REVIEW

Probiotics for the prevention and treatment of *Clostridium difficile* in older patients

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Abstract

Clostridium difficile infection (CDI) is the leading cause of nosocomial diarrhoea in older people, causing substantial morbidity and mortality. The fact that CDI is almost exclusively a disease of older people and the debilitated indicates that patient susceptibility is a major determinant of who gets CDI. It would help efforts to combat this disease if we better understood and could reduce patient susceptibility. In this regard, several strategies are currently under investigation. The use of probiotics for CDI has received particular attention in the medical and lay media. Patients and their carers often ask doctors about them. In this review article, we describe the pathogenesis of CDI before looking at the ageing host in more detail. We discuss how probiotics may work and review the current evidence for their use in CDI.

Keywords: probiotics, Clostridium difficile, immunesenescence, antibiotic-associated diarrhoea, intestinal microflora, older people

Introduction

Huge efforts are being made to combat the spread of *Clostridium difficile* infection (CDI) in hospitals. Although rates have fallen markedly in the UK, it remains the leading healthcare-associated infection affecting older people [1].

Clostridium difficile was described as a cause of antibiotic-associated diarrhoea (AAD) in 1978 [2]. Rates of CDI increased through the 1990s, but the disease emerged dramatically after around 2000, when two major changes in epidemiology occurred. First, CDI rates rose exponentially throughout North America and Europe. In the UK, rates peaked in 2007 with over 50,000 cases reported, 80% being in patients aged over 65 [3]. Second, the clinical syndrome associated with CDI changed. Initial reports of increased severity and mortality came from Quebec in Canada [4]. Novel strains of *C. difficile* ribotype 027 which were resistant to fluoroquinolone antibiotics became rapidly dispersed across North America and Europe and were linked to severe disease [5]. In the UK, ribotype 027 *C. difficile* was associated with notorious outbreaks at Stoke Mandeville and Maidstone Hospitals and came to account for over 40% of *C. difficile* isolates from English hospitals

by 2007 [6, 7]. A huge effort across the NHS backed by the Department of Health has been made to reduce CDI rates. Key components include improved infection control practice, rapid diagnosis and isolation of cases, use of 'root-cause analysis' and antibiotic stewardship policies focused on reducing use of 'high-risk' antibiotics. Nevertheless, there were still over 20,000 cases of CDI in the NHS in 2010. While the prevalence of ribotype 027 strains is now falling in the UK, other virulent ribotypes are now being described [8].

Search strategy and selection criteria

A search of BioMed Central Journals, Cochrane Library—Cochrane Database of Systematic Reviews (Wiley), Global Health (Ovid Technologies), MEDLINE (CSA), PubMed, SCOPUS-V.4 (Elsevier) and Web of Knowledge was conducted during March 2012. Keywords included: probiotic, *Clostridium difficile*, AAD, intestinal microflora, immunesenescence, elderly. References were searched by hand and further relevant papers identified from their citations. The pubmed search identified 37 original papers describing the use of probiotics in AAD or CDI with seven additional

papers identified manually. From these, 16 original research papers describing intervention studies were identified and included in the review.

The ageing host

The fact that CDI almost exclusively affects older people is only partly explained by healthcare contact and burden of predisposing diseases. It is now clear that ageing itself predisposes to CDI. The gut microflora develops during infancy and remains stable through adult life. Colonisation resistance is the process by which the normal microflora competes for nutrients and receptor-binding sites and prevents pathogen overgrowth [9]. However, in old age the faecal microflora becomes less diverse with an overall reduction in anaerobic bacteria and bifidobacteria [10]. A range of changes in intestinal physiology accompany ageing and may predispose to CDI. These include increased mucosal permeability and declines in secretory IgA, defensins and gastric acid [11]. Failure of adequate pathogen clearance can result in chronic low-grade activation of the immune system often referred to as 'inflammaging', which has been linked to an increased incidence of diabetes and increased frailty [12].

The immune system exhibits age-related changes in both innate and adaptive immune responses known as 'immunosenescence' which have been reviewed elsewhere [13]. In brief, older people have a reduced number and functionality of phagocytic cells. The humoral response is compromised by reductions in the number of B lymphocytes, in antibody diversity and antibody affinity. T cell receptor diversity is also reduced.

Pathogenesis of CDI

CDI occurs when changes in gut physiology and microflora allow ingested spores to reach the colon, germinate and become established through specific adhesins. Diarrhoea is toxin mediated with disruption to the colonic epithelial integrity resulting in epithelial detachment, fluid accumulation and tissue destruction [14]. The two *C. difficile* Toxins, A and B, are very similar and apparent differences in function may relate to receptor specificity [15]. Toxin A may not be necessary for virulence since virulent Toxin A–B + strains are now well described [16]. Antibodies to these toxins provide some protection from disease, and only around one-third of patients develop symptoms after infection [17, 18]. Strains such as the 'hypervirulent' 027 strains produce larger quantities of Toxins A and B but also synthesise an additional 'Binary' Toxin the role of which is not established.

Management of CDI

Metronidazole and vancomycin remain first-line treatment. In fulminant disease, colectomy may be required, although pooled intravenous immunoglobulin has been used in refractory cases [19]. Disease recurrence is a major challenge affecting around 20–30% of patients despite successful initial treatment with metronidazole or vancomycin [20]. Faecal transplantation may have a role in patients experiencing multiple recurrences [21]. Recent data indicate that many recurrences are indeed new infections in a patient who has remained susceptible rather than true recurrence of the same strain, further emphasising the importance of patient susceptibility in this setting [22].

Fidaxomicin is a non-absorbable macrocyclic antibiotic that is now licenced in Europe and the USA for severe CDI. Fidaxomicin appears to have less effect on the gut microflora than vancomycin or metronidazole and in a phase 3 non-inferiority study showed equivalent clinical cure and a 45% relative reduction in the relapse rate compared with vancomycin [23].

Probiotics: nomenclature and mechanisms of action

The term probiotic refers to live micro-organisms which when administered in adequate amounts confer a health benefit to the host. In 2002, the Food and Agricultural Organisation and World Health Organisation released guidelines outlining requirements for probiotic classification (summarised in Figure 1). The probiotic effect may be strain-specific, and correct naming of a probiotic strain frequently includes the gene and species (e.g. *Lactobacillus rhamnosus*) and a specific strain-identifying name. Of note, the term prebiotic refers to non-digestible food ingredients consumed with the aim of stimulating the growth or activity of bacteria in the gastrointestinal tract and some products containing pro- and prebiotics are marketed and termed synbiotics.

The majority of probiotics studied are species of *Lactobacillus* and *Bifidobacterium*, both of which are part of the normal gut flora. However, one of the first strains described was *E. coli* Nissle 1917 which was identified in the stool of a soldier who survived an outbreak of dysentery in the First World War. The yeast *Saccharomyces boulardii* has also been studied extensively. It is not part of the human gut flora but colonises the skin of fruit such as lychees.

Although use of probiotics has been described in a wide range of conditions including atopy and bacterial vaginosis, the great majority of experience relates to gastro-intestinal disease, particularly in children. A range of potential mechanisms for the beneficial effects of probiotics have been proposed. These include stabilisation of epithelial tight junctions, action of bacteriocins, immunomodulation and modulation of cell signalling pathways, displacement of pathogenic bacteria from the epithelial surface, alteration of gut pH by fermentation and induction of opioid and cannabinoid receptors [24].

Strain Identification
- Effects are strain specific
- Allow accurate surveillance and epidemiological studies
- Phenotypic eg. PFGE and genotypic eg. 16sRNA techniques should be used

Screening potential strains: *in vitro*
Mechanism of action
- Resistance to bile and gastric acid
- Adherence to mucus and human epithelial cell lines
- Limit pathogen adherence

Safety
- Antibiotic resistance patterns
- Assessment of toxin production

Efficacy studies: *in vivo*
- Animal studies should be used if appropriate prior to human trials eg. Establish level of infectivity in immunocompromised animal models
- In randomised placebo controlled trials, the placebo should be the same as the test strain in terms of vehicle of delivery and starter cultures
- Phase 2 studies should incorporate a quality of life tool

Figure 1. Guidelines for the evaluation of probiotics adapted from the joint FAO/WHO guidelines [53].

Saccharomyces boulardii may have specific beneficial properties. In particular, it secretes a protease that hydrolyses Toxin A and appears to induce a range of anti-inflammatory responses and secretion of IgA, including IgA specific for *C. difficile* toxin into the colon. These properties have been reviewed by Pothoulakis [25].

Probiotic use in clinical practice

The best evidence for the clinical efficacy of probiotics exists for the prevention of necrotising enterocolitis in preterm infants [26]. In the adult setting, different probiotic preparations have been studied in inflammatory bowel disease [27]. Notably, three randomised placebo-controlled trials have shown equivalence of *E. coli* Nissle 1917 to mesalazine in maintaining remission of ulcerative colitis and the combination probiotic preparation VSL3 has shown benefit in pouchitis [28].

In the past decade, a number of studies have studied the role of probiotics in AAD generally or CDI specifically. These can be divided broadly into studies looking at prevention and treatment.

Primary prevention

Multiple published studies describe the use of probiotics to prevent AAD and two relevant meta-analyses have been performed. D'Souza *et al.* identified nine placebo-controlled studies, seven in adults and two in children with a total of 1,214 subjects; four using *S.-boulardii*, four *Lactobacillus* species preparations and one strain of *Enterococcus* species. All but one study of *S. boulardii* indicated a protective effect and the combined odds ratio in favour of active treatment was 0.37 [95% confidence interval (CI) 0.26–0.53] [29]. Sazawal *et al.* identified 18 studies describing the prevention of AAD and one, the prevention of CDI specifically. The largest study included in the earlier meta-analysis was excluded because it was written in French but 10 studies published after 2001 were included. All showed a positive effect and the authors concluded that probiotics significantly reduce AAD by 52% (95% CI: 35–65%) [30]. Both analyses suggested a bias away from the publication of negative results, but Sazawal *et al.* estimated that 330 unpublished negative trials would have to exist to overturn their findings. Nevertheless, both meta-analyses should be interpreted with caution given the inconsistency in study design between trials including the definition of AAD, duration of follow-up, the age of patients recruited and the type and number of probiotic strains used.

Since 2006, there have been 10 randomised controlled trials investigating the role of probiotics in the prevention of AAD, with seven suggesting a benefit [31–37]. In a small Swedish study, patients in the active group experienced fewer gastrointestinal symptoms including loose watery stool (odds ratio: 0.69; 95% CI: 0.52–0.92) and nausea (odds ratio: 0.51; 95% CI: 0.30–0.85) compared with the placebo group, although there was no difference in

AAD [38]. The remaining studies were methodologically flawed, lacking an appropriate matched placebo, inadequate dosing of the active product and limited follow-up [39, 40].

There have been fewer studies specifically addressing the effect of probiotics on CDI which is usually measured as a secondary outcome (Table 1). In a study by Hickson et al., 135 patients were randomised to receive a probiotic (*Lactobacillus casei* DN-114001, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*) or placebo milkshake drink twice daily for the duration of antibiotic use and the following 7 days. An absolute risk reduction of 17% (7–27%) occurred with no cases of CDI in the probiotic group. However, this

study only recruited 8% of the total screened population due to stringent exclusion criteria, suggesting these results may not be applicable to the wider hospital community [32]. Recently, the first dose-response effect study was conducted at a single centre in China. Patients were randomised to two probiotic capsules containing *Lactobacillus acidophilus* CL1285® and *Lactobacillus casei* LBC80R® (Pro-2), single probiotic capsule and placebo capsule (Pro-1) or two placebo capsules. The incidence of CDI was lowest in the Pro-2 group (1.2%) with higher rates seen in the Pro-1 (9.4%) and placebo groups (23.8%). The overall rates of CDI were greater in this study compared with those in

Table 1. Probiotic trials: primary prevention of CDI

Author	n	Probiotic strain used	Study design	CDI rate probiotic (%)	CDI rate placebo (%)	Comments
Gao et al. [31]	255	*Lactobacillus acidophilus* CL1285® + *Lactobacillus casei* LBC80R®	50–70 years of age. Product given for the duration of antibiotics + 5 days. Follow-up: 21 days post-cessation of product	1.2[a]	23.8	Probiotic blend showed positive dose-ranging effect (100 billion c.f.u. versus 50 billion); study only single-centred and only recruited patients receiving high-risk antibiotics
Sampalis et al. [33]	437	*Lactobacillus acidophilus* CL1285® + *Lactobacillus casei* LBC80R®	>18 years of age. Product given for the duration of antibiotics + 5 days. Follow-up: 21 days post cessation of product	6.2	13.3	Multi-centre study in Canada. Patients in the active group had a shorter duration of AAD and a significantly reduced incidence ($P = 0.067$)
Safdar et al. [34]	40	*Lactobacillus acidophilus*	>18 years of age. Product given for the duration of antibiotics + 14 days. Follow-up: not defined	0 (0/3)	25 (1/4)	This was a small pilot study and therefore underpowered. Only one CDT positive case of diarrhoea out of total of seven tested
Beausoleil et al. [36]	89	*Lactobacillus acidophilus* CL1285® + *Lactobacillus casei*	Age limits not defined; mean age in the active group 68.8 ± 14.5 Product given for the duration of antibiotics only. Follow-up: 21 days post-cessation of product	2.3	15.6	Although there was a significant difference in the rate of AAD between the groups, this was not seen for CDI due to the small numbers of patients with CDI
Hickson et al. [32]	135	*Lactobacillus casei* DN114001, *Lactobacillus Bulgaricus, Streptococcus thermophilus*	≥50 years of age. Product given for the duration of antibiotics + 7 days. Follow-up: 4 weeks post-discharge	0	17	The NNT was six and there was a significant difference in the rate of CDI ($P = 0.001$) Only 8% of the screened population were recruited; therefore, it is difficult to generalise these findings to the wider hospital population
Plummer et al. [51]	138	*Lactobacillus acidophilus, Bifidobacterium bifidum*	Elderly patientsPrimary outcome was the rate of CDIProduct given for 20 daysFollow-up: patients were not followed up	3	7	Lower rate of CDI in the probiotic group (2.9 versus 7.25% placebo); poor recruitment to trial resulted in reduced statistical power
Surawicz et al. [52]	180	*Saccharomyces boulardii*	Only mean age reported in study (47.8 years ± 21). Product given for the duration of antibiotics + 2 weeks. Follow-up: mean duration of 17.3 ± 8.6 days	3	5	Lower rate of CDI in the probiotic group (9.4 versus 31% placebo; $P = 0.07$). Intervention tested in much younger population

The primary outcome in all studies except Plummer et al. was the rate of AAD. CDI was measured as a secondary outcome.
[a]This rate was seen in Pro-2 group = two probiotic capsules; rate in Pro-1 group = placebo + probiotic capsule was 9.4%.

Europe and North America; possibly reflecting the exclusive inclusion of patients receiving antibiotics associated with an increased risk of CDI [31].

Secondary prevention

Five studies have investigated probiotic use as a secondary prophylaxis against CDI relapse, with only two showing a significant effect. The first study established that in patients who had experienced a relapse of CDI, *S. boulardii* in combination with standard CDI treatment prevented further disease relapse [relative risk (RR) 0.43, 95% CI: 0.2–0.97] [41]. The second study, carried out by the same group, replicated these results and demonstrated that high-dose vancomycin (2 g/day) and probiotic was the most effective combination. A lower dose of vancomycin (500 mg/day) reduced CDI recurrence (21 versus 62% placebo), but at the expense of a longer mean duration of treatment [42]. The remaining studies are limited by small sample sizes and therefore underpowered, making it difficult to draw a meaningful conclusion [43–45].

Treatment

There has never been a trial investigating probiotics as the sole treatment for CDI. A Cochrane systematic review looked at four studies investigating the use of probiotics as an adjunct to first line conventional antibiotic treatment [46]. Only one study showed patients treated with *S. boulardii* were statistically less likely to experience a relapse of CDI versus placebo (RR: 0.59; 95% CI: 0.35–0.98) [41]. The remaining studies were too small to draw any significant conclusions. The authors concluded that no recommendation can be made for the use of probiotics as treatment for CDI.

Safety

Although endocarditis has been reported following the consumption of *L. rhamnosus* [47], no cases of systemic bacteraemia have been reported in trials using a probiotic test strain in otherwise healthy adults, including trials involving older people. Concerns remain about the use of probiotics in severely immunocompromised patients but the significance of this is not clear. In a multicentre study carried out in intensive care, 298 patients with severe pancreatitis were randomised to receive a multispecies probiotic preparation or placebo. The rate of intestinal ischaemia and mortality rate were higher in the active group versus placebo (16 versus 6% placebo, RR: 2.53 95% CI: 1.22–5.25) [48]. However, in contrast, a study involving mechanically ventilated patients in a similar setting found no difference in 28-day mortality between the active multi-species probiotic group (25.3%) and placebo (23.7%) [49]. Therefore, the exact nature of immune compromise may be significant as

probiotics have been used in pre-term neonates and HIV patients with no reported serious adverse events [50].

Key points

- Older people remain at risk of CDI due to recurrent hospital admissions and repeated courses of antibiotics on a background of an ageing immune system.
- Probiotics may expedite the recovery of the microflora following damage by antibiotics, thus limiting AAD and overgrowth of *C. difficile*.
- They are generally a safe and relatively cheap intervention.
- There is no evidence to support probiotic use for the treatment of CDI.
- Several studies have suggested their role in the prevention of AAD and CDI; however, they have been underpowered with small sample sizes.
- A large well-designed multicentre study in older people is needed and should address the impact on both the host microflora and immune response, to establish if probiotics can be used to prevent CDI in this susceptible group of patients.

Conflicts of interest

Supplementary data

Supplementary data mentioned in the text is available to subscribers in *Age and Ageing* online.

References

The very long list of references supporting this review has meant that only those most important are listed here and are represented by bold type throughout the text. The full list of references is available on Supplementary data in *Age and Ageing* online, Appendix 1.

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