.8)
• Piperacillin	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.4)	1 (0.2)
• Piperacillin plus tazobactam	18 (8.9)	126 (8.5)	24 (12.6)	51 (9.3)	24 (4.6)
Cephalosporins	59 (29.1)	602 (40.7)	26 (13.6)	18 (3.3)	10 (1.9)
First generation	10 (4.9)	113 (7.6)	6 (3.1)	15 (2.7)	7 (1.3)
• Cefalexin	10 (4.9)	112 (7.6)	6 (3.1)	15 (2.7)	7 (1.3)
• Cefradine	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Second generation	52 (25.6)	517 (35.0)	22 (11.5)	3 (0.5)	0 (0.0)
• Cefaclor	13 (6.4)	38 (2.6)	0 (0.0)	0 (0.0)	0 (0.0)
• Cefixime	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
• Cefuroxime	45 (22.2)	507 (34.3)	22 (11.5)	3 (0.5)	0 (0.0)
Third generation	2 (1.0)	16 (1.1)	0 (0.0)	0 (0.0)	3 (0.6)
• Cefotaxime	1 (0.5)	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
• Ceftazidime	0 (0.0)	11 (0.7)	0 (0.0)	0 (0.0)	3 (0.6)
• Ceftriaxone	1 (0.5)	4 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)

continued

TABLE 31 Antibiotic exposure according to centre. The number and percentage of participants who received therapy with the antibiotic during the period 7 days before recruitment to the end of follow-up at 8 weeks (*continued*)

	ABMUHB			CDDFT	
Antibiotic (classes and drugs)	Singleton, *n* (%)	Morriston, *n* (%)	Princess of Wales, *n* (%)	Durham, *n* (%)	Darlington, *n* (%)
Other antibiotics	153 (75.4)	1036 (70.0)	138 (72.3)	332 (60.7)	295 (56.6)
Carbapenems and other beta-lactams	8 (3.9)	43 (2.9)	4 (2.1)	6 (1.1)	1 (0.2)
Ertapenem	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)
Imipenem	0 (0.0)	5 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)
Meropenem	8 (3.9)	39 (2.6)	4 (2.1)	5 (0.9)	1 (0.2)
Tetracyclines	36 (17.7)	235 (15.9)	26 (13.6)	74 (13.5)	62 (11.9)
Demeclocycline	0 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Doxycycline	36 (17.7)	226 (15.3)	26 (13.6)	69 (12.6)	55 (10.6)
Lymecycline	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Oxytetracycline	0 (0.0)	4 (0.3)	0 (0.0)	6 (1.1)	4 (0.8)
Tetracycline	0 (0.0)	2 (0.1)	0 (0.0)	1 (0.2)	3 (0.6)
Aminoglycosides	7 (3.4)	271 (18.3)	28 (14.7)	16 (2.9)	56 (10.7)
Gentamicin	6 (3.0)	271 (18.3)	28 (14.7)	16 (2.9)	56 (10.7)
Tobramycin	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Macrolides	45 (22.2)	144 (9.7)	34 (17.8)	152 (27.8)	125 (24.0)
Azithromycin	0 (0.0)	2 (0.1)	4 (2.1)	9 (1.6)	9 (1.7)
Clarithromycin	39 (19.2)	98 (6.6)	26 (13.6)	137 (25.0)	113 (21.7)
Erythromycin	9 (4.4)	52 (3.5)	5 (2.6)	12 (2.2)	6 (1.2)
Clindamycin	2 (1.0)	16 (1.1)	1 (0.5)	2 (0.4)	11 (2.1)
Sulphonamides and trimethoprim	40 (19.7)	267 (18.1)	30 (15.7)	88 (16.1)	45 (8.6)
Co-trimoxazole	0 (0.0)	5 (0.3)	0 (0.0)	1 (0.2)	0 (0.0)
Trimethoprim	40 (19.7)	262 (17.7)	30 (15.7)	87 (15.9)	45 (8.6)
Metronidazole	22 (10.8)	217 (14.7)	21 (11.0)	30 (5.5)	23 (4.4)
Quinolones	43 (21.2)	229 (15.5)	16 (8.4)	41 (7.5)	36 (6.9)
Ciprofloxacin	38 (18.7)	224 (15.1)	15 (7.9)	31 (5.7)	20 (3.8)
Levofloxacin	5 (2.5)	7 (0.5)	1 (0.5)	11 (2.0)	11 (2.1)
Moxifloxacin	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.4)	5 (1.0)
Norfloxacin	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Glycopeptides	6 (3.0)	133 (9.0)	9 (4.7)	11 (2.0)	19 (3.6)
Teicoplanin	4 (2.0)	102 (6.9)	8 (4.2)	11 (2.0)	18 (3.5)
Vancomycin	2 (1.0)	43 (2.9)	1 (0.5)	0 (0.0)	1 (0.2)
TB drugs	1 (0.5)	31 (2.1)	3 (1.6)	6 (1.1)	5 (1.0)
Ethambutol	0 (0.0)	0 (0.0)	2 (1.0)	1 (0.2)	0 (0.0)
Rifampicin	1 (0.5)	31 (2.1)	3 (1.6)	6 (1.1)	5 (1.0)
Streptomycin	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)

TABLE 31 Antibiotic exposure according to centre. The number and percentage of participants who received therapy with the antibiotic during the period 7 days before recruitment to the end of follow-up at 8 weeks (*continued*)

	ABMUHB			CDDFT	
Antibiotic (classes and drugs)	Singleton, *n* (%)	Morriston, *n* (%)	Princess of Wales, *n* (%)	Durham, *n* (%)	Darlington, *n* (%)
Others	5 (2.5)	56 (3.8)	8 (4.2)	17 (3.1)	5 (1.0)
Daptomycin	0 (0.0)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Linezolid	0 (0.0)	2 (0.1)	1 (0.5)	0 (0.0)	0 (0.0)
Nitrofurantoin	5 (2.5)	41 (2.8)	4 (2.1)	17 (3.1)	4 (0.8)
Sodium fusidate	0 (0.0)	12 (0.8)	4 (2.1)	0 (0.0)	1 (0.2)

TABLE 32 Serious adverse events classified according to MedDRA PT, severity classification and intervention arm[a]

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
SAE resulted in death	79 (5.4)	64 (4.4)	143 (4.9)
Pneumonia	15 (1.0)	12 (0.8)	27 (0.9)
General physical health deterioration	10 (0.7)	6 (0.4)	16 (0.5)
Obstructive airways disorder	9 (0.6)	7 (0.5)	16 (0.5)
Lung neoplasm malignant	3 (0.2)	5 (0.3)	8 (0.3)
Cardiac failure	4 (0.3)	3 (0.2)	7 (0.2)
Cerebrovascular accident	4 (0.3)	3 (0.2)	7 (0.2)
Metastatic neoplasm	2 (0.1)	3 (0.2)	5 (0.2)
Sepsis	3 (0.2)	2 (0.1)	5 (0.2)
Cardiac arrest	2 (0.1)	2 (0.1)	4 (0.1)
Pleural effusion	2 (0.1)	1 (0.1)	3 (0.1)
Cardiac failure congestive	2 (0.1)	1 (0.1)	3 (0.1)
Elderly	2 (0.1)	1 (0.1)	3 (0.1)
Myocardial infarction	1 (0.1)	2 (0.1)	3 (0.1)
Pulmonary embolism	1 (0.1)	2 (0.1)	3 (0.1)
Renal failure chronic	2 (0.1)	1 (0.1)	3 (0.1)
Respiratory failure	2 (0.1)	1 (0.1)	3 (0.1)
Urinary tract infection	0 (0.0)	3 (0.2)	3 (0.1)
Gastrointestinal haemorrhage	2 (0.1)	0 (0.0)	2 (0.1)
Peptic ulcer perforation	1 (0.1)	0 (0.0)	1 (0.0)
Renal failure	0 (0.0)	2 (0.1)	2 (0.1)
Aortic aneurysm rupture	0 (0.0)	1 (0.1)	1 (0.0)
Bacterial sepsis	0 (0.0)	1 (0.1)	1 (0.0)
Bladder cancer	1 (0.1)	0 (0.0)	1 (0.0)

continued

TABLE 32 Serious adverse events classified according to MedDRA PT, severity classification and intervention arm[a] (*continued*)

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
Bladder neoplasm	1 (0.1)	0 (0.0)	1 (0.0)
Bronchiectasis	0 (0.0)	1 (0.1)	1 (0.0)
Cardiac death	1 (0.1)	0 (0.0)	1 (0.0)
Chronic renal failure	1 (0.1)	0 (0.0)	1 (0.0)
Creutzfeldt–Jakob disease	1 (0.1)	0 (0.0)	1 (0.0)
Dementia	1 (0.1)	0 (0.0)	1 (0.0)
Diverticulitis	1 (0.1)	0 (0.0)	1 (0.0)
Duodenal ulcer perforation	0 (0.0)	1 (0.1)	1 (0.0)
Hiatus hernia, obstructive	0 (0.0)	1 (0.1)	1 (0.0)
Hyponatraemia	0 (0.0)	1 (0.1)	1 (0.0)
Implant site infection	1 (0.1)	0 (0.0)	1 (0.0)
Ischaemic heart disease	1 (0.1)	0 (0.0)	1 (0.0)
Left ventricular failure	0 (0.0)	1 (0.1)	1 (0.0)
Lower gastrointestinal haemorrhage	1 (0.1)	0 (0.0)	1 (0.0)
Lung infection – pseudomonal	1 (0.1)	0 (0.0)	1 (0.0)
Pleural neoplasm	1 (0.1)	0 (0.0)	1 (0.0)
Pulmonary fibrosis	0 (0.0)	1 (0.1)	1 (0.0)
Pulmonary oedema	0 (0.0)	1 (0.1)	1 (0.0)
Insufficient details to classify	1 (0.1)	0 (0.0)	1 (0.0)
SAE was life-threatening	6 (0.4)	5 (0.3)	11 (0.4)
Cardiac arrest	1 (0.1)	2 (0.1)	3 (0.1)
Acute renal failure	1 (0.1)	0 (0.0)	1 (0.0)
Gastric cancer	1 (0.1)	0 (0.0)	1 (0.0)
Lung neoplasm malignant	0 (0.0)	1 (0.1)	1 (0.0)
Metastatic neoplasm	0 (0.0)	1 (0.1)	1 (0.0)
Pulmonary oedema	1 (0.1)	0 (0.0)	1 (0.0)
Sepsis	1 (0.1)	0 (0.0)	1 (0.0)
Small intestinal obstruction	1 (0.1)	0 (0.0)	1 (0.0)
Subdural haemorrhage	0 (0.0)	1 (0.1)	1 (0.0)
SAE prolonged or required hospitalisation	222 (15.1)	223 (15.2)	445 (15.1)
Pneumonia	35 (2.4)	38 (2.6)	73 (2.5)
Obstructive airways disorder	17 (1.2)	15 (1.0)	32 (1.1)
Fall	18 (1.2)	13 (0.9)	31 (1.1)
Urinary tract infection	14 (1.0)	9 (0.6)	23 (0.8)
Cerebrovascular accident	6 (0.4)	10 (0.7)	16 (0.5)
Wound infection	5 (0.3)	7 (0.5)	12 (0.4)

TABLE 32 Serious adverse events classified according to MedDRA PT, severity classification and intervention arm[a] (*continued*)

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
Cellulitis	10 (0.7)	2 (0.1)	12 (0.4)
Lower gastrointestinal haemorrhage	3 (0.2)	7 (0.5)	10 (0.3)
Sepsis	4 (0.3)	6 (0.4)	10 (0.3)
Cardiac failure congestive	6 (0.4)	3 (0.2)	9 (0.3)
Abdominal pain	4 (0.3)	3 (0.2)	7 (0.2)
Anaemia	5 (0.3)	3 (0.2)	8 (0.3)
Myocardial infarction	7 (0.5)	1 (0.1)	8 (0.3)
Angina pectoris	4 (0.3)	3 (0.2)	7 (0.2)
Cardiac failure	3 (0.2)	4 (0.3)	7 (0.2)
Dyspnoea	4 (0.3)	3 (0.2)	7 (0.2)
Gastrointestinal haemorrhage	4 (0.3)	2 (0.1)	6 (0.2)
Pleural effusion	1 (0.1)	4 (0.3)	5 (0.2)
Viral infection	3 (0.2)	3 (0.2)	6 (0.2)
Bacterial sepsis	2 (0.1)	3 (0.2)	5 (0.2)
Chest pain	4 (0.3)	1 (0.1)	5 (0.2)
Cholecystitis	2 (0.1)	2 (0.1)	4 (0.1)
Cholelithiasis	4 (0.3)	1 (0.1)	5 (0.2)
Gastroenteritis	3 (0.2)	2 (0.1)	5 (0.2)
Joint dislocation reduction	2 (0.1)	2 (0.1)	4 (0.1)
Lung infection – pseudomonal	1 (0.1)	3 (0.2)	4 (0.1)
Urinary retention	1 (0.1)	3 (0.2)	4 (0.1)
Diverticulitis	4 (0.3)	0 (0.0)	4 (0.1)
Drug hypersensitivity	0 (0.0)	4 (0.3)	4 (0.1)
Haemorrhagic diathesis	1 (0.1)	2 (0.1)	3 (0.1)
Pulmonary oedema	2 (0.1)	2 (0.1)	4 (0.1)
Constipation	1 (0.1)	2 (0.1)	3 (0.1)
General physical health deterioration	2 (0.1)	1 (0.1)	3 (0.1)
Pulmonary embolism	1 (0.1)	2 (0.1)	3 (0.1)
Renal failure	1 (0.1)	1 (0.1)	2 (0.1)
Upper gastrointestinal haemorrhage	3 (0.2)	0 (0.0)	3 (0.1)
Acute coronary syndrome	0 (0.0)	2 (0.1)	2 (0.1)
Arrhythmia	2 (0.1)	0 (0.0)	2 (0.1)
Bladder catheter management	0 (0.0)	2 (0.1)	2 (0.1)
Convulsion	2 (0.1)	0 (0.0)	2 (0.1)
Decubitus ulcer	0 (0.0)	2 (0.1)	2 (0.1)
Deep-vein thrombosis postoperative	1 (0.1)	1 (0.1)	2 (0.1)

continued

TABLE 32 Serious adverse events classified according to MedDRA PT, severity classification and intervention arm[a] (*continued*)

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
Dehydration	0 (0.0)	2 (0.1)	2 (0.1)
Haematoma	2 (0.1)	0 (0.0)	2 (0.1)
Haematuria	0 (0.0)	2 (0.1)	2 (0.1)
Hepatic cirrhosis	1 (0.1)	1 (0.1)	2 (0.1)
Hypercalcaemia	0 (0.0)	1 (0.1)	1 (0.0)
Ischaemic heart disease	1 (0.1)	1 (0.1)	2 (0.1)
Metastatic neoplasm	2 (0.1)	0 (0.0)	2 (0.1)
Oesophageal neoplasm	1 (0.1)	0 (0.0)	1 (0.0)
Organ failure	1 (0.1)	1 (0.1)	2 (0.1)
Pulmonary fibrosis	0 (0.0)	2 (0.1)	2 (0.1)
Pyelonephritis	0 (0.0)	2 (0.1)	2 (0.1)
Social problem	2 (0.1)	0 (0.0)	2 (0.1)
Transient ischaemic attack	2 (0.1)	0 (0.0)	2 (0.1)
Upper respiratory tract infection	0 (0.0)	2 (0.1)	2 (0.1)
Wound dehiscence	0 (0.0)	2 (0.1)	2 (0.1)
Adverse drug reaction	0 (0.0)	1 (0.1)	1 (0.0)
Agitation	0 (0.0)	1 (0.1)	1 (0.0)
Anorectal varices haemorrhage	1 (0.1)	0 (0.0)	1 (0.0)
Aortic surgery	1 (0.1)	0 (0.0)	1 (0.0)
Appendiceal abscess	0 (0.0)	1 (0.1)	1 (0.0)
Arterial thrombosis limb	0 (0.0)	1 (0.1)	1 (0.0)
Arthritis infective	1 (0.1)	0 (0.0)	1 (0.0)
Atrial fibrillation	1 (0.1)	0 (0.0)	1 (0.0)
Bile duct T-tube removal	0 (0.0)	1 (0.1)	1 (0.0)
Bile duct stent removal	0 (0.0)	1 (0.1)	1 (0.0)
Bone trimming	0 (0.0)	1 (0.1)	1 (0.0)
Brain neoplasm	0 (0.0)	1 (0.1)	1 (0.0)
Bronchial fistula repair	1 (0.1)	0 (0.0)	1 (0.0)
Bronchospasm	1 (0.1)	0 (0.0)	1 (0.0)
Cardiac pacemaker replacement	0 (0.0)	1 (0.1)	1 (0.0)
Cardiac pacemaker revision	0 (0.0)	1 (0.1)	1 (0.0)
Cholecystectomy	1 (0.1)	0 (0.0)	1 (0.0)
Colostomy	0 (0.0)	1 (0.1)	1 (0.0)
Cystoscopy	1 (0.1)	0 (0.0)	1 (0.0)
Deep-vein thrombosis	0 (0.0)	1 (0.1)	1 (0.0)
Dementia	1 (0.1)	0 (0.0)	1 (0.0)

TABLE 32 Serious adverse events classified according to MedDRA PT, severity classification and intervention arm[a] (*continued*)

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
Diabetic foot infection	0 (0.0)	1 (0.1)	1 (0.0)
Dialysis related complication	1 (0.1)	0 (0.0)	1 (0.0)
Diarrhoea	0 (0.0)	1 (0.1)	1 (0.0)
Duodenal ulcer haemorrhage	1 (0.1)	0 (0.0)	1 (0.0)
Eating disorder	1 (0.1)	0 (0.0)	1 (0.0)
Eczema	1 (0.1)	0 (0.0)	1 (0.0)
Foreign-body aspiration	1 (0.1)	0 (0.0)	1 (0.0)
Gallbladder empyema	1 (0.1)	0 (0.0)	1 (0.0)
Gangrene	1 (0.1)	0 (0.0)	1 (0.0)
Gastroenteritis norovirus	0 (0.0)	1 (0.1)	1 (0.0)
Gastrointestinal examination	0 (0.0)	1 (0.1)	1 (0.0)
Groin abscess	1 (0.1)	0 (0.0)	1 (0.0)
Haematoma infection	1 (0.1)	0 (0.0)	1 (0.0)
Heart valve replacement	1 (0.1)	0 (0.0)	1 (0.0)
Herpes zoster ophthalmic	0 (0.0)	1 (0.1)	1 (0.0)
Hip fracture	0 (0.0)	1 (0.1)	1 (0.0)
Hyperkalaemia	1 (0.1)	0 (0.0)	1 (0.0)
Hypertension	1 (0.1)	0 (0.0)	1 (0.0)
Hypocalcaemia	1 (0.1)	0 (0.0)	1 (0.0)
Hypoglycaemia	0 (0.0)	1 (0.1)	1 (0.0)
Hypokalaemia	0 (0.0)	1 (0.1)	1 (0.0)
Hyponatraemia	0 (0.0)	1 (0.1)	1 (0.0)
Implant site infection	1 (0.1)	0 (0.0)	1 (0.0)
Infected bites	1 (0.1)	0 (0.0)	1 (0.0)
International normalised ratio abnormal	0 (0.0)	1 (0.1)	1 (0.0)
Intestinal polyp	1 (0.1)	0 (0.0)	1 (0.0)
Ischaemic limb pain	1 (0.1)	0 (0.0)	1 (0.0)
Joint injection	0 (0.0)	1 (0.1)	1 (0.0)
Joint surgery	1 (0.1)	0 (0.0)	1 (0.0)
Leg amputation	0 (0.0)	1 (0.1)	1 (0.0)
Limb crushing injury	0 (0.0)	1 (0.1)	1 (0.0)
Liver abscess	0 (0.0)	1 (0.1)	1 (0.0)
Lung neoplasm malignant	0 (0.0)	1 (0.1)	1 (0.0)
Malnutrition	0 (0.0)	1 (0.1)	1 (0.0)
Mediastinal abscess	0 (0.0)	1 (0.1)	1 (0.0)
Mouth haemorrhage	0 (0.0)	1 (0.1)	1 (0.0)

continued

TABLE 32 Serious adverse events classified according to MedDRA PT, severity classification and intervention arm[a] (*continued*)

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
Multiple myeloma	1 (0.1)	0 (0.0)	1 (0.0)
Muscle swelling	0 (0.0)	1 (0.1)	1 (0.0)
Musculoskeletal chest pain	0 (0.0)	1 (0.1)	1 (0.0)
Myalgia	1 (0.1)	0 (0.0)	1 (0.0)
Nephrectomy	0 (0.0)	1 (0.1)	1 (0.0)
Nephrolithiasis	1 (0.1)	0 (0.0)	1 (0.0)
Non-Hodgkin's lymphoma	1 (0.1)	0 (0.0)	1 (0.0)
Oesophagogastroscopy	1 (0.1)	0 (0.0)	1 (0.0)
Oral discharge	0 (0.0)	1 (0.1)	1 (0.0)
Otitis externa	1 (0.1)	0 (0.0)	1 (0.0)
Pain in extremity	1 (0.1)	0 (0.0)	1 (0.0)
Pancreatitis	0 (0.0)	1 (0.1)	1 (0.0)
Peptic ulcer haemorrhage	1 (0.1)	0 (0.0)	1 (0.0)
Peptic ulcer perforation	1 (0.1)	0 (0.0)	1 (0.0)
Peritonitis	1 (0.1)	0 (0.0)	1 (0.0)
Pneumonia aspiration	0 (0.0)	1 (0.1)	1 (0.0)
Pneumothorax	1 (0.1)	0 (0.0)	1 (0.0)
Renal failure chronic	0 (0.0)	1 (0.1)	1 (0.0)
Shunt infection	0 (0.0)	1 (0.1)	1 (0.0)
Skin graft infection	0 (0.0)	1 (0.1)	1 (0.0)
Skin ulcer	1 (0.1)	0 (0.0)	1 (0.0)
Supraventricular tachycardia	1 (0.1)	0 (0.0)	1 (0.0)
Syncope	0 (0.0)	1 (0.1)	1 (0.0)
Trigeminal neuralgia	0 (0.0)	1 (0.1)	1 (0.0)
Umbilical hernia, obstructive	0 (0.0)	1 (0.1)	1 (0.0)
Urethral stent insertion	0 (0.0)	1 (0.1)	1 (0.0)
Ventricular tachycardia	1 (0.1)	0 (0.0)	1 (0.0)
Viral upper respiratory tract infection	0 (0.0)	1 (0.1)	1 (0.0)
Volvulus	1 (0.1)	0 (0.0)	1 (0.0)
Wound complication	0 (0.0)	1 (0.1)	1 (0.0)
Wound haematoma	0 (0.0)	1 (0.1)	1 (0.0)
Wound infection	1 (0.1)	0 (0.0)	1 (0.0)
Insufficient details to classify	0 (0.0)	1 (0.1)	1 (0.0)
SAE resulted in persistent or significant disability or incapacity	2 (0.1)	2 (0.1)	4 (0.1)
Cerebrovascular accident	1 (0.1)	2 (0.1)	3 (0.1)
Urinary tract infection	1 (0.1)	0 (0.0)	1 (0.0)

TABLE 32 Serious adverse events classified according to MedDRA PT, severity classification and intervention arm[a] (*continued*)

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
Other significant medical event	6 (0.4)	5 (0.3)	11 (0.4)
Upper gastrointestinal haemorrhage	1 (0.1)	1 (0.1)	2 (0.1)
Cardiac failure	0 (0.0)	1 (0.1)	1 (0.0)
Cholelithiasis	1 (0.1)	0 (0.0)	1 (0.0)
Dysentery	1 (0.1)	0 (0.0)	1 (0.0)
Haemorrhoids	1 (0.1)	0 (0.0)	1 (0.0)
Incision site haematoma	0 (0.0)	1 (0.1)	1 (0.0)
Joint dislocation reduction	1 (0.1)	0 (0.0)	1 (0.0)
Lower gastrointestinal haemorrhage	1 (0.1)	0 (0.0)	1 (0.0)
Psoas abscess	0 (0.0)	1 (0.1)	1 (0.0)
Renal failure	0 (0.0)	1 (0.1)	1 (0.0)

a Diagnoses for SAEs were based on all available clinical evidence. SAEs were classified according to an organ system wherever possible. For example, 'cellulitis' was classified as 'skin and subcutaneous tissue disorders' in preference to 'infections and infestations'. SAEs were allocated to the most appropriate PT of the MedDRA.[42]

b Percentages represent the number of participants with at least one SAE divided by the number of participants in the treatment arm.

TABLE 33 Serious adverse events that resulted in the IMP being withdrawn or discontinued temporarily according to MedDRA PT[a] and intervention arm

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
IMP withdrawn	8 (0.5)	6 (0.4)	14 (0.5)
Lower gastrointestinal haemorrhage	1 (0.1)	1 (0.1)	2 (0.1)
Pneumonia	2 (0.1)	0 (0.0)	2 (0.1)
Acute renal failure	1 (0.1)	0 (0.0)	1 (0.0)
Cerebrovascular accident	0 (0.0)	1 (0.1)	1 (0.0)
Cholelithiasis	1 (0.1)	0 (0.0)	1 (0.0)
Drug hypersensitivity	0 (0.0)	1 (0.1)	1 (0.0)
Duodenal ulcer perforation	0 (0.0)	1 (0.1)	1 (0.0)
Haemorrhagic diathesis	0 (0.0)	1 (0.1)	1 (0.0)
Metastatic neoplasm	0 (0.0)	1 (0.1)	1 (0.0)
Peptic ulcer perforation	1 (0.1)	0 (0.0)	1 (0.0)
Sepsis	1 (0.1)	0 (0.0)	1 (0.0)
Upper gastrointestinal haemorrhage	1 (0.1)	0 (0.0)	1 (0.0)
IMP withdrawn temporarily	44 (3.0)	46 (3.1)	90 (3.1)
Pneumonia	4 (0.3)	5 (0.3)	9 (0.3)
Urinary tract infection	3 (0.2)	3 (0.2)	6 (0.2)
Cerebrovascular accident	3 (0.2)	2 (0.1)	5 (0.2)
Obstructive airways disorder	3 (0.2)	2 (0.1)	5 (0.2)
Angina pectoris	2 (0.1)	2 (0.1)	4 (0.1)
Fall	1 (0.1)	3 (0.2)	4 (0.1)
Wound infection	1 (0.1)	3 (0.2)	4 (0.1)
Cholecystitis	1 (0.1)	1 (0.1)	2 (0.1)
Myocardial infarction	3 (0.2)	0 (0.0)	3 (0.1)
Viral infection	3 (0.2)	0 (0.0)	3 (0.1)
Abdominal pain	1 (0.1)	1 (0.1)	2 (0.1)
Bacterial sepsis	1 (0.1)	1 (0.1)	2 (0.1)
Cardiac arrest	0 (0.0)	2 (0.1)	2 (0.1)
Cellulitis	2 (0.1)	0 (0.0)	2 (0.1)
Gastrointestinal haemorrhage	2 (0.1)	0 (0.0)	2 (0.1)
Haematoma	2 (0.1)	0 (0.0)	2 (0.1)
Joint dislocation reduction	1 (0.1)	1 (0.1)	2 (0.1)
Lung infection – pseudomonal	1 (0.1)	1 (0.1)	2 (0.1)
Appendiceal abscess	0 (0.0)	1 (0.1)	1 (0.0)
Bronchospasm	1 (0.1)	0 (0.0)	1 (0.0)
Cardiac pacemaker revision	0 (0.0)	1 (0.1)	1 (0.0)
Chest pain	1 (0.1)	0 (0.0)	1 (0.0)
Cholelithiasis	1 (0.1)	0 (0.0)	1 (0.0)

TABLE 33 Serious adverse events that resulted in the IMP being withdrawn or discontinued temporarily according to MedDRA PT[a] and intervention arm (*continued*)

PT	Probiotic (*N* = 1470), *n* (%)[b]	Placebo (*N* = 1471), *n* (%)[b]	Total (*N* = 2941), *n* (%)[b]
Deep-vein thrombosis postoperative	1 (0.1)	0 (0.0)	1 (0.0)
Diabetic foot infection	0 (0.0)	1 (0.1)	1 (0.0)
Diverticulitis	1 (0.1)	0 (0.0)	1 (0.0)
Dysentery	1 (0.1)	0 (0.0)	1 (0.0)
Gastroenteritis	1 (0.1)	0 (0.0)	1 (0.0)
Hepatic cirrhosis	0 (0.0)	1 (0.1)	1 (0.0)
Incision site haematoma	0 (0.0)	1 (0.1)	1 (0.0)
International normalised ratio abnormal	0 (0.0)	1 (0.1)	1 (0.0)
Ischaemic limb pain	1 (0.1)	0 (0.0)	1 (0.0)
Joint injection	0 (0.0)	1 (0.1)	1 (0.0)
Leg amputation	0 (0.0)	1 (0.1)	1 (0.0)
Lower gastrointestinal haemorrhage	0 (0.0)	1 (0.1)	1 (0.0)
Nephrectomy	0 (0.0)	1 (0.1)	1 (0.0)
Pain in extremity	1 (0.1)	0 (0.0)	1 (0.0)
Pneumonia aspiration	0 (0.0)	1 (0.1)	1 (0.0)
Psoas abscess	0 (0.0)	1 (0.1)	1 (0.0)
Pulmonary embolism	0 (0.0)	1 (0.1)	1 (0.0)
Pulmonary oedema	1 (0.1)	0 (0.0)	1 (0.0)
Sepsis	0 (0.0)	1 (0.1)	1 (0.0)
Shunt infection	0 (0.0)	1 (0.1)	1 (0.0)
Skin graft infection	0 (0.0)	1 (0.1)	1 (0.0)
Small intestinal obstruction	1 (0.1)	0 (0.0)	1 (0.0)
Upper gastrointestinal haemorrhage	1 (0.1)	0 (0.0)	1 (0.0)
Upper respiratory tract infection	0 (0.0)	1 (0.1)	1 (0.0)
Viral upper respiratory tract infection	0 (0.0)	1 (0.1)	1 (0.0)
Wound dehiscence	0 (0.0)	1 (0.1)	1 (0.0)
Wound haematoma	0 (0.0)	1 (0.1)	1 (0.0)

a Diagnoses for SAEs were based on all available clinical evidence. SAEs were classified according to an organ system wherever possible. For example, 'cellulitis' was classified as 'skin and subcutaneous tissue disorders' in preference to 'infections and infestations'. SAEs were allocated to the most appropriate PT of the MedDRA.[42]

b Percentages represent the number of participants with at least one SAE divided by the number of participants in the treatment arm.

EME
HS&DR
HTA
PGfAR
PHR

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