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THE USE OF PROBIOTICS IN THE PREVENTION AND TREATMENT OF *CLOSTRIDIUM DIFFICILE* DIARRHEA

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EXECUTIVE SUMMARY

Background: *Clostridium difficile*-associated diarrhea (CDAD) is the most common form of nosocomial diarrhea. A small, but increasing, percentage of cases may also experience megacolon, perforation, colectomy, shock, or death. Patients with CDAD have been found to stay an average of 8 days longer in hospital and incur substantially greater costs. In Quebec, *C. difficile* infection has elicited particular concern due to a recent increase in the number of reported cases, and the proportion of complicated cases with a high case-fatality. Traditionally it has been treated with antibiotics such as metronidazole or vancomycin, but in about 20% of patients these treatments provide only a temporary relief and there is a recurrence of CDAD that can continue for years. The search for alternative therapies has led to the use of probiotics, or naturally occurring microorganisms such as *lactobacillus* and yeast, which are believed to act through restoring the balance of the natural bacterial flora of the colon.

Objective: To evaluate the evidence in favor of the use of probiotics for prevention and treatment of CDAD in adults.

Methods: We compiled information from systematic reviews, observational studies and randomized controlled trials on prevention and treatment of CDAD in adults using probiotics. Comparisons from randomized controlled trials were summarized in the form of risk differences and 95% confidence intervals.

Results: *Prevention:* We identified only one randomized trial of the probiotic *Lactobacillus acidophilus* for prevention of CDAD. This study found a slightly lower number of *C. difficile* toxin positive cases in the intervention group, but the small numbers (2 cases in the intervention and 5 in the control group) preclude any conclusion. Five related studies of different probiotics, while focusing on the prevention of antibiotic-associated diarrhea (AAD) as the primary outcome (not CDAD), included some cases of *C. difficile*. The small numbers of CDAD cases in these studies limit conclusions but did not provide any suggestion of benefit.

Treatment: Of the 3 randomized studies of the use of probiotics for the treatment of CDAD, one study using the probiotic *L. plantarum 299v* was inconclusive due to a very small sample size (21 patients). The other two studies evaluated the probiotic yeast *S. boulardii* and the overall results were inconclusive. Each reported a beneficial effect in a sub-group of patients – one in patients with a history of CDAD, and the other in patients using high-doses of vancomycin - but such weak evidence can only be interpreted as hypothesis generating.

Safety: Probiotics seem to have a good safety profile, though there have been some case reports of fungemia and bacterimia, particularly among immunocompromised patients.

Conclusion: There is very little evidence relating to the use of probiotics for either prevention or treatment of CDAD. Available evidence does not support the administration of probiotics with antibiotics to prevent the development of CDAD, and is inadequate to justify its introduction as a treatment for developed CDAD at the MUHC. The suggestive, but as yet inconclusive, evidence of benefit with probiotics in the treatment of AAD suggests direction for future studies.

Recommendation: It is recommended that the MUHC does not adopt the use of probiotics for the prevention or treatment of CDAD at the present time. The literature should be re-evaluated as more evidence becomes available.

GLOSSARY

AAD: Antibiotic Associated Diarrhea

C. difficile: *Clostridium difficile*

CCOHTA: Canadian Coordinating Office for Health Technology Assessment

CDAD: *Clostridium difficile* Associated Diarrhea

CFU: Colony Forming Units. For ease of comparison we use a common unit of 1 billion (10^9) CFU for all probiotics.

CHUS: Centre Hospitalier de l'Université de Sherbrooke

CI: Confidence Interval

ELISA: Enzyme-Linked ImmunoSorbent Assay

INAHTA: International Network of Agencies for Health Technology Assessment

MUHC: McGill University Health Centre

NHPD: Natural Health Products Directorate

PCR: Polymerase Chain Reaction

RCT: Randomized Controlled Trial

PMC: Pseudo Membranous Colitis

ICU: Intensive Care Unit

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1. CLOSTRIDIUM DIFFICILE ASSOCIATED DIARRHEA

1.1 Epidemiology of CDAD

Clostridium difficile-associated diarrhea (CDAD) is the most common form of nosocomial diarrhea[1]. About 30%-50% of cases of antibiotic-associated diarrhea and about 10% of cases of nosocomial diarrhea are attributed to the pathogen *Clostridium difficile* (*C. difficile*) [2]. CDAD can manifest as lower abdominal discomfort, diarrhea, colitis, fulminant pseudomembranous colitis (PMC), toxic megacolon and, in severe cases, can result in death[3;4]. About 3.2% of patients with CDAD have complications requiring colectomy, while in 1-2% patients it results in death [5;6]. One study found that among patients requiring a colectomy for toxic megacolon or perforation, the mortality rate was 35-50%[2].

CDAD is more common in hospitals and long-term facilities, with estimates from the United States of 25-60 per 100,000 occupied bed-days, compared to 7.7 cases per 100,000 person-years in the community[1]. A surveillance study in 1997 found similar results in Canadian hospitals with an incidence of 38-95 cases per 100,000 patient-days and 3.4 to 8.4 cases per 1,000 admissions [6].

1.2 Costs associated with CDAD

Length of hospital stay for patients with CDAD is found to increase by 8 days among adult inpatients and 36 days in geriatric patients[2]. McFarland et al found that costs are particularly high for patients with recurrent CDAD due to the long duration of the disease, the costs involved in diagnosis, treatment, hospitalizations, and recurrent treatments[7]. They found that while the average cost of the first episode was US $1,914, the average cost for subsequent episodes was US $3,103 totaling on average to US $10,970 for patients with multiple episodes. Another study from the US estimated that CDAD cases incurred 54% higher costs compared to patients without the disease[8]. Most studies so far have been able to estimate costs due to increased length of stay, but have not considered other potentially cost generating factors such as ward closure, loss of bed days and infection control measures[9].

1.3 Risk factors for CDAD

The two primary risk factors identified by most systematic reviews on CDAD are: 1) hospitalization or admission to a long-term care facility, and 2) exposure to antibiotics in the previous 6 weeks. Other independent risk factors of recurrent CDAD are increasing age, increased severity of underlying disease, and low serum antibody response to toxin A[6]. The use of gastric acid-inhibitors has also been proposed as a possible risk factor. In a recent study by Dial et al the use of proton pump inhibitors, which are potent inhibitors of gastric acid production, were found to be significantly associated with CDAD in two different observational studies after adjustment for antibiotic use and admission to hospital [10,11].

Transmission of *C. difficile* occurs through the fecal-oral route[1], and, in hospitalized patients transmission can occur through other colonized or infected patients, contaminated surfaces and objects in hospitals, or through the hospital personnel whose hands can be colonized by the bacteria[6]. In the absence of an outbreak, the acquisition rate has been estimated between 4-21%, but this acquisition remains asymptomatic in 63% of cases[2]. Acquisition rates as high as 32% have been reported during outbreaks illustrating the explosive potential of *C. difficile* dissemination in the hospital setting[2].

In healthy individuals, the bacterial flora in the colon usually protects against the colonization by *C. difficile*[6]. However, alterations of the bacterial flora, such as through the use of antibiotics, antineoplasic or immunosuppressive drugs, may permit the colonization by *C. difficile*[1]. More than 90% of *C. difficile* infections occur during or after antibiotic use[2]. Certain antibiotics have been found to increase the risk of *C. difficile*, particularly broad-spectrum antibiotics such as cephalosporins, clindamycin and penicillins associated with a beta-lactamase inhibitor[3]. However, all antibiotics, including vancomycin and metronidazole that are traditionally used to treat CDAD (see Section 1.5), have been associated with an increased risk [1]. A combination of antibiotics and long duration of the course increases the risk of CDAD[2]. While hospitalization is considered a risk factor for acquiring *C. difficile*, out-patients receiving antibiotics for common, non-life threatening infections such as sinusitis or upper respiratory tract infections have also been found to be at high risk[4]. The initial symptoms of *C. difficile*-associated diarrhea

(CDAD) may start as early as the first day of the antibiotic therapy, or within 6 weeks or more after the end of the antibiotic course[1] in patients harbouring the bacteria.

Although most of the published literature identifies antibiotic use and hospitalization as the risk factors for CDAD, there is some evidence to the contrary. A recent review article by Thomas et al identifies several biases in articles reporting a relation between antibiotic use and CDAD[13], while a study by Wilcox found that only 50% of *C. difficile* cases had taken antibiotics in the previous month and only 32% had been hospitalized in the previous 6 months[12].

1.4 CDAD in Quebec

The incidence and severity of CDAD has been increasing in Quebec. Data from Med-Echo, the provincial hospitalization database, reveal that the number of cases of *C. difficile* infection has gone up from 3262 in the year 2000 to 7004 in the year 2003[14]. This has been confirmed by two recent studies. A retrospective chart review study of all cases of CDAD diagnosed at the Centre hospitalier universitaire de Sherbrooke (CHUS) between January 1991 and December 2003 revealed that the annual incidence of CDAD increased from 35.6 cases per 100,000 population in 1991 to 156.3 cases per 100,000 population in 2003[15]. The increased incidence was more noticeable in patients older than 65 years, with a 10-fold increase during the same period from 70 per 100,000 population to 860 per 100,000 population. The investigators also believe that the severity of the disease has worsened with the number of complicated cases increasing from 7.1% to 18.2%, and the 30-day case-fatality rate increasing from 4.7% to 13.8%. The classes of antibiotics that were associated with the greatest increase in incidence of CDAD per 1000 patient-days of antibiotic use were macrolides, cephalosporins, clindamycin and quinolones. The authors suggest three possible reasons for this increase: 1) the greater number of elderly patients admitted to the hospital and the greater number of patients with numerous comorbidities, 2) the decreasing investment in Quebec hospitals during the last decade has resulted in conditions of hygiene that facilitate the transmission of pathogens such as *C. difficile*, and 3) a more virulent, more transmissible strain appears to have emerged over the decade. In another study by Loo and colleagues, the case fatality

rate associated with CDAD in 10 Montreal-area hospitals was 8.6% between January to June 2004[14].

1.5 Traditional methods for treatment and prevention

The most important first step in treatment is cessation of the inciting antibiotic if medically appropriate[6]. This is sufficient for mild cases of the disease. For more severe cases, CDAD is normally treated with oral metronidazole (250mg QID or 500mg BID for 10-14 days), or oral vancomycin (125mg QID for 10-14 days)[6]. Metronidazole is the preferred initial choice because of the greater cost of vancomycin and because widespread use of vancomycin has resulted in increased resistance of enterococci[6]. Although these antibiotics are effective in the treatment of CDAD, recurrence may occur in about 5-20% of patients 3 to 28 days after the antibiotic has been discontinued [6;7]. For some patients with multiple relapses tapered and pulsed antibiotic therapy with vancomycin or metronidazole has been used[6]. For patients not responding to antibiotics surgical intervention may be required particularly when colonic perforation or toxic megacolon are suspected[6].

Preventive measures to avoid transmission of the disease include hand-washing, isolation and barrier precautions, cleaning of the physical environment during the symptomatic disease period with agents that kill the bacteria and its spores such as hypochlorite solution[6]. Hand hygiene and use of gloves have been demonstrated to prevent transmission of *C. difficile* in hospitalized patients[6]. The isolation of patients with CDAD in private rooms used together with other measures was also effective in decreasing the transmission of *C. difficile* [6]. Other reported means of preventing CDAD are antibiotic restriction, and passive and active immunization, however each of these strategies present with varying degrees of efficacy[6]. Guidelines for the prevention of outbreaks of *C. difficile* in hospitals and other health-care facilities have been prepared by the Provincial Infectious Diseases Advisory Committee of Ontario (http://www.health.gov.on).

1.6 C. difficile

C. difficile is a spore-forming, anaerobic, gram positive bacteria. Children are common asymptomatic intestinal carriers of *C. difficile*, but the number of people carrying the bacteria decreases with age, and it is estimated that the prevalence of *C. difficile* carriers among healthy adults is 0-3% in the United States[1]. Some strains of *C. difficile* are non-toxinogenic and do not cause disease. Clinical symptoms develop in only about 30% of carriers, while asymptomatic carriers are found to be at a decreased risk for the development of CDAD [1;16]. Clinically significant strains of *C. difficile* produce at least two exotoxins: toxin A, which is primarily an enterotoxin, and toxin B, a cytotoxin[1]. *C. difficile* toxin B has been isolated from stools of more than 95% of PMC cases and from 15-25% cases of antibiotic-associated diarrhea[2]. Though Toxin A is considered to play a more critical role in the pathogenesis of CDAD because of its association with extensive tissue damage, the description of Toxin A negative/Toxin B positive strains suggest that Toxin A is not necessary for virulence[6].

1.7 Diagnosing CDAD

The presence of *C. difficile* is generally based on the detection of toxin A or toxin B in the stool. Testing of a single stool specimen is generally sufficient to establish a diagnosis. Approximately 5% to 20% of patients need more than one stool assay to detect the toxin. Tests are usually conducted on diarrheal stool specimens as there is no value to testing stools of asymptomatic patients unless an outbreak is being investigated[6].

Tissue culture assay for cytotoxicity of toxin B is considered the "gold standard" for diagnosing *C. difficile* with a sensitivity of around 80%-100% and a specificity of 99% [1;6]. Increasingly, enzyme-linked immunosorbent assays (ELISAs) are being used which can be performed more rapidly than culture. These have been developed to detect either toxin A or B in stool and have a sensitivity of 81-94% and a specificity of 92-98%. Test kits that are able to detect both toxins are more sensitive because they are able to identify cases of toxin A negative/toxin B positive CDAD[6]. Stool cultures are not generally performed because of the possibility of detecting asymptomatic strains that are non-toxigenic and because the procedure is labour intensive. However, this procedure is

required to determine the strain of the *C. difficile* for investigation of an outbreak[6]. Polymerase-chain reaction (PCR) tests have been reported to have a very high sensitivity but poor specificity due to difficulty in distinguishing between asymptotic carriage and symptomatic infection[1]. PMC may be diagnosed by direct visualization of colonic mucosa via sigmoidoscopy or colonoscopy.

2 PROBIOTICS

2.1 Probiotics used for prophylaxis and treatment of CDAD

A probiotic is a live microorganism or a microbial mixture that is administered to beneficially affect the host by improving its microbial balance[17]. The term "biotherapeutic agent" is used to describe a microbe having specific therapeutic activity against a specific disease[17]. Probiotics that have been proposed for prophylaxis and treatment of AAD include various bacteria (*Bifidobacterium*, *Lactobacillus* GG, *Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus plantarum 299v, enteroccus faecium (SF68)*) and yeasts (*Saccharomyces boulardii, Saccharomyces cerevisiae*).

2.2 Mechanism of action

As previously mentioned, disturbance of the normal colonic flora predispose the patients to colonisation by *C. difficile* [6]. Probiotics are believed to restore the equilibrium in the altered gastrointestinal microflora, thus protecting against the colonization by pathogens[18]. Probiotics may be preferable to vaccines due to the immediate onset of action[4]. Several possible mechanisms have been proposed to explain how probiotics work including immune stimulation, inhibition of epithelial and mucosal adherence of the pathogen, enzymatically modifying toxin receptors, production of antimicrobial substances, competition for nutrients, stimulation of immunoglobulin A and trophic effects on intestinal mucosa [4;19].

2.3 Recovery of probiotics in stool samples

Probiotics are essentially a way of delivering active constituents, such as enzymes, to targets in the gastrointestinal tract[20]. Thus the beneficial effect of a probiotic depends on its ability to preserve these active constituents against the acidity of the stomach and deliver them to the target site. Pharmacokinetic studies of *S. boulardii* in healthy volunteers have shown that it reaches steady state levels 10^8 CFU/g after 3 days of oral dosing with 0.5g/day (10^{10} CFU/g) twice a day[21]. Following cessation of dosing *S. boulardii* declined rapidly. Similar results were observed for *L. acidophilus*. In contrast, a special human strain of *L. casei* has been found to persist in healthy volunteers for up to 7 days after dosing was stopped.

As part of a randomized study on *S. boulardii* for the treatment of recurrent CDAD, Elmer et al measured *S. boulardii* concentrations at various times in stools of patients[21]. Patients in the intervention group received 1g of lyophilized *S. boulardii* per day containing about 10×10^9 CFUs along with either vancomycin or metronidazole. Of the 50 intervention group patients, 41 (82%) had detectable stool concentrations of *S. boulardii* ranging from 1.5×10^3 to 6.2×10^7 CFUs per gram. Further, they found that the *S. boulardii* concentration was higher in patients who did not have a recurrence of CDAD (1×10^6 CFU per g compared to 1.5×10^4 CFU per g). These differences were not explained by age, gender or antibiotic type/dose. The same study found that *S. boulardii* was cleared by 94% of patients by the third day after treatment was discontinued.

2.4 Formulation

Probiotics are commonly available either as lyophilized capsules or in the form of a fermented drink. They may be prescribed or purchased over the counter. In Canada, the Natural Health Products Directorate (NHPD) oversees the regulations concerning probiotics. For the probiotic to be effective it has to contain a certain minimum number of CFUs per dose [22]. For prescription probiotics, the current daily intake recommended by the NHPD is limited to 5 billion CFU per day for five consecutive days. Over-the-counter products on the other hand can have over 50 billion CFUs per dose. Several articles, including one from Canada, have reviewed commercially available products such as food

supplements, yoghurt and fermented milk[22;23]. In all articles it was found that the actual number of CFUs could be much lower than advertised and that product labels often reported incorrect information on the bacterial species. This could be misleading to consumers if the beneficial effect of the probiotic is dependent on the individual strain.

2.5 Cost

The cost of various probiotic products was summarized as follows in a recent report to the Pharmacy and Therapeutics Committee of the MUHC:[24]

Probiotic	Brand	Cost	Cost per dosage form
Lactobacillus rhamnosus GG	Bacid	50 caps = $28.33	$0.56/cap
Ferments Lactiques Caps(Unclear content)	Proboclac	500 mg/cap x 60 caps = $16.28	$0.27/ cap
Lactobacillus acidophilus and Casei	Bio-K+ (liquid)	50 billion/btl x 6 bottles = $13.59	$2.26 / bottle
(Lactobacillus acidophilus 15 billion / caps and Lactobicillus acidophilus Kaps extra-strength 30 billion/ caps)	Bio-Kaps	15 billion/cap x 6 caps = $13.19 30 billion/cap x 6 caps = $19.19	$2.20 / cap $3.20 / cap
Sacchromyces Boulardii	Florastor	250 mg/cap x 50 caps = $27.97	$0.56 / cap

2.6 Side-effects

In general probiotics have been reported to have a good safety profile. There have been case reports of infections, particularly fungemia and bacterimia. Most reported cases seem to be in immunocompromised patients. A recent review article reported that 30 cases of fungemia have been reported following treatment with *S. boulardii* and 2 cases of bacterimia following treatment with *Lactobacillus rhamnosus*[25]. All patients who had fungemia had an in-dwelling vascular catheter and were being treated in an ICU. The majority of infections were noticed when *S. boulardii* was used in a sachet form that had to be poured into enteral nutrition formulas or drinks, or when capsules of *S. boulardii* were opened. Clustering of cases in certain ICUs suggests local hazards may be responsible. The two cases of bacterimia associated with *Lactobacillus* were observed in one patient with non-insulin-dependent diabetes and another patient with mild mitral valve regurgitation. Salminen et al caution that even though they did not notice any correlation between increased probiotic use of *L. rhamnosus* GG and the incidence of *Lactobacillus* bacterimia [26], the widespread use of immunosuppressive therapy and anti-microbial agents ineffective against lactobacilli might increase the importance of commonly used probiotic *Lactobacillus* strains as pathogens[27].

Other side-effects that could theoretically be associated with living microorganisms, such as deleterious metabolic activity, excessive immune stimulation or

gene transfer, have not been observed in humans. In order for a bacterial probiotic drug to be effective when given concurrently with antibiotics, it should be resistant to the antibiotics. This property raises the question as to whether resistance genes can be transferred to the bacteria for which the antibiotics are being used, and what impact the transfer would have on subsequent antimicrobial therapy of those antibiotics. Thus, there is a possibility that use of probiotics could hasten the development of antibiotic-resistant bacterial strains[20]

Probiotic products that contain strains or species of bacteria that have been associated with infections and/or have a high risk of developing antibiotic resistance are not approved by Health Canada. [28]

3 *PREVENTION* OF CDAD USING PROBIOTICS

3.1 Evidence from RCTs

There has only been one randomized controlled trial evaluating the efficacy of probiotics on prevention of CDAD. We report comparisons between the intervention and control groups using risk differences and 95% confidence intervals. The confidence intervals were calculated using the hybrid score method with continuity correction proposed by Newcombe[29].

3.1.1 Plummer et al, 2004, Int Microb[30]:

Study design: This study had a double-blind, placebo-controlled, randomized design. There were 69 patients in each arm recruited from patients presenting with acute emergencies at a hospital and needing treatment with an antibiotic. The probiotic treatment comprised 1 capsule per day, with 20×10^9 CFU of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* per capsule, for 20 days. Patients received antibiotics for different durations up to a maximum of 20 days. The intervention was begun within 36 hours of antibiotic prescription. Stool samples were collected at enrollment, at the end of the antibiotic therapy, and following any occurrence of diarrhea. Stool samples were first

tested for *C. difficile* and positive samples were further tested for the presence of *C. difficile* toxins A and B.

Results: Using the stool sample at enrollment the authors found an "elevated" number of asymptomatic *C. difficile* positive-toxin negative patients, 8/138 (6%). This was attributed to the fact that these patients had probably received antibiotic therapy or been hospitalized prior to the current hospitalization. The authors note that there was an increase in *C. difficile* associated problems after the admission of these patients but they do not give any details. Only one of these patients went on to develop diarrhea during the study.

Results obtained while antibiotic therapy was ongoing (i.e. based on stool samples of patients who developed diarrhea during treatment) are summarized below:

Outcome	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
Diarrhea	15/69 (21.7%)	15/69 (21.7%)	0% (-13.7%, 13.7%)
Diarrhea + *C. difficile* toxin (CDAD)	2/69 (2.9%)	5/69 (7.2%)	4.3% (-3.8%, 13.2%)

Despite very small numbers of CDAD cases, and a statistically non-significant result, the authors conclude that there was a "much greater" proportion of toxin-positive patients in the placebo group, and a "close relationship" between incidence of diarrhea and presence of the toxin. Though they do not present results on positive culture status they conclude that "many" of the patients receiving the probiotic were in the asymptomatic state.

Results obtained after antibiotic therapy (i.e. based on stool samples of all patients following antibiotic treatment) are summarized below:

Outcome	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
C. difficile positive	11/69 (13.0%)	9/69 (15.9%)	2.8% (-8.7%, 15.3%)

Among *C. difficile* positive patients :			
Toxin positive	5/11 (45.5%)	7/9 (77.8%)	32.3% (-15.8%, 65.2%)
Toxin positive + diarrhea	2/11 (18.2)	6/9 (66.7%)	48.5% (-0.1%, 77.0%)

In an analysis conducted among *C. difficile* positive patients only, the authors conclude that there were "more" asymptomatic *C. difficile* positive patients in the placebo group, though 6 asymptomatic patients in the intervention group compared to 2 in the placebo group provides only feeble support to this statement.

Finally, using results from an earlier study by Wilcox that suggested that treatment of each patient with CDAD costs £4,000, they calculated a 50% reduction in costs associated with the use of probiotics based on the results from the first table above.

Interpretation: The authors conclusion of a beneficial effect of the intervention is unfortunately not supported by the numbers presented. The wide confidence interval around the risk difference suggests the possibility of no effect or even a negative effect of the intervention. One of the main drawbacks of the study design was the short follow-up. The literature suggests that CDAD can develop up to six weeks after stopping antibiotic treatment, whereas this study was terminated immediately after antibiotic use. The target of 200 patients in each arm was not achieved, limiting the number of CDAD cases. The authors claim that the target sample size was determined by a power calculation to detect a 50% benefit but unfortunately do not report the power they wanted to achieve. Further, it was not possible to replicate their results with reasonable guess values for the power. Another drawback of the study was that only *C. difficile* positive patients were tested for the presence of the toxin, therefore it is not known if there were *C. difficile* negative-toxin positive patients and whether these patients had diarrhea. There was no mention of how compliance was assessed or if there were any side-effects. Finally, no statistical analyses were performed to adjust for differences between the groups in antibiotic type, dose or duration, length of stay in hospital and previous history of CDAD. These differences might arise by chance despite randomization and should be adjusted for, given they are important risk factors of CDAD.

Conclusion: This is a poorly presented paper in which the authors conclusion go beyond the data. It provides no evidence of a beneficial effect of probiotics.

We also reviewed studies focusing on AAD as the primary outcome if CDAD was measured a secondary outcome. We identified five such studies. In three there was no suspicion of therapeutic benefit of probiotics on CDAD, and in two despite the lower CDAD rate in patients receiving probiotics, risk differences were not statistically significant. In none of these studies was the CDAD subgroup sufficiently large to either prove or disprove the benefit of probiotics in the prevention of CDAD. (In no study were there more than 10 such patients). As regards the effect of probiotics on AAD, in three trials there was a statistically significant benefit of the intervention. In two studies there was no difference between intervention and control groups. These results are summarized in Appendix B. All studies reviewed were conducted among adult subjects. We also reviewed two meta-analyses of the use of probiotics for the prevention of AAD. Though both concluded a beneficial effect of probiotics there were several methodological problems with these analyses as explained in Appendix B.

4 *TREATMENT* OF CDAD USING PROBIOTICS

4.1 Evidence from open trials

We found four reports of the use of probiotics in non-comparative studies of adults with *C. difficile*. In a letter to the Lancet in 1987, Gorbach et al describe the successful treatment of 5 patients with recurrent CDAD using *Lactobacillus GG*[31]. All patients had multiple recurrences following antibiotic therapy within a 10-day period. Following treatment with *Lactobacillus GG* there was no recurrence for periods ranging from 4 months to 4 years. Similar results were reported by Schellenberg et al in another letter describing the treatment of three patients with brewer's yeast (*Saccharomyces cerevisiae*)[32]. Follow-up times in this report were between 6 to 8 weeks. A study by Surawicz et al reported cessation of CDAD in 11/13 patients treated with *Saccharomyces boulardii*[33]. A study by Bennett et al compared three different doses of lactobacillus[34].

They found that when patients were given four capsules per day, each with 1×10^9 lactobacilli, for 14 days there was no recurrence of CDAD during a two month follow-up period. There were 3/9 recurrences among patients given one or two capsules for 10 days and 2/9 among patients given two capsules for 21 days. Re-treatment following the recurrence was successful for 3/5 patients.

4.2 Evidence from RCTs

So far only three randomized controlled trials have appeared in the literature regarding the use of probiotics for CDAD[35-37]. Another study (the TUMMY trial) was reported as having commenced in 2000 but its results have not yet been published[38]. Given the heterogeneity between studies in the study population and the type of probiotic used it was decided not to perform a meta-analysis. We report comparisons between the intervention and control groups using risk differences and 95% confidence intervals calculated using the continuity-corrected method proposed by Newcombe [29].

4.2.1 McFarland et al, 1994, JAMA[35]:

Study design: This was a multi-centre, double-blinded, randomized placebo controlled trial. Patients were recruited by referrals from physicians across the United States, by advertisement and by screening for *C. difficile* at the clinical microbiology laboratories of the participating centers. Patients were included only if they had diarrhea (either uncomplicated or psuedomembranous colitis) and were positive on at least one *C. difficile* assay (culture, toxin A or toxin B). All patients received either vancomycin, metronidazole or both depending on their physician's assessment. The intervention group received a lyophilized capsule of *S. boulardii* twice a day for a period of 4 weeks. The probiotic preparation had 30×10^9 colony forming units (CFU) of *S. boulardii*. The probiotic treatment was required to overlap with the antibiotic treatment for a minimum of 4 days. Patients maintained a standardized daily diary of stool frequency and consistency, adverse reactions etc. for an 8-week period. They were contacted each week by telephone to verify the contents of their diaries. At the end of the probiotic treatment they were tested for *C. difficile*. A patient was considered to have recurrent CDAD if they

developed diarrhea and had at least one positive *C. difficile* assay (culture, toxin A or toxin B). Statistical analyses used the intention-to-treat approach.

Results: Analysis within sub-groups defined by whether patients were first-time or recurrent CDAD cases reveals greater use of vancomycin among the latter. Though the authors conclude that there was no statistically significant difference in the type of antibiotic between the intervention and placebo groups, it is possible that an important difference is obscured by small sample size. Of the 124 eligible patients recruited 104 (83.9%) completed the trial. The analysis of recurrent CDAD is summarized below:

Sub-group	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
First-instance CDAD	6/31 (19.3%)	8/33 (24.2%)	4.9% (-17.6%, 26.4%)
Recurrent CDAD	9/26 (34.6%)	22/34 (64.7%)	30% (2.3%, 50.6%)
Overall	15/57 (26.3%)	30/67 (44.8%)	18.4% (0.3%, 34.9%)

Thus, these results show a statistically significant lower rate of recurrence of CDAD in the treated group versus the controls. These results were further adjusted for age, gender, severity of the enrollment CDAD episode, duration of CDAD, type of antibiotic and comorbidity, but none of these covariates were found to be important. Among those who failed to respond to the treatment, those in the intervention group had fewer daily stools than those in the control group. There was no difference in the time to recurrence of CDAD in the two groups, with most failures occurring within 2 weeks of discontinuing the antibiotic. The effectiveness of *S. boulardii* was similar among patients with diarrhea, colitis or PMC.

At the end of the antibiotic treatment, a similar percentage of patients in both groups were *C. difficile* positive (12% intervention vs. 19% placebo). When the probiotic/placebo was terminated, there was significantly lower number of toxin B positive cases (6.7% intervention vs 30% placebo) but there was no difference in culture positivity. These results were based on only 61.3% of the sample. Finally, there were a

small, but statistically significantly larger, number of patients who reported increased thirst and constipation after treatment with the probiotic.

Interpretation: The main conclusion of the authors was that there was a statistically significant, beneficial effect of *S. boulardii* on recurrent CDAD, particularly among patients who have had at least one prior episode of CDAD. However, the wide confidence interval around this result suggests there isn't sufficient evidence to conclude a clinical benefit. The large beneficial effect among the sub-group with a history of CDAD is impressive but it is not clear why a similar large benefit was not observed in patients with an initial CDAD episode considering there were a similar number of patients in both sub-groups. Patients with a previous episode had been treated in the past with various antibiotics and one or more were treated with the probiotic *L. casei,* though interestingly, none had been treated with *S. boulardii*. Two important measures of severity that were not adjusted for were the time-elapsed since the last CDAD episode and the length of stay in hospital. This raises the possibility that more patients in the intervention group may have initially had a less severe case of CDAD. Another concern is the dependence on patient diaries to assess diarrhea, though any inaccuracy is likely to have been non-differential between the two study groups.

Conclusion: This was a well-designed, appropriately analyzed study. A marginally significant overall beneficial effect of the intervention was found, being almost completely restricted to a subset of patients with severe, recurrent CDAD, most of whom had been recipients of high-dose vancomycin. The numbers were not substantial resulting in an overall risk difference ranging from 0.3% to 34.9 %.

4.2.2 Surawicz et al, 2000, Clin Inf Dis[36]:

Study design: This study was also conducted by the group that did the first RCT for *S. boulardii* (McFarland et al). They improve upon their earlier design by administering the same antibiotic to intervention group patients and their controls for the same duration of 10 days. Patients received either high-dose vancomycin (2 g/day), low-dose vancomycin (500mg/day) or metronidazole (1 g/day). The antibiotic treatment was determined after the randomization. Patients were included if they had diarrhea, were *C. difficile* positive (i.e. either by culture or toxin A or B) and had at least one occurrence of CDAD in the

previous year. The probiotic was administered for 28 days in a capsule form at a dosage of 1g/day (2 250mg capsules). Patients kept daily diaries with details of stool frequency, adverse reactions etc for an 8-week period. Failure was defined as recurrence of CDAD during this time.

Results: The patients in the high-dose vancomycin group were found to have more severe CDAD with colitis, PMC or the presence of fecal leukocytes. There was no *C. difficile* persistence in the high-dose vancomycin group following antibiotic therapy, compared to 13% persistence in the low-dose vancomycin group and 80% persistence in the metronidazole group. The results on recurrent CDAD are summarized below:

Sub-group	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
High-dose vancomycin	3/18 (16.7%)	7/14 (50%)	33% (-0.3%, 62%)
Low-dose vancomycin	23/45 (51.1%)	17/38 (44.7%)	6.4% (-16.2%, 28.1%)
Metronidazole	13/26 (50.0%)	13/27 (48.2%)	1.9% (-25.8%, 29.2%)
Overall	39/79 (49.4%)	37/79 (46.8%)	2.5% (-13.6%, 18.5%)

Thus, overall there was no difference between the intervention and placebo groups in the proportion of patients with recurrent CDAD. Only in the group of patients taking high dose vancomycin was there a statistically significant benefit (The authors report a p-value of 0.05). Comparing the three sub-groups on placebo it appears that there was no difference between vancomycin and metronidazole. The median time to recurrence was also greater in the intervention group (12 days compared to 7 days) and the average number of recurrences was significantly lower (0.17 vs 0.78, p-value=0.03). There was no significant difference between the *S. boulardii* and placebo groups at this time or at the 8-week follow-up. There was no significant difference in the number of adverse reactions reported by the two groups, with a mean (standard deviation) of 1.9 (1.8) in the intervention group compared to 2.0 (1.7) on placebo.

Interpretation: The authors conclude there is a beneficial effect of *S. boulardii* in patients treated with high-dose vancomycin even though this is in a small group of 32 patients. However, it is not apparent that there is a clinically significant benefit of probiotics given the wide confidence interval. Interestingly, the previous study by this group also found a beneficial effect in a group of patients with one or more previous CDAD episodes – a group in which patients were more likely to use vancomycin than metronidazole. As in the previous study the observed effect is surprising because these patients had more serious manifestations of CDAD than other sub-groups. A limitation of the study was that the results were not adjusted for the severity and number of previous CDAD episodes, the time since the last episode and the length of stay in hospital, though this is unlikely to make a difference given the small sample size. No data were reported on compliance.

Conclusion: This is also a well-designed study whose main limitation is a small sample size. It finds no evidence of a therapeutic benefit of probiotics overall, but a positive effect of borderline statistical significance in a small subgroup of patients who received high-dose vancomycin.

4.2.3 Wullt et al, 2003, Scand J inf Dis[37]:

Study design: This was a multi-center, double-blind, placebo-controlled trial conducted in Sweden over a 2 year period. Patients were included if they had ongoing diarrhea and a positive *C. difficile* toxin (A or B) assay within 6 days of enrollment, if they had had CDAD within the previous 2 months and were not being treated with a list of drugs including vancomycin and metronidazole at the time of enrolment. Patients were randomized to received 400mg of metronidazole for 10 days in combination with either the intervention or placebo. The intervention was given in the form a fruit drink containing oats fermented with *L. plantarum 299v* (50×10^9 CFU/day) once a day for 38 days. Cure was measured in two ways: 1) clinical cure: cessation of diarrhea, 2) bacteriological cure: negative assay for *C. difficile* toxin. If a patient reported a clinical recurrence, they were subjected to a toxin assay within 2 days of onset of symptoms. Compliance was assessed by presence of *L. plantarum 299v* in faeces.

Results: A total of only 21 patients completed the study across the 9 centers over the two-year period. The primary results are summarized below:

Outcome	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
Clinical cure within 5-10 days	11/12 (91.7%)	9/9 (100%)	8.3% (-29.6%, 40.3%)
Bacteriological cure on days 11-13	7/12 (8.3%)	7/9 (77.8%)	19.4% (-25.8%, 54.3%)
Bacteriological recurrence on days 37-41*	5/11 (45%)	3/9 (33%)	-12.1% (-32.9%, -50.7%)
Clinical recurrence at day 70*	4/11 (36%)	6/9 (67%)	30.3% (-17.7%, 64.5%)

* Among those who were clinically cured by 5-10 days.

Interpretation: The main problem with this study is the small sample size that makes it impossible to conclude anything. They did not reach their target sample size of 40 patients in each arm. The higher rate of bacteriological recurrence apparently did not result in clinical recurrences leading the authors to conclude that "the lactobacilli either counteract the pathogenesis of *C. difficile* or have a positive overall impact on the microflora that prevents clinical recurrences". However, they do not provide any numbers to support this. The separation of definitions of bacteriological and clinical cure makes it difficult to compare these results from other studies measuring one combined outcome. The authors report good compliance and no apparent side-effects in the intervention group.

Conclusion: The numbers involved in this study were too small to allow any conclusions. There is no suggestion of therapeutic benefit.

5. DISCUSSION

Incidence of CDAD has become more frequent in Quebec hospitals over the last decade and the number of severe cases is increasing as well. This is a debilitating illness with a high cost, particularly among patients with recurrent CDAD for whom there is no satisfactory treatment with vancomycin or metronidazole. Probiotics that serve to restore the balance in the normal colonic flora that may have been disrupted by the prior use of antibiotics, have been proposed as an alternative to antibiotics.

While there is some evidence of the beneficial effects of probiotic interventions in infectious diarrheas[39], in antibiotic associated diarrheas in adults[40;41], and in children[42], the purpose of this report was to evaluate the published evidence on the use of probiotics for prevention or treatment of diarrheas associated with *C. difficile* infection (CDAD) in adults. We reviewed RCTs that either measured CDAD as a primary or a secondary outcome and observational studies where CDAD was the first outcome. We also reviewed a recent report prepared by the Pharmacy and Therapeutics Committee of the MUHC, which concluded that use of probiotics is not recommended due to lack of sufficient evidence from RCTs.

One factor that makes it difficult to compare results across studies, whether the outcome is CDAD or AAD, is the variability in the type of probiotic used and its concentration. For example, while in the study for AAD by Thomas et al the intervention had 10 billion *Lactobacillus GG* and was given for 14 days, in the study by Beausoleil et al the intervention had 50 billion *Lactobacillus acidophilus* and *L. casei* and was given for a variable duration. Another concern in comparing studies is the variability in the method of diagnosis of CDAD. While some studies required both diarrhea and toxin to be present, others distinguished between presence of diarrhea, presence of *C. difficile* and presence of toxin. The poor sensitivity and specificity of tests for *C. difficile* and for toxin presence may explain why we observe several combinations of results on these three criteria (ex. toxin present but no diarrhea, diarrhea and *C. difficile* but no toxin etc.).

In our review of probiotic formulations we found that there can be great variability in the advertised and actual number of CFU in the different products. While Health Canada requires probiotic capsules to have a maximum of 5 billion CFU per capsule, probiotics are also distributed in the form of fermented drinks that may have a

much higher number of microorganisms exceeding 50 billion CFU per dose. Some of the negative studies used the same dose of probiotic for adult patients that was used in positive studies of pediatric patients. This suggests that the lack of evidence of a benefit may relate to use of a probiotic dose that is not strong enough. Another reason for the lack of benefit in the prevention studies is that the antibiotics may be killing the probiotics. There has been a small number of case reports of fungemia and bacterimia among patients prescribed probiotics.

6. CONCLUSION

The results of the limited evidence we have found can be summarized as follows:

Prevention: In this report we have reviewed one randomized controlled trial evaluating a probiotic (mixture of *L. acidophilus* and *Bifidobacterium bifidium*) for the prevention of CDAD. This study had very few CDAD cases – less than 10 out of 138 patients in both groups combined – and results were neither statistically nor clinically significant. No evidence of therapeutic benefit in prevention of CDAD was revealed in the analysis of small subgroups of CDAD patients in five studies in which prevention of AAD (not CDAD) was the primary outcome. Thus, we found no evidence to support the prior use of probiotics in the prevention of CDAD.

Treatment: We reviewed three studies evaluating different probiotics (*S. boulardii* and *L. plantarum 299v*) for the treatment of recurrent or first-time CDAD. In one study no beneficial effect of probiotics was found. In two there was a small statistically significant therapeutic effect in a small subgroup of patients, one with recurrent severe CDAD, and the other in patients who were receiving high-dose vancomycin. In both cases the probiotic was *S. boulardii* and the patient sub-group consisted of severe CDAD cases.

These results clearly do not constitute sufficient evidence of benefit of probiotics to justify a change of therapeutic policy at the MUHC at this time. They do suggest however that there is need for a larger trial in severe CDAD patients, particularly those being treated with vancomycin.

7. RECOMMENDATION

It is recommended that the MUHC does not adopt the use of probiotics for the prevention or treatment of CDAD at the present time. The literature should be reevaluated as more evidence becomes available.

APPENDIX A: SEARCH METHODOLOGY

The search methodology involved the following two steps:

Step 1: Articles for this report were identified using combinations of the following search terms:

(Probiotic OR Probiotics OR Lactobacillus OR lactic-acid OR Saccharomyces OR yeast OR boulardii OR Bifidobacterium OR SF68)

AND

(Clostridium OR difficile OR diarrhea OR antibiotic-associated)

AND

Patients

in the following on-line databases:

Database	Webpage
MEDLINE - National Library of Medicine (USA)	www.health.library.mcgill.ca/database/medline.htm
PubMed – National Library of Medicine (USA)	http://www.ncbi.nlm.nih.gov/PubMed/
CCOHTA – Canadian Coordinating Office for Health Technology Assessment	www.ccohta.ca
INAHTA – International Network of Agencies for Health Technology Assessment	www.inahta.org
HEN - Health Evidence Network (WHO Regional Office for Europe)	www.who.dk/eprise/main/WHO/Progs/HEN/Home
The Cochrane Collaboration	http://www.cochrane.org

Step 2: Bibiliographies of articles selected in Step 1 were searched.

APPENDIX B: Randomized controlled trials and meta-analyses evaluating probiotics for preventing antibiotic-associated diarrhea

About 20% of patients who take antibiotics experience antibiotic-associated diarrhea (AAD)[43]. The risk of diarrhea depends on the type of antibiotic and the duration for which it is taken. Between 5-50% of antibiotic-associated diarrhea is attributed to *Clostridium difficile* (*C. difficile*). Several randomized controlled trials have evaluated different probiotics for the prevention of AAD. Some of these measured *C. difficile* presence as a secondary outcome. In this appendix we summarize results from five such studies and one meta-analysis. One of the main concerns about these studies is that since *C. difficile* was not a primary outcome it was not systematically evaluated in all patients. All studies had very small numbers of *C. difficile* patients making results statistically non-significant even when there appeared to be a slight benefit. A drawback of these studies was that no adjustment was made for the variability in the antibiotic (type, dosage or duration) and length of stay in hospital between intervention and placebo groups. While the randomization of patients and the double-blinded design would theoretically achieve a similar distribution of these variables in the two groups, small sample sizes raise the possibility of an imbalance. Another ignored covariate is the history of CDAD. While some studies take care to exclude patients who had CDAD a few months prior to the start of the study, they may still have included patients prone to CDAD.

C.1 Surawicz et al, 1989, Gastro[43]:

Study design: This was the first double-blind, randomized, placebo-controlled study to look at the use of *S. boulardii* for the prevention of AAD in an acute care setting. A 2:1 randomization design was used. Patients were excluded if they had diarrhea during the week preceding enrollment, were taking vancomycin or metronidazole, were on antibiotic therapy for less than 3 days or using antifungal antibiotics that could inactivate the yeast. Patients were followed for a minimum of 8 days. The probiotic intervention was 1g of lyophilized *S. boulardii* per day. It was administered within 2 days of starting the antibiotic therapy for up to 2-weeks following the last antibiotic dose. Stool frequency and drug intake was recorded by nursing staff at the hospital and by the patient following discharge. Stool samples for *C. difficile* culture were collected at enrollment, day 5 and

every 10 days thereafter till the completion of the study. Only *C. difficile* positive stools were tested for toxin B.

Results: The main results are summarized below:

Outcome	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
AAD	11/116 (9.5%)	14/64 (21.8%)	12.4% (0.2%, 24.7%)
Diarrhea among *C. difficile* positive*	3/32 (9.4%)	5/16 (31.3%)	21.9% (-0.4%, 50.0%)
Acquired *C. difficile* after enrollment	22/81 (27%)	5/36 (14%)	-13.3% (-25.7%, 4.7%)
Diarrhea among toxin positive**	2/14 (14.3%)	1/5 (20%)	5.7% (-29.4%, 57.2%)

* Mix of patients with asymptomatic *C. difficile* at enrollment and those who acquired it in hospital.

** Mix of patients with toxin B at enrollment and those who acquired it in hospital.

Only 138 out of 180 patients who submitted sufficient stool samples to determine the time of onset of *C. difficile*. Of these 48 were *C. difficile* positive at atleast one point point. Among these 48 patients there was a large difference between the intervention and control groups in the percentage with diarrhea, however it was not statistically significant. There was also no significant difference between study groups in the percentage of patients who acquired *C. difficile* among the 117 patients who did not have it at enrollment. No results were presented on the effect of *S. boulardii* on cytotoxicity. There were no side effects of either *S. boulardii* or placebo.

Interpretation: The study concluded that the intervention caused significant reduction in AAD, but did not prevent the acquisition of *C. difficile*. There are several problems with the *C. difficile*-related analyses making it impossible to draw any conclusions about the effect of the probiotic on CDAD: 1) authors do not say how the 42 patients for whom sufficient *C. difficile* cultures were not available were different from the 138 patients analyzed, 2) they report diarrhea among a mix of asymptomatic *C. difficile* and other

patients. Since the former are known to be at a lower risk of CDAD it is possible that the beneficial effect of the intervention was due to a larger number of these cases in the intervention group. 3) they do not report what percentage of patients who were *C. difficile* negative developed diarrhea, and since they tested only *C. difficile* positive patients for toxin presence the actual percentage of patients with toxin B and diarrhea is not known. A more stringent definition of CDAD requiring patients to have have both diarrhea and toxin B might have altered their conclusions.

C.2 McFarland et al 1995, Am J Gastro[44]:

Study design: This was a randomized, double-blind, placebo controlled trial evaluating the use of *S. boulardii* for preventing diarrhea among patients taking beta-lactam antibiotics, which have been associated with the highest frequency of AAD. Patients were required to not have diarrhea at enrollment and to be taking at least 1 beta-lactam antibiotic for at least 48 hours prior to enrollment. The probiotic was administered in a capsule form (2 250mg capsules twice a day or 30×10^9 CFUs). The study drug was started within 72 hours of the beta-lactam and continued for 3 days after the antibiotic was discontinued. Follow-up was a period of 7 weeks after that. Patients maintained a daily diary with details of stool frequency, medications taken and adverse reactions. They were contacted weekly by telephone after discharge from the hospital. Stool samples were collected and tested for *C. difficile* at enrollment, at the end of the intervention and at any time that diarrhea occurred.

Results: The results are summarized in the table below:

Outcome	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
AAD	7/97 (7.2%)	14/96 (14.6%)	7.4% (-0.2%, 17.2%)
Diarrhea among *C. difficile* positive	3/10 (30%)	4/14 (28.6%)	-1.4% (-37.1%, 29.2%)

The main result of the study was that *S. boulardii* was associated with a significant decrease in AAD (though we not able to replicate the p-value of 0.02). However, the rate

of *C. difficile* positivity, and the incidence of diarrhea in the 24 *C. difficile* positive patients, was almost identical in the intervention and placebo groups. In a multivariate logisitic regression model, *S. boulardii* continued to have a significant protective effect (RR=0.29, 95%CI=0.08,0.98) after adjusting for days of antibiotic use and patient's age.

Interpretation: There were too few cases of AAD to make a definitive conclusion. From the data observed it appears there was no clinically significant benefit of probiotics on preventing CDAD.

C.3 Lewis et al, 1998, J Infec[45]:

Study design: This was a randomized, placebo-controlled trial of *S. boulardii*. It is not clear whether physicians treating the patients were blinded. Patients were elderly in acute care. They were required to have been prescribed antibiotics within the preceding 24 hours and not to have taken antibiotics within the previous 6 weeks. The intervention was administered throughout the time the patient received antibiotics. The probiotic was administered in capsule form (113mg twice daily). Patients were assessed daily to monitor bowel habits, compliance and side-effects. Stool samples were analyzed for *C. difficile* toxin by a cell culture technique every 4 days or if the subject developed diarrhea.

Results: The main results are as follows:

Outcome	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
Diarrhea	7/33 (21.2%)	5/36 (13.9%)	-7.3% (-25.0%, 10.5%)
C. difficile toxin positive	5/33 (15.2%)	3/36 (8.3%)	-6.8% (-22.6%, 8.0%)

There was no statistically or clinically significant benefit of the probiotic on either AAD or CDAD. Patients who were *C. difficile* toxin positive had a longer course of antibiotics, received more types of antibiotics, stayed longer in hospital and were more likely to develop diarrhea. There were no side-effects attributable to *S. boulardii*. The authors mention that a possible reason for the lack of an effect may have been because

the probiotic dose was not large enough. They cite the study by Adam et al [46] as having used double the dose used by them.

Interpretation: The small number of patients with the outcomes of interest (diarrhea, *C. difficile* positive, toxin positive) makes it impossible to conclude anything from this study. Study groups were not compared on the outcome diarrhea+toxin positive, which has been used to define CDAD in the literature.

C.4 Thomas et al, 2001, Mayo Clin Proc[47]:

Study design: This was a randomized, double-blind, placebo-controlled trial conducted between July 1998 and October 1999. Patients were required to have received either an intravenous or an oral antibacterial agent for the treatment of an infection. Patients were excluded if they were on an antibiotic for more than 24 hours prior to enrollment or at any time within the prior 2 weeks, had *C. difficile* colitis within the previous 3 months. Randomization was within strata defined by bowel movement frequency, use of beta-lactams and age at entry. Intervention group patients were given 1 capsule of *Lactobacillus* GG (10×10^9 CFUs) twice daily for 14 days. The primary outcome measured was diarrhea in the first 21 days after enrollment. Two secondary outcomes measured were the proportion of patients who had additional testing to determine the cause of the diarrhea and the number of patients with CDAD after enrollment. Patients maintained a daily diary with details of stools, side-effects and number of study pills taken. Patients were also contacted by telephone every week.

Results: The 12% of patients who did not complete the study were slightly older. The results are summarized below:

Outcome	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
Diarrhea	39/133 (29.3%)	40/134 (29.9%)	0.5% (-10.5%, 11.3%)
Additional testing to determine cause of diarrhea	2/133 (1.5%)	0/134 (0%)	-1.5% (-5.0%, 1.0%)
CDAD	2/133 (1.5%)	3/134 (2.2%)	0.7% (-3.0%, 4.5%)

There was no beneficial effect of the probiotic on AAD or CDAD. There was no significant difference between intervention and control groups within strata defined by bowel movements, beta-lactam use or age. There was no effect on the results after adjustment for duration of antibiotic use. Most patients (87.3%) who had diarrhea had it while on antibiotics. The compliance rate was estimated to be 86.2% in the *Lactobacillus* GG group. There was no statistically significant difference in the adverse events reported by the two groups.

Interpretation: Since only 5 of the 267 patients were *C. difficile* positive, (2 intervention, 3 placebo) this study provides no information on the efficacy of probiotics in the prevention of *C difficile*. The authors suggest that the lack of effect may have been because the *Lactobacillus* did not survive to colonize the colon due to the higher dose of intravenous antibiotics given to their patients initially. They note that the dose of the probiotic may have been sub-therapeutic as it is the same dose that has been shown to reduce AAD successfully in children. One drawback of this study is that since CDAD was only a secondary outcome *C. difficile* does not seem to have been tested for systematically in all patients. The *C. difficile* positive results were obtained retrospectively from a chart review. Authors do not provide information on how *C. difficile* was tested for and whether these patients had diarrhea. If not all patients were tested it is possible that some CDAD patients were missed, though this is likely to be non-differential between study groups.

C.5 Beausoleil et al, 2004, Abstract submitted to Am J Gastro meeting:

Study design: This was a randomized, double-blind, placebo-controlled trial for assessing the efficacy of a probiotic intervention in the prevention of AAD. It was carried out at Maisonneuve-Rosemont hospital with funding from a Montreal-based company, BioK+. Patients in the intervention group received *lactobacilli*-fermented milk with 50×10^9 CFUs of *Lactobacillus acidophilus* and *L. casei* daily. The treatment lasted the duration of the antibiotic course and patients were followed for a further 21 days. Clinical data on patients and details of stool frequency and consistency during follow-up were obtained from hospital, medical and pharmacy records.

Results: The results of the study are summarized below:

Outcome	Intervention X/N (%)	Placebo X/N (%)	Risk Difference (95% CI) (Placebo-Intervention)
Diarrhea	7/41 (17.1%)	16/43 (37.2%)	20.1% (0.5%, 36.9%)
Diarrhea + *C. difficile* toxin (CDAD)	1/41 (2.4%)	7/43 (16.3%)	13.8% (-1.1%, 29.0%)
Median days of hospitalization	8	10	-

The authors concluded that the intervention significantly reduced the risk of AAD and CDAD. The numbers on CDAD were obtained from an advertisement in the press and not from the abstract. Using these numbers we were not able to replicate the p-value of 0.028 in the abstract. There was no difference in the proportion of patients who reported side-effects in the two groups.

Interpretation: Based on the limited information in the abstract we cannot evaluate the appropriateness of the study design or analysis. Though the authors conclude a benefit of the probiotic for preventing AAD and CDAD based on statistical significance, the wide confidence intervals calculated by us for the risk differences suggests that the benefit may not be clinically significant for either outcome.

C.6 D'Souza et al, 2002, BMJ[41]

A recent meta-analysis summarized results from nine randomized controlled trials evaluating the use of probiotics for prevention of AAD. This article was considered as some of the studies included measured CDAD as a secondary outcome. The authors carried out separate analyses for studies using *S. boulardii* and for the studies using either *lactobacilli* or *enterococci*. In both groups there was a similar beneficial effect of the probiotic on AAD: 0.39 (95% CI 0.25, 0.62) for the yeast and 0.32 (95% CI 0.19, 0.61) for *lactobacilli*. Of the nine studies only three studies showed a statistically significant beneficial effect on AAD. These three studies happen to carry the greatest weight accounting for 66.2% of the weight in total. Of the remaining six studies, five

demonstrated a benefit that was not statistically significant. One study found the placebo performed better for treating AAD, though this result was not statistically significant.

There are several problems with this meta-analysis raising questions about its conclusion in favor of probiotics for preventing AAD. There was variability across studies in the type of the probiotic, its dose and duration. For example, among the positive studies that used *S. boulardii* the probiotic regimen was reported as 4 capsules a day [46]in one, and 1g per day (Surawicz) in the other. In the third study showing a beneficial effect the probiotic regimen was 1-2 capsules of *lactobacillus* GG for 10 days. This study, which accounted for 21.2% of the weight, was the conducted among children. The study showing a non-significant negative effect was conducted among elderly. There was also a lot of variability in the antibiotic type, dose and duration in the different studies. Finally, though some of the studies included did test for CDAD these results were not included in the meta-analysis. Thus despite the conclusion of a positive benefit of probiotics for AAD, we cannot conclude anything about their effect on preventing CDAD from this study.

C.7 Cremonini et al, 2002, Aliment Pharmacol Ther[40] :

Another meta-analysis of studies on prevention of AAD using probiotics that was published at the same time as the paper by D'Souza et al. is the report by Cremonini and colleagues[40]. This analysis included two studies not considered by D'Souza et al and did not include four of the studies considered by them. Neither of the additional studies included any mention of CDAD. As in the meta-analysis by D'Souza et al the authors concluded that there was a beneficial effect of probiotics on preventing AAD (RR: 0.40 (95% CI 0.27-0.57)), and that the main methodological problem was the pooling of results from heterogenous studies: results from studies using different types of probiotics (differences in micro-organism, dose and duration), with different antibiotic regimens and differing age groups of the patient population.

Summary:

In summary, three of the five individual studies and the meta-analysis by D'Souza et al found a statistically significant beneficial effect of a probiotic treatment for AAD.

However, there was no evidence of benefit of probiotic therapy on CDAD in four of these studies, and in one which was reported only in abstract, there was a small therapeutic benefit which did not reach statistical significance. We were able to obtain all articles reviewed in the meta-analysis except the one by Adam et al. We reviewed those that were conducted in adults and that presented any information on *C. difficile*. Even if the study by Adam et al [46] satisfied these criteria it is possible that its results are not relevant to our report given the study was conducted in 1976 when treatment and diagnostic standards for CDAD were very different from current practice.

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