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ORIGINAL RESEARCH

Probiotics are effective at preventing *Clostridium difficile*-associated diarrhea: a systematic review and meta-analysis

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Introduction: *Clostridium difficile* infection (CDI) is the leading cause of antibiotic-associated diarrhea. CDI has increased in incidence and severity over the past decade, and is a growing worldwide health problem associated with substantial health care costs and significant morbidity and mortality. This meta-analysis examines the impact of probiotics on the incidence of *Clostridium difficile*-associated diarrhea (CDAD) among children and adults, in both hospital and outpatient settings.

Methods: A comprehensive literature search of all published randomized control trials (RCTs) assessing the use of probiotics in the prevention of CDAD in patients receiving antibiotic therapy was conducted, and the incidence of CDAD was analyzed.

Results: Twenty-six RCTs involving 7,957 patients were analyzed. Probiotic use significantly reduced the risk of developing CDAD by 60.5% (relative risk [RR] =0.395; 95% confidence interval [CI], 0.294–0.531; $P<0.001$). Probiotics proved beneficial in both adults and children (59.5% and 65.9% reduction), especially among hospitalized patients. *Lactobacillus*, *Saccharomyces*, and a mixture of probiotics were all beneficial in reducing the risk of developing CDAD (63.7%, 58.5%, and 58.2% reduction).

Conclusion: Probiotic supplementation is associated with a significant reduction in the risk of developing CDAD in patients receiving antibiotics. Additional studies are required to determine the optimal dose and strain of probiotic.

Keywords: probiotics, *Clostridium difficile*-associated diarrhea, antibiotic-associated diarrhea

Introduction

Clostridium difficile, a Gram-positive, spore forming, and toxin-producing anaerobic rod bacterium, is the leading cause of hospital- and community-acquired antibiotic-associated diarrhea (AAD) in the Western world.[1,2] *C. difficile* infection (CDI) is a growing worldwide health problem associated with substantial health care costs and significant morbidity and mortality.[2] The incidence of CDI in the United States is rapidly increasing, and is estimated to affect approximately 1% of all hospitalized patients, increasing length of stay by 55%, and US health care system costs by USD1–3 billion annually.[1,3,4]

C. difficile-associated diarrhea (CDAD) occurs most often in elderly and hospitalized patients receiving broad-spectrum antibiotics.[2,5–8] Antibiotic exposure is considered the most significant risk factor for CDI, and several drugs have been implicated in high CDAD rates including cephalosporins, fluoroquinolones, penicillins, and clindamycin.[1,9–11] Other CDAD risk factors include the use of proton-pump inhibitors, H_2 antagonists, methotrexate,

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and the presence of existing gastrointestinal pathologies, such as inflammatory bowel disease.[1,9]

Most approaches for the prevention of CDI to date have focused on limiting the spread of CDI. The most common methods are early detection and isolation, contact precautions, and appropriate hand hygiene. A number of studies have focused on the role of environmental cleaning to eradicate CDI in the health care environment, including the use of environment disinfectants as well as chlorhexidine patient baths, but have shown limited success.[12,13]

More recently, probiotics have been proposed for the prevention and treatment of a variety of gastrointestinal conditions, including diarrhea. Normal intestinal flora is an important barrier against pathogenic bacteria, and disruption of this normal flora with antibiotic use can lead to diarrhea.[14,15] Probiotics are live microbial food supplements and have been hypothesized to counteract disturbances in intestinal flora and reduce colonization by pathogenic bacteria.[14,15] Various species of probiotics have been studied, with the most common being within the *Lactobacillus* and *Bifidobacterium* genus. More recently, *Saccharomyces boulardii*, a yeast, has also been considered a probiotic.[15]

Hempel et al[16] reported a 42% reduction in the risk of developing AAD with the use of probiotics (relative risk [RR] =0.58; 95% confidence interval [CI], 0.50–0.68; $P<0.001$). In a meta-analysis by Johnston et al,[17] a 66% reduction in the risk of CDAD with the use of probiotics (RR =0.34; 95% CI, 0.24–0.49; $P<0.001$) was observed. A Cochrane Review reported similar results with a 64% reduction in the risk of CDAD.[18]

A significant number of more contemporary randomized controlled trials (RCTs) not included in the Johnston et al study[17] or the Cochrane Review have recently been published, which address probiotic use, and have yielded conflicting results.[19–22] Shan et al[20] studied probiotic supplementation in 283 children between the ages of 6 months and 14 years (139 receiving *S. boulardii* and 144 in the control group), and reported one case of CDAD in the probiotic group and eight cases in the control group (RR =0.13; 95% CI, 0.02–1.05, $P<0.05$). Conversely, Allen et al[19] published results from the largest RCT, involving 2,941 hospitalized patients >65 years (1,470 patients receiving probiotics and 1,471 patients in the control group) across five hospitals in the United Kingdom, and reported no significant reduction in CDAD incidence with the use of a multistrain preparation of *Lactobacillus* and *Bifidobacterium* (RR =0.71; 95% CI, 0.34–1.47; $P=0.35$).

Given the high morbidity and mortality associated with CDI, and conflicting results among published RCTs, this meta-analysis provides an updated analysis on the efficacy of probiotics in the prevention of CDAD, for both adults and children, in the hospital and outpatient settings.

Methods
Study selection

A comprehensive search of all published RCTs evaluating probiotics to prevent CDAD was conducted using PubMed, Cochrane Central Registry of Controlled Trials, and Google Scholar (1966–2015). Additional citations were searched, using the references of the articles retrieved from prior publications. The last search was conducted on October 10, 2015, and only articles written in English were considered. Keywords used in the search included combinations of "probiotics", "*Lactobacillus*", "*Bifidobacterium*", "*Saccharomyces*", "*Clostridium difficile*", "*Clostridium difficile*-associated diarrhea", "antibiotic associated diarrhea", and "diarrhea". The following inclusion criteria were used: RCTs comparing the use of any strain or dose of a specified probiotic with a placebo or "no intervention" control group, probiotics initiated within 3 days of starting antibiotics and continued for at least the entire duration of antibiotic treatment. In case of duplicate publications, only the most recent and updated report of the clinical trial was included.

Data extraction

Articles retrieved from the searches were assessed for eligibility, and data pertaining to patients, intervention, control groups, outcomes, and methodology were abstracted (Figure 1). The clinical outcome of interest was incidence of CDAD (diarrhea and positive stool cytotoxin assay or culture), among children and adults, in both the hospital and outpatient setting.

Statistical analysis

For each trial, RR with a 95% CI for CDAD was calculated. Meta-analysis of the pooled data was performed using the Comprehensive Meta-Analysis software Version 3 (Biostat, Englewood, NJ, USA). For studies reporting zero events in any group, a continuity correction factor of 0.5 was adopted to calculate the RR and variance. If there was a zero event in both groups, the RR was not calculable and the study was excluded from the meta-analysis. Both the fixed-effects model and random-effects model were considered, depending on the heterogeneity of the included studies. To assess the heterogeneity between studies, both Cochrane's Q statistic and I^2 statistic were used. Heterogeneity was considered statistically significant when $P<0.05$ or $I^2>50$. If heterogeneity
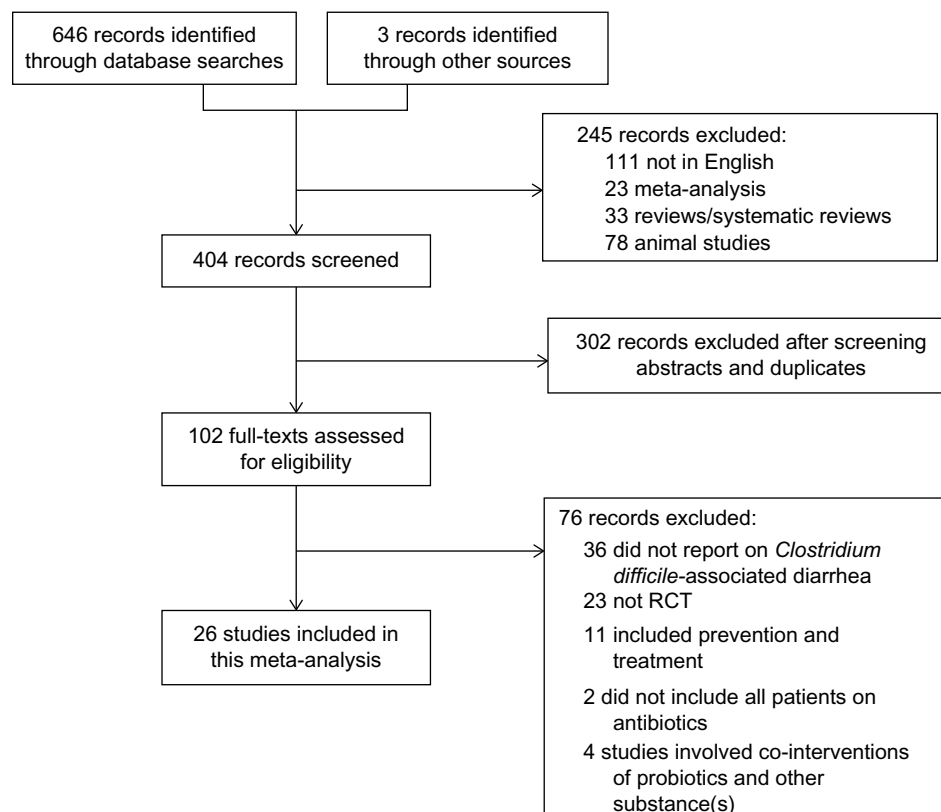
Figure 1 CONSORT diagram of the study selection process.
Abbreviations: RCT, randomized control trial; CONSORT, Consolidated Standards of Reporting Trials.

was observed, data were analyzed using a random-effects model. Conversely, in the absence of heterogeneity, a fixed-effects model was assumed. The publication bias regarding the RR of CDAD in patients receiving probiotics was first visually evaluated by a funnel plot, and further evaluated using Egger's and Begg's tests. A two-tailed *P*-value of <0.05 was considered statistically significant. Subgroup analysis was performed based on the genus of probiotics (*Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, and a mixture of probiotics), the age of the patient (pediatric patients <18 years and adults), and the health care setting (hospitalized inpatient or outpatient).

Results
Demographic characteristics of the studies
A total of 26 RCTs evaluating the use of probiotics in the prevention of CDAD were identified, involving a total of 7,957 patients (Table 1). Approximately 4,124 patients received probiotic supplementation, and 3,833 patients received either placebo or "no treatment". Among patients who received probiotics, 2,273 received *Lactobacillus*, 1,757 received *Saccharomyces*, and 3,927 received a mixture of probiotics.

None of the studies compared *Bifidobacterium* alone with a placebo group.

Effects of probiotics on CDAD
Data on the incidence of CDAD (defined as diarrhea and positive stool cytotoxin assay or culture) in both the probiotic group and the placebo group were reported in all trials. Fewer patients in the probiotics group developed CDAD, compared to the control group who received placebo or no supplement (62/4,124 [1.5%] versus 145/3,833 [3.8%]). There was no significant heterogeneity between trials (P=0.751, I^2=0.000), and a fixed-effects model was assumed. Meta-analysis showed a significantly lower risk of developing CDAD in the probiotics group compared to the control group (RR =0.395; 95% CI, 0.294–0.531; P<0.001; Figure 2).

Subgroup analysis identified that *Lactobacillus*, *Saccharomyces*, or a mixture of probiotics were beneficial in reducing the risk of developing CDAD (RR =0.363; 95% CI, 0.225–0.585; P<0.001 for *Lactobacillus*; RR =0.415; 95% CI, 0.217–0.796; P=0.008 for *Saccharomyces*; and RR =0.418; 95% CI, 0.263–0.664; P<0.001 for mixture of probiotics; Figure 3). Probiotics were beneficial for both adults (RR =0.405; 95% CI, 0.294–0.556; P<0.001)

Table 1 Characteristics of all published randomized control trials evaluating the use of probiotics for the prevention of CDAD (1966–2015)

Study	Total number of patients (numbers on probiotics, numbers on placebo)	Age of patients (adults versus pediatrics)	Health care setting (inpatient versus outpatient)	Probiotic used	Age (years), (mean ± SD)
Surawicz et al (1989)[49]	180 (116; 64)	Adults	Inpatient	Saccharomyces boulardii	Probiotic: 48.6 / Placebo: 45.4
McFarland et al (1995)[48]	193 (97; 96)	Adults	Inpatient	Saccharomyces boulardii	Probiotic: 40.7±16.0 / Placebo: 42.3±17.7
Arvola et al (1999)[50]	119 (61; 58)	Children	Mix	Lactobacillus GG	Probiotic: 4.7 (range: 2 weeks–11.8 years) / Placebo: 4.4 (range: 2 weeks–12.8 years)
Thomas et al (2001)[44]	267 (133; 134)	Adults	Inpatient	Lactobacillus GG	Probiotic: 57.2±18.0 / Placebo: 54.4±17.4
Plummer et al (2004)[51]	138 (69; 69)	Adults	Inpatient	Lactobacillus acidophilus, Bifidobacterium bifidum	Patients >18 years
Duman et al (2005)[42]	376 (196; 180)	Adults	Outpatient	Saccharomyces boulardii	Probiotic: 45.68±12.7 / Placebo: 44.65±13.9
Kotowska et al (2005)[52]	246 (119; 127)	Children	Mix	Saccharomyces boulardii	Probiotic: 39.3±29 months / Placebo: 39.3±41 months
Can et al (2006)[53]	151 (73; 78)	Adults	Inpatient	Saccharomyces boulardii	Range: 25–50
Beausoleil et al (2007)[45]	89 (44; 45)	Adults	Inpatient	Lactobacillus acidophilus CL1285 and Lactobacillus casei	Probiotic: 68.8±14.5 / Placebo: 72.9±13.4
Cindoruk et al (2007)[46]	124 (62; 62)	Adults	Outpatient	Saccharomyces boulardii	Probiotic: 45.82±13.35 / Placebo: 47.56±13.53
Hickson et al (2007)[54]	113 (57; 56)	Adults	Inpatient	Lactobacillus casei imunitass, Lactobacillus bulgaricus, Streptococcus thermophilus	All patients: 74
Rafiq et al (2007)[55]	100 (45; 55)	Adults	Inpatient	Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacterium bifidum, Streptococcus thermophilus	Patients >18
Miller et al (2008; a)[56]	189 (95; 94)	Adults	Inpatient	Lactobacillus GG	Patients >18
Miller et al (2008; b)[56]	316 (157; 159)	Adults	Inpatient	Lactobacillus GG	Patients >18
Ruszczynski et al (2008)[57]	240 (120; 120)	Children	Mix	Lactobacillus rhamnosus	Probiotic: 18.6±13.7 months / Placebo: 32.6±41 months
Safdar et al (2008)[58]	40 (23; 17)	Adults	Inpatient	Lactobacillus acidophilus	Probiotic: 66.56±14.53 / Placebo: 72.47±11
Wenus et al (2008)[59]	63 (34; 29)	Adults	Inpatient	Lactobacillus rhamnosus GG, Lactobacillus acidophilus La-5, Bifidobacterium Bb-12	Probiotic: 58.8±16.5 / Placebo: 56.2±18.7
Gao et al (2010)[60]	255 (171; 84)	Adults	Inpatient	Lactobacillus acidophilus CL1285 and Lactobacillus casei LBC80R	Probiotic: 60±6 / Placebo: 60±6
Lonnermark et al (2010)[47]	163 (80; 83)	Adults	Mix	Lactobacillus plantarum	Probiotic: median =47 / Placebo: median =43
Sampalis et al (2010)[61]	437 (216; 221)	Adults	Mix	Lactobacillus acidophilus CL1285, Lactobacillus casei	Probiotic: 59.5±18.1 / Placebo: 58.1±19.1

Study	N (groups)	Population	Setting	Probiotic organisms	Age
Pozzoni et al (2012)[62]	204 (106; 98)	Adults	Inpatient	*Saccharomyces boulardii*	Probiotic: 79.9±9.9 Placebo: 78.5±9.7
Allen et al (2013)[19]	2,941 (1,470; 1,471)	Adults	Inpatient	*Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium lactis*	Probiotic: median (IQR) =77.2 (70.8–83.6) Placebo: median (IQR) =77.0 (71.3–83.5)
Selinger et al (2013)[63]	122 (61; 61)	Adults	Inpatient	*Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii bulgaricus, Streptococcus thermophilus*	Probiotic: 57.9 Placebo: 57.0
Shan et al (2013)[20]	283 (139; 144)	Children	Inpatient	*Saccharomyces boulardii*	Probiotic: 49.8±36 months Placebo: 48.7±34 months
Wong et al (2014)[21]	158 (76; 82)	Adults	Inpatient	*Lactobacillus casei*	Probiotic: 52.5 Placebo: 51
Ouwehand et al (2014)[22]	450 (304; 146)	Adults	Inpatient	*Lactobacillus acidophilus, Lactobacillus paracasei, Bifidobacterium lactis Bi-07, Bifidobacterium lactis Bi-04*	Probiotic high dose: 50.5±11.0 Probiotic low dose: 49.3±11.4 Placebo: 50.0±11.0

Abbreviations: CDAD, *Clostridium difficile*-associated diarrhea; GG, Gorbach/Goldin (a strain of *Lactobacillus rhamnosus* isolated by Dr Sherwood Gorbach and Dr Barry Goldin); IQR, interquartile range; SD, standard deviation.

and children (RR =0.341; 95% CI, 0.153–0.759; *P*=0.008; Figure 4). Hospitalized patients were more likely to benefit from probiotic use compared to outpatients (RR =0.390; 95% CI, 0.283–0.538; *P*<0.001 versus RR =0.306; 95% CI, 0.013–7.470; *P*=0.468; Figure 5).

Subgroup analysis based on type of *C. difficile* testing

Most studies utilized stool culture and cytotoxin testing, or enzyme immunoassay (EIA) in diagnosing *C. difficile.* One study used EIA but stated that polymerase chain reaction was available if required. A few studies were nonspecific and either did not state the technique utilized or simply stated that *C. difficile* was diagnosed according to hospital protocol.

Subgroup analysis based on the testing method (stool culture and cytotoxin versus EIA) identified that probiotics were beneficial in reducing the risk of CDAD with both the stool cytotoxin (RR =0.271; 95% CI, 0.131–0.561; *P*<0.001) and EIA testing methods (RR =0.431; 95% CI, 0.288–0.647; *P*<0.001). There was no significant difference between the benefit derived from either testing method (*P*=0.536).

Publication bias

A funnel plot was used to qualitatively assess for publication bias, and Egger's and Begg's tests were conducted to calculate publication bias. There was no obvious evidence of asymmetry on the funnel plot (Figure 6). Furthermore, there was no evidence of publication bias for the primary end point of this study (RR of CDAD in patients receiving probiotics) by either the Egger's (*P*=0.748) or Begg's test (*P*=0.747).

Discussion

C. difficile was first described by Hall and O'Toole[23] as part of the intestinal microflora in neonates, and represents the leading cause of AAD.[24,25] Prior to the year 2000, the rate of CDI in the USA did not vary greatly from year to year and has been relatively stable at 30–40 cases per 100,000 population.[26] In 2001, a sudden spike in CDAD rates occurred, rising to approximately 50 cases per 100,000 population, and this incidence has continued to rise by approximately 25% each year.[26] In a retrospective analysis of the US National Hospital Discharge Survey between 2001 and 2010, Reveles et al[27] reported that the incidence of CDI among hospitalized patients nearly doubled from 4.5 CDI discharges per 1,000 total adult discharges in 2001 to 8.2 CDI discharges per 1,000 adult discharges in 2010. Overall mortality also increased from 6.6% in 2001 to 7.2% in 2010.[27]
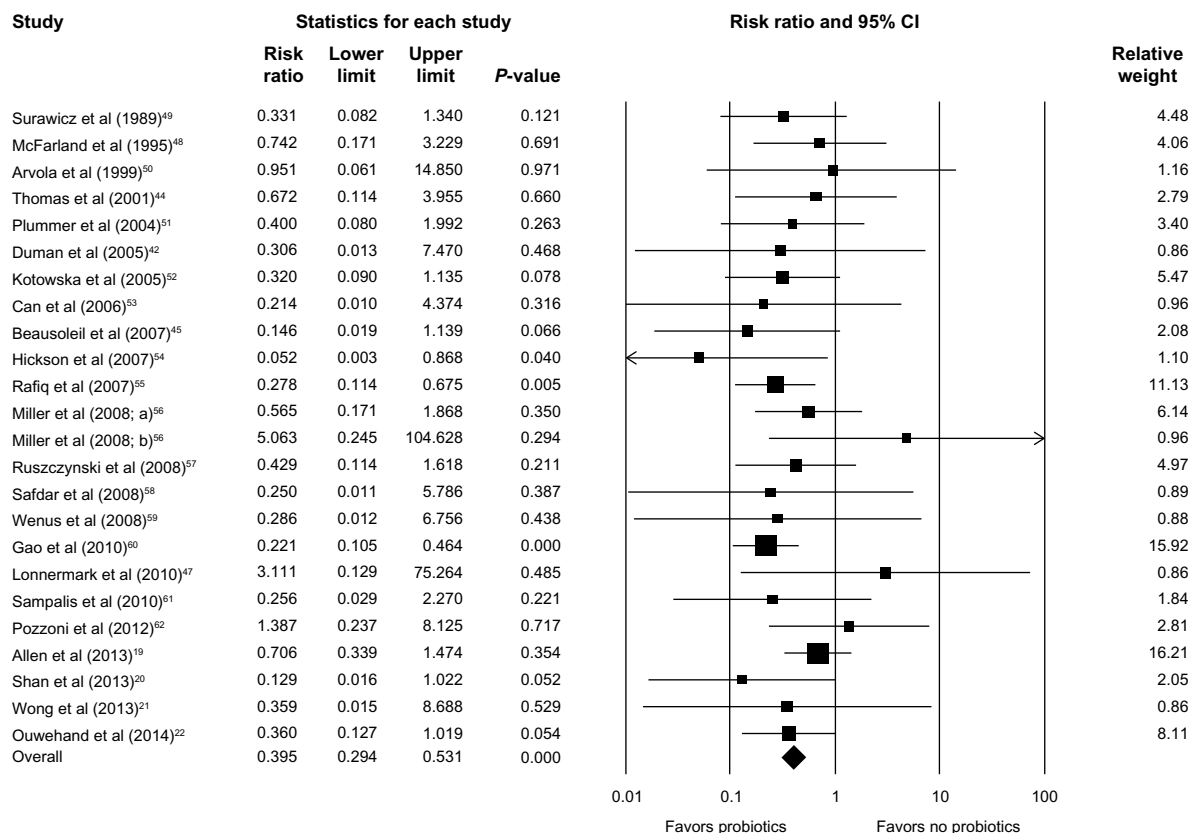
Study	Statistics for each study				Risk ratio and 95% CI	Relative weight
	Risk ratio	Lower limit	Upper limit	*P*-value		
Surawicz et al (1989)[49]	0.331	0.082	1.340	0.121		4.48
McFarland et al (1995)[48]	0.742	0.171	3.229	0.691		4.06
Arvola et al (1999)[50]	0.951	0.061	14.850	0.971		1.16
Thomas et al (2001)[44]	0.672	0.114	3.955	0.660		2.79
Plummer et al (2004)[51]	0.400	0.080	1.992	0.263		3.40
Duman et al (2005)[42]	0.306	0.013	7.470	0.468		0.86
Kotowska et al (2005)[52]	0.320	0.090	1.135	0.078		5.47
Can et al (2006)[53]	0.214	0.010	4.374	0.316		0.96
Beausoleil et al (2007)[45]	0.146	0.019	1.139	0.066		2.08
Hickson et al (2007)[54]	0.052	0.003	0.868	0.040		1.10
Rafiq et al (2007)[55]	0.278	0.114	0.675	0.005		11.13
Miller et al (2008; a)[56]	0.565	0.171	1.868	0.350		6.14
Miller et al (2008; b)[56]	5.063	0.245	104.628	0.294		0.96
Ruszczynski et al (2008)[57]	0.429	0.114	1.618	0.211		4.97
Safdar et al (2008)[58]	0.250	0.011	5.786	0.387		0.89
Wenus et al (2008)[59]	0.286	0.012	6.756	0.438		0.88
Gao et al (2010)[60]	0.221	0.105	0.464	0.000		15.92
Lonnermark et al (2010)[47]	3.111	0.129	75.264	0.485		0.86
Sampalis et al (2010)[61]	0.256	0.029	2.270	0.221		1.84
Pozzoni et al (2012)[62]	1.387	0.237	8.125	0.717		2.81
Allen et al (2013)[19]	0.706	0.339	1.474	0.354		16.21
Shan et al (2013)[20]	0.129	0.016	1.022	0.052		2.05
Wong et al (2013)[21]	0.359	0.015	8.688	0.529		0.86
Ouwehand et al (2014)[22]	0.360	0.127	1.019	0.054		8.11
Overall	0.395	0.294	0.531	0.000		

0.01　　0.1　　1　　10　　100

Favors probiotics　　　　Favors no probiotics

Figure 2 Forest plot evaluating the RR of CDAD associated with probiotic use.
Abbreviations: RR, relative risk; CI, confidence interval; CDAD, *Clostridium difficile*-associated diarrhea.
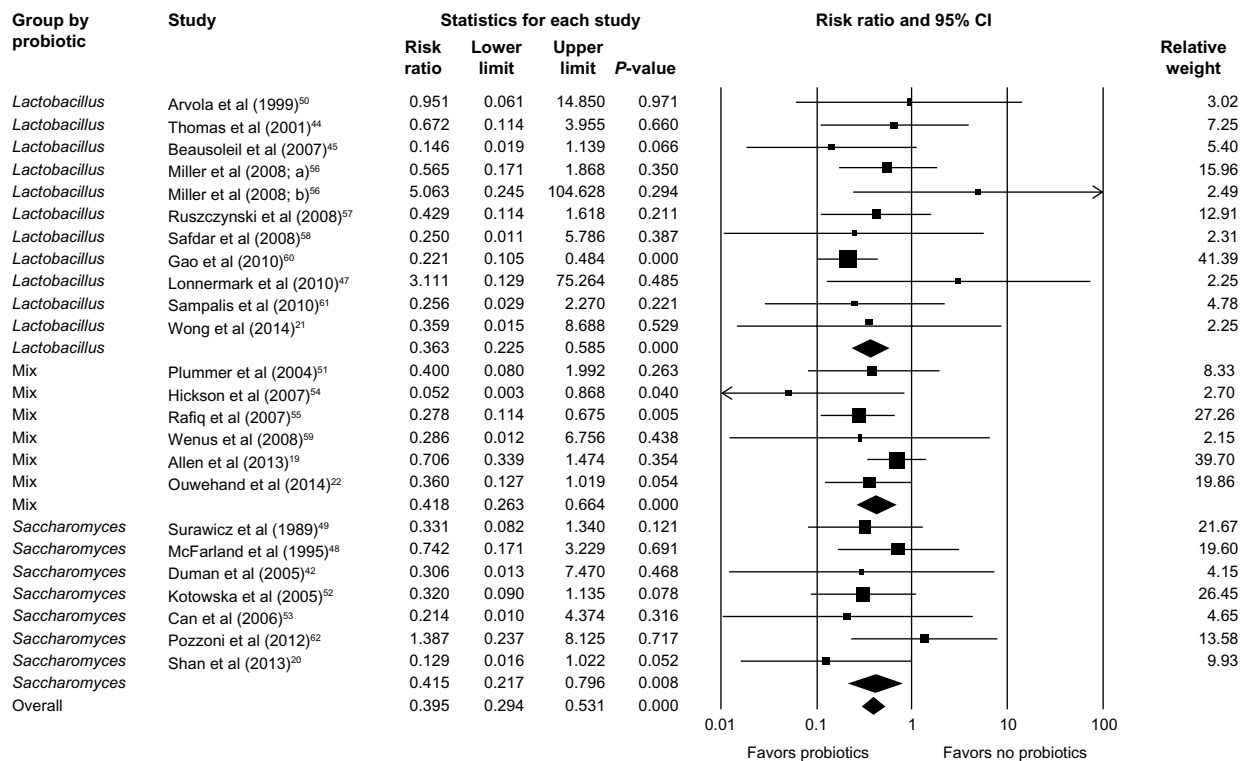
Group by probiotic	Study	Statistics for each study				Risk ratio and 95% CI	Relative weight
		Risk ratio	Lower limit	Upper limit	*P*-value		
Lactobacillus	Arvola et al (1999)[50]	0.951	0.061	14.850	0.971		3.02
Lactobacillus	Thomas et al (2001)[44]	0.672	0.114	3.955	0.660		7.25
Lactobacillus	Beausoleil et al (2007)[45]	0.146	0.019	1.139	0.066		5.40
Lactobacillus	Miller et al (2008; a)[56]	0.565	0.171	1.868	0.350		15.96
Lactobacillus	Miller et al (2008; b)[56]	5.063	0.245	104.628	0.294		2.49
Lactobacillus	Ruszczynski et al (2008)[57]	0.429	0.114	1.618	0.211		12.91
Lactobacillus	Safdar et al (2008)[58]	0.250	0.011	5.786	0.387		2.31
Lactobacillus	Gao et al (2010)[60]	0.221	0.105	0.484	0.000		41.39
Lactobacillus	Lonnermark et al (2010)[47]	3.111	0.129	75.264	0.485		2.25
Lactobacillus	Sampalis et al (2010)[61]	0.256	0.029	2.270	0.221		4.78
Lactobacillus	Wong et al (2014)[21]	0.359	0.015	8.688	0.529		2.25
Lactobacillus		0.363	0.225	0.585	0.000		
Mix	Plummer et al (2004)[51]	0.400	0.080	1.992	0.263		8.33
Mix	Hickson et al (2007)[54]	0.052	0.003	0.868	0.040		2.70
Mix	Rafiq et al (2007)[55]	0.278	0.114	0.675	0.005		27.26
Mix	Wenus et al (2008)[59]	0.286	0.012	6.756	0.438		2.15
Mix	Allen et al (2013)[19]	0.706	0.339	1.474	0.354		39.70
Mix	Ouwehand et al (2014)[22]	0.360	0.127	1.019	0.054		19.86
Mix		0.418	0.263	0.664	0.000		
Saccharomyces	Surawicz et al (1989)[49]	0.331	0.082	1.340	0.121		21.67
Saccharomyces	McFarland et al (1995)[48]	0.742	0.171	3.229	0.691		19.60
Saccharomyces	Duman et al (2005)[42]	0.306	0.013	7.470	0.468		4.15
Saccharomyces	Kotowska et al (2005)[52]	0.320	0.090	1.135	0.078		26.45
Saccharomyces	Can et al (2006)[53]	0.214	0.010	4.374	0.316		4.65
Saccharomyces	Pozzoni et al (2012)[62]	1.387	0.237	8.125	0.717		13.58
Saccharomyces	Shan et al (2013)[20]	0.129	0.016	1.022	0.052		9.93
Saccharomyces		0.415	0.217	0.796	0.008		
Overall		0.395	0.294	0.531	0.000		

0.01　　0.1　　1　　10　　100

Favors probiotics　　　　Favors no probiotics

Figure 3 Forest plot evaluating the RR of CDAD associated with various species of probiotic use.
Abbreviations: RR, relative risk; CI, confidence interval; CDAD, *Clostridium difficile*-associated diarrhea.
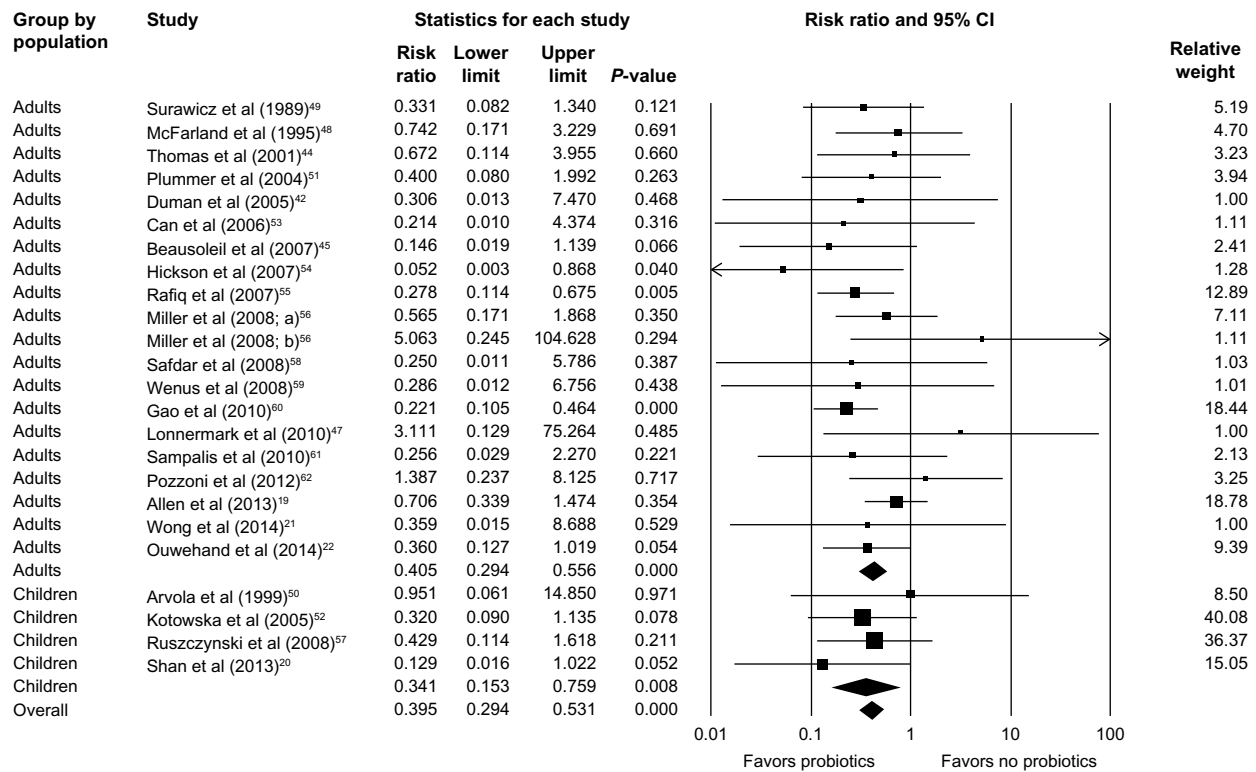
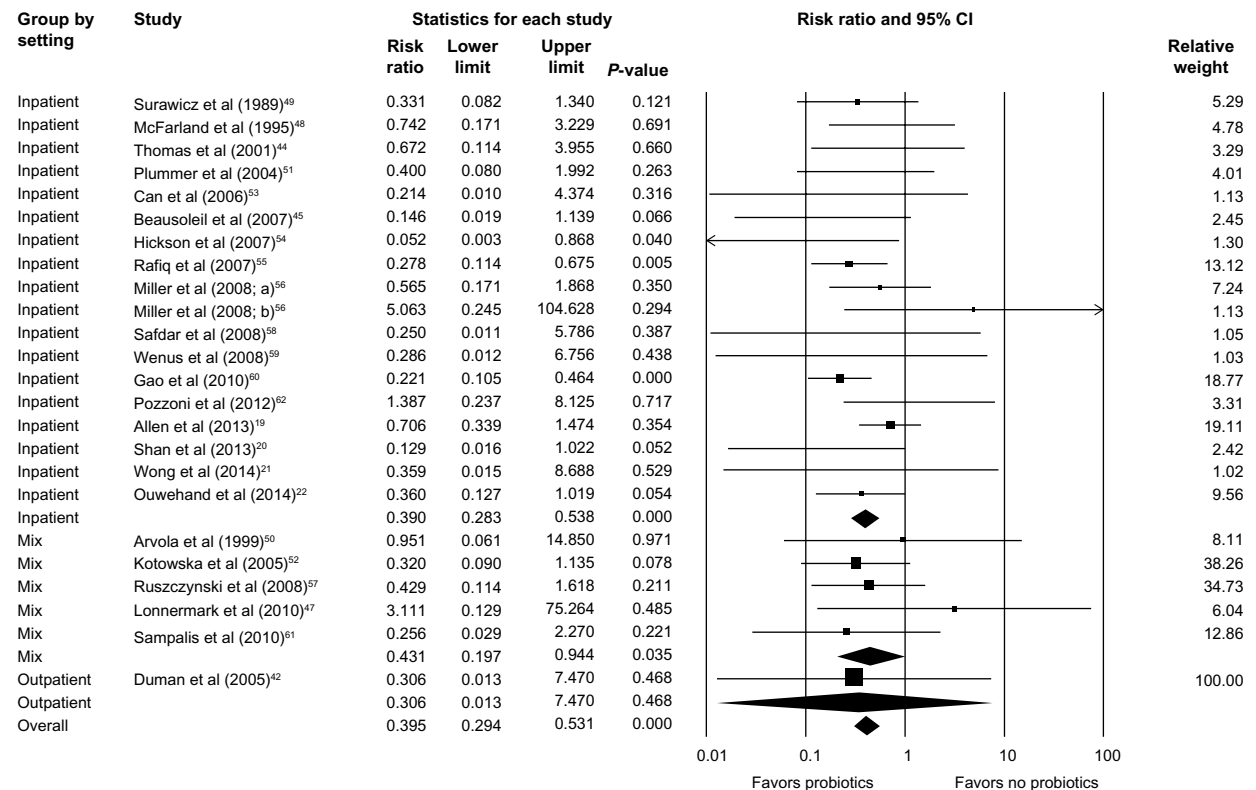| Group by population	Study	Risk ratio	Lower limit	Upper limit	P-value	Risk ratio and 95% CI	Relative weight
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Adults	McFarland et al (1995)[48]	0.742	0.171	3.229	0.691		4.70
Adults	Thomas et al (2001)[44]	0.672	0.114	3.955	0.660		3.23
Adults	Plummer et al (2004)[51]	0.400	0.080	1.992	0.263		3.94
Adults	Duman et al (2005)[42]	0.306	0.013	7.470	0.468		1.00
Adults	Can et al (2006)[53]	0.214	0.010	4.374	0.316		1.11
Adults	Beausoleil et al (2007)[45]	0.146	0.019	1.139	0.066		2.41
Adults	Hickson et al (2007)[54]	0.052	0.003	0.868	0.040		1.28
Adults	Rafiq et al (2007)[55]	0.278	0.114	0.675	0.005		12.89
Adults	Miller et al (2008; a)[56]	0.565	0.171	1.868	0.350		7.11
Adults	Miller et al (2008; b)[56]	5.063	0.245	104.628	0.294		1.11
Adults	Safdar et al (2008)[58]	0.250	0.011	5.786	0.387		1.03
Adults	Wenus et al (2008)[59]	0.286	0.012	6.756	0.438		1.01
Adults	Gao et al (2010)[60]	0.221	0.105	0.464	0.000		18.44
Adults	Lonnermark et al (2010)[47]	3.111	0.129	75.264	0.485		1.00
Adults	Sampalis et al (2010)[61]	0.256	0.029	2.270	0.221		2.13
Adults	Pozzoni et al (2012)[62]	1.387	0.237	8.125	0.717		3.25
Adults	Allen et al (2013)[19]	0.706	0.339	1.474	0.354		18.78
Adults	Wong et al (2014)[21]	0.359	0.015	8.688	0.529		1.00
Adults	Ouwehand et al (2014)[22]	0.360	0.127	1.019	0.054		9.39
Adults		0.405	0.294	0.556	0.000		
Children	Arvola et al (1999)[50]	0.951	0.061	14.850	0.971		8.50
Children	Kotowska et al (2005)[52]	0.320	0.090	1.135	0.078		40.08
Children	Ruszczynski et al (2008)[57]	0.429	0.114	1.618	0.211		36.37
Children	Shan et al (2013)[20]	0.129	0.016	1.022	0.052		15.05
Children		0.341	0.153	0.759	0.008		
Overall		0.395	0.294	0.531	0.000		

0.01   0.1   1   10   100

Favors probiotics   Favors no probiotics

Figure 4 Forest plot evaluating the RR of CDAD associated with probiotic use in adult and pediatric populations.
Abbreviations: RR, relative risk; CI, confidence interval; CDAD, *Clostridium difficile*-associated diarrhea.

Group by setting	Study	Risk ratio	Lower limit	Upper limit	P-value	Risk ratio and 95% CI	Relative weight
Inpatient	Surawicz et al (1989)[49]	0.331	0.082	1.340	0.121		5.29
Inpatient	McFarland et al (1995)[48]	0.742	0.171	3.229	0.691		4.78
Inpatient	Thomas et al (2001)[44]	0.672	0.114	3.955	0.660		3.29
Inpatient	Plummer et al (2004)[51]	0.400	0.080	1.992	0.263		4.01
Inpatient	Can et al (2006)[53]	0.214	0.010	4.374	0.316		1.13
Inpatient	Beausoleil et al (2007)[45]	0.146	0.019	1.139	0.066		2.45
Inpatient	Hickson et al (2007)[54]	0.052	0.003	0.868	0.040		1.30
Inpatient	Rafiq et al (2007)[55]	0.278	0.114	0.675	0.005		13.12
Inpatient	Miller et al (2008; a)[56]	0.565	0.171	1.868	0.350		7.24
Inpatient	Miller et al (2008; b)[56]	5.063	0.245	104.628	0.294		1.13
Inpatient	Safdar et al (2008)[58]	0.250	0.011	5.786	0.387		1.05
Inpatient	Wenus et al (2008)[59]	0.286	0.012	6.756	0.438		1.03
Inpatient	Gao et al (2010)[60]	0.221	0.105	0.464	0.000		18.77
Inpatient	Pozzoni et al (2012)[62]	1.387	0.237	8.125	0.717		3.31
Inpatient	Allen et al (2013)[19]	0.706	0.339	1.474	0.354		19.11
Inpatient	Shan et al (2013)[20]	0.129	0.016	1.022	0.052		2.42
Inpatient	Wong et al (2014)[21]	0.359	0.015	8.688	0.529		1.02
Inpatient	Ouwehand et al (2014)[22]	0.360	0.127	1.019	0.054		9.56
Inpatient		0.390	0.283	0.538	0.000		
Mix	Arvola et al (1999)[50]	0.951	0.061	14.850	0.971		8.11
Mix	Kotowska et al (2005)[52]	0.320	0.090	1.135	0.078		38.26
Mix	Ruszczynski et al (2008)[57]	0.429	0.114	1.618	0.211		34.73
Mix	Lonnermark et al (2010)[47]	3.111	0.129	75.264	0.485		6.04
Mix	Sampalis et al (2010)[61]	0.256	0.029	2.270	0.221		12.86
Mix		0.431	0.197	0.944	0.035		
Outpatient	Duman et al (2005)[42]	0.306	0.013	7.470	0.468		100.00
Outpatient		0.306	0.013	7.470	0.468		
Overall		0.395	0.294	0.531	0.000		

0.01   0.1   1   10   100

Favors probiotics   Favors no probiotics

Figure 5 Forest plot evaluating the RR of CDAD associated with probiotic use in both inpatient and outpatient populations.
Abbreviations: RR, relative risk; CI, confidence interval; CDAD, *Clostridium difficile*-associated diarrhea.
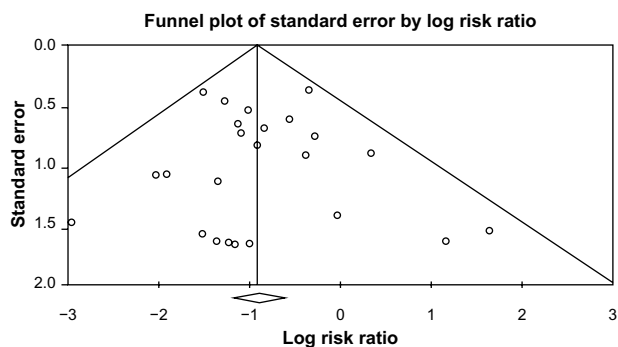
Funnel plot of standard error by log risk ratio

Figure 6 Funnel plot assessing publication bias (analyzing the effect of probiotic supplementation on the incidence of CDAD).
Abbreviation: CDAD, *Clostridium difficile*-associated diarrhea.

The rise in CDI has been attributed to various factors, including antibiotic resistance and the emergence of new *C. difficile* strains. The toxogenic *C. difficile* NAP1/BI/027 strain was discovered in 2002, and found to be associated with more severe presentations, including toxic megacolon, leukemoid reaction, severe hypoalbuminemia, septic shock, and death.[26,28–30] Several epidemic outbreaks occurred throughout North America during the mid-1900s and mid-2000s, which were attributable to this hypervirulent *C. diffile* strain.[31,32] According to the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America, metronidazole remains the initial drug of choice for mild-to-moderate CDI, and oral vancomycin for severe CDI.[26,28] However, recurrence and relapse of CDI, even after repeated cycles of antibiotic therapy, has emerged as a major public health problem.[33] Fecal microbiota transplantation has been increasingly studied.[34–36] Cammarota et al[34] conducted an RCT involving 39 patients with recurrent CDI (20 patients receiving fecal transplantation and 19 patients receiving vancomycin) and reported significantly higher rates of resolution with the use of fecal transplantation (90% versus 26%, *P*<0.0001).

Given the high morbidity and mortality of the CDI, and the rising incidence despite adequate antibiotic therapy, efforts to prevent rather than treat CDI are paramount. Several approaches have been suggested to prevent the transmission of *C. difficile*; however, the mainstay remains early detection and isolation, contact precautions, and appropriate hand hygiene. The use of environmental cleaning disinfectants and chlorhexidine patient baths has also been studied, but has shown only limited success.[12,13]

Probiotics, which are living commensal microorganisms and part of the normal host intestinal flora, has been shown to exert a protective effect on the gastrointestinal tract. The mechanism by which probiotics work has not been fully elucidated, but various mechanisms have been proposed.

Commensal bacteria inhibit enteric pathogens and may help suppress the growth and invasion of pathogenic bacteria, thereby improving intestinal barrier function.[37] Probiotics also modulate proinflammatory cytokines, which help regulate immune responses and maintain homeostasis.[37,38] Probiotic supplementation may also allow the acquisition and subsequent population of the gastrointestinal tract with normal commensal bacterial flora, modulating the inflammatory balance, and as a result, decrease the development of CDAD in patients receiving antibiotics.[37,38]

Probiotics have been extensively studied and shown to have a therapeutic role in various gastrointestinal conditions, including diarrhea. Ford et al[39] published a meta-analysis, which included 23 RCTs involving 2,575 patients with irritable bowel syndrome, and reported that 21% of patients experienced improved symptoms with probiotics (RR =0.79; 95% CI, 0.70–0.89; *P*<0.01). Shen et al[40] published a meta-analysis including 12 RCTs involving 723 patients with inflammatory bowel disorder (649 had ulcerative colitis, 74 had Crohn's disease). Probiotics were effective in inducing remission in patients with ulcerative colitis (RR =1.80; 95% CI, 1.36–2.39; *P*=0.01); however, it was less effective in patients with Crohn's disease (RR =0.89; 95% CI, 0.70–1.13; *P*=0.35).[40] Salari et al[41] published a meta-analysis that included 19 RCTs including 3,867 children with acute diarrhea, and reported a significant reduction in the duration of diarrhea (weighted mean difference [WMD] =–0.67; 95% CI, –0.95 to –0.38; *P*<0.0001) and the duration of fever (WMD =–0.18; 95% CI, –0.34 to –0.02; *P*=0.0246).

Although CDI is closely linked to the use of broad-spectrum antibiotics that disrupt the normal intestinal flora, probiotics as adjunct therapy along with antibiotics has been considered in the prevention of CDI and its complications. The current meta-analysis found that probiotics are associated with a 60.5% reduction in the incidence of CDAD.[17,18] The use of *Lactobacillus*, *Saccharomyces*, and a mixture of different probiotics all significantly reduced the risk of CDAD (63.7%, 58.5%, and 58.2% risk reduction, respectively). There was a 59.5% risk reduction among adults and a 65.9% risk reduction among children. A 61% risk reduction was observed in hospitalized inpatients. In the lone study addressing outpatient treatment with probiotics, a 69.4% reduction was observed but was not statistically significant.[42]

The efficacy of probiotics has been widely studied in a variety of RCTs; however, the adverse events for probiotic therapy are not well documented. In a comprehensive systematic review conducted by the Agency for Healthcare Research and Quality, a third of the 622 published studies

provided only nonspecific statements indicating that probiotics were well-tolerated, while most other articles indicated only the presence or absence of one or more specific adverse events, but failed to provide specific details.[43] Although some case studies have reported fungemia, bacteremia, and sepsis associated with probiotic use, the incidences of these adverse events are inconsistent and not statistically significant across studies.[43] Most studies included in this meta-analysis provided minimal nonspecific statements about adverse events, although some studies did report no statistical significance between patients receiving probiotics and the control group with respect to nausea, abdominal cramping, constipation, and urticaria.[44–47] Several studies even noted that probiotics were associated with decrease in length of stay, fever, and nausea/vomiting.[44,48]

Although the results of this meta-analysis are significant, there are limitations to these results, given the variation and heterogeneity of the RCTs analyzed. The enrollment criteria used in each study differed with regard to patient age, comorbidities, and health care setting. The specific strain, dosage, and duration of probiotic also differed between studies, as well as the concurrent antibiotic regimen of the patients. The test used for diagnosing CDI and the follow-up period also varied between studies. Additional studies to determine the optimal dose and particular strain of probiotic as well as the long-term effects of probiotics are required. Most existing studies have included individuals of all age groups, and very few trials have specifically studied elderly patients who are at highest risk of developing CDI and CDAD. Furthermore, almost all studies explicitly stated that immunocompromised patients and patients with a history of major gastrointestinal surgery were excluded from the study, and the remainder were nonspecific as to whether or not these patients were included. Additional randomized placebo-controlled trials more clearly assessing the safety and efficacy of probiotics in this particular population are needed.

Conclusion

Despite the aforementioned limitations, this study found that probiotic supplementation is a valuable adjunct in the routine care of patients receiving antibiotic therapy. Given the high morbidity and mortality associated with CDI and CDAD, the significant reduction in the incidence of CDAD achieved with probiotic supplementation and the apparent lack of significant negative side effects should prompt physicians to consider these readily available, low-cost supplements as an effective and potentially routine therapy for patients receiving antibiotic therapy.

Disclosure

The authors report no conflicts of interest in this work.

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