Probiotics for the Prevention of Antibiotic Associated Diarrhea and Clostridium Difficile Infection among Hospitalized Patients: A Systematic Review and Meta-Analysis

R Pattani MD, VA Palda MD MSc, SW Hwang MD MPH, PS Shah, MD MSc

Corresponding author and Guarantor: Reena Pattani.
Dr. Pattani is a resident in Internal Medicine at the University of Toronto, Toronto, Ontario.
Email: reena.pattani@mail.utoronto.ca (may be published)
Department of Medicine, St. Michael's Hospital
30 Bond Street
Toronto, ON M5B 1W8
Phone: (647) 984-6037

Dr. Palda is an Associate Professor in the Department of Medicine and the Institute for Health Policy, Management and Evaluation, University of Toronto, Toronto, Ontario.
Email: va.palda@utoronto.ca

Dr. Hwang is an Associate Professor in the Department of Medicine, University of Toronto and Research Scientist at St. Michael's Hospital, Toronto, Ontario.
Email: hwangs@smh.ca

Dr. Shah is an Associate Professor in the Department of Pediatrics and the Institute for Health Policy, Management and Evaluation, University of Toronto, Toronto, Ontario.
Email: pshah@mtsinai.on.ca

Author Contributions:

R Pattani participated in designing the study methodology, contributed to data extraction and analysis, and was the principal writer of the manuscript. VA Palda conceived the project, outlined the qualitative study methods, and contributed to data extraction and analysis. SW Hwang assisted in designing study methods and in the data extraction and analysis. PS Shah outlined quantitative methods, conducted the quantitative analysis, and participated in writing the manuscript. All of the authors reviewed and edited drafts of the manuscript for important intellectual content and each author approved the final version of the manuscript.

Conflicts of Interest:

None

Acknowledgements: We thank Ms. Elizabeth Uleryk for her help in developing the search strategy, undertaking the search, and writing the literature search methods section in Appendix 1.

Abstract Word Count: 311
Manuscript Word Count: 3000
Figures: 3
Tables: 3

ABSTRACT

Background: Antibiotic associated diarrhea (AAD) and Clostridium difficile infection (CDI) are associated with high morbidity, mortality, and health care costs. Probiotics may mitigate the existing disease burden. Our objective was to systematically review and meta-analyze studies of co-administration of probiotics with antibiotics for prevention of AAD and CDI in adult inpatients.

Methods: Systematic searches of MEDLINE (1946 – May 2012), EMBASE (1980 – May 2012) and Cochrane Central Register of Controlled Trials (CCTR) were undertaken to identify relevant publications on May 31, 2012. We searched for randomized controlled trials published in the English language of adult inpatients receiving antibiotics and randomized to the co-administration of probiotics or to usual care with or without the use of placebo. Studies were included if they reported on either AAD or CDI as outcomes. Data were extracted on predetermined criteria evaluating study characteristics, methods, and risk of bias. Trials were given a global rating of good, fair, or poor by at least two reviewers. Meta-analyses were performed using a random effect model, and pooled relative risks (RR) and 95% confidence intervals (CI) were calculated.

Results: Sixteen trials were included in this review. Four studies were good quality, five were fair quality, and seven were poor quality. Pooled analyses revealed significant reduction in the risk of AAD (RR 0.61, 95% CI 0.47, 0.79) and CDI (RR 0.37, 95% CI 0.22, 0.62) among patients randomized to the co-administration of probiotics. The number needed to treat for benefit was 11 (95% CI 8, 20) for AAD and 14 (95% CI 8, 50) for CDI. With subgroup analysis,

significant reductions in both AAD and CDI rate were retained in the subgroups of good quality trials, trials assessing a primarily Lactobacillus-based formulation, and trials for which the follow-up period was less than 4 weeks.

Interpretation: Probiotics used concurrently with antibiotics reduce the risk of AAD and CDI.

Introduction

A rise in the use of antibiotics has resulted in a marked increase in antibiotic-associated diarrhea (AAD) and Clostridium Difficile Infection (CDI) (1). There is a spectrum of adverse sequelae associated with CDI, including diarrhea, electrolyte abnormalities, sepsis and septic shock, toxic megacolon requiring colectomy, ICU admission, and death (2). In response to this devastating infection, non-antibiotic strategies such as toxin-binding agents, active immunization, Intravenous Immune Globulin (IVIG) administration, and fecal transplantation have been attempted with variable success (3). Many hospitals are emphasizing infection control measures and antimicrobial stewardship to mitigate disease burden (4). The administration of probiotics with antibiotics has also been studied as a preventive intervention against AAD and CDI.

Randomized controlled trials (RCTs) assessing probiotics for the prevention of AAD and CDI have been marred by low case volumes. Existing systematic reviews and meta-analyses (5-9) have grouped disparate populations such as inpatients with outpatients or adults with children, and have considered clinically distinct entities as combined outcomes such as prevention and treatment or AAD and CDI.

Given the high morbidity of AAD and CDI among inpatients, we conducted a systematic review and meta-analysis to evaluate the efficacy of probiotics administered with antibiotics in reducing these outcomes. AAD and CDI were examined as separate outcomes and we limited our review to adult inpatients, because hospitalization is a potent risk factor for CDI colonization (10).

Methods

Data Sources and Searches

This review is reported according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (11). Systematic searches of MEDLINE (1946 – May 2012), EMBASE (1980 – May 2012) and Cochrane Central Register of Controlled Trials (CCTR) were undertaken to identify relevant publications on May 31, 2012. We employed a sensitive search strategy (Appendix 1) using broad keywords to identify the condition of interest (clostridium difficile, antibiotic-associated diarrhea, and phrase variants) as well as the intervention of interest ("probiotics" and specific probiotic genera). A manual search of the reference lists of identified manuscripts was also performed in order to identify and retrieve relevant research studies.

Study Selection

One extractor screened all abstracts for relevance to the topic. Among relevant articles, two independent reviewers screened abstracts for possible inclusion. We included randomized controlled trials (RCTs) published in the English language of adult inpatients (hospitalized patients admitted to medical, surgical, or acute care of the elderly wards) receiving antibiotics who were randomized to the co-administration of probiotics or to usual care, with or without the use of placebo. To be included in the review, the study must have reported either AAD or CDI prevention as outcomes. AAD and CDI were defined as the

number of patients who developed diarrhea and C. difficile positivity by toxin assay or stool culture, respectively, while on antibiotics, divided by the number of patients with available endpoints. We contacted primary authors to obtain original data for our quantitative analysis if the necessary data were not reported in an included publication. If the authors could not provide it, their studies were included in the systematic review but excluded from meta-analysis. We excluded studies of probiotics to prevent CDI recurrence in patients previously diagnosed with CDI. We further excluded trials in which antibiotics were used for Helicobacter pylori eradication, as this represents a distinct clinical endpoint of treatment augmentation and is a condition for which management occurs almost exclusively in the outpatient setting. We excluded studies that were pilot trials of feasibility or tolerability because these studies did not define AAD or CDI incidence as outcomes of interest. We also excluded studies presented only at conferences, studies of before-after comparison, as well as non-randomized comparison and cohort studies. Letters, commentaries, reviews, and editorials were excluded if they did not contain original data.

Data Extraction and Assessment of Risk of Bias

The included manuscripts received a full-text review by two reviewers. Risk of bias among included studies was assessed on the basis of the US Preventative Services Task Force (USPSTF) recommendations which includes domains of randomization, blinding, comparable groups, adequate follow-up (>80%), clear interventions and outcomes, intention to treat analysis, and

adequate study power (12). A data extraction form was used to record the findings from each trial. Studies were given a rating of good, fair or poor by two reviewers based on a predetermined global quality rating scale combining the aforementioned criteria (Appendix 2). Disagreement on quality rating was resolved by a third reviewer.

Data Synthesis

Meta-analytic software (RevMan 5.0 from the Cochrane Collaboration) was used to synthesize the results. Relative risk (RR), risk difference (RD) and number needed to treat to benefit or to harm (NNTB/H), with their respective 95% confidence intervals (CI) were calculated using the Der-Simonian Laird method. Mantel Haenszel method was used to determine the weighting of the studies in the meta-analyses because rare events were being assessed. We expected clinical and statistical heterogeneity among the studies. Thus, we used the random effect model for meta-analyses because it accounts for random variability both within studies and among studies. Subgroup analyses were planned a priori to assess the effect on results of study quality (good vs. fair vs. poor), type of probiotic (Lactobacillus-based vs. Saccharomyces boulardii-based), and follow-up duration (< 4 weeks or ≥ 4 weeks). No adjustments were made for multiple analyses. *Post-hoc* meta-regression was performed to identify independent effects of type of probiotics.

Heterogeneity and publication bias assessment

Clinical heterogeneity was assessed for population characteristics, type of probiotic supplementation, and quality of studies. Statistical heterogeneity was assessed using Cochrane Q test and by calculating I-squared (I^2) values. A funnel plot was created to assess for the possibility of publication bias.

Source of Funding and Ethics Approval

No external funding was received for this review. Data are available in published articles, thus no ethics approval was necessary.

Results

Sixteen studies (13-28) were included. Details of the selection process are shown in Figure 1. Baseline characteristics of these studies are reported in Table 1. Only five studies were multi-center (14, 16-18, 27) and the majority of studies were conducted in the United States or the United Kingdom. Among all of the trials, the range of mean ages among patients randomized to probiotic was 33 – 79.9 years and to placebo was 33 – 78.5 years. Male patients comprised 43 – 89% of participants in the probiotics groups and 40 – 94.9% in the placebo groups; however, the upper limits for enrolled males were influenced by one study (25). The majority of studies included fewer than 75% males.

All but one of the sixteen studies (19) examined AAD as a primary outcome. Only one trial (18) assessed two endpoints of AAD with different definitions, and in that case, the definition that most closely approximated the outcome definitions in other studies was used for meta-analysis. One study (13)

examined a dose-response relationship using a Lactobacillus acidophilus and Lactobacillus casei co-formulation. Patients were randomized to a high-dose probiotic group, a low-dose probiotic group, or a placebo group. For the purpose of this meta-analysis, data from the low dose and placebo groups were used, since this comparison most closely approximated the dosing regimens of the other included RCTs. Ten studies (13-15, 17-20, 23, 24, 28) used a Lactobacillus-based probiotic, five studies (16, 21, 22, 25, 26) evaluated Saccharomyces boulardii, and one study (27) assessed Enterococcus species. Thirteen studies (13-17, 19-22, 24-26) sought to evaluate CDI as an outcome, with one having CDI as the primary endpoint (19). Of the thirteen studies evaluating CDI, four were initially excluded. Two of these studies (21, 24) did not report CDI event rates because there was insufficient data to detect a difference. An additional two studies (16, 20) did not identify CDI cases according to their original study protocols. Primary authors of these studies were contacted, but only one publication's original data could be obtained to generate outcome information that was comparable to the other included studies (24). Therefore all four studies were included in the systematic review and only the one study for which original data were acquired (24) was ultimately added in the meta-analysis.

Meta-analysis of included studies demonstrated statistically significant reduction in the risk of AAD (RR 0.61, 95% CI 0.47, 0.79; I^2=44%; RD -0.09, 95% CI -0.13, -0.05; NNTB 11, 95% CI 8, 20). For CDI, there was a large drop-off in the number of patients with available endpoints. The event rates were 18 [3.1%] of 574 patients in the intervention arm and 55 [10.3%] of 533 patients in the

placebo arm (RR 0.37, 95% CI 0.22, 0.62; I^2=0%; RD -0.07, 95% CI -0.12, -0.02; NNTB 14, 95% CI 8, 50). The forest plot displaying the effect size by trial as well as the aggregate effect size is shown in Figure 2. It should be noted that due to small sample sizes and the rarity of outcomes, several of the studies have confidence intervals that cross unity. Studies were heterogeneous in sample size and the funnel plot (Figure 3) demonstrates a moderate degree of publication bias.

The quality rating of all of the studies is provided in Table 2. The results of subgroup analyses by study quality, probiotic type, and follow-up duration are reported in Table 3.

When stratified by study quality, the four good quality studies (13-16) demonstrated reduction in AAD and CDI with the use of probiotics. They shared features that led to their high rating: clear inclusion criteria, interventions, and outcomes. These studies used validated scales or precise qualitative explanations to define the outcome measures and had reasonable long-term follow-up between 3 to 7 weeks. The fair quality studies (17-21), when pooled, demonstrated reduction in AAD and CDI that was not significant. These studies received a lower quality rating because of a lack of clarity or validity in their outcomes measures, with the use of very liberal, subjective criteria for AAD and CDI that may have resulted in over-reporting. Specifically for CDI, two of the studies (19, 21) tested for C. difficile toxin on formed stool, which may have led to the inclusion of cases of C. difficile colonization as opposed to the clinically relevant outcome of C. difficile infection. All but one (22) of the seven poor quality

studies (22-28) showed statistically significant RR in AAD with the use of probiotics. Four of the poor quality trials (22, 24-26) assessed CDI as a secondary outcome, and none of them demonstrated significant risk reduction. In general, these poor quality studies were limited by unclear interventions and outcomes. These studies lacked formal reporting of key study methods, such as randomization process, blinding methods, and duration of the intervention or follow-up.

When studies were pooled by type of probiotic, reductions in AAD and CDI were observed regardless of whether a primarily Lactobacillus-based probiotic or an S. boulardii-based formulation was used. However, only the combined analysis of Lactobacillus-based formulations resulted in a reduction that was statistically significant. The similarity in effect size between both groups has some biologic plausibility, given that probiotic utility is thought to derive its benefit, at least in part, from re-colonizing the gastrointestinal tract with “normal”, non-pathogenic flora, rather than from species-specific effects.

The literature suggests that AAD and CDI can occur after just one dose of antibiotics and up to several weeks after completion of therapy (29). As such, an adequate follow-up period is needed to ensure that most cases are appropriately identified. Our subgroup analysis by follow-up period was dichotomized to before or after 4 weeks, because this time frame reflects a practical and clinically applicable cutoff for ongoing patient surveillance. While the effect size observed was more robust with longer follow-up, only reduction in AAD, and not CDI, remained significant. Statistical heterogeneity was moderately increased for the

subgroup of patients that had follow-up ≥ 4 weeks, I^2 of 54% for AAD and 57% for CDI.

Post-hoc meta-regression analysis by type of probiotic confirmed the findings of subgroup analysis. Specifically, the primarily Lactobacillus-based formulation remained significantly effective in reducing AAD. Due to wide variability in duration of follow-up, we were unable to perform meta-regression of duration of follow-up as a continuous measure.

No life-threatening adverse probiotic effects were reported in these RCTs. Despite case reports of toxicity among patients with extenuating circumstances (30-32), probiotics had an excellent safety profile; the most common side effect was gastrointestinal upset.

Discussion

Probiotics can confer health benefits in several ways: by creating nutrient competition, altering gut flora favorably, serving as a barrier against pathogen-receptor binding, elaborating immunomodulators (such as IgA) or trophic factors, and reducing osmotic diarrhea (33). With recent epidemiologic patterns showing a rise in AAD and CDI occurrence among healthier, previously spared populations, as well as among patients most vulnerable to its complications (34-36), there is an urgent need to find innovative solutions for prevention.

Our findings indicate that probiotics given concurrently with antibiotics reduce the risk of AAD and CDI. The results of our meta-analysis are concordant with several prior systematic reviews and meta-analyses (5-9), which have varied

in the patients assessed and outcomes defined. A recent meta-analysis by Hempel et al (8) assessed probiotics in both the prevention and treatment of AAD and reported benefit. This study included 82 trials of significant heterogeneity; in addition to examining both prevention and treatment trials, they assessed trials of both inpatients and outpatients, evaluated all age groups, and included 24 trials in which patients were receiving antibiotics for H. pylori eradication.

One of the strengths of this review is the emphasis placed on ensuring that comparable outcome definitions were used in meta-analyzing the data. This was achieved by carefully selecting the study arms to include for trials that had more than two intervention groups, and by contacting primary authors for original data when needed. These actions will mitigate some of the impact of the clinical heterogeneity observed between the trials. Another major strength is that the review focused on a specific patient population: inpatients. Reducing the incidence of CDI will improve individual patient outcomes while curtailing spread in the high-risk setting of hospitals. Thus these results have implications for the health of other, non-infected inpatients. Finally, this meta-analysis shows that benefit is retained regardless of study quality, type of probiotic used, and follow-up duration. However, the results are only significant for both AAD and CDI concurrently for the subgroups of good quality studies, studies assessing Lactobacillus-based formulations, and studies in which the follow-up was < 4 weeks.

Several limitations in the individual studies and in our meta-analysis merit discussion. A notable limitation among some of the more recent studies is the

high baseline rates of AAD and CDI observed in the placebo arms. Three of the recent, good quality RCTs (13-15) reported AAD rates of 34 – 44% and CDI rates at 16 – 24% in the control groups. These high baseline event rates may have facilitated the detection of a significant effect size despite a small sample size. The extent to which this may have been influenced by different local practices in antimicrobial stewardship and environmental infection control is unknown, and thus their effect sizes may not be duplicated in other settings where baseline rates of AAD and CDI are lower.

The included trials shared certain methodological issues that also limit broad interpretation of the results. Some of the studies had lower enrolment than planned for detecting the expected differences, a factor of particular importance for the negative trials (20, 21). Almost all of the studies that we assessed excluded patients that might otherwise be considered candidates for a hospital-wide intervention like probiotics. For example, patients who had received a course of antibiotics as an outpatient in the weeks preceding trial enrolment were excluded to avoid inclusion of cases of community-associated CDI. Furthermore, patients with preexisting gastrointestinal pathology were excluded to avoid inclusion of patients suffering from diarrhea not related to antibiotics. These steps may limit the interpretation of how probiotics will affect a more inclusive inpatient population. Two of the fair quality studies (18, 21) reported possible probiotic under-dosing. A high rate of attrition > 20%, was observed in four studies (22-24, 26), necessitating a poor quality rating.

Our meta-analysis also suffers from some important limitations. There was evidence of moderate publication bias as demonstrated in Figure 3. Three trials were excluded because they were not in English (37-39). Furthermore, among all of the patients assessed for AAD, 1200 patients did not have endpoints for CDI.

We chose to convey outcome information using RDs and NNTs. We acknowledge the limitation of utilizing NNTs to convey outcome information given the clinical and statistical heterogeneity among the studies included in this review. Admittedly, NNT is difficult to interpret when such heterogeneity exists, and so we caution readers and decision-makers against using this information without putting it in the context of the variability of the studies and considering the local prevalence of CDI in their population.

Conclusions

Our findings illuminate the benefits of probiotics in preventing both AAD and CDI among the specific patient population of inpatient adults requiring antibiotics. On the basis of the current review, probiotics are recommendable in such patients in the absence of contraindications; however, prevalence of AAD and CDI should be taken into consideration before making guidelines. The literature does not clearly indicate a favored choice of probiotic, although there is stronger evidence for Lactobacillus-based formulations.

Many health care providers have been hesitant to adopt probiotics in routine practice despite impressive effect sizes. This may be because of the small sample sizes in the individual trials, high baseline rates of AAD and CDI in

the larger, more recent trials, the clinical and statistical heterogeneity between trials, and the publication bias seen in this and other meta-analyses. While there may be a signal towards clinical equipoise, future RCTs should strive to recruit more patients and strengthen power in order to help bring probiotics to the bedside. Other research that will add to our current knowledge might address whether there is greater benefit with the use of combination therapy over single-species probiotic formulations. The hypothesis of a dose-response effect requires further validation.

1. Valiquette L, Low D, Pépin J, McGeer A. Clostridium difficile infection in hospitals: a brewing storm. CMAJ 2004 Jul 6;171(1):27-9.

2. Hurley BW, Nguyen CC. The spectrum of pseudomembranous enterocolitis and antibiotic-associated diarrhea. Arch Intern Med. 2002 Oct 28;162 (19):2177-84.

3. Bauer MP, van Dissel JT. Alternative strategies for Clostridium difficile infection. Int J Antimicrob Agents. 2009 Mar;33 Suppl 1:S51-6.

4. Bartlett JG. A call to arms: the imperative for antimicrobial stewardship. Clin Infect Dis. 2011 Aug;53 Suppl 1:S4-7.

5. Cremonini F, Di Caro S, Nista EC, Bartolozzi F, Capelli G, Gasbarrini G, et al. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. Aliment Pharmacol Ther. 2002 Aug;16(8):1461-7.

6. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am J Gastroenterol. 2006 Apr;101(4):812-22.

7. Dendukuri N, Costa V, McGregor M, Brophy JM. Probiotic therapy for the prevention and treatment of Clostridium difficile-associated diarrhea: a systematic review. CMAJ 2005 Jul 19;173(2):167-70.

8. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JNV, Shanman R, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA. 2012 May 9;307(18):1959-69.

9. Johnson S, Maziade PJ, McFarland LV, Trick W, Donskey C, Currie B, et al. Is primary prevention of Clostridium difficile infection possible with specific probiotics? Int J Infect Dis. 2012 Nov; 16(11):e786-92.

10. Loo VG, Bourgault A, Poirier L, Lamothe L, Michaud S, Turgeon N, et al. Host and Pathogen Factors for Clostridium difficile Infection and Colonization. N Engl J Med. 2011;365(18):1693-703.

11. Moher D, Liberati A, Tetzlaff J, Altman DG, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. Open Medicine 2009;3(3):123-30.

12. Harris RP, Helfand M, Woolf SH, Lohr KN, Mulrow CD, Teutsch SM, et al. Current methods of the US Preventive Services Task Force: a review of the process. Am J Prev Med 2001;20:21-35.

13. Gao XW, Mubasher M, Fang CY, Reifer C, Miller LE. Dose-response efficacy of a proprietary probiotic formula of Lactobacillus acidophilus CL1285 and Lactobacillus casei LBC80R for antibiotic-associated diarrhea and Clostridium difficile-associated diarrhea prophylaxis in adult patients. Am J Gastroenterol. 2010 Jul;105(7):1636-41.

14. Hickson M, D'Souza AL, Muthu N, Rogers TR, Want S, Rajkumar C, et al. Use of probiotic Lactobacillus preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. BMJ. 2007 Jul 14;335(7610):80.

15. Beausoleil M, Fortier N, Guenette S, L'Ecuyer A, Savoie M, Franco M, et al. Effect of a fermented milk combining Lactobacillus acidophilus Cl1285 and

Lactobacillus casei in the prevention of antibiotic-associated diarrhea: a randomized, double-blind, placebo-controlled trial. Can J Gastroenterol. 2007 Nov;21(11):732-6.

16. McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Moyer KA, Melcher SA, et al. Prevention of beta-lactam-associated diarrhea by Saccharomyces boulardii compared with placebo. Am J Gastroenterol 1995;90 (3):439-48.

17. Psaradellis E, Dylewski J, Sampalis J. Efficacy of BIO K+ CL1285 in the reduction of antibiotic-associated diarrhea - A placebo controlled double-blind randomized, multi-center study. Arch Med Sci 2010;6 (1):56-64.

18. Song HJ, Kim J-Y, Jung S-A, Kim S-E, Park H-S, Jeong Y, et al. Effect of probiotic Lactobacillus for the prevention of antibiotic-associated diarrhea: a prospective, randomized, double-blind, multicenter study. J Korean Med Sci. 2010 Dec;25(12):1784-91.

19. Plummer S, Weaver MA, Harris JC, Dee P, Hunter J. Clostridium difficile pilot study: effects of probiotic supplementation on the incidence of C. difficile diarrhoea. Int Microbiol. 2004 Mar;7(1):59-62.

20. Thomas MR, Litin SC, Osmon DR, Corr AP, Weaver AL, Lohse CM. Lack of effect of Lactobacillus GG on antibiotic-associated diarrhea: A randomized, placebo-controlled trial. Mayo Clin Proc. 2001 Sep;76 (9):883-9.

21. Lewis SJ, Potts LF, Barry RE. The lack of therapeutic effect of Saccharomyces boulardii in the prevention of antibiotic-related diarrhoea in elderly patients. J Infect. 1998 Mar;36(2):171-4.

22. Pozzoni P, Riva A, Bellatorre A, Amigoni M, Redaelli E, Ronchetti A, et al. Saccharomyces boulardii for the Prevention of Antibiotic-Associated Diarrhea in Adult Hospitalized Patients: A Single-Center, Randomized, Double-Blind, Placebo-Controlled Trial. Am J Gatroenterol. 2012 Jun;107(6):922-31.

23. Cimperman L, Bayless G, Best K, Diligente A, Mordarski B, Oster M, et al. A randomized, double-blind, placebo-controlled pilot study of Lactobacillus reuteri ATCC 55730 for the prevention of antibiotic-associated diarrhea in hospitalized adults. J Clin Gastroenterol 2011 October;45 (9):785-9.

24. Wenus C, Goll R, Loken EB, Biong AS, Halvorsen DS, Florholmen J. Prevention of antibiotic-associated diarrhoea by a fermented probiotic milk drink. Eur J Clin Nutr. 2008 Feb;62 (2):299-301.

25. Can M, Besirbellioglu BA, Avci IY, Beker CM, Pahsa A. Prophylactic Saccharomyces boulardii in the prevention of antibiotic-associated diarrhea: a prospective study. Med Sci Monit. 2006 Apr;12(4):PI19-22.

26. Surawicz CM, Elmer GW, Speelman P, McFarland LV, Chinn J, Van Belle G. Prevention of antibiotic-associated diarrhea by Saccharomyces boulardii: A prospective study. Gastroenterology 1989;96 (4):981-8.

27. Wunderlich PF, Braun L, Fumagalli I, D'Apuzzo V, Heim F, Karly M, et al. Double-blind report on the efficacy of lactic acid-producing Enterococcus SF68 in the prevention of antibiotic-associated diarrhoea and in the treatment of acute diarrhoea. J Int Med Res. 1989 Jul;17(4):333-8.

28. Gotz V, J.A. R, Moss J, Murray HW. Prophylaxis against ampicillin-associated diarrhea with a lactobacillus preparation. Am J Hosp Pharm. 1979 Jun;36(6):754-7.

29. Kelly C, Pothoulakis C, LaMont J. Clostridium Difficile Colitis. N Engl J Med 1994 Jan 27;330(4):257-262.

30. Rautio M, Jousimies-Somer H, Kauma H, Pietarinen I, Saxelin M, Tynkkynen S, et al. Liver abscess due to a Lactobacillus rhamnosus strain indistinguishable from L. rhamnosus strain GG. Clin Infect Dis. 1999 May;28(5):1159-60.

31. Mackay A, Taylor M, Kibbler C, Hamilton-Miller J. Lactobacillus endocarditis caused by a probiotic organism. Clin Microbiol Infect. 1999 May;5(5):290-292.

32. Luong M, Sareyyupoglu B, Nguyen M, Silveira F, Shields R, Potoski B, et al. Lactobacillusprobiotic use in cardiothoracic transplant recipients: a link to invasive Lactobacillus infection? Transpl Infect Dis. 2010;12(6):561-4.

33. Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. Nat Rev Gastroenterol Hepatol. 2010 Sep;7(9):503-14.

34. Rates of Clostridium Difficile Infection Among Hospitalized Patients Aged > 65 by Age Group. MMWR. 2011;60:1171 [cited 2012 Dec 1]. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6034a7.htm?s_cid=mm6034a7_w.

35. Cober ED, Malani PN. Clostridium difficile infection in the "oldest" old: clinical outcomes in patients aged 80 and older. J Am Geriatr Soc. 2009 Apr;57(4):659-62.

36. Serrano PE, Khuder SA, Fath JJ. Obesity as a Risk Factor for Nosocomial Infections in Trauma Patients. J Am Coll Surg. 2010 July;211 (1):61-7.

37. Stein GY, Nanim R, Karniel E, Moskowitz I, Zeidman A. Probiotics as prophylactic agents against antibiotic-associated diarrhea in hospitalized patients. Harefuah 2007 Jul;146 (7):520-2, 75.

38. Cots JM. Consumption of probiotics can reduce the incidence of antibiotic-associated diarrhea. Formacion Medica Continuada en Atencion Primaria. 2008 Mar 01;15 (3):201.

39. Li D, Wang H, Tan M, Shao Y. Use of probiotics for prevention of antibiotic-associated diarrhea in elderly patients. Chinese Journal of Gastroenterology. 2010;15 (3):154-6.

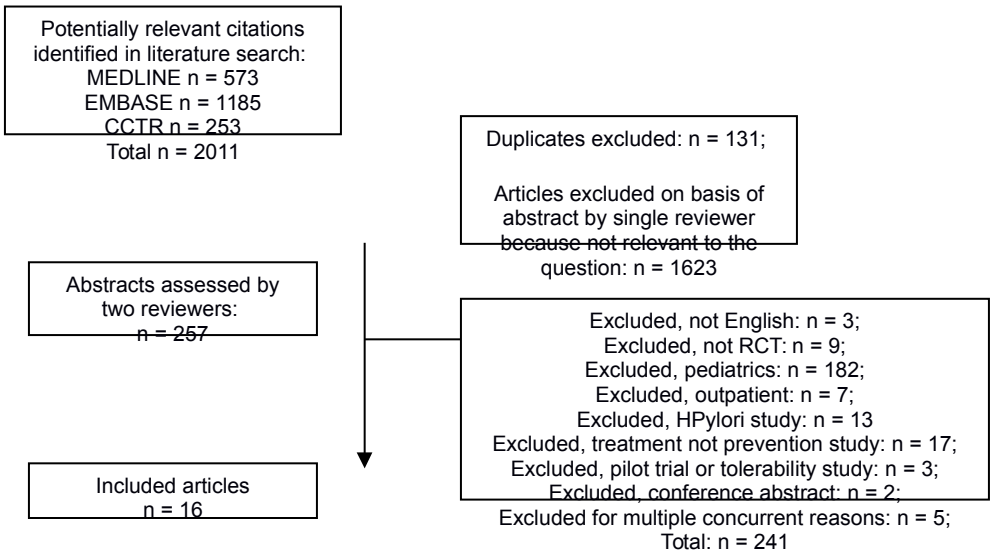
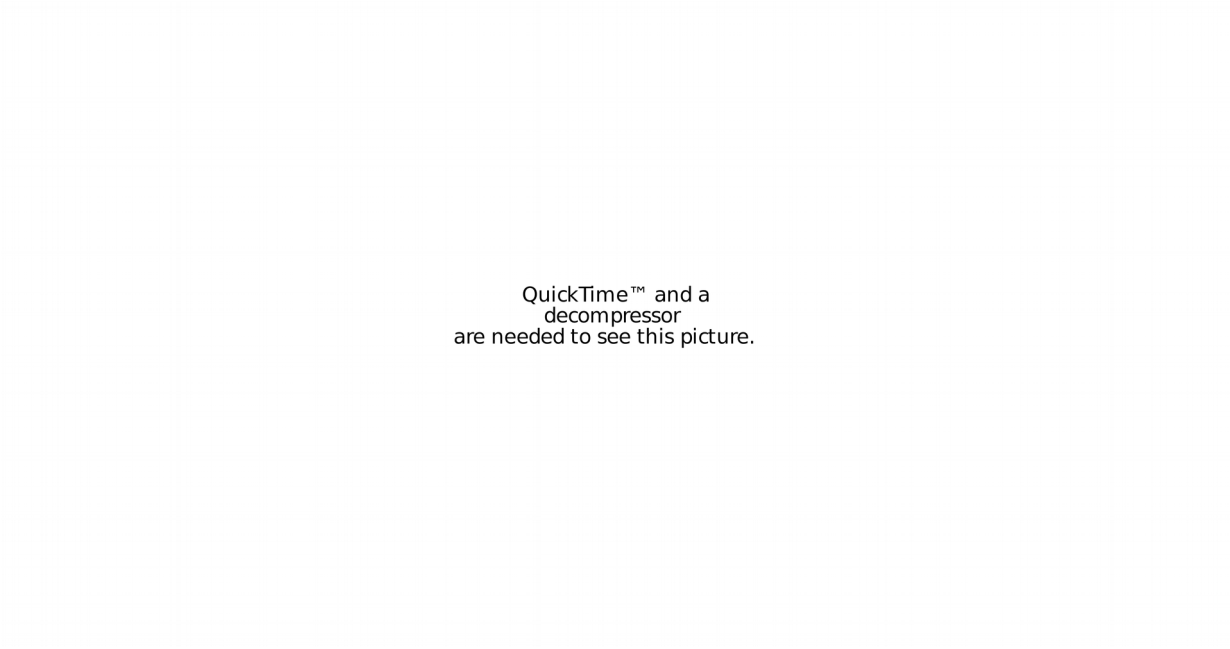


Figure 1: Flow diagram of study selection process

Figure 3: Funnel plot of 15 studies included in the meta-analysis for the outcome of AAD, demonstrating moderate publication bias.

Figure 3: Funnel plot of 15 studies included in the meta-analysis for the outcome of AAD, demonstrating moderate publication bias.

(a) AAD



(b) CDI

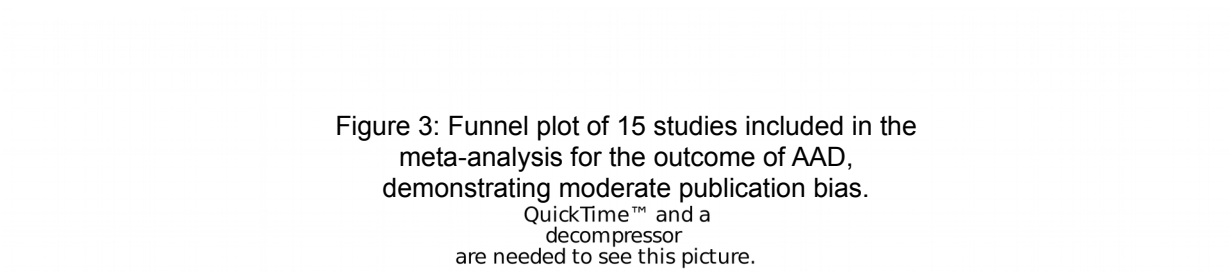


Figure 3: Funnel plot of 15 studies included in the meta-analysis for the outcome of AAD, demonstrating moderate publication bias.

Figure 2: Meta-analysis of all randomized controlled trials demonstrating effect of probiotics on (a) AAD and (b) CDI

Figure 3: Funnel plot of 15 studies included in the meta-analysis for the outcome of AAD, demonstrating moderate publication bias.

Source	Characteristics of Study Population	Probiotic Agent & Duration	Additional Follow-Up	Primary & Secondary Outcome	N randomized Total (Probiotic, Placebo)	N analyzed Total (Probiotic, Placebo)	Attrition Primary Outcome (%)
GOOD QUALITY							
Gao et al, 2010	Adult inpatients Mean age: 60 yrs (both groups), Gender: 51% vs. 50% male (probiotic vs. placebo) Single centre, China	Lb-A, Lb-C within 36 hr to 5 d post-antibiotics	21 d	AAD CDI	255 (high 86 / low 85, 84)	AAD: 255 (high 86 / low 85, 84) CDI: 255 (high 86 / low 85, 84)	0
Hickson et al, 2007	Adult inpatients on orthopedic, medical, and care of the elderly wards Mean age: 73.7 vs 73.9 yrs (probiotic vs. placebo) Gender: 43% vs. 48% male (probiotic vs. placebo) Three hospitals, United Kingdom	Lb-C, ST, LB-B within 48 hr to 7 d post-antibiotics	28 d	AAD CDI	135 (69, 66)	AAD: 113 (57, 56) CDI: 109 (56, 53)	16
Beausoleil et al, 2007	Adult inpatients Mean age: 68.8 vs 72.9 yrs (probiotic vs. placebo) Gender: 45.5% vs. 51.1% male (probiotic vs. placebo) Single tertiary care center, Canada	Lb-A, Lb-C within 48 hr for duration of antibiotics	21 d	AAD CDI	89 (44, 45)	AAD: 89 (44, 45) CDI: 89 (44, 45)	0
McFarland et al, 1995	Adult inpatients receiving at least 1 β-lactam antibiotic Mean age: 40.7 vs. 42.3 yrs (probiotic vs. placebo) Gender: 63.9% vs 65.6% male (probiotic vs. placebo) Four centers, United States	SB within 72 hr to 3 days post-antibiotics	31-46 d	AAD CDI *	193 (97, 96)	AAD: 193 (97, 96) CDI: 24 (10, 14)	0
FAIR QUALITY							
Psaradellis et al, 2010	Adult inpatients in emergency dept. or ward Mean age: 59.5 vs. 58.1 yrs (probiotic vs. placebo) Gender: 54.2% vs. 48.4% (probiotic vs. placebo) Eight centers, Canada	Lb-A, Lb-C within 24 hr to 5 days post-antibiotics	21 d	AAD CDI	472 (233, 239)	AAD: 437 (216, 221) CDI: 46 (16, 30)	8
Song et al, 2010	Adult inpatients Mean age: 61 vs. 60 yrs (probiotic vs. placebo) Gender: 61.2% vs. 62.2% male (probiotic vs. placebo)	Lb-A, Lb-R within 48 hr for 14 d	14 d	AAD-1 AAD-2	214 (103, 111)	AAD-1: 214 (103, 111) AAD-2: 214 (103, 111)	0

	Ten tertiary hospitals, Korea						
Plummer et al, 2004	Adult inpatients on medical, and care of the elderly wards Baseline characteristics not provided (mean age, gender, etc.) Single centre, United Kingdom	Lb-A, BB within 36 hr for 20 d	0 d	CDI	138 (69, 69)	CDI: 138 (69, 69)	0
Thomas et al, 2001	Adult inpatients on a medical ward Mean age: 57.2 vs. 54.4 yrs (probiotic vs. placebo) Gender: 51.1% vs. 56.0% (probiotic vs. placebo) Single centre, United States	Lb-R within 24 hr for 14 d	≥ 7 d	AAD CDI *	302 (152, 150)	AAD: 267 (133, 134) CDI: 267 (133, 134)	12
Lewis et al, 1998	Adult inpatients on a medical ward Mean age: 75 vs. 77 yrs (probiotic vs. placebo) Baseline gender characteristics not provided Single centre, United Kingdom	SB within 24 hr for duration of antibiotics	Treatment Duration	AAD CDI *	72 (not indicated)	AAD: 69 (33, 36) CDI: 69 (33, 36)	4
POOR QUALITY							
Pozzoni et al, 2012	Adult inpatients Mean age: 79.9 vs. 78.5 yrs (probiotic vs. placebo) Gender: 49.6% vs. 50.0% (probiotic vs. placebo) Single centre, Italy	SB within 48 hr for 7 days post-antibiotics	84 d	AAD CDI	275 (141, 134)	AAD: 204 (106, 98) CDI: 204 (106, 98)	26
Cimperman et al, 2011	Adult inpatients on medical wards Mean age: 42.8 vs. 63.6 yrs (probiotic vs. placebo) Gender: 54% vs. 40% male (probiotic vs. placebo) Single centre, United States	Lb-Reut within 96 hr for 28 d	Treatment Duration	AAD	31 (15, 16)	23 (13, 10)	26
Wenus et al, 2008	Adult inpatients Mean age: 58.8 vs. 56.2 yrs (probiotic vs. placebo) Gender: 65.2% vs. 51.2% male (probiotic vs. placebo) Single centre, Norway	Lb-R, BB-12, Lb-A within 72 hr for 14 d	Treatment Duration	AAD CDI	87 (46, 41)	AAD: 63 (34, 29) CDI: 55 (Not Reported)	28

Can et al, 2006	Adult inpatients receiving chemotherapy Mean age not provided, range: 25-50 yrs (both groups) Gender: 89.0% vs. 94.9% male (probiotic vs. placebo) Single centre, Turkey	SB within 48 hr, duration not noted	28 d	AAD CDI	151 (73, 78)	AAD: 151 (73, 78) CDI: 151 (73, 78)	0
Surawicz et al, 1989	Adult inpatients Mean age: 48.8 vs. 45.4 yrs (probiotic vs. placebo) Gender: 66% vs. 73% male (probiotic vs. placebo) Single centre, United States	SB within 48 hr to 14 days post-antibiotics	Treatment Duration	AAD CDI	318 (Not Reported)	AAD: 180 (116, 64) CDI: 138 (91, 47)	43
Wunderlich et al, 1989	Adult inpatients Mean age: 33 yrs overall Gender: 48% male overall Five centres, Switzerland	Enterococcus SF 68 for 7 d	Treatment Duration	AAD	45 (23, 22)	45 (23, 22)	0
Gotz et al, 1979	Adult inpatients on medical wards receiving Ampicillin Mean age: 64 vs. 65 yrs (probiotic vs. placebo) Gender: 36.1% vs. 51.2% male (probiotic vs. placebo) Single centre, United States	Lb-A, Lb-B within 24 hr for 5 d	Treatment Duration	AAD	98 (48, 50)	AAD: 79 (36, 43)	19

Table 1: Source Table
Lb-A Lactobacillus Acidophilus, Lb-B Lactobacillus Bulgaricus, Lb-R Lactobacillus Rhamnosus, Lb-Reut Lactobacillus Reuteri Lb-C Lactobacilus Casei, BB Bifidobacterium Bifidum, Bifidobacterium Bifidum 12 BB-12, ST Streptococcus Thermophilus, SB S Boulardii; AAD-1 loose or watery stools more than 3 times per day for at least 2 days within 14 days of enrolment, AAD-2 more than 2 times per day over same period; NR Not reported
* Not included in Meta-analysis

RCT	Randomized	Blinded	Comparable Groups (Start and End)	F/u > 80%	Clear Interventions	Clear, relevant Outcomes	Valid, reliable, equal measures used	Intention to Treat Analysis	Confounders noted	Power calculation reported and appropriate
GOOD QUALITY										
Gao et al, 2010	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hickson et al, 2007	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Beausoleil et al, 2007	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
McFarland et al, 1995	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
FAIR QUALITY										
Psaradellis et al, 2010	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No
Song et al, 2010	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Plummer et al, 2004	Yes	Yes	Not Reported	Yes	Yes	Yes	Yes	Yes	No	No
Thomas et al, 2001	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No
Lewis et al, 1998	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
POOR QUALITY										
Pozzoni et al, 2012	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	No
Cimperman et al, 2011	Yes	Yes	No	No	Yes	Yes	Yes	No	No	No
Wenus et al, 2008	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Can et al, 2006	Yes	Yes	Not Reported	Yes	Yes	Yes	No	Yes	No	No
Surawicz et al, 1989	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Wunderlich et al, 1989	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Gotz et al, 1979	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No

Table 2: Risk of bias among included studies

Subgroup		# of Studies	Probiotic (N events, N analyzed)	Placebo (N events, N analyzed)	RR (95% CI)	RD (95% CI)	I^2 (%)
Table 3: Subgroup Analyses							
AAD							
All studies		**15**	**178, 1169**	**266, 1127**	**0.61 (0.47, 0.79)**	**-0.09, (-0.13, -0.05)**	**44**
Study Quality	Good	4	45, 283	86, 281	0.54 (0.39, 0.73)	-0.14 (-0.21, -0.06)	0
	Fair	4	97, 485	118, 502	0.85 (0.67, 1.08)	-0.03 (-0.08, 0.01)	3
	Poor	7	36, 401	62, 344	0.42 (0.23, 0.76)	-0.11 (-0.18, -0.04)	42
Probiotic Type*	Lactobacillus	9	134, 721	207, 733	0.64 (0.48, 0.84)	-0.11 (-0.17, -0.04)	35
	S Boulardii	5	42, 425	53, 372	0.68 (0.37, 1.24)	-0.05 (-0.11, 0.00)	53
Follow-Up	< 4 weeks	10	146, 823	208, 789	0.57 (0.41, 0.79)	-0.09 (-0.14, -0.04)	29
	≥ 4 weeks	5	32, 346	58, 338	0.47 (0.23, 0.94)	-0.09 (-0.18, -0.01)	54
CDI							
All studies		**9**	**18, 574**	**55, 533**	**0.37 (0.22, 0.62)**	**-0.07 (-0.12, -0.02)**	**0**
Study Quality	Good	3	9, 185	36, 182	0.24 (0.08, 0.73)	-0.15 (-0.21, -0.09)	29
	Fair	2	3, 85	9, 99	0.42 (0.12, 1.52)	-0.05 (-0.11, 0.02)	0
	Poor	4	6, 304	10, 252	0.47 (0.18,1.24)	-0.02 (-0.05, 0.01)	0
Probiotic Type	Lactobacillus	6	12, 304	46, 310	0.33 (0.18, 0.60)	-0.09 (-0.15, -0.04)	0
	S Boulardii	3	6, 270	9, 223	0.49 (0.17, 1.40)	-0.02 (-0.06, 0.02)	2
Follow-Up	< 4 weeks	6	15, 339	42, 304	0.35 (0.20, 0.62)	-0.07 (-0.11, -0.03)	0
	≥ 4 weeks	3	3, 235	13, 229	0.31 (0.03, 2.77)	-0.05 (-0.13, 0.03)	57