Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial

Stephen J Allen, Kathie Wareham, Duolao Wang, Caroline Bradley, Hayley Hutchings, Wyn Harris, Anjan Dhar, Helga Brown, Alwyn Foden, Michael B Gravenor, Dietrich Mack

**Summary**

**Background** Antibiotic-associated diarrhoea (AAD) occurs most frequently in older (≥65 years) inpatients exposed to broad-spectrum antibiotics. When caused by *Clostridium difficile*, AAD can result in life-threatening illness. Although underlying disease mechanisms are not well understood, microbial preparations have been assessed in the prevention of AAD. However, studies have been mostly small single-centre trials with varying quality, providing insufficient data to reliably assess effectiveness. We aimed to do a pragmatic efficacy trial in older inpatients who would be representative of those admitted to National Health Service (NHS) and similar secondary care institutions and to recruit a sufficient number of patients to generate a definitive result.

**Methods** We did a multicentre, randomised, double-blind, placebo-controlled, pragmatic, efficacy trial of inpatients aged 65 years and older and exposed to one or more oral or parenteral antibiotics. A computer-generated randomisation scheme was used to allocate participants (in a 1:1 ratio) to receive either a multistrain preparation of lactobacilli and bifidobacteria, with a total of 6 × 10¹⁰ organisms, one per day for 21 days, or an identical placebo. Patients, study staff, and specimen and data analysts were masked to assignment. The primary outcomes were occurrence of AAD within 8 weeks and *C difficile* diarrhoea (CDD) within 12 weeks of recruitment. Analysis was by modified intention-to-treat. This trial is registered, number ISRCTN70017204.

**Findings** Of 17 420 patients screened, 1493 were randomly assigned to the microbial preparation group and 1488 to the placebo group. 1470 and 1471, respectively, were included in the analyses of the primary endpoints. AAD occurred in 159 (10·8%) participants in the microbial preparation group and 153 (10·4%) participants in the placebo group (relative risk [RR] 1·04; 95% CI 0·84–1·28; p=0·71). CDD was an uncommon cause of AAD and occurred in 12 (0·8%) participants in the microbial preparation group and 17 (1·2%) participants in the placebo group (RR 0·71; 95% CI 0·34–1·47; p=0·35). 578 (19·7%) participants had one or more serious adverse event; the frequency of serious adverse events was much the same in the two study groups and none was attributed to participation in the trial.

**Interpretation** We identified no evidence that a multistrain preparation of lactobacilli and bifidobacteria was effective in prevention of AAD or CDD. An improved understanding of the pathophysiology of AAD is needed to guide future studies.

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**Introduction** Antibiotic-associated diarrhoea (AAD) occurs most frequently in older (≥65 years) inpatients exposed to broad-spectrum antibiotics, the risk increases progressively with longer treatment courses, and it can occur up to 12 weeks after antibiotic exposure. The frequency of diarrhoea varies according to the antibiotic used, occurring in 2–20% of patients given cephalosporins, fluoroquinolones, macrolides, or tetracycline, 5–10% given ampicillin, and 10–25% given co-amoxiclav. Additional recognised risk factors for AAD include prolonged hospital stay, treatment with proton pump inhibitors, use of a nasogastric tube, previous hospital admission, and previous gastrointestinal surgery. The main mechanism by which antibiotics cause diarrhoea is thought to be through impaired resistance to pathogens as a result of disruption of the gut microbial flora and subsequent changes in the metabolism of carbohydrates, short-chain fatty acids, and bile acids. AAD is usually a mild and self-limiting illness but 15–39% of cases are caused by *Clostridium difficile*, which can result in pseudomembranous colitis, toxic megacolon, and high case-fatality. Although some investigations have failed to identify high-risk antibiograms, *C difficile* is associated with *C difficile* diarrhoea (CDD). Additionally, cumulative antibiotic exposure increases risk. Of great concern...
since 2003 has been an increased frequency and severity of CDD associated with emergence of the hyper-virulent 027 strain. This concern has lead to concerted efforts to prevent infection through improved environmental hygiene, handwashing, antibiotic stewardship, and isolation of patients with diarrhoea.19

In view of the proposed underlying disease mechanisms, several trials have assessed microbial preparations that might prevent or ameliorate AAD through anti-pathogen effects, such as secretion of bacteriocins, competition for nutrients and binding sites, and enhancement of the immunological barrier function and integrity of the gut mucosa.15 Meta-analyses have provided some evidence for the efficacy of microbial preparations in prevention of AAD.22 However, substantial statistical heterogeneity in pooled results, attributable to variation in individual study results, undermined the findings.

Our hypothesis was that the administration of a microbial preparation would reduce the frequency of AAD and CDD in an at-risk population. We aimed to do a pragmatic efficacy trial in older inpatients who would be representative of those admitted to National Health Service (NHS) and similar secondary care institutions and to recruit a sufficient number of patients to generate a definitive result. On the basis of previous evidence,16 we selected a high-dose, multi-strain preparation of lactobacilli and bifidobacteria, the genera most frequently assessed in clinical trials. In this report, we have used the term microbial preparation and avoided probiotic, on the basis that the effect of the intervention on prevention of AAD was unknown.19

Methods
Study design and participants
We did a multicentre, randomised, double-blind, placebo-controlled, two-group trial and have reported the trial protocol previously.23 Inpatients aged 65 years or older and exposed to one or more oral or intravenous antibiotics in the preceding 7 days, or about to start antibiotic treatment, were recruited by research nurses from medical and surgical wards of three hospitals in south Wales (Abertawe Bro Morgannwg University Health Board; ABMUHB) and two hospitals in northeast England (County Durham and Darlington Foundation Trust; appendix p 1). Exclusion criteria were existing diarrhoea, immunocompromised sufficiently to need isolation or barrier nursing, illness needing high dependency or intensive care, prosthetic heart valve, CDD in the previous 3 months, inflammatory bowel disease that had needed specific treatment in the previous 12 months, suspected acute pancreatitis (abdominal pain with serum amylase or lipase more than three times the institutional upper limit of normal), known abnormality or disease of mesenteric vessels or coeliac axis, jejunal tube in situ or receiving jejunal feeds, previous adverse reaction to microbial preparations, and unwillingness to discontinue existing use of microbial preparations. In practice, patients who were nil by mouth or severely ill and not expected to survive for the period of follow-up were also not invited to join the study.

Patients provided signed informed consent or, when assessed to be unable to do so, signed assent was provided by relatives or carers. The Research Ethics Committee for Wales approved the study on Nov 27, 2008 (No 08/MRE09/18).

Randomisation and masking
Eligible patients were allocated sequentially by research nurses in a 1:1 ratio to the two groups (placebo or microbial preparation) of the study, according to a computer-generated random sequence, stratified by centre and using blocks of variable size. The allocation sequence was generated by the independent statistician and not available to any member of the research team until databases had been completed and locked. Patients, study staff, and specimen and data analysts were masked to assignment. In view of the established safety record of lactobacilli and bifidobacteria15 there was no provision for emergency unmasking of participants and copies of the allocation sequence were not held at the recruiting centres.

Procedures
The microbial preparation was a lyophilised powder in a vegetarian capsule containing $6 \times 10^{10}$ live bacteria: two strains of Lactobacillus acidophilus (CUL60, National Collection of Industrial, Food and Marine Bacteria [NCIMB] 30157; and CUL21, NCIMB 30156) and two strains of bifidobacterium (Bifidobacterium bifidum CUL20, NCIMB 30153; and Blactis CUL34, NCIMB 30172). Identical placebo capsules contained inert maltodextrin powder. The dose was one capsule per day for 21 days with food and, when possible, between antibiotic doses. Unused capsules were collected opportunistically from some participants at the point of use for quantitative bacterial culture by an independent laboratory.

Research nurses collected baseline demographic data, characteristics of patients, and details of antibiotic therapy. Participants were followed up by research staff daily during hospital admission and weekly by phone call after discharge. We had intended that follow-up would continue for 8 weeks after stopping antibiotics. In practice, prolonged follow-up for participants on long courses of antibiotics was not feasible and follow-up was discontinued at 8 weeks after recruitment. Changes to antibiotic treatment, the occurrence of diarrhoea, gastrointestinal symptoms, adverse events, and compliance with the trial interventions were recorded on standard forms.

We defined diarrhoea as three or more loose stools (consistency 5–7 on the Bristol Stool Form Scale) in a 24 h period or as stools described as looser than normal in participants unable to use the scale. Stool samples...
were collected only during episodes of diarrhoea and were analysed for *Salmonella* spp, *Shigella* spp, *Campylobacter* spp, *Escherichia coli* O157, and ova, cysts, and parasites in a wet film according to routine laboratory practice. Detection of viruses was done according to the clinical context and during suspected diarrhoea outbreaks. In ABMUHB, detection of *C difficile* toxins was by an in-house tissue culture assay with confirmation by enzyme immunoassay (Premier Toxins A&B; Meridian Bioscience, Cincinnati, OH, USA). In the two hospitals in northeast England, the VIDAS *Clostridium difficile* A & B assay (bioMérieux SA, Marcy l’Etoile, France) was used until June 2010, when detection of glutamate dehydrogenase (C. DIFF QUIK CHEK; TECHLAB, Blacksburg, VA, USA) was used in conjunction with the toxin assay. Hospital laboratory records were reviewed for occurrence of diarrheal stools positive for *C difficile* toxins until 12 weeks after recruitment.

**Statistical analysis**

The primary outcomes were the occurrence of AAD within 8 weeks and CDD within 12 weeks of recruitment. AAD was diarrhoea occurring in association with antibiotic therapy and without detection of diarrhoeal pathogens or an alternative explanation (eg, laxative treatment). Patients with AAD and a positive stool *C difficile* toxin assay were diagnosed as CDD.

Secondary outcomes were severity and duration of AAD and CDD, abdominal symptoms, serious adverse events, duration of hospital stay, the acceptability of the microbial preparation, and quality of life. CDD was managed by the patient’s clinical team and severity of the episode classified according to UK national guidelines from information collected from case records. Quality of life was assessed by the generic 12-item short form survey (SF12 v2), which was administered by research nurses at baseline, and 4 and 8 weeks. Additionally, we...
had intended to modify instruments validated to measure quality of life in treatment-induced diarrhoea in people with HIV and older patients with faecal incontinence. In practice, we decided that completion of additional questionnaires was too onerous for older inpatients and these instruments were not pursued. We estimated that AAD would occur in 20% and CDD in 4% of participants allocated to the placebo group. At the 5% significance level, 2478 participants (1239 in each group) were needed to detect a 50% reduction in CDD in the active group with 80% power and this sample size would provide a power of more than 99% to detect a CDD in the active group with 80% power and this sample size would provide a power of more than 99% to detect a 25% reduction in AAD in the active group. We intended to recruit 2974 participants to allow for 10% dropout and 10% loss to follow-up.

Antibiotics were classified according to British National Formulary categories, indications for antibiotic treatment according to the System Organ class, and serious adverse events according to Preferred Terms of the Medical Dictionary for Regulatory Activities.

For the primary endpoint analysis, we calculated relative risks (RRs) and odds ratios (ORs) together with their 95% CIs using a generalised linear model that included treatment as one predictor. We analysed secondary endpoints in the same way. Additionally, we did a covariate-adjusted analysis for the primary outcome analysis by logistic regression, controlling for ten prespecified potential risk factors for AAD (centre, age, sex, antibiotic class, duration of antibiotic treatment, antacid therapy, nasogastric tube in-situ, previous gastrointestinal surgery, recent previous hospital admission, and duration of hospital stay). We summarised continuous variables using number of observations, median and IQR, or mean and SD, depending on variable distributions; we summarised categorical variables by the number and percentage of events. We also used χ² tests and Mann-Whitney methods for comparative purposes.

We calculated SF12 v2 quality-of-life subscales and component summary scores with imputation of missing values when possible. SF12 v2 subdomain, physical component summary score, and mental component summary score were allocated a value of 0 for the lowest (worst) score and 100 for the highest (best) score. We used mixed model analysis to assess change from baseline in SF12 v2 physical component summary and mental component summary scores at 4 weeks and 8 weeks in the two study groups. Baseline score was used as a covariate and treatment, visit, and interaction between the treatment and visit as fixed effects; participant was a random effect. Incomplete observations were assumed to be missing at random.

We did the analysis of study outcomes and safety in a modified intention-to-treat population, excluding the small number of participants who withdrew shortly after randomisation, did not receive the interventions, and did not have follow-up data. We also did a per-protocol analysis, excluding participants who did not receive any doses of the trial interventions or in whom compliance was unclear, and in those who took all 21, 14 or more, or seven or more doses of the trial interventions. We used SAS (version 9.2) for data analyses.

This trial is registered, number ISRCTN70017204.

### Role of the funding source

The institutions funding the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study. SJA had final responsibility for the decision to submit for publication.

### Results

Recruitment was done between Dec 1, 2008, and Feb 28, 2012. 2981 of 17 420 (17·1%) patients assessed for inclusion were recruited of whom 2941 (98·7%) were included in the analysis according to treatment...
allocated (figure 1). The main reason for non-recruitment was participants who declined to take part (9068, 52·1%), mainly because of unwillingness to take an additional medication.

Baseline characteristics were generally much the same in the 1470 participants assessed in the microbial preparation group and the 1471 in the placebo group (table 1). Indications for antibiotic treatment were much the same in the two study groups, with the most common indication being respiratory, thoracic, and mediastinal disorders (appendix p 2). Exposure to antibiotics was similar in the two groups (table 2). Median number of days between hospital admission and starting an antibiotic was 0 (IQR 0–1) in both groups (p=0·35). Non-antibiotic drug treatment was common starting an antibiotic was 0 (IQR 0–1) in both groups (data not shown). Median duration of hospital admission was similar in the microbial preparation and the 1471 in the placebo group. CDD was an uncommon cause of AAD (195/266, 73·3%) and CDD (22/29, 75·9%) occurred with CDD was identified as having pseudomembranous colitis, needed colectomy, had a recurrence, or died from the illness.

Table 2: Antibiotic therapy by class and treatment group

| Antibiotic Class                   | Microbial preparation (n=1470) | Placebo (n=1471) |
|-----------------------------------|-------------------------------|------------------|
| Penicillins                        |                               |                  |
| Amy*                              | 1052 (71·6%)                  | 1061 (72·1%)     |
| Benzylopenicillin                  | 315 (7·8%)                    | 99 (6·7%)        |
| Penicillase resistant penicillin—fluoroquinolones | 322 (21·9%)                  | 310 (21·1%)      |
| Broad spectrum penicillins (amoxicillin, ampicillin, co-amoxiclav) | 822 (55·9%)                  | 829 (56·4%)      |
| Anti-pseudomonas penicillins (pipercillin, piperacillin plus tazobactam) | 127 (8·6%)                   | 118 (8·0%)       |
| Cephalosporins                    |                               |                  |
| Amy*                              | 359 (24·4%)                   | 356 (24·2%)      |
| First generation (cefalexin, cefadine) | 77 (5·2%)                    | 74 (5·0%)        |
| Second generation (cefuroxime, cefaclor, cefixime) | 290 (19·7%)                  | 304 (20·7%)      |
| Third generation (ceftaxime, ceftazidime, ceftriaxone) | 11 (0·7%)                    | 10 (0·7%)        |
| Other antibiotics                  |                               |                  |
| Carbapenems and other β-lactams (ertapenem, imipenem, meropenem) | 33 (2·2%)                    | 29 (2·0%)        |
| Tetracyclines (demeclocycline, doxycycline, lymecycline, oxytetacycline, tetracycline) | 211 (14·4%)                  | 222 (15·1%)      |
| Aminoglycosides (gentamicin, tobramycin) | 182 (12·4%)                  | 196 (13·3%)      |
| Macrolides (azithromycin, clarithromycin, erythromycin) | 249 (16·9%)                  | 251 (17·1%)      |
| Clindamycin | 18 (1·2%)                   | 14 (1·0%)        |
| Co-trimoxazole or trimethoprim | 228 (15·5%)                   | 242 (16·5%)      |
| Metronidazole | 171 (11·6%)                  | 142 (9·7%)       |
| Quinolones (ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin) | 185 (12·6%)                  | 180 (12·2%)      |
| Glycopeptides (teicoplanin, vancomycin) | 103 (7·0%)                   | 75 (5·1%)        |
| Tuberculosis drugs (ethambutol, rifampicin, streptomycin) | 26 (1·8%)                    | 20 (1·4%)        |
| Others (daptomycin, linezolid, nitrofurantoin, sodium fusidate) | 38 (2·6%)                    | 53 (3·6%)        |

Coadministration of antibiotic therapy

| Combination antibiotic therapy | Microbial preparation (n=1470) | Placebo (n=1471) |
|-------------------------------|-------------------------------|------------------|
| 1 class only                  | 310 (21·1%)                   | 310 (21·1%)      |
| 2 classes                     | 407 (27·7%)                   | 397 (27·0%)      |
| ≥3 classes                    | 752 (51·2%)                   | 764 (51·9%)      |

Duration of antibiotic therapy

| Duration of antibiotic therapy | Microbial preparation (n=1470) | Placebo (n=1471) |
|-------------------------------|-------------------------------|------------------|
| One dose                      | 123 (9·3%)                    | 123 (8·8%)       |
| 1–6 days treatment            | 389 (27·7%)                   | 398 (28·5%)      |
| 7–13 days treatment           | 402 (28·6%)                   | 426 (30·5%)      |
| ≥14 days treatment            | 482 (34·3%)                   | 451 (32·3%)      |

Data are number (%) of participants who received therapy with the antibiotic during the period 7 days before recruitment to the end of follow-up at 8 weeks. * Some participants received more than one antibiotic in these classes. **Duration of therapy was known in 1406 participants in the microbial preparation group and 1398 in the placebo group.
participants in the microbial preparation group and ten (0.7%) patients in the placebo group (RR 0.70; 95% CI 0.27–1.84). In the microbial preparation group, six patients had norovirus diarrhoea and one was diagnosed with non-specific colitis. In the placebo group, six patients had norovirus diarrhoea, one had diarrhoea after taking laxatives, two had drunk a large volume of fruit juice, and one had abnormal clotting and melaena.

Compliance with the trial interventions was known for 1462 participants in the microbial preparation and 1465 in the placebo group and was much the same in both study groups (figure 3). 777 (53.1%) participants in the microbial preparation group and 766 (52.3%) in the placebo group were observed or reported as taking all 21 doses. The corresponding figures for 14 or more doses were 1104 (75.5%) and 1106 (75.5%). Accounting for compliance in covariate analysis did not materially alter the risk of AAD (OR 1.02; 95% CI 0.80–1.30) or CDD (0.66; 0.30–1.47). 34 unused microbial preparation capsules collected at the point of use all contained at least 1.62×10¹⁰ viable bacteria and 33 placebo capsules tested were sterile.

578 (19.7%) participants had one or more serious adverse event; the frequency of serious adverse events was much the same in the two study groups (appendix pp 6–10). The most common events were respiratory, thoracic, and mediastinal disorders (83 of 1470 [5.6%] vs 87 of 1471 [5.9%]); gastrointestinal disorders (44 [3.0%] vs 35 [2.4%]); and cardiac disorders (42 [2.9%] vs 28 [1.9%]) in the microbial preparation and placebo groups, respectively. No serious adverse event was attributed to participation in the trial.

SF-12 v2 mental component summary, physical component summary, and subscale scores were similar at baseline and, with the exception of vitality, tended to increase either by 4 or 8 weeks. Changes from baseline were much the same in each group (appendix p 11).

Discussion
Administration of a high dose preparation of lactobacilli and bifidobacterium did not show the effect of prevention of AAD in our trial of nearly 3000 older inpatients. Analysis of secondary outcomes including diarrhoea severity, frequency of abdominal symptoms, length of hospital stay, and quality of life showed no evidence of a beneficial effect attributable to the microbial preparation. Accounting for potential risk factors for AAD and compliance with the trial interventions did not significantly change the findings. Per-protocol analysis produced consistent results with the intention-to-treat analysis.

As far as we are aware, our pragmatic study done in busy NHS hospitals is the largest trial so far for this problem (panel, figure 4). By contrast with many previous trials, we confirmed the viability of the microbes at the point of use. Our study had several weaknesses. Although we attempted to minimise the exclusion criteria
to patients clearly predisposed to diarrhoea and those who might be at specific risk from bacterial supplements,\(^5,13\) we recruited fewer than one in five eligible patients. The main reason for non-participation was the unwillingness of people already receiving medicines to take an additional preparation. This practical difficulty needs to be considered when developing novel interventions for older patients with many comorbidities. Ethnic diversity was low in our study but was representative of the local older populations.\(^4\) Despite the low conversion rate, we recruited from a range of medical and surgical wards in five hospitals and the baseline characteristics, comorbidity, and indications for antibiotic treatment suggest that our findings are relevant to older inpatients in NHS and similar secondary care settings.

Our trial suggests that properties common to many so-called probiotic bacteria, such as the production of lactic acid, are not effective against AAD in older inpatients.

### Panel: Research in context

**Systematic review**

Several meta-analyses of trials of microbial preparations in the prevention of AAD have suggested a beneficial effect\(^13,22–25,28\), including the most comprehensive review so far (63 trials; 11,811 participants), which reported that microbial preparations reduced the risk of AAD (random effects analysis: RR 0.58; 95% CI 0.50–0.68).\(^22\) However, as in other reviews, the clinical trials included varied substantially in participant characteristics, the microbial preparations tested, antibiotic exposure, and trial settings, and the reliability of this pooled result was undermined by large statistical heterogeneity ($I^2$=54%). Subgroup analyses accounting for these factors did not explain the heterogeneity. As in a Cochrane review\(^28\) of microbial preparations in the prevention of AAD in children, trial design and reporting were often poor.\(^27\)

For the prevention of CDD, efficacy of the microbial preparation in our study was consistent with the findings of a meta-analyses (20 trials, 3818 people; random effects model: RR 0.58; 95% CI 0.50–0.68).\(^22\) However, as in other reviews, the clinical trials included varied substantially in participant characteristics, the microbial preparations tested, antibiotic exposure, and trial settings, and the reliability of this pooled result was undermined by large statistical heterogeneity ($I^2$=80%). Subgroup analyses accounting for these factors did not explain the heterogeneity. As in a Cochrane review\(^28\) of microbial preparations in the prevention of AAD in children, trial design and reporting were often poor.\(^27\)

For CDD, we identified only four trials that either studied older patients\(^29\) or the participants recruited had an average age of older than 65 years\(^30–32\) (figure 4). Although the pooled result showed a statistically significant risk reduction in AAD in patients receiving microbial preparations, the difference was small and unlikely to be of clinical significance. Furthermore, despite limiting the scope of the studies, substantial statistical heterogeneity ($I^2$=90%) undermines the reliability of this finding.

For CDD, we identified only one previous trial that has reliably reported outcomes in this age group;\(^33\) CDD was reduced in participants receiving a combination of Lactobacillus casei DN-114 001, L bulgaricus, and Streptococcus thermophilus (none of 56; 0%) compared with those assigned placebo (nine of 53; 17.0%). However, the frequency of CDD in the control group was high (17.0%) and patients were highly selected.\(^30,33\)

**Interpretation**

Administration of a high dose preparation of lactobacilli and bifidobacteria did not show the effect of prevention of AAD in our trial of nearly 3000 older inpatients. Overall, we believe that there is insufficient evidence to support the use of any microbial preparation for the prevention of AAD in older inpatients.
The design of further intervention studies is hampered by a poor understanding of the pathophysiology of AAD. Potentially important but largely unknown factors include the mechanisms by which specific antibiotics cause diarrhoea and how these mechanisms might be affected by characteristics of the pretreatment enteric flora, which varies between individuals and is affected by age, chronic disease, frailty, diet, residence, and care setting. Also, whether specific strains of microbes possess specific anti-diarrhoeal mechanisms needs further investigation.

Many episodes of AAD were of short duration and we failed to obtain stool samples for testing in about 40% of participants with diarrhoea. When reported, this issue has also been a problem in smaller trials with shorter follow-up. Although these missing samples probably resulted in some missed cases of CDD in our study, the low frequency of CDD (0·99% overall) is consistent with falling rates in England, Wales, and other regions associated with other approaches to prevention of AAD. The proportion of stools tested in our study was much the same in the two groups and, in view of the absence of efficacy of the microbial preparation against AAD, it seems unlikely that the missing stool analyses have biased the estimate of intervention effect against CDD. Overall, further assessment of novel interventions for the prevention of CDD needs to take account of its falling frequency in some settings so that potential benefits are balanced with potential risks and cost.

Our findings do not provide statistical evidence to support recommendations for the routine use of microbial preparations for the prevention of AAD and CDD. Further trials of microbial preparations should only be done when there is supporting evidence that one or more specific microbes act against identified underlying pathophysiological mechanisms for AAD and CDD in a specific population group.

Conflicts of interest
SJA has done research in probiotics supported by Cultech, UK, has been an invited guest at the Yakult Probiotic Symposium, and has received research funding from Yakult, UK. The other authors declare that they have no conflicts of interest.

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References
1 McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. *Dig Dis* 1998; 16: 292–307.
2 Bartlett JG. Clinical practice. Antibiotic-associated diarrhea. *N Engl J Med* 2002; 346: 334–39.
3 Antunes LG, Han J, Ferreira RB, Lolig P, Borchers CH, Finlay BB. Effect of antibiotic treatment on the intestinal metabolome. *Antimicrob Agents Chemother* 2011; 55: 1949–503.
4 Badger VO, Ledeboer NA, Graham MB, Edmiston CE Jr. *Clostridium difficile* epidemiology, pathogenesis, management, and prevention of a recalcitrant healthcare-associated pathogen. *JPEN J Parenter Enteral Nutr* 2012; 36: 645–62.
5 Weiss K, Bergeron L, Bernatchez H, Goyette M, Savoie M, Thirion D. *Clostridium difficile*-associated diarrhoea rates and global antibiotic consumption in five Quebec institutions from 2001 to 2004. *Int J Antimicrob Agents* 2007; 30: 309–14.
6 Thomas C, Stevenson M, Riley TV. Antibiotics and hospital-acquired *Clostridium difficile*-associated diarrhoea: a systematic review. *J Antimicrob Chemother* 2001; 51: S139–50.
7 Bartlett JG, Gerding DN. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2008; 46 (supp 1): S12–18.
8 Stevens V, Dumuyati G, Fine LS, Fisher SG, van Wijngaarden E. Cumulative antibiotic exposures over time and the risk of *Clostridium difficile* infection. *Clin Infect Dis* 2011; 53: 42–48.
9 Warny M, Pepin J, Fang A, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 2005; 366: 1079–84.
10 UK Department of Health. *Clostridium difficile* infection: how to deal with the problem. London: Department of Health, 2008.
11 Ohlund CL, Macnaburtin WK. Probiotic bacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol* 2010; 298: G807–19.
12 McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic-associated diarrhoea and the treatment of *Clostridium difficile* disease. *Am J Gastroenterol* 2006; 101: S12–22.
13 FAO, WHO. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. 2001. ftp://ftp.fao.org/esn/food/probioto_report_en.pdf (accessed April 10, 2013).
14 Allen SJ, Wareham K, Bradley C, et al. A multicentre randomised controlled trial evaluating lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea in older people admitted to hospital: the PLACIDE study protocol. *BMJ Infect Dis* 2012; 12: 108.
15 Hempel S, Newberry S, Ruelaz A, et al. Safety of probiotics used to reduce risk and prevent or treat disease. *Evid Rep Technol Assess (Full Rep)* 2011; 200: 1–645.
16 Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 1997; 32: 920–24.
17 Ware JE Jr, Kosinski M, Turner-Bowker DM, Gandek B. User’s Manual for the SF-12 v2 Health Survey (with a Supplement Documenting SF-12 v2 Health Survey). Lincoln, RI: QualityMetric Incorporated, 2002.
18 Thielman NM, Rust PF, Guerrant RL. Criterion-related validity of a diarrhea questionnaire in HIV-infected patients. *Dig Dis Sci* 2002; 47: 1423–26.
19 Rockwood TH, Church JM, Fleshman JW, et al. Fecal Incontinence Quality of Life Score: quality of life instrument for patients with fecal incontinence. *Dis Colon Rectum* 2000; 43: 9–16.
20 British Medical Association and Royal Pharmaceutical Society. British National Formulary. London: BMA and RPS, 2012.
21 ICH Secretariat. MedDRA term selection: points to consider. 2011. http://meddrarmsso.com/subscribe_library_ptc.asp (accessed June 25, 2013).
22 Hempel S, Newberry SJ, Maher AR, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA 2012; 307: 1959–69.
23 Cremolini F, Videlock EJ. Probiotics are associated with a decreased risk of antibiotic-associated diarrhea. Evid Based Med 2013; 18: 71–72.
24 Ritchie ML, Romaknuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. PLoS One 2012; 7: e34938.
25 Videlock EJ, Cremolini F. Meta-analysis: probiotics in antibiotic-associated diarrhea. Aliment Pharmacol Ther 2012; 35: 1355–69.
26 McFarland LV. Diarrhoea associated with antibiotic use. BMJ 2007; 335: 54–55.
27 Johnston BC, Ma SS, Goldenberg JZ, et al. Probiotics for the prevention of Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. Ann Intern Med 2012; 157: 878–88.
28 Johnston BC, Goldenberg JZ, Vandvik PO, Sun X, Guyatt GH. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database Syst Rev 2011; 11: CD004827.
29 Stockenhuber A, Kanhuber C, Leeb G, et al. Preventing antibiotic-associated diarrhea using a probiotic Lactobacillus casei preparation. Gut 2008; 57: (suppl 1): A20.
30 Beausoleil M, Fortier N, Guenette S, et al. Effect of a fermented milk combining Lactobacillus acidophilus CII285 and Lactobacillus casei in the prevention of antibiotic-associated diarrhea: a randomized, double-blind, placebo-controlled trial. Can J Gastroenterol 2007; 21: 732–36.
31 Beniwal RS, Arena VC, Thomas L, et al. A randomized trial of yogurt for prevention of antibiotic-associated diarrhea. Dig Dis Sci 2003; 48: 2077–82.
32 Hickman M, D’Souza AL, Muthu N, et al. Use of probiotic Lactobacillus preparation to prevent diarrhea associated with antibiotics: randomized double blind placebo controlled trial. BMJ 2007; 335: 80.
33 Besselink MG, van Santvoort HC, Buskens E, et al, and the Dutch Acute Pancreatitis Study Group. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomized, double-blind, placebo-controlled trial. Lancet 2008; 371: 651–59.
34 Office for National Statistics. Statistical bulletin: 2011 census: key statistics for England and Wales, March 2011. http://www.ons.gov.uk/ons/guide-method/census/2011/index.html (accessed June 25, 2013).
35 Arumugam M, Raes J, Pelletier E, et al, and the MetaHIT Consortium. Enterotypes of the human gut microbiome. Nature 2011; 473: 174–80.
36 Claesson MJ, Cusack S, O’Sullivan O, et al. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc Natl Acad Sci USA 2011; 108 (suppl 1): 4586–91.
37 Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012; 488: 178–84.
38 Health Protection Agency. Results from the mandatory Clostridium difficile reporting scheme. 2012. http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/ClostridiumDifficile/EpidemiologicalData/MandatorySurveillance/ cdiffMandatoryReportingScheme/ (accessed June 25, 2013).
39 Public Health Wales. All Wales Commentaries: Clostridium difficile reports. 2012. http://www.wales.nhs.uk/sites3/page.cfm?orgid=379&pid=18490 (accessed June 25, 2013).
40 Kanerva M, Mentula S, Virolainen-Julkunen A, Kärki T, Möttönen T, Lyytikäinen O, and the Hospital Infction Surveillance Team. Reduction in Clostridium difficile infections in Finland, 2008–2010. J Hosp Infect 2013; 83: 127–31.