doi: 10.1111/j.1471-0307.2009.00518.x

REVIEW

The potential of probiotic fermented milk products in reducing risk of antibiotic-associated diarrhoea and *Clostridium difficile* disease

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Currently, the burden of infective antibiotic-associated diarrhoea (AAD) and Clostridium difficile*-associated disease (CDAD) is placing considerable strain on health services. The potential of probiotics has been investigated, particularly whether they may help reduce the vulnerability of the elderly to these diseases. Probiotic mechanisms of activity include helping to maintain the normal intestinal microbiota and supporting its ability to resist colonisation by pathogens as well as immune modulation. Despite conflicting results from meta-analyses, there is now increasing evidence that certain* Lactobacillus *probiotic strains may reduce the risk of AAD and CDAD. Several recent studies report positive results with probiotic-fermented dairy products.*

Keywords Antibiotic-associated diarrhoea, *Clostridium difficile*, Fermented milk products, *Lactobacillus*, Probiotic.

PROBIOTIC-FERMENTED MILK PRODUCTS

The probiotic concept started to become established at the beginning of the 20th century with Metchnikoff's theory that long-term health benefit could result from using saccharolytic bacteria, particularly lactic acid producers, to replace proteolytic and toxin-producing organisms in the large intestine (Metchnikoff 1907; Hamilton-Miller 2008). The practical aspects of this idea were also investigated by Cohendy (1906a, 1906b) who reported that consumption of milk fermented with *Lactobacillus delbrueckii* var. *bulgaricus* (then known as the Bulgarian bacillus) reduced putrefactive gut fermentation and helped to establish a predominantly Gram-positive gut microbiota. Subsequent studies with other *Lactobacillus* species, bifidobacteria and fermented milk products gave further credence to the theory, leading to the development of one of the first probiotic products in 1930 by Minoru Shirota (Tissier 1906; Rettger and Cheplin 1921; Shortt 1999).

Many probiotic-fermented milk products as well as other types of probiotic food and drinks, powders, tablets and capsules are now available. *Lactobacillus* and *Bifidobacterium* strains still predominate in these products, but probiotics are also known in species of *Bacillus*, *Enterococcus*, *Escherichia*, *Lactococcus, Propionibacterium*, *Streptococcus* and the yeast *Saccharomyces*.

Scientific evidence and range of probiotic benefit

The scientific evidence for probiotics in food was evaluated in 2001 (Joint FAO/WHO Expert Consultation 2001), followed by publication of guidelines (Joint FAO/WHO Working Group 2002) which included a probiotic definition still widely accepted today: 'Live micro-organisms which when administered in adequate amounts confer a health benefit on the host'.

There is now a growing body of scientific evidence, primarily based on human trials and mechanistic studies, for probiotic benefits of certain strains. Many early trials investigated reduction of risk for diarrhoeal disease but research has now diversified into several aspects of health and disease ranging from modulation of the gut microbiota and support of the body's natural defences to irritable bowel syndrome (IBS), constipation, inflammatory bowel disease (IBD), diarrhoea, *Helicobacter pylori*, allergy and atopic disease, cancer and disorders relating to liver disease and obesity (Friedman 2005; Khedkar and Ouwehand 2006; Baker and Day 2008; Candy *et al.* 2008; Canny and McCormick 2008; Farnworth 2008).

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JL has received academic funding from Yakult UK Ltd.

SDT International Journal of Dairy Technology

ANTIBIOTIC-ASSOCIATED DIARRHOEA AND *CLOSTRIDIUM DIFFICILE*

The problem of antibiotic-associated diarrhoea

Reports of the prevalence of infective antibiotic-associated diarrhoea (AAD) range from 5% to 25%, a variation that can be attributed to differences in inclusion criteria, clinical setting and frequency of stool analysis potentially leading to a reduced proportion of pathogen-positive samples (Wistrom *et al.* 2001; Asha *et al.* 2006; Gougoulias *et al.* 2007).

There are four main categories of risk factors for the disease: medications given, host factors, environmental factors and procedures undergone by the patient (McFarland 2008b). Clindamycin, aminopenicillins, second or third generation cephalosporins and fluoroquinolones are antibiotics most associated with AAD and *Clostridium difficile*-associated disease (CDAD) (Thomas *et al.* 2003; Muto *et al.* 2005; Owens *et al.* 2008). Use of cytotoxic drugs has been shown to increase the risk for all infective causes of AAD, yet antacids were found to increase the risk only for *Clostridium perfringens* AAD (Asha *et al.* 2006). The general use of proton pump inhibitors (PPIs) has been suggested as a potential risk factor for CDAD because these reduce the acid barrier of the stomach to intestinal colonisation (Dial *et al.* 2004; Jayatilaka *et al.* 2007; Cunningham and Dial 2008) but not all studies show agreement (McFarland 2008b).

Age is a common risk factor; AAD rates increase for patients over 65 years (McFarland 2008c). Female patients are also at increased risk of *C. perfringens* AAD (Asha *et al.* 2006). Length of hospital stay as well as type of ward, prevalence of AAD on the ward and local standards of hygiene are further risk factors (Dubberke *et al.* 2007) that may be minimised through effective infection control procedures (Department of Health and Health Protection Agency 2009). Medical interventions such as surgery and the requirement of feeding tubes also increase risk (Gougoulias *et al.* 2007).

There have been only a few studies that have specifically examined the financial burden for health services. Wilcox *et al.* (1996) examined 50 patients with CDAD, estimating costs for antibiotic treatment, laboratory testing and the hotel cost of side rooms, and found that 94% of the additional cost in managing each *C. difficile* infection case was due to the increased length of stay. The total additional cost per case of *C. difficile* was greater than £4000 ($5717/€4406) compared with a noninfected patient. This increased cost can be used to justify expenditure on enhanced laboratory diagnosis (for example 7 day testing protocols), hospital cleaning, increased personnel costs and any interventions that may reduce the risk of disease.

The causative agents: *Clostridium difficile* and other pathogens

Clostridium difficile occupies a prominent position in both media and clinical attention as an aetiological agent of AAD, with current data suggesting this species is responsible for up to 33% of cases (Spencer 1998; Asha *et al.* 2006). Other species and genera of bacteria, however, have also been implicated. *Clostridium perfringens* enterotoxin, shown to be present in 11–15% of faecal samples of AAD patients (Hancock 1997; Pituch *et al.* 2007), is often found in conjunction with *C. difficile* toxin in AAD cases (McFarland 2008b) but epidemiological studies of *C. perfringens* AAD have not been performed (Asha *et al.* 2006). Following its identification as a cause of enterocolitis in the 1950s, several recent studies have suggested that *Staphylococcus aureus* is responsible for up to 7.3% of AAD (Flemming and Ackermann 2007), and this pathogen caused 60 cases of nosocomial AAD in France over 2 years (Gravet *et al.* 1999). Other potential causes of AAD are *Klebsiella oxytoca*, *Candida albicans* and *Salmonella* species but further research is needed to confirm this.

Clostridium difficile infections were first reported in the 1970s, some time after the organism was first described in 1935 (Hall and O'Toole 1935; Bartlett *et al.* 1978; Larson *et al.* 1978). This Gram-positive, spore-forming obligate anaerobe is found in approximately 3–5% asymptomatic adults and 66% infants (Viscidi *et al.* 1981; Brazier 2008; http://www.hpa.org.uk). Due to its anaerobic nature, it predominantly affects the large bowel (Durai 2007). Up to six different toxins may be produced but the main virulence factors are an enterotoxin (toxin A) and a cytotoxin (toxin B) (Brazier 2008; McFarland 2008a). Following antibiotic treatment, the colonisation resistance of the normal gut microbiota is depressed, and if a patient is exposed to *C. difficile*, the ingested spores will germinate in the terminal ileum and the pathogen will grow in the colonic lumen. Overgrowth of endogenous *C. difficile* may also occur in asymptomatic carriers as a result of decreased colonisation resistance during or after a period of antibiotic treatment. The toxins produced by the pathogen damage the gut tissue and disrupt the epithelial tight junctions. The pathogen may adhere to the colonic epithelium, releasing toxins and enzymes that damage the tissue, with further damage caused by recruited neutrophils. *Clostridium difficile* infection is associated with a range of enteric symptoms ranging from mild diarrhoea to serious and life-threatening conditions (pseudomembranous colitis, toxic megacolon and bowel perforation), which would be caused if there are successive cycles of

growth, toxin production and neutrophil recruitment in the colon (Borriello 1998).

The spores can survive for long periods, thus standard infection control procedures along with transmission-based precautions are extremely important in reducing cross-infection on the ward. The following measures are advised (Durai 2007):

1) strict hygiene and hand washing between examining patients;
2) frequent washing of wards, cleaning with chlorine-based or other sporocidal agent;
3) prompt laboratory diagnosis by toxin detection and faecal culture;
4) isolation and other infection control procedures;
5) strict control of antibiotic use, minimising broad spectrum use, particularly quinolones and cephalosporins.

New and more detailed guidelines for healthcare professional have recently been published in the UK by the Department of Health and Health Protection Agency (2009).

DETECTION AND REPORTING OF AAD AND CDAD

Diagnosis of CDAD is usually confirmed by *C. difficile* toxin detection in faecal samples using commercial enzyme-linked immunosorbent assay (ELISA) diagnostic kits, sometimes also with traditional culture methods. Recent studies in the UK, Europe and USA, however, have shown that current lab testing based on ELISA detection of *C. difficile* toxins may miss up to 50% of positive patients (Reller *et al.* 2007; Fenner *et al.* 2008). A commercial assay has recently been developed based on detection in faecal samples of glutamate dehydrogenase, an enzyme specific for *C. difficile*. Positive samples are then confirmed for toxin by ELISA, which allows final results for 92% of specimens with a turnaround time of 4 h (Fenner *et al.* 2008).

The polymerase chain reaction (PCR) ribotyping method developed by the UK Anaerobe Reference Laboratory in Cardiff is now the most widely accepted typing method for *C. difficile*. This uses specific primers complementary to sites within the RNA operon, targeting the amplification process at the spacer regions between the 16S and 23S ribosomal RNA regions, and ascribing a three-figure nomenclature to each distinct pattern of amplicons (Stubbs *et al.* 1999). Further methods to differentiate within ribotypes are currently being developed.

In the UK there is now mandatory reporting of CDAD. Surveillance studies in other European countries, USA and Canada indicate a substantial increase in CDAD over the last 10 years. The majority (75%) of infections in the UK have been shown to be caused by three types: PCR ribotypes 001, 027 and 106 (Brazier 2008). Type 027 has been implicated in major outbreaks in several countries and seems to cause more serious illness, possibly because it produces higher levels of toxin A and B as well as an additional binary toxin (McFarland 2008a). Surveillance has also shown that the incidence of cases in the community is rising, as well as cases in populations originally not thought at risk (children, young adults and peripartum women).

PROBIOTIC STUDIES RELATING TO AAD AND CDAD

Probiotic mechanisms of activity relevant for AAD and CDAD

The three main defences in the gut are the commensal intestinal bacteria, the mucosal barrier of the epithelium and mucus layer and the gut-associated lymphoid tissue. The commensal bacteria, particularly beneficial genera such as bifidobacteria and lactobacilli, are important for the colonisation resistance of the gut against pathogens (Gibson and Wang 1994), which involves several mechanisms, including the lowering of gut pH by short chain fatty acid production, competition for nutrients and colonisation sites, direct antagonism by natural antimicrobials, and immune stimulation.

The toxins of *C. difficile* probably evolved to give the species a selective advantage in the gut but normally the growth of the organism is suppressed by the commensal microbiota. When antibiotics disrupt this bacterial defence, it is easier for such pathogens to grow (Borriello 1998). The rationale for using probiotics to reduce CDAD risk is based on this knowledge, coupled with the understanding that the basis for most probiotic activity is modulation of the composition (and metabolic activity) of the commensal microbiota and modulation of the immune response (Gougoulias *et al.* 2007).

Review of evidence for probiotic-fermented milk products in reducing risk of AAD and CDAD

When examining the evidence in general for probiotics with regard to the reduction of risk of AAD and CDAD, meta-analyses and systematic reviews conducted to date provide conflicting evidence. From 25 randomised controlled trials (RCTs), probiotics as powders, capsules or other formulations (notably *Saccharomyces boulardii*, *L. rhamnosus* GG or mixtures) significantly reduced the relative risk (RR) of AAD (RR = 0.43, 95% CI: 0.31, 0.58, $P < 0.001$). From six randomised trials, probiotics (*S. boulardii*) had significant efficacy for CDAD (RR = 0.59, 95% CI: 0.41, 0.85, $P = 0.005$) (McFarland 2006). The preventative effect towards AAD was supported by a further

meta-analysis of 34 RCTs showing a reduction in AAD by 52% (95% CI: 35–65%) in those receiving the probiotic (Sazawal *et al.* 2006), and by a review of nine RCTs in which the odds ratio was 0.37, in favour of probiotics being more effective than placebo (IC95: 0.26–0.53; $P < 0.001$) (D'Souza *et al.* 2002). Doron *et al.* (2008) recently reviewed five meta-analyses which suggested that probiotics showed an overall reduction in the risk of AAD when probiotics were co-administered with antibiotics. A systematic review examining the efficacy of *L. rhamnosus* GG in preventing AAD concluded that of six trials selected, four found a significant reduction in risk of AAD (Hawrelak *et al.* 2005).

Conversely, there was no evidence to support clinical use of probiotics from a systematic review of the eight trials selected where prevention or treatment of CDAD by probiotics was the primary or secondary outcome (Dendukuri *et al.* 2005). The trials selected included a range of probiotic organisms including *L. acidophilus*, *Bifidobacterium bifidum*, *S. boulardii*, *Lactobacillus plantarum* and *L. rhamnosus* GG. Dendukuri *et al.* (2005) concluded that better designed and larger studies were required.

There are, however, potential problems in using meta-analyses to estimate the efficacy of probiotics in general. By definition, the health benefits of each probiotic strain must be supported by its own body of evidence. Individual strains are likely to differ in their specific mechanisms of action, thus some may show little or no efficacy in reducing the risk of AAD and/or CDAD. Furthermore, the conditions considered in some meta-analyses vary widely, as have the patient characteristics (Dendukuri and Brophy 2007). The degree of statistical heterogeneity found in many probiotic studies is a further reason why meta-analyses may not be appropriate. Information on the numbers of probiotic micro-organisms given as an intervention, which would provide useful dose–response data (McCartney 2002), is also excluded from the analyses. These limitations should be kept in mind when attempting to apply a general conclusion to the treatment of an individual patient. When further studies have been conducted with probiotic-fermented milk products, it might then be possible to conduct meta-analysis focusing on specific products, as carried out, for example, with *Lactobacillus* GG concerning benefit against acute diarrhoea in children (Szajewska *et al.* 2007). One review on CDAD has also focused particularly on *Lactobacillus* GG and *S. boulardii* (Segarra-Newnham 2007).

Reviews highlighting examples of trials using probiotics are often more appropriate to assess the relative merits of different genera and species of probiotic organisms for specific outcomes. Gougoulias *et al.* (2007) reviewed 18 trials and concluded that administration of probiotics showed an overall reduction in the risk of AAD and the number of relapses in recurrent CDAD. The most reproducible results were obtained predominantly with *S. boulardii* and *L. rhamnosus* GG. Surawicz (2008) performed a similar review and from the trials examined noted that there was merit in the approach of using probiotics in AAD, CDAD and recurrent CDAD but that various probiotic organisms have variable efficacy. These papers list all trials conducted to date in an unbiased manner, with each probiotic organism clearly identified for each outcome.

Studies with probiotic-fermented dairy products

Early reports of *L. rhamnosus* GG suggested that this strain might be more effective as a fermented milk powder than as a freeze-dried powder (Goldin *et al.* 1992; Saxelin 1997). Due to the scope of this journal, trials with fermented milk products will be described in more detail (Table 1), focusing on studies that investigated prevention rather than treatment of the disease. Efficacy of a strain or product may not necessarily be the same in both cases.

Some probiotic studies use formulations comprising more than one bacterial strain; in these cases the effectiveness of any individual organism is not clear. Ordinary yoghurts containing live bacteria should not be considered probiotic unless labelled as such. The lactic acid bacterial starter cultures used to make yoghurt may not necessarily survive transit through the gut. To be called probiotic, a product must have scientific evidence of health benefit (Del Campo *et al.* 2005; Conway *et al.* 2007).

Sullivan *et al.* (2003) compared the effect of clindamycin on the intestinal microbiota in subjects ($n = 24$) ingesting yoghurt with added probiotic strains (*L. acidophilus*, *Bifidobacterium lactis* and *Lactobacillus* F19) with that of a control group taking a placebo yoghurt. It was found that in both groups, numbers of Gram-positive bacteria (apart from enterococci) decreased, and there was a rise in Gram-negative bacteria (apart from *Escherichia coli*). In the probiotic group, numbers of lactobacilli and bacteroides remained stable, whereas there was a decrease in these bacteria in the placebo group.

Four recent studies with probiotic-fermented dairy products have reported promising results for AAD; all included certain improvements in their trial designs, in relation to statistical power considerations, definition of endpoints and continued monitoring of trial participants after antibiotic therapy was terminated (Meier 2005).

Table 1 Summary of trials using probiotic-fermented milk products in relation to AAD and/or CDAD

Probiotic strain (s)	*Target group and intervention*	*Results*	*References*
Lactobacillus acidophilus; Bifidobacterium lactis; Lactobacillus F19	Healthy subjects (n = 24) on 150 mg clindamycin for 7 days, and probiotic or placebo yoghurt for 14 days	Analysis of faecal flora showed increase in enterococci and drop in some Gram-positive bacteria for both groups. Lactobacilli and bifidobacteria numbers maintained in probiotic group but dropped in placebo group.	Sullivan *et al.* (2003)
L. acidophilus CL1285*; Lactobacillus casei* Bio-K	Hospital patients (n = 89) prescribed antibiotics, given either a probiotic fermented milk drink or placebo within 48 h of antibiotics and during treatment. Exclusion criteria included treatment with vancomycin or aminogycoside	AAD developed in 7/44 (15.9%) of probiotic group and 16/45 (35.6%) of control group (P = 0.05).	Beausoleil *et al.* (2007)
L. casei DN 11-4001	Hospital patients (n = 135; >50 years) prescribed antibiotics. Probiotic yoghurt drink (2 bottles) or milkshake (placebo) within 48 h of antibiotics and one week after finishing the medication. Exclusion criteria included high risk antibiotics	AAD developed in 7/57 (12%) of the probiotic group and 19/56 (34%) of control group (P = 0.007). CDAD developed in 0 (0%) of the probiotic group and 9/53 (17%) of control group (P = 0.001).	Hickson *et al.* (2007)
L. casei Shirota	Healthy children (n = 88; 12–144 months) requiring antibiotics for mild infection. One daily bottle of *L. casei* Shirota fermented milk drink or placebo until antibiotics finished	AAD developed in 0 (0%) of probiotic group; 6 (15%) of control group (P = 0.01)	Martinez *et al.* (2003);
L. casei Shirota	Hospital patients (n = 678; mean age 71) on one bottle of *L. casei* Shirota fermented milk drink during antibiotics and for 3 days after course finished. Control group not given probiotic.	AAD developed in 17/340 (5%) of probiotic group and 63/338 (18%) of control group (P < 0.001). CDAD developed in 0 (0%) of probiotic group and 21/338 (6%) of control group (P < 0.0001).	Stockenhuber *et al.* (2008)
Lactobacillus rhamnosus GG, *Lactobacillus johnsonii* La-5; *B. lactis* Bb12	Patients (n = 87) on antibiotics given fermented milk drink with the probiotic strains LGG, La-5 and Bb-12, or a placebo with heat-killed bacteria for 14 days.	AAD developed in 2/46 (5.9%) of probiotic group and 8/41 (27.6%) of placebo group (P = 0.035).	Wenus *et al.* (2008)

AAD, antibiotic-associated diarrhoea; CDAD, *Clostridium difficile*-associated disease.

A randomised double-blind placebo controlled trial of Hickson *et al.* (2007) in 135 elderly hospital patients (mean age 74 years) assessed the efficacy of a probiotic yoghurt drink containing the probiotic strain *Lactobacillus casei* DN-114 001 and two yoghurt cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), consumed twice daily during a course of antibiotics and for 1 week afterwards, in preventing AAD and CDAD. Statistically significant reductions for both the primary endpoint of AAD incidence (P = 0.007) and the secondary endpoint, CDAD incidence, (P = 0.001) were reported for the probiotic group. The positive outcome of the trial led to the conclusion that the probiotic intervention had the potential to decrease morbidity, healthcare costs and mortality if used routinely in patients aged over 50, but there was criticism of the low generalisability because a large number of the potential population was excluded from the analysis (135 enrolled from 1760 patients screened for inclusion) (Wilcox and Sandoe 2007).

Beausoleil *et al.* (2007) presented findings on the reduction of AAD incidence using a fermented milk product with *L. acidophilus* CL1285 and *L. casei* in a randomised, double blind, placebo-controlled trial. From 89 recruited participants, 44 were randomly assigned to the *Lactobacillus* group and seven (15.9%) went on to develop AAD compared with 16 out of 45 patients (35.6%) in the control group. The statistical analysis used indicated P = 0.05, which is at the limit of statistical significance. A comparison was performed for CDAD occurrence (one case in the *Lactobacillus* group and seven in the control group), but the small sample size and low number of *C. difficile* toxin-positive cases limited the strength of conclusions that the authors could make and indicated the need for a larger trial. There was no significant difference in mean hospital stay between the two groups (P = 0.15). Trial participants did not have

stool culture for the *Lactobacillus* subsp. performed which when included in research lends credibility to results obtained, because poor compliance otherwise unnoticed might lead to a false-negative outcome (Aggett *et al.* 2005). Elegant methods for the identification and enumeration of probiotic bacteria from human faeces have been described (Tuohy *et al.* 2007). Nevertheless, as this study was placebo-controlled and randomised it strengthened the evidence for the role of *Lactobacillus* subsp. in the prevention of AAD.

Wenus *et al.* (2008), in a short communication, found that the administration of *L. rhamnosus* GG, *L. acidophilus* La-5 and *Bifidobacterium* B-12 conferred a preventative effect for AAD in a randomised, double blind, placebo-controlled trial. Out of 853 eligible patients 87 were recruited and were randomised to receive the probiotic drink ($n = 46$) or an equivalent tasting placebo ($n = 41$). Following drop-outs or withdrawals the numbers of participants in each group was reduced by 12. Two (5.9%) patients in the probiotic group and eight (27.6%) patients in the placebo group developed AAD ($P = 0.035$). The risk of AAD was reduced by 79% (RR = 0.21, 95% CI: 0.05–0.93).

Interim results of a large open-label trial with an *L. casei* Shirota-fermented milk drink, reported at the 2008 United European Gastroenterology conference, were notable in that there was no exclusion of high-risk antibiotics or PPIs (Stockenhuber *et al.* 2008). Antibiotic therapy for patients in the trial, taking either single or multiple antibiotics, included penicillins, cephalosporins, quinolones, clindamycin and vancomycin, administered orally or parenterally. The patients, all over 50, were recruited from three wards, with 340 in the probiotic group (mean age: 71 years) and 338 in the control group (mean age: 69 years). The groups were matched for antibiotic regime, age, sex, severity of any particular disease, morbidity, duration of hospitalisation and PPI therapy. Patients were excluded if they had diarrhoea on admission or in the previous month, if they had taken high-risk antibiotics within 1 month of admission and if they experienced recurrent diarrhoea or chronic intestinal disease associated with diarrhoea. The primary outcome was AAD occurrence; the secondary outcome was diagnosis of *C. difficile* infection by toxin detection. A further interesting feature was the fact that the probiotic was consumed by all the patients on the wards during the intervention period, even those not on antibiotics, as well as the staff on the wards during this time. After 6 months, 63 (18.6%) patients in the control group had developed AAD compared with 17 (5%) in the group consuming the *L. casei* Shirota probiotic ($P < 0.001$). Furthermore, 21 (6.2%) of the control group developed *C. difficile* toxin-positive diarrhoea, whereas only one patient in the probiotic group developed this ($P < 0.001$). In fact, it was later discovered that this patient was noncompliant and had not consumed the probiotic. There is also one previous report of this strain being effective in preventing AAD in children (Martinez *et al.* 2003).

A PRACTICAL EXPERIENCE OF USING PROBIOTIC-FERMENTED MILK DRINKS IN A HOSPITAL

The proposition that potential pathogens such as *C. difficile* can be denied territory in the normal gut flora by administering probiotic is becoming extremely persuasive, given the body of available evidence described above. It would seem logical therefore to provide this protection before any damage occurs to the normal flora by antibiotic administration. Nonetheless, most studies to date do not appear to have made use of this principle and have allowed prior antibiotic administration for up to 72 h before the commencement of probiotics (Beausoleil *et al.* 2007; Hickson *et al.* 2007; Wenus *et al.* 2008). When probiotics were considered as part of an infection prevention strategy at the West Hertfordshire Hospitals NHS Trust, the infection control consultant took the approach that it was better to give probiotics to vulnerable elderly patients *before* they were given any antibiotic medication. Thus, one bottle of low calorie (i.e. suitable for diabetics) fermented milk drink containing the probiotic strain *L. casei* Shirota was administered daily to all patients on four care of the elderly wards on two sites, irrespective of whether they were receiving antibiotics or not, starting from October 2007. Antibiotic policy aiming to reduce administration of the high-risk antibiotics was already in place, as was more focused hygienic practice. An isolation ward for patients with *C. difficile* diarrhoea was also opened at the same time as the probiotic was introduced. This combination of tactics resulted in an overall drop in cases acquired by Trust hospitals in both sites. In the 26-month period before the probiotic was used, *C. difficile* cases averaged 10.8 cases per month, but in the subsequent 27-month period (after introducing the isolation ward and the probiotic), average cases dropped to 3.4 cases per month (Figure 1).

The other arms of the new and stricter infection prevention strategy (antibiotic policies; focused hygienic practice; isolation ward) had been applied to all patients in the Trust hospitals. Probiotics, however, had only been offered on Care of the Elderly wards, and were not available to the patients on other wards. A decision was therefore taken in January 2008 to extend probiotic administration to all patients over 65 years of age in the

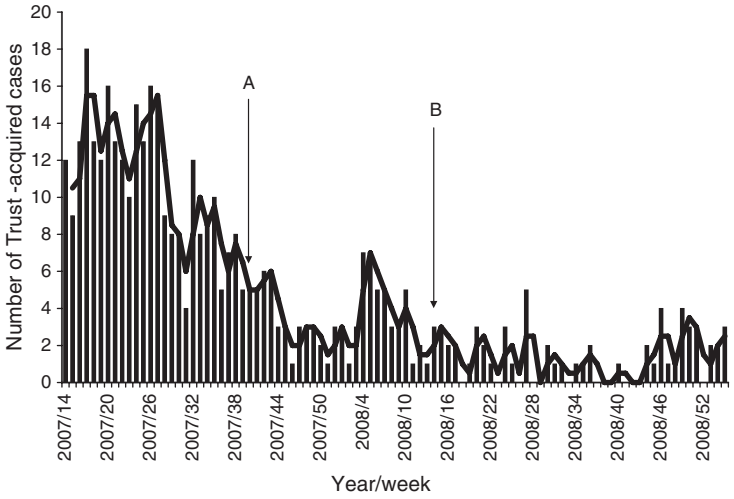

Figure 1 Incidence of *Clostridium difficile* cases in the West Hertfordshire Hospitals NHS Trust: weekly data from April 2007 to January 2009. From point A (1 October 2007) the probiotic-fermented milk drink with *Lactobacillus casei* Shirota was given to all patients over 65 years on four Care of the Elderly wards. (Isolation wards were introduced 1 week later for all the Trust). From point B (31 March 2008) the probiotic was given to all patients aged over 65 years in the West Hertfordshire Hospitals NHS Trust.

Trust's hospitals, irrespective of which ward they were in. Analysis of the data over the next 12-month period showed that this policy further reduced the incidence of cases of *C. difficile* to an average of 1.4 cases per month. During a 7-week period in September/October 2008, only one patient suffered *C. difficile* diarrhoea across the whole Trust (Figure 1). A change in the overall profile of patients who were affected was also observed. When examining the profile of patients who contracted *C. difficile*, it was observed that the average *C. difficile* cases per month changed from a ratio of 71% more cases in elderly patients than in younger patients in the period April 2007 to June 2008 (i.e. before introduction of the probiotic) to 18% more elderly cases than younger cases after the probiotic began to be offered on the elderly wards (Figure 2). This apparent increase in the proportion of cases in younger patients may be an indication of the improved gut colonisation resistance of the elderly patients who were receiving the probiotic.

Compliance with taking probiotics among the ward population that did not contract *C. difficile* related diarrhoea was approximately 90%. In contrast, analysis of the patients acquiring *C. difficile* diarrhoea during 2008 also found that, with one exception, all had either been noncompliant or only partially compliant in taking their daily probiotic. The one fully compliant patient developed relatively mild diarrhoea and made a rapid recovery. Reasons for noncompliance included dementia (and consequent difficulties with oral feeding), and the necessity to be nil-by-mouth for reasons

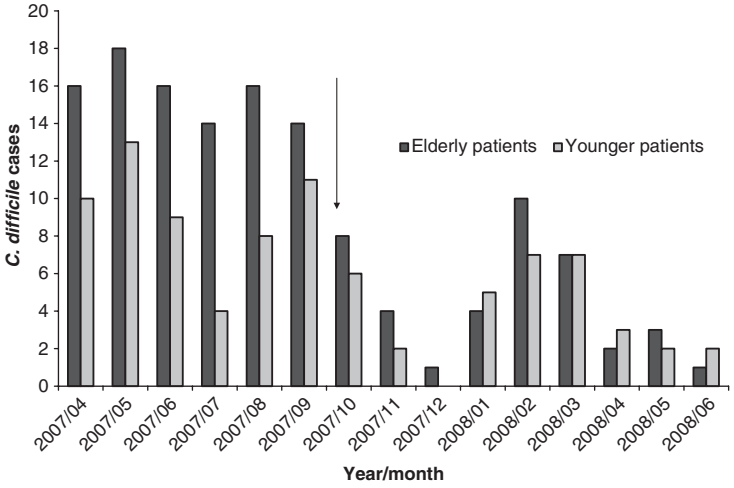

Figure 2 Graph to show the change in patient profile for *Clostridium difficile* cases in the West Hertfordshire Hospitals NHS Trust after introduction of the probiotic to all patients over 65 years of age on four Care of the Elderly wards (indicated by the arrow).

ranging from recent gut surgery to neurological problems with swallowing. The fact that some patients who were partially compliant developed *C. difficile* diarrhoea suggests that protection, to be effective, needed to be maintained by regular daily intake of the probiotic. Sustained protective colonisation does not seem to occur with intermittent consumption. This study therefore suggests that truly prophylactic administration is much more effective in patients at risk rather than simply administering probiotic concurrent with antibiotic. It is worth speculating also that giving probiotic to the entire ward population may reduce the overall incidence of asymptomatic *C. difficile* carriage, with a consequent and beneficial reduction in environmental shedding of *C. difficile* spores. Further ward-based studies might shed light on this interesting possibility but the potential of this combined approach is highlighted by comparing numbers of reported *C. difficile* over this period for 45 Trusts across England which also had reported 100–200 cases of *C. difficile* between April and June 2007. When comparing this with their reporting of cases for the period July to September 2008, overall there was an average reduction of 53% of cases. The West Hertfordshire Hospitals NHS Trust achieved 90% reduction of cases, the greatest improvement shown by any of these Trusts.

CONCLUSIONS

Studies to date show that there is a growing body of research to suggest that certain probiotic-fermented milk products may be worth considering as part of an infection prevention strategy against *C. difficile*. Prophylactic use of these products may potentially reduce not just morbidity and mortality, but also healthcare costs. Based on the findings of their study using a daily intervention of two bottles of a probiotic yoghurt drink for elderly patients on antibiotics, Hickson *et al.* (2007) estimated that this might achieve substantial hospital savings, due to the potential reduction in patients requiring increased length of hospital stay and vancomycin treatment.

The data described in this review indicate the need for further well-designed trials, which should, for example, consider the use of appropriate placebos that would allow the adequate blinding of all patients and study personnel. There may also be value in collecting and analysing data on the antibiotics each patient receives, for example: what, how, and how long for.

The studies described in detail above also highlight a difference in approach to using probiotics to prevent *C. difficile*. In most studies the probiotics have been given to patients within 48 h of receiving antibiotics, but for at risk groups such as the elderly hospitalised patient, there may be value in trying to maintain colonisation resistance in the gut by giving a probiotic before the patient receives any antibiotic treatment. The onset of AAD is often in the week after the antibiotic course finishes, which suggests that the patient should continue taking a probiotic during this time.

Several age-related physiological and histological changes occur in the gut that make older people more vulnerable to infection. The intestinal microbiota appears to change in later life, with numbers of beneficial species dropping and a possible rise in numbers of harmful species (Mitsuoka 1992; Hopkins and Macfarlane 2002; Hopkins *et al.* 2002; Woodmansey *et al.* 2004). This age group is prone to constipation, probably with a reduced consumption of fewer prebiotic-containing vegetables, and older people also have a weakened immune system. All of this suggests that probiotics that help maintain gut health and function may be beneficial before and during any inpatient stay, whether or not on antibiotics (Hopkins and Macfarlane 2002; Tuohy *et al.* 2004; van Tongeren *et al.* 2005).

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