PREVENTION OF ***CLOSTRIDIUM DIFFICILE*** INFECTION

Prevention of *Clostridium difficile* infection: a systematic review and critical appraisal of clinical practice guidelines and an independent participant data meta-analysis on probiotics for prophylaxis in adults and children administered antibiotics

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LAY ABSTRACT

Clostridium difficile infection (CDI) is a common hospital-associated infection, and prevention is of high priority. We reviewed clinical practice guidelines on CDI prevention to summarize their recommendations, and assess the quality of guideline development and reporting. Furthermore, we analysed patient data from randomized clinical trials to obtain an overall estimate (meta-analysis) of whether using a novel strategy, probiotic prophylaxis, is effective and safe. The guidelines had several limitations, importantly that authors were not transparent about how recommendation were developed, and recommendations were not always linked to evidence. Although no guideline recommended using probiotics to prevent CDI, our advanced analysis of previously conducted trials suggested that it was an effective intervention, reducing infections by approximately 76%, and was not associated with differences in serious adverse events compared to participants not receiving probiotics. In summary, guidelines on CDI prevention should be improved, and probiotics may be considered as an additional strategy.

ABSTRACT

Clostridium difficile infection (CDI) prevention is of high priority. We reviewed clinical practice guidelines (CPGs), and conducted an individual participant data meta-analysis (IPMDA) of randomized controlled trials (RCTs) to assess effectiveness and safety of probiotic prophylaxis.

For CPGs, we rated quality, summarized recommendations with their strength and author-reported evidence, then re-evaluated evidence. For the IPDMA, we pooled RCTs investigating probiotics versus control for CDI prevention among antibiotic consumers, using generalized linear mixed models. Our outcomes were CDI and serious adverse events (SAEs). We adjusted for age, sex, hospitalization status, and exposure to high risk antibiotics. We assessed study risk of bias and confidence in estimates of effect.

Five international guidelines were evaluated, and all scored poorly for applicability, stakeholder involvement, and rigor of development. Recommendations were not always linked to evidence, and guideline authors were not transparent about how evidence limitations impacted their decisions. None of the guidelines recommended probiotics.

Fourteen studies contributed data, with one pending. Probiotics reduced CDI among all studies and the adjusted model. No covariates were significantly associated with CDI. Subgroups suggested that high incidence did not affect probiotic effectiveness, and high-dose, multi-strain probiotics were more beneficial. Our estimate was robust to sensitivity analyses. Probiotics did not significantly affect SAE odds among all studies and the adjusted model. Increasing age was a significantly associated with SAEs. No SAEs were reportedly probiotics-

related. For both outcomes, estimates were similar from data of obtained and not obtained studies. Confidence in estimates was moderate for both outcomes, due to low event rates.

Current guidelines on CDI prevention did not adhere well to validated standards for development and reporting, most notably due to insufficient links between recommendations and supporting evidence. Our preliminary analysis suggests that probiotic prophylaxis is useful and safe for CDI prevention.

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LIST OF ABBREVIATIONS AND SYMBOLS

AGREE = Appraisal of Guidelines Research & Evaluation

CDI = *Clostridium difficile* Infection

CI = Confidence Interval

CPG = Clinical Practice Guideline

GEE = Generalized Estimating Equations

GLMM = Generalized Linear Mixed Models

GRADE = Grading Quality of Evidence and Strength of Recommendations

IPC = Infection Prevention and Control

IPDMA = Individual Participant Data Meta-Analysis

ITS = Interrupted Time Series

OCEBM = Oxford Centre for Evidence Based Medicine Levels of Evidence

PICO = Patient(s), Intervention(s), Comparator(s), Outcome(s)

RCT = Randomized Controlled Trial

SAE = Serious Adverse Event

DECLARATION OF ACADEMIC ACHIEVEMENT

I was the main contributor and first author of all studies. The names and affiliations of collaborators are provided at the beginning of each study.

THESIS OUTLINE

This thesis examined *Clostridium difficile* infection prevention, by evaluating the content and quality of current clinical practice guidelines, as well as analysing participant data from controlled trials on probiotic prophylaxis. The first chapter introduces the main disease-related and methodology concepts relevant to the thesis: *C. difficile* infection, clinical practice guidelines, probiotics, and individual participant data meta-analysis. The second chapter is a systematic review of clinical practice guidelines, with two parts. First, the recommendations are summarized, and the scientific evidence underlying recommendations is reviewed and classified using the Oxford Centre for Evidence Based Medicine Levels of Evidence[1]. Second, the overall quality of development and reporting is assessed with the Appraisal of Guidelines for Research & Evaluation Instrument[2]. The third chapter is an individual participant data meta-analysis of the efficacy and safety of probiotics for reducing *C. difficile* infection in adults and children concurrently administered antibiotics, for which we pooled 10 studies and determined an adjusted effect estimate, examined participant subgroups, and conducted sensitivity analyses.

THESIS OBJECTIVES

The objectives of this thesis are to investigate the current clinical practice guidelines (CPGs) on the prevention of *C. difficile* infection (CDI), and to evaluate the usefulness of probiotics as a prevention strategy. We addressed the following research questions:

1. What are the available CPGs on CDI prevention, and what is their quality of development and reporting?
2. What are the recommendations made by CDI prevention CPGs, and were they reflective of the currently available evidence, with consideration of evidence quality?
3. Are probiotics an effective prevention strategy for adults and children taking antibiotics, based on findings from individual participant data from randomized controlled trials?
4. Are there subgroups of participants who have differential estimates of effect from probiotic prophylaxis?

CHAPTER I: INTRODUCTION

Clostridium difficile infection

Pathophysiology and risk factors for infection

Clostridium difficile is a rod-shaped, gram-positive, spore forming bacterium[3]. There are non-toxigenic and toxigenic strains, the latter of which may produce toxins TcbA and TcbB, as well as binary toxin CDT[4]. Some strains are more virulent than others, producing considerably higher concentrations of toxins[5]. *C. difficile* spores can be shed from both colonized patients (carriers) and patients with CDI, and are highly transmissible via the fecal-oral route[6,7]. The spores can survive for up to five months outside of the body, and are resistant to alcohol, heat, acid, and antibiotics[8].

Exposure and uncontrolled growth of the toxigenic bacteria may result in *C. difficile* infection (CDI). Exposure to toxigenic or nontoxigenic strains may also result in asymptomatic colonization by *C. difficile.* Colonization prevalence ranges from 10-37% among infants under two years of age, and 3-21% among older children and adults[9-12]. Active surveillance for colonization is not routine, however a recent review has suggested that patients colonized by *C. difficile* at hospital admission have an estimated 5.9 times higher risk of developing CDI[13]. The severity of outcomes of CDI range from mild or severe diarrhea, to pseudomembranous colitis and toxic megacolon[14].

CDI may be hospital-acquired or community-associated[15]. Although community-associated CDI rates are rising, it is most commonly a hospital-acquired infection (HAI)[16]. The use of antibiotics is the most important risk factor for CDI. Almost all antibiotics have been

linked with CDI; however, studies have shown that that broad-spectrum antibiotics such as clindamycin, 3rd and 4th generation cephalosporins, and fluoroquinolones carry the most risk[17,18]. In addition to the pharmacological antibiotic class, increased risk has been observed for longer duration of antibiotic exposure, and, more recently, hospital ward prescribing practices[19-21]. Additional risk factors are recent history of hospitalization or long-term care facility exposure, older age (over 65 years), certain comorbidities (e.g. inflammatory bowel disease, use of immunosuppressants, malignancy), treatment with gastric acid reducing agents, and disease pressure (i.e. exposure to endemic versus epidemic CDI settings)[21-29]. Recent findings have demonstrated a rise in CDI cases among patient groups previously considered at low risk, such as pregnant women and children[30].

Burden of illness

The incidence of *C. difficile* has increased in recent years[31]. Currently, CDI is the most common HAI in North America[24,32]. Surveillance data estimates CDI incidence in Europe, Canada, United States, Australia, and New Zealand to range between 2.45 to 7.5 per 10,000 patient days, or 9 to 80 per 10,000 patient admissions, with higher rates observed in outbreak settings[24,27,32-34]. In some countries, however, there have been reports of a recent decline in CDI, such as Finland and the United Kingdom[35,36].

Patients with CDI have a high risk of intensive care unit admissions, colectomy, and death[24]. Severe cases of CDI and CDI-attributable mortality has been rising[31]. Among hospitalized patients, a recent review found that mortality due to CDI is 4.5-5.7% in endemic periods, and up to 16.7% during outbreaks[37]. A study of population-level disease burden in

Ontario, Canada, which estimated health-adjusted life years (an estimate of years of healthy life lost and years lost to premature mortality), indicated that *C. difficile* is the 9th most burdensome infectious disease in the province[38].

Nurses from the United States and France who care for patients with *C. difficile* were surveyed in a recent qualitative study, and their most common challenge was the considerable time burden of practicing contact precautions combined with management of frequent and uncontrollable diarrhea[39]. For healthcare systems, prevention and management of CDI is a significant economic burden. A recent review of economic evaluations of the direct costs associated with CDI worldwide found that attributable mean CDI costs ranged from $8,911 to $30,049 for hospitalized patients[40]. Costs are higher for treating recurrences, and for complicated CDI that may require surgical intervention[40].

Diagnosis and treatment

C. difficile infection is diagnosed through laboratory analysis of stool samples, or with histological/pathological evidence of pseudomembranous colitis or toxic megacolon[41]. There is no single gold standard laboratory test for *C. difficile.* Diagnosis is can be done by *C. difficile* cytotoxicity assay, enzyme immunoassay (EIA) for glutamate dehydrogenase (GDH) produced by *C. difficile*, EIA for toxin (A and/or B), and nucleic acid amplification test (NAAT)/polymerase chain reaction (PCR) for *C. difficile* toxin genes (A and/or B), or a combination of these[41]. Recently, a survey of Western European countries has suggested that under-diagnosis of CDI due to absence of clinical suspicion, compounded by misdiagnosis related to suboptimal methods is still a problem[42].

Primary CDI infection is treated with metronidazole or vancomycin, and secondary (recurring) infection with vancomycin[43]. Treatment failure is an increasing issue. Reports show that approximately 22% of patients fail on metronidazole, and 14% on vancomycin[44]. Following successful treatment for CDI, 20-30% of patients experience recurrence within two weeks[45]. Recurrence may be due to the same strain or a different strain[46]. McFarland *et al.* found that patients with two or more recurrences have more than double the risk for subsequent recurrence[47]. Fidoxamycin is an approved treatment strategy that was found to be non-inferior to vancomycin and reduced risk of recurrence, however it is costly[48,49]. A novel approach for treating recurrent and severe CDI is fecal microbiota transplantation[50]. An additional prevention strategy currently researched is the administration of an oral liquid formulation of non-toxigenic *C. difficile* spores for recurrent infection[51].

Prevention

Prevention of primary *C. difficile* infection is focused on interventions to reduce transmission (i.e. spread of bacteria), including surveillance, isolating symptomatic patients, practicing contact precautions and good hand hygiene, and environmental cleaning with sporicidal agents[52]. In addition, antibiotic stewardship programs are one of the most effective interventions[53]. Several novel prevention strategies are being investigated, such as probiotics for primary infection, as well as vaccines and monoclonal antibodies for recurrent infection[54-56].

The efficacy, safety, and cost-effectiveness of each intervention must be considered, as decision makers need to know where time and costs should be allocated. Assessing efficacy is a challenge for non-pharmaceutical interventions for two reasons. First, a large proportion of

infection prevention literature is on behavioural/policy change interventions, which are commonly quasi-experimental (non-randomized) designs that have considerable risk of bias[57]. Second, interventions are commonly implemented as 'bundles,' i.e. multiple interventions, to control an outbreak or reduce high endemic levels of CDI, and analysed retrospectively. Thus, it is difficult to estimate the relative effectiveness of each individual intervention for reducing overall CDI rates.

Clinical practice guidelines on the prevention of *Clostridium difficile* infection

Definition and purpose of clinical practice guidelines

Clinical practice guidelines (CPGs) are defined as "statements that include recommendations intended to optimize patient care that are informed by a systematic review of evidence and an assessment of the benefits and harms of alternative care options[58]." With the abundance of medical literature available, it is often difficult for healthcare providers to keep up to date. CPGs collate and appraise the available evidence, and serve as a guidance to healthcare providers, assisting with critical decision making for optimizing patient care. A CPG is useful in a number of situations, including when there is (1) uncertainty or conflicting opinions about managing aspects patient care, (2) evidence regarding a potentially effective disease treatment, (3) need to collate scientific knowledge and expertise on a subject, and/or (4) an iatrogenic disease or intervention that carries significant risks or costs[59]. However, compliance with CPGs across clinical settings and healthcare providers vary, despite their availability and the emphasis on evidence-based medicine[60].

Guideline development methodology

Research has shown that adherence to CPGs may reduce inappropriate practice variation, enhance translation of research into practice, and improve healthcare quality and safety[58]. As such, it is important that guidelines are high-quality and trustworthy. Guideline development requires considerable costs and resources, and creating poor guidelines may cause undue harm. In order to have sufficient expertise and financial support, guidelines are commonly developed by government agencies, international organizations, clinical specialty societies, disease or population-specific organizations, and other private organizations[58]. Many of these groups have proposed standards for guideline developers[61].

Previously developed criteria may be summarized as follows: CPGs should (1) be developed by a knowledgeable, multidisciplinary panel of key stakeholders, (2) be based on an explicit and transparent process that minimizes distortions, biases, and conflicts of interest, (3) be transparent about funding and author conflicts of interest, both financial and intellectual, (4) have a scope and objectives, (5) be based on a systematic review of the existing evidence, (6) provide a clear explanation of the logical relationships between alternative care options and health outcomes, (7) provide ratings of both the quality of evidence and the strength of the recommendations, (8) consider important patient subgroups and patient preferences, as appropriate, (9) be peer reviewed, and (10) be reconsidered and revised as appropriate when important new evidence warrants modifications of recommendations[58,61].

Assessment of guideline development and reporting

It is imperative to assess the quality of guidelines[62,63]. The gold standard for guideline appraisal is the Appraisal of Guidelines, Research and Evaluation (AGREE) instrument[62], which was recently updated as the AGREE II[2]. The instrument is comprised of 23 items within six domains: scope and purpose, stakeholder involvement, rigour of development, clarity of presentation, applicability, and editorial independence. Each item is scores 1-7 on a Likert scale, from strongly disagree (1) to strongly agree (7). The standardized score for each domain is calculated by subtracting the minimum possible score from the obtained score, and dividing by the difference of the maximum possible score and the minimum possible score. This is then converted into a percentage, which demonstrates the percentage of the domain that was addressed by the guideline.

Probiotics for ***Clostridium difficile*** infection prevention

Definition, mechanism, and safety of probiotics

Probiotics are live microbial preparations that, when taken in sufficient quantities, may offer a health benefit on the host[64,65]. The mechanism of probiotics vary by species and strain, but generally they have enzymatic and antimicrobial activity, ability to enhance the intestinal barrier, and immunomodulation effects[65-67]. They may be taken alone or in combination with prebiotics, which are non-digestible fibers that are thought to modulate the effects of probiotics in the gastrointestinal (GI) tract[68]. Taken together, they are termed synbiotics.

A systematic review of randomized controlled trials and observational studies that have used probiotics found that there have been no serious adverse events associated with their

use[69]. Common side effects are mild to moderate such as bloating, flatulence, abdominal cramps, abdominal distension, and tend to resolve on their own. However, there have been concerns regarding bacteremia and fungemia[35,70-72]. Generally these conditions tend to occur in immunocompromised individuals.

Application of probiotics for CDI prevention

Probiotics have been investigated for prevention and treatment of numerous health conditions. In particular, they have been investigated as an infection prevention strategy[73]. There have been a number of reviews on randomized controlled trials (RCTs) and observational studies that look at the effect of probiotics on prevention of necrotizing enterocolitis in premature infants, CDI in adults and children, and respiratory tract infections, ventilator associated pneumonia, urinary tract infections, and surgical site infections in adults[56,74-78].

The prevention of CDI is one of the most promising uses of probiotics. The biological rationale of this intervention is that probiotics attenuate the microflora-disrupting effects of antibiotics, which are the most common risk factor for CDI. A recent systematic review and meta-analysis of 23 RCTs demonstrated that administering probiotics concurrently with antibiotics reduces the relative risk of CDI by 64% (95% CI 49-74%) in adults and children administered antibiotics[56]. However, the study did not have sufficient power, thus the certainty in the estimate of effect was considered moderate.

Current limitations

There is considerable evidence supporting the use of probiotics for certain health conditions. Routine use, however, is uncommon. There have been several reasons reported that may explain this discrepancy. First, the aforementioned safety concerns remains one of the key concerns for widespread implementation of probiotics. Second, it is unclear which patient groups the probiotics should be administered to, such as older or younger age, hospitalized or not, and other patient risk factors (e.g. patients who are immunocompromised and/or with severe comorbidities). Third, the relative effectiveness of probiotics in low incidence settings has been debated[79]. Lastly, there are general concerns regarding the lack of information on the specific strain of probiotic and dose to use for each health condition, as most clinical trials have been conducted using different products, with doses ranging from 1 million to 900 billion colony forming units (CFU) per day.

Individual participant data meta-analysis

Description of study design

Meta-analysis methods involve combining quantitative data from several related studies to estimate the overall results of the study question, most commonly the treatment effect of an intervention. The majority of meta-analyses are based on published study data, i.e. aggregate data, which are summary measures of participant groups, such as blood pressure mean and standard deviation, or relative risk and 95% confidence intervals of mortality. An alternative approach is to obtain the individual participant data from the trialists, and conduct an individual participant data meta-analysis (IPDMA).

IPDMAs are currently considered the gold standard for estimating treatment effect[80]. This research study design is increasingly used in healthcare research, as it allows for estimating how the treatment effect is modified by study level characteristics, such as study location or treatment dose, and participant level characteristics, such as age, sex, presence of comorbidities, and other risk factors pertinent to the outcome of interest[80]. It is important to note, however, that the individual studies' bias in design or conduct must be taken into account[81].

Strengths and limitations

An IPDMA analysis has several strengths. First, it allows one to estimate the treatment effect while controlling for confounders, e.g. participant s' baseline risk factors[82]. For aggregate data, reviewers may conduct meta-regression, however this analysis is at risk of ecologic fallacy[83]. Second, IPDMAs allow for handling of missing participant data, and reviewers can conduct sensitivity analyses to test the robustness of the effect estimate[80]. Third, it allows for incorporation of unpublished data, if accessible[80].

There are also a number of limitations. First, conducting an IPDMA is time and resource intensive. It is important to have strong rationale why an IPDMA is needed compared to a conventional, aggregate data meta-analysis[81]. Second, it is imperative to garner the willingness of potential collaborators to participate and to estimate the amount of IPD that can be obtained from the available trials, in order to minimize publication bias[80,84]. Although the benefits of sharing data from clinical trials has been widely recognized, there are concerns over participant identification, misuse of data, and financial burden on the researchers[85,86]. Third,

given the statistical complexity of an IPDMA, appropriate training and advice should be sought[81].

Analysis of individual participant data

Individual participant data that is somehow clustered, such as different trials within an IPDMA or different hospitals within a multicenter trial, often cannot be analysed as a single trial. This is because the participants within a trial are more similar to each other than to participants from other trials, and thus are not a true independent sample. To model binary outcome data that is clustered, one may use a random effects model (i.e. mixed effects model), also called multilevel or hierarchical models, or a population average model (i.e. generalized estimating equations models [GEEs])[87]. In a random effects model, parameter estimates are based on each cluster, whereas for the population average model, parameter estimates are averaged. Generalized linear mixed models (GLMMs) are a family of models for analysing binary clustered data which allow for incorporation of heterogeneity both between studies and within studies[88]. The GEE approach, on the other hand, assumes equal correlation between observations within a cluster.

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PREVENTION OF *CLOSTRIDIUM DIFFICILE* INFECTION: A SYSTEMATIC REVIEW AND CRITICAL APPRAISAL OF CLINICAL PRACTICE GUIDELINES

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Abstract

Background: *Clostridium difficile* infection (CDI) is the most common cause of hospital-acquired infectious diarrhea. Prevention efforts are of high priority, and numerous clinical practice guidelines provide recommendations. We summarized the recommendations and analysed the quality of guidelines on the prevention of CDI in a hospital setting.

Methods: We searched medical databases and grey literature for guidelines on CDI prevention published January 2004-January 2015. Three reviewers independently screened articles and rated the quality of guidelines using the AGREE II instrument, which is comprised of 23 items within six domains. Each item was rated 1-7, and for each guideline we calculated the score for each domain as a percentage of its maximum possible score and standardized range. We extracted and summarized recommendations and the quality of evidence using the Oxford Levels of Evidence.

Results: Of 2,578 articles screened, five guidelines met the inclusion criteria: three from the United States, one from Europe (comprising 11 countries), and one from the United Kingdom. All guidelines addressed CDI prevention in hospitals, such as antibiotic stewardship, hypochlorite solutions, probiotic prophylaxis, and bundle strategies. Based on the median AGREE II scores and interquartile ranges, the level of clarity of presentation 75.9% (75.9-79.6%), scope and purpose 74.1% (68.5-85.2%), and editorial independence 63.9% (47.2-66.7%) were acceptable. Low scores were found for applicability (43.1% (19.4-55.6%), stakeholder involvement (40.7% (38.9-44.4%)), and in particular rigor of development 18.1% (17.4-35.4%).

Conclusions: The available guidelines on CDI prevention did not adhere well to reporting standards endorsed by the AGREE II group, and recommendations were not consistent with the

quality of evidence. The poorest scores were for rigor of development due to insufficient links between recommendations and supporting evidence.

Introduction

Clostridium difficile infection (CDI) is the most common cause of hospital-acquired infectious diarrhea, and is of increasing concern in the community[1-3]. The incidence of CDI varies by country and between clinical settings, though the rate and severity of CDI has been increasing over the past decade in high-income countries[4,5]. CDI risk depends on patient characteristics, such as older age[6], and antibiotic exposure[7-9]. Symptoms of CDI range from mild diarrhea to more severe conditions, including pseudomembranous colitis and toxic megacolon[2]. Despite fairly successful treatment rates, approximately 18-20% of patients experience recurrence within 8 weeks after the first episode[3]. Based on Canadian data, the disease-attributable mortality rate is approximately 5.3-10% in endemic situations, and upwards of 17% in outbreak settings[1]. In the United States, the cost of treating CDI ranges from $8,911 to $30,049 per case for primary infection, and from $13,655 to $18,067 per case for CDI recurrences[10,11]. To reduce the CDI incidence, there has been an increased emphasis on infection prevention and control, with efforts for generating evidence as well as developing and adhering to clinical practice guidelines (CPGs)[12].

The aim of CPGs is to provide evidence-based recommendations for patient care[13]. A number of organizations have published guides for the development of CPGs (e.g. Institute of Medicine, World Health Organization, Scottish Intercollegiate Guidelines Network), however numerous studies have consistently shown that guideline recommendations often do not follow these criteria[14]. To assess the quality of the development and reporting of CPGs, the gold standard is the Appraisal of Guidelines for Research and Evaluation (AGREE) instrument, which has demonstrated validity and reliability[15]. Guideline development is an arduous process, and

the content and quality varies between CPGs on the same topic, particularly regarding the evidence collection and assessment, and formulation of recommendations[14,16].

Due to the morbidity, mortality and costs associated with CDI, the guidelines on its prevention and control, and the scientific evidence on which they are based, deserves close evaluation. The objectives of this study were to systematically identify and review the available CPGs on the prevention of CDI. We assessed the quality of CPG development and reporting, summarized the current recommendations, and evaluated the quality of the supporting evidence for each recommendation.

Methods

Literature search

Using a comprehensive search strategy developed with a librarian, we searched MEDLINE (1946-2015) and EMBASE (1974-2015), using subject terms and key words, up to January of 2015 (Supplementary Table 1S). In addition, we searched 10 grey literature sources: National Guidelines Clearinghouse (NGC; from the Agency for Healthcare Research and Quality in the United States, AHRQ), Turning Research into Practice (TRIP), Canadian Medical Association (CMA), National Institute for Health and Care Excellence (NICE), Scottish Intercollegiate Guidelines Network (SIGN), Guidelines International Network (GIN), and Google Scholar. Furthermore, we searched for relevant CPG on the websites of the Centre for Disease Control (CDC), European Centre for Disease Control (ECDC), American Gastroenterology Association (AGA), and the Institute for Clinical Systems Improvement (ICSI). Finally, we searched the bibliographies of the included studies. There were no language or publication status restrictions.

Study selection

We included studies that (1) were clinical practice guidelines, defined as documents developed by a nationally recognized committee, a publically funded institution, or medical society, that provide recommendations for the prevention of CDI, (2) contained an explicit methodology section outlining its development (e.g. definition of a search strategy, evidence quality assessment, method used to create recommendations), and (3) were 'de novo' publications, or the most recent version of the guideline. We excluded guidelines on prevention of hospital-acquired infections (HAIs) that were not exclusive to *C. difficile*. One reviewer (LL)

screened titles and abstracts, and potentially eligible full-text articles were retrieved. Using a standard form, two reviewers (LL, FA) independently screened the full-text studies for eligibility. Disagreements were resolved through consensus, and a third party methodologist (BCJ, DM) was consulted if needed.

Data extraction and quality assessment

Three reviewers (BS, FA, LL) independently extracted data from the included CPGs, using a standardized and pilot-tested extraction form. Prior to beginning data abstraction, reviewers conducted calibration exercises with methodology experts (AS, BCJ) to help ensure consistency and validity of abstraction between reviewers. We extracted guideline characteristics, including title, year, authors/organization(s), whether it is a novel publication or update, and the country of development. Using the AGREE II instrument, the same three reviewers independently rated guideline development and reporting based on 23 items across six domains: 1) scope and purpose, 2) stakeholder involvement, 3) rigor of development, 4) clarity of presentation, 5) applicability, and 6) editorial independence (Supplementary Table 2S)[17]. Each item was rated on a 7-point Likert scale, and inter-rater differences were discussed. Differences of three points for a given item were permitted. If not achieved, a third party methodologist (BCJ, DM) was consulted. An overall score of 1-7 was given to each guideline, and were categorized (recommended, recommended with modifications, or not recommended).

Quality appraisal of evidence used in guidelines

One reviewer (LL) extracted the recommendations for prevention and control of CDI, along with the strength of each recommendation, and the evidence cited to support each

recommendation, when available. Ten percent of recommendations, and their associated evidence, were randomly selected and checked by a second reviewer (BS). In three of the guidelines, articles referenced in the recommendation statement were extracted as reported by authors. For two guidelines, references were at the end of chapters[18] or from supplement text[19], thus two reviewers came to consensus as to which references were likely used for the specific recommendation, thus introducing some level of subjectivity. We used the Oxford Center for Evidence Based Medicine (OCEBM) Levels of Evidence to rate the quality of evidence of each citation supporting each recommendation[20] (Supplementary Table 4S), which we modified for study designs found in infection prevention and control (IPC) literature (Supplementary table 5S). Each study was extracted and rated from 1 to 5, where 1 represents the best methodological design (e.g. meta-analysis of randomized trials), and 5 represents the poorest design, (e.g. ecological studies). The design can be rated down due to study quality, imprecision, indirectness (study PICO does not match questions PICO), because of inconsistency between studies, or because the absolute effect size is very small, or graded up if there is a large or very large effect size[21].

Data analysis

Agreement for the full-text screening was measured using the Kappa statistic and associated 95% confidence interval (CI)[22]. For each guideline, we calculated the AGREE II score for each domain as a percentage of the maximum possible score for that domain, and its standardized range. A score of 60% was chosen as a threshold of acceptable quality, as found in previous literature[23]. For domains across all CPGs, we calculated the median score and the interquartile (IQR) range. Inter-rater agreement for AGREE II scores were calculated using the

intra-class correlation coefficient (ICC), with 95% confidence intervals (CI)[24]. Agreement of 0.01-0.20 was considered as poor, 0.21-0.40 as fair, 0.41-0.60 as moderate, 0.61-0.80 as substantial, and 0.81-1.00 as very good[25]. All analyses were conducted using Microsoft Excel 2013 (Redmond, Washington).

Results

Literature search

A total of 2,684 potentially eligible articles were identified through our primary database search, and 19 through the grey literature search. After removing duplicates, 2,578 articles were screened, of which 33 were selected for full-text review (Figure 1). Five CPGs were included in the final review (Kappa = 0.84; 95% CI 0.53-1.00). A third author was consulted to resolve a disagreement on one occasion. Of the excluded studies, 16 were not guidelines, six did not address prevention, four were previous versions of included guidelines, one was inaccessible, and one was a position statement regarding existing guidelines rather than an original document.

Guideline characteristics

The included CPGs were developed by the 1) American College of Gastroenterology (ACG)[26], 2) Association for Professionals in Infection Control and Epidemiology (APIC)[18], 3) European Society of Clinical Microbiology and Infectious Disease (ESCMID)[27], 4) United Kingdom Health Protection Agency/Department of Health (HPA/DH)[19], and 5) Society for Healthcare Epidemiology of America/Infectious Diseases Society of America (SHEA/IDSA)[28] (Table 1). The guidelines were published between 2008 and 2014. Although four of the five guidelines were an update from a previous version, two had updated treatment and management but not prevention information, thus we used the earlier version[19,27]. Three guidelines were from the United States[26,28,18], one from Europe (comprising 11 countries)[27,29], and one from the UK[19]. The overall reviewer's agreement for the evaluation with the AGREE II instrument was very good (ICC: 0.88; 95% CI 0.83-0.913). Authors resolved all disagreements amongst themselves.

Guideline recommendations

Guideline authors searched for general prevention-related literature, rather than proposing research questions and conducting formal systematic reviews. The median number of recommendations per guideline was 40 (range=9-67). None of the guidelines explained how the recommended strategies were selected. We reviewed 202 total recommendations related to prevention across guidelines, and authors with knowledge of infection prevention strategies (DM, FA, LL) discussed which key strategies and individual recommendations to include. We categorized the overall strategies as follows: (1) surveillance, (2) antibiotic stewardship, (3) hand hygiene, (4) patient isolation and personal equipment, (5) protective clothing, (6) environmental cleaning, (7) probiotics, and (8) staff, patient, and visitor education. We reported on 22 groups of key recommendations. When available, we listed each recommendation's 1) status: whether it was recommended, not recommended, or authors considered it to be unclear, 2) strength: based on system reported in the guideline methodology, 3) author-assessed evidence: based on system reported, and 4) reviewer-assessed evidence using the OCEBM levels: (Table 2).

Quality appraisal of underlying evidence

For the 22 recommendations, there were 76 guideline statements across the five CPGs, and 180 unique studies supporting them. The majority of recommendations referenced previously conducted strategy-specific reviews or guidelines (e.g. hand hygiene, isolation precautions). These reviews were not always systematic, and were published in 2007 or earlier, thus considered outdated for use in the three newer guidelines[18,26,28]. The majority of reviews (systematic and not systematic) and individual studies referenced consisted of before-after

studies, very few of which were controlled trials. Often, studies that implemented 'bundle' strategies (i.e. multiple interventions) and/or were conducted to control outbreaks were used to support individual strategies. We found only two randomized controlled trials (RCTs) with CDI incidence as an outcome. One assessed the impact of treatment of asymptomatic patients, and the other evaluated the use of reusable thermometers. The use of probiotics was the only preventive measure that was assessed in a meta-analysis of RCTs with CDI incidence as an outcome, based on more than 20 studies[30].

Quality appraisal of guidelines

Domain 1: Scope and Purpose

The median score for this domain was 74.1% (IQR 68.5-85.2%), indicating that approximately 74% of the criteria for scope and purpose were met. All guidelines met the threshold of 60% for this domain. Limitations included insufficient details about the population of interest, such as disease severity, comorbidities, and whether any populations were excluded. Strategies for management in situations with increased CDI incidence or outbreaks was reported in all guidelines, though to varying degrees.

Domain 2: Stakeholder Involvement

The median score for this domain was 40.7% (IQR 38.9-44.4%). None of the guidelines scored above 60%. Guideline author panels included professionals from many disciplines but did not describe each authors' role in the guideline development process. Furthermore, none of the guidelines sought values and preferences of the target population (e.g. advocacy groups).

Lastly, only HPA/DH explicitly defined target users (e.g. clinicians, trusts, clinical directors) and how they may use the guideline[19].

Domain 3: Rigor of Development

This was the lowest scoring domain, with a median of 18.1% (IQR 17.4-35.4%). None of the guidelines scored above 60%, and none of the guidelines outlined questions for their literature review. Only ESCMID had conducted a systematic search for evidence, although the selection criteria were not specified[27]. None of the guidelines reported how the recommendations were selected (e.g. Nominal Group Technique, Delphi Method, Consensus Conferences[31]), although SHEA/IDSA reports they were chosen "by consensus"[28]. All but APIC used an approach to assign a strength to their recommendation based on the evidence available[18]. Both ACG and SHEA/IDSA used a modified version of GRADE methods[26,28], ESCMID used a system by the Healthcare Infection Control Practices Advisory Committee (HICPAC)[27], and HPA/DH created a system[19] (Supplementary Table 2S). Only ESCMID provided a transparent account of their grading of the scientific literature, using the OCEBM system[27]. Guidelines did not report how the evidence affected their development of recommendations. However, SHEA/IDSA broadly mentioned the methodological issues in the literature and reported that despite lack of level 1 evidence, antibiotic stewardship was an essential recommendation[28]. In addition, HPA/DH provided a detailed list of research gaps that need to be addressed[19]. Finally, only SHEA/IDSA stated a procedure for updating the guideline[28].

Domain 4: Clarity of Presentation

This domain was well addressed by guidelines, with a median score of 75.9% (IQR 75.9-79.6%). The only guideline that did not meet the 60% threshold was the APIC guideline, which scored poorly because specific recommendations were not well outlined throughout the document[18].

Domain 5: Applicability

The median score for this domain was 43.1% (IQR 19.4-55.6%). None of the guidelines scored above 60% in this domain. The most common issue was failing to address the potential resource implications (e.g. costs) for guideline implementation, followed by few descriptions of facilitators and barriers to guideline implementation. However, SHEA/IDSA included a separate section regarding implementation strategies[28].

Domain 6: Editorial Independence

The median score for editorial independence was 63.9% (IQR 47.2-66.7%), with three of the guidelines meeting the 60% threshold[26-28]. Of the two guidelines that scored poorly, HPA/DH did not include any information on the competing interests of authors[19], and APIC had an industry sponsorship (cleaning agent) that we felt may have influenced the focus of the guideline[18].

Overall Evaluation

The overall median score for guidelines was 4 out of 7. One CPG was categorized as not recommended for use in prevention of CDI[26], and the other four were categorized as

"recommended, with modifications." A summary of limitations and actions to improve guideline quality can be found in Supplementary Table 6S.

Discussion

Major findings of this study

Among the five clinical practice guidelines identified, we found that although the recommendations were similar across guidelines, they were developed inconsistently, and each guideline had serious methodological limitations. Based on AGREE II guideline development standards, none of the guidelines met the quality thresholds for all six domains. The poorest scores were for rigor of development, stakeholder involvement and applicability, and insufficient links between recommendations and supporting evidence. Importantly, the CPGs were not transparent about how the limitations of the evidence impacted their recommendations, with a few exceptions[28].

The Rigor of Development domain was the lowest scoring domain across guidelines. Good-quality, trustworthy CPGs are contingent on clear research questions and a systematic review of the evidence[31]. None of the CPGs outlined their questions a priori, and only one guideline conducted a systematic review, though with limitations (no inclusion/exclusion criteria, no screening results reported)[27]. Guidelines frequently referenced existing reviews that were outdated, and did not utilize all of the evidence available to them before drafting recommendations. Quality assessment of evidence supporting recommendations was available in four CPGs, however it was transparent in only one[27]. Although recommendations were relatively consistent across guidelines, authors of all but one guideline[28] did a poor job reporting their consensus methodology. In addition, recommendations were mostly consistent across guidelines despite poor reporting (transparency) of evidence to recommendations, and incongruence between the quality of evidence and recommendations among all guidelines. For

example, strong recommendations were often made on low level evidence (Table 2), whereas the prevention strategy with the highest quality evidence, probiotics, were not recommended or deemed unresolved by the four guidelines. This may suggest that guideline panels depended on non-systematic, consensus-based methods to develop recommendations, and citing selected evidence as applicable.

The Applicability domain was also poorly addressed, particularly regarding costs and barriers/facilitators to implementation. However, one of the newest guidelines[28] had a very comprehensive strategy for CPG implementation, suggesting that panels are recognizing its importance. It is important to keep in mind, however, that guideline should be rigorously developed and trustworthy, before considering facilitating its application.

The Editorial Independence domain scored well, although none of the guidelines were led by a methodologist, as suggested by guidelines development experts[32]. There was a conflict of interest issue in one guideline we found, a CPG sponsored by Clorox, which is a company that makes sodium hypochlorite-based cleaning solutions[18]. We suspect that this sponsorship may have influenced the guideline recommendations, as they dedicated the majority of the guideline to discussing cleaning strategies cantered around hypochlorite solutions, whereas the SHEA/IDSA guideline reported this as an area of controversy[28].

In an evaluation of the underlying evidence behind the recommendations, we found three major limitations. First, the majority of infection prevention and control literature were quasi-experimental studies, which are prone to a number of potential biases, including maturation effects, selection bias, and confounding[33]. Second, interventions were often

conducted during outbreaks, which are vulnerable to regression to the mean artefacts[34]. Third, it was common to implement 'bundle' strategies, i.e. multiple interventions. While conducting such a study is sometimes the only feasible option[33], it is invalid to extrapolate the effectiveness of an individual strategy based on these studies, which was a common issue among guideline recommendations. Importantly, we found that none of the guidelines discussed how the limitations of the body of evidence impacted the decisions of assigning strengths of recommendations.

Previous work on this topic

There are a number of handbooks on the development of CPGs, which provide guidance on establishing transparency, management of conflict of interest, group composition, systematically reviewing evidence, rating and articulating recommendations, external review, and updating the CPG[35]. Despite the availability of these handbooks, they are not often followed by guideline development groups across numerous disease areas[36].

To our knowledge, this is the only critical appraisal of infection prevention and control CPGs. Previous guideline reviews of other disease areas have reported similar limitations, particularly in rigor of development, applicability, and editorial independence[16,37]. Notably, other guideline reviews have also remarked on the similarity of the recommendations made by guidelines despite considerably different methodologies[38]. A possible reason for this may be that CPGs are still reliant on expert-based recommendations, which are then supported by selective evidence rather than based on a systematic search for evidence. The current gold

standard for recommendation development, the GRADE approach, was only used in two guidelines, and was considerably modified in both[26,28] (Supplementary Table 2S).

Two previous reviews on CDI prevention and control studies have commented on similar limitations of the available literature, such as the lack of RCTs and controlled time-series designs, as well as the tendency to implement multiple strategies to control outbreaks[39,40]. However, in the absence of high-quality evidence, poor or indirect evidence should still be used, and authors should be transparent about these limitations and how this impacted recommendation development. It has been suggested that when there is poor quality evidence, this is where clinicians need guidance most from CPGs[31]. A novel decision support tool to assist guideline developers to systematically and transparently develop recommendation from available evidence has been proposed[41].

Strengths and limitations

Our study had some limitations. Firstly, while AGREE II is a robust guideline appraisal instrument[42], the quality might have been underestimated due to incomplete reporting of methods. However, there is universal agreement that transparent reporting of methodology is key for creating trustworthy guidelines[43]. Secondly, we used the OCEBM Levels of Evidence instrument to rate the evidence for each recommendation, however this is a crude measure, limited due to frequent variability in quality across similar study designs. We attempted to account for this by modifying ratings to accommodate the types of quasi-experimental studies encountered. For example, we considered that an interrupted time series (ITS) study with a historical control was a level 3 study, whereas a prospective ITS with a concurrent control group

was level 2. Thirdly, we only checked 10% of data for the recommendations (8/77 individual CPG recommendations from the 22 consorted topics), however the second reviewer did not find differences in the extractions, thus we feel confident in our methodological approach.

Our study also had several strengths. First, we conducted a comprehensive search, including both medical databases and 10 grey literature sources. Second, three reviewers appraised each guideline, each with either methodology expertise or clinical expertise, and the team had high concordance in AGREE II scores. Third, we analysed the cited evidence underlying each recommendation, which has rarely been evaluated for CPGs[44].

Conclusion

There is a considerable need for high quality CPGs, as guidelines are often used to guide patient care. Research has suggested that CPGs may reduce inappropriate practices, bridge the gap of research and clinical application, and improve the overall quality and safety of healthcare services[31]. Future guidelines of CDI prevention should be developed using validated methodological standards. Furthermore, there is a need for higher quality primary research on this topic in order to better inform recommendations.

Funding sources/sponsors

This study was unfunded.

Conflicts of interest

The authors have no known conflicts of interest to declare.

Figures

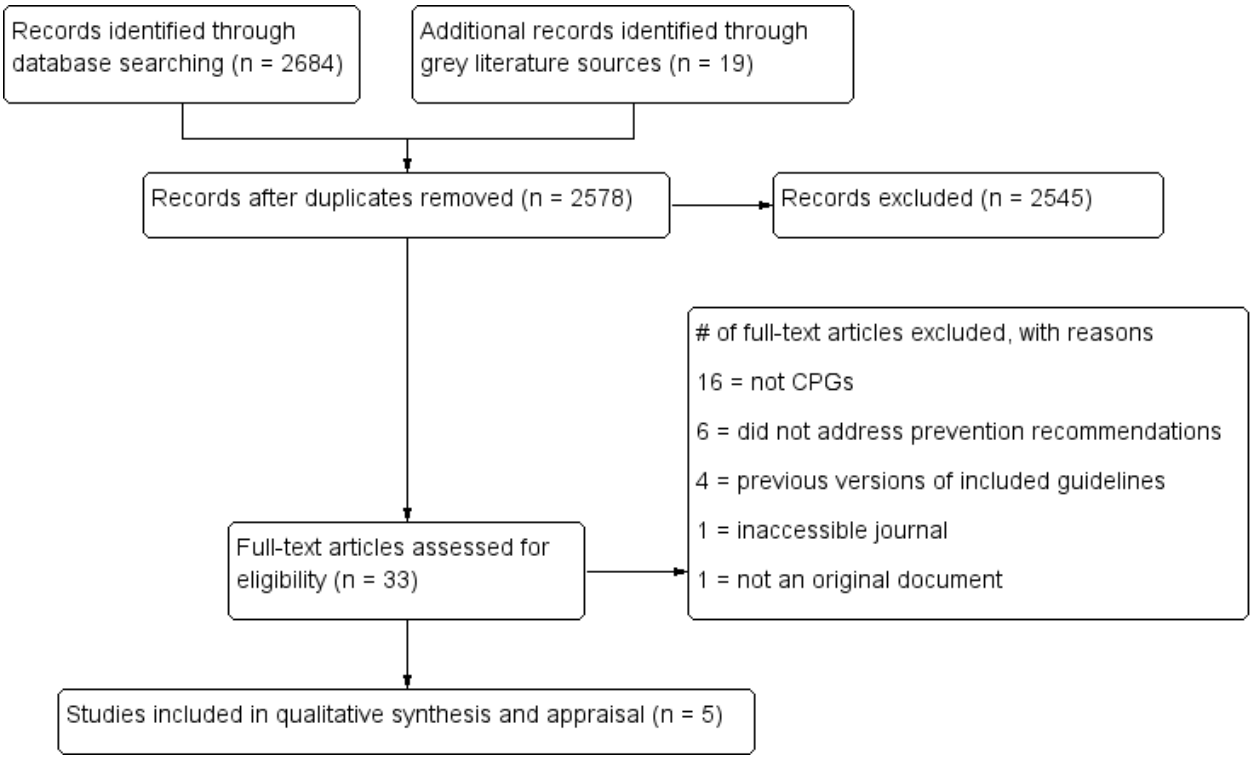

Figure 1. PRISMA study flow diagram.

Tables

Table 1. Characteristics, recommendations and quality assessment across guidelines					
GUIDELINES	**ACG (2013)**	**APIC (2013)**	**ESCMID (2009)**	**HPA/DH (2008)**	**SHEA/IDSA (2014)**
Organization(s)	ACG	APIC	ECDC, ESCMID	NHS, PHE	AHA, APIC, IDSA, SHEA
Country	United States	United States	Europe	United Kingdom	United States
Source of funding	None	Industry	No statement	No statement	National agency
Novel publication or update	Novel	Update	Novel*	Novel*	Update
Number of recommendations	9	19	40	93	25

Acronyms: ACG = American College of Gastroenterology; AHA = American Hospital Association; APIC = Association of Professionals in Infection Control and Epidemiology; DH = Department of Health; ECDC = European Centre for Disease Control and other collaborators; EPA = Environmental Protection Agency; ESCMID = European Society for Clinical Microbiology and Infectious Diseases; HPA = Health Protection Agency; IDSA = Infectious Diseases Society of America; NHS = National Health Service; PHE = Public Health England; SHEA = Society for Healthcare Epidemiology of America.

Notes: * = Has been updated, however update does not include new information on prevention

Table 2. Recommendations across guidelines, their associated strength, and evidence assessment by authors and by study reviewers.

	AJG 2013				APIC 2013		ESCMID 2009				HPA/DH 2008			SHEA/IDSA 2014			
RECOMMENDATION	**I**	**SR**	**E**	**L**	**I**	**L**	**I**	**SR**	**E**	**L**	**I**	**SR/ E**	**L**	**I**	**SR**	**E**	**L**
Educate HCPs, staff, patients, and their families on CDI	-	-	-	-	✓	2,3,4,5	✓	IA	1a,2b ,4,5	4	✓	B	3	✓	1	III	2,3,4
Only test diarrheal patients for *C. difficile*, unless ileus present	✓	S	H[1]	4,5	-	-	✓	IB	2b,3b ,4	4,5	✓	B	-	✓	3	II	5
Do not repeat testing, unless recurrence is suspected	-	-	-	-	-	-	✓	IB	3b,4	4,5	✓	B	-	✓	3	III	-
Determine baseline rate and threshold to identify high incidence	-	-	-	-	✓	3,5	✓	IB	2b,2c	4,5	✓	B	4	✓	1	III	3,4
***Store fecal samples from CDI cases for typing; compare isolates**	-	-	-	-	-	-	✓	IB	1b,3b ,4	5	✓[2]	C	5	-	-	-	-
Use antimicrobial stewardship; monitor CDI patients' antibiotics	✓	S	H	3,4, 5	✓	3,4,5	✓	IB	1a,2b ,3b,4	2,3, 4	✓	B	2,3,4, 5	✓	1	II	2,3,4, 5
***Minimize prescription of high-risk antimicrobials**	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	2	II	2,4
Use alcohol based hand rubs	-	-	-	-	✓	3,4,5	X[3]	IB	2b,2c	4,5	X	B	3,4,5	✓	1[4]	III	3,4,5
Use soap and water	-	-	-	-	✓	3,4,5	✓	IB	2a,2b ,2c,4	3,4, 5	✓	A	3,5	✓	1	III	3,4,5
***Use soap and water only**	-	-	-	-	✓	3,4,5	-	-	-	-	-	-	-	✓	2	III	-
Suspected or known CDI patients should be in a private room or with other CDI patients	✓	S	H	5	✓	2,4,5	✓	IB	1b,2b ,4	3,4	✓	B	5	✓	1	III	-

Isolation can be discontinued 48 hours after symptoms resolve	-	-	-	-	-	-	✓	II	4	4,5	✓	C	5	✓	1	III	5
***Isolate all patients with diarrhea while awaiting test result**	-	-	-	-	✓	4,5	-	-	-	-	✓	B	5	✓	2	III	5
***Consider isolating CDI patient until discharge**	-	-	-	-	✓	5	-	-	-	-	-	-	-	✓	2	III	-
***Cohorted patients should be managed by designated staff**	-	-	-	-	✓	-	✓	IB	1b,4	3,4	-	-	-	-	-	-	-
Use disposable equipment; dedicate non-disposable equipment	✓	S	M	2[5]	✓	3	✓	IA[5],IB	1b,2b,2c,4	2[5],3,4,5	-[6]	-	-	✓	1	III	3,5
Gloves and gowns for staff of known or suspected CDI patient	✓	S	M	3[7]	✓	3,4,5	✓	IB	1a,1b,2b,4	3,4,5	✓	B	-	✓	1	II[7], III	3,4
Gloves and gowns for visitors of known or suspected CDI patient	✓	S	M	3[7]	✓	2,4,5	-	-	-	-	✓	A[8]	-	U	-	-	2
Use EPA registered disinfectant with *C. difficile*-sporicidal label claim or 1,000 ppm chlorine-containing cleaning agents	✓[9]	S	H	3,4,5	✓	2,3,4,5	✓	IB	2b,2c,4	3,4,5	✓	B	3,4,5	✓[10]	2	III	4
***Use bleach solution for daily disinfection and discharge cleaning**	-	-	-	-	✓	2,3,4,5	-	-	-	-	✓	B	3,4,5	U	2	III	4
***Use of alternate methods of disinfection (ultraviolet light, HPV)**	-	-	-	-	✓	3,4,5	-	-	-	-	✓	B	4	U	-	-	3,4,5
Use probiotics for prophylaxis	X	S	L	2	-	-	U	-	-	1,2	X	-	1,2	U	-	-	1,2

Acronyms: ACG = American College of Gastroenterology; AHA = American Hospital Association; APIC = Association of Professionals in Infection Control and Epidemiology; DH = Department of Health; E = Evidence (assigned by guideline authors); ECDC = European Centre for Disease Control and other collaborators; EPA = Environmental Protection Agency; ESCMID = European Society for Clinical Microbiology and Infectious Diseases; H = High quality of evidence; HPA = Health Protection Agency; I = Inclusion of recommendation; IDSA = Infectious Diseases Society of America; L = Oxford Centre for Evidence Based Medicine Level (assigned by reviewers); Lo = Low quality of evidence; M = Moderate quality of evidence; NHS = National Health Service; PHE = Public Health England; S = Strong recommendation; SHEA = Society for Healthcare Epidemiology of America; SR = Strength of recommendation.

Notes: ✓ = Recommended; X = Not recommended; U = Unclear; - = Not mentioned; * = Recommendation specific for a high incidence/outbreak environment. The APIC 2013 guideline did not assign a strength to each recommendation, nor did the authors assign evidence quality for each recommendation; thus, these were omitted. The HPA/DH guideline had a joint measure of evaluating both the strength and evidence assessment; thus, these are combined. 1 = Authors combined recommendation for not screening (OCEBM level 4 and 5) with not treating asymptomatic patients (OCEBM level 2); 2 = Storage of fecal samples in non-outbreak settings is recommended; 3 = ABHR should not be the only hand hygiene measure; 4 = Considered an area of controversy; 5 = Referring to disposable thermometers only; 6 = No specific recommendation, however does discuss that environmental contamination has been linked to spread of *C. difficile* via personal equipment, and also that replacing electronic thermometers with single-use disposable thermometers has been associated with significant reductions in CDI; 7 = Referring to gloves only; 8 = Part of combined recommendation of glove/apron use and handwashing; likely the higher evidence grade is for handwashing; 9 = Recommends 5,000 ppm or greater; 10 = Data are conflicting as to whether inactivation of spores is necessary to prevent *C. difficile* transmission, especially in an endemic setting.

Table 3. Methodological quality of included guidelines: AGREE II domain-standardized scores.

AGREE Domain	ACG 2013	APIC 2013	ESCMID 2009	HPA/DH 2008	SHEA/IDSA 2014
Scope and Purpose (%)	63.0	85.2	68.5	85.2	74.1
Stakeholder Involvement (%)	38.9	27.8	40.7	44.4	50.0
Rigor of Development (%)	18.1	15.3	48.6	17.4	35.4
Clarity of Presentation (%)	75.9	53.7	88.9	79.6	75.9
Applicability (%)	4.2	58.3	19.4	55.6	43.1
Editorial Independence (%)	77.8	47.2	63.9	30.6	66.7
Overall recommendation	NR	RWM	RWM	RWM	RWM

Acronyms: ACG = American College of Gastroenterology; APIC = Association of Professionals in Infection Control and Epidemiology; DH = Department of Health; ECDC = European Centre for Disease Control and other collaborators; ESCMID = European Society for Clinical Microbiology and Infectious Diseases; HPA = Health Protection Agency; IDSA = Infectious Diseases Society of America; NR = Not recommended; PHE = Public Health England; RWM = Recommended, with modifications; SHEA = Society for Healthcare Epidemiology of America.

Supplementary tables

Table 1S. MEDLINE Search strategy (1946-January 13 2015).

#	Searches	Results
1	exp *Clostridium difficile*/	5528
2	exp Enterocolitis, pseudomembranous/	6388
3	Clostridium diff*.mp.	9874
4	C diff*.mp.	5470
5	CDAD.mp.	598
6	or/1-5	13979
7	exp Practice Guideline/	19541
8	exp Practice Guidelines as Topic/	82427
9	Guideline*.mp.	289332
10	Guidance*.mp.	66440
11	Recommend*.mp.	417330
12	(polic* adj5 (statement* or document* or development*)).mp.	11030
13	(consensus adj5 (statement* or document* or development*)).mp.	16449
14	(Polic* adj5 statement*).mp.	1831
15	(Polic* adj5 document*).mp.	1560
16	(Polic* adj5 development).mp.	6718
17	(Polic* adj5 paper*).mp.	1822
18	(Consens?s adj5 statement*).mp.	4316
19	(Consens?s adj5 document*).mp.	1494
20	(Consens?s adj5 development*).mp.	13016
21	(Consens?s adj5 paper*).mp.	604
22	or/7-21	711495
23	6 and 22	720

Table 2S. AGREE II Instrument	
Domain	**Item**
Scope and purpose	1. The overall objective(s) of the guideline is (are) specifically described.
	2. The health question(s) covered by the guideline is (are) specifically described.
	3. The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described.
Stakeholder involvement	4. The guideline development group includes individuals from all the relevant professional groups.
	5. The views and preferences of the target population (patients, public, etc.) have been sought.
	6. The target users of the guideline are clearly defined.
Rigor of development	7. Systematic methods were used to search for evidence.
	8. The criteria for selecting the evidence are clearly described.
	9. The strengths and limitations of the body of evidence are clearly described.
	10. The methods for formulating the recommendations are clearly described.
	11. The health benefits, side effects and risks have been considered in formulating the recommendations.
	12. There is an explicit link between the recommendations and the supporting evidence.
	13. The guideline has been externally reviewed by experts prior to its publication.
	14. A procedure for updating the guideline is provided.
Clarity of presentation	15. The recommendations are specific and unambiguous.
	16. The different options for management of the condition or health issue are clearly presented.
	17. Key recommendations are easily identifiable.
Applicability	18. The guideline describes facilitators and barriers to its application.
	19. The guideline provides advice and/or tools on how the recommendations can be put into practice.
	20. The potential resource implications of applying the recommendations have been considered.
	21. The guideline presents monitoring and/ or auditing criteria.

Table 2S. AGREE II Instrument	
Domain	**Item**
Editorial independence	22. The views of the funding body have not influenced the content of the guideline.
	23. Competing interests of guideline development group members have been recorded and addressed.
Overall Guideline Assessment	1. Rate the overall quality of this guideline.
Overall Guideline Assessment	2. I would recommend this guideline for use.

Table 3S. Systems of evidence review and recommendation development used in guidelines		
Guideline	**System for summarizing evidence**	**System for assigning strength to recommendation**
American Journal of Gastroenterology	Modified GRADE High: if further research is unlikely to change our confidence in the estimate of the effect Moderate: if further research is likely to have an important impact and may change the estimate Low: if further research is very likely to change the estimate	Modified GRADE Strong: when the evidence shows the benefit of the intervention or treatment clearly outweighs any risk Conditional: when uncertainty exists about the risk – benefit ratio
Association of Professionals in Infection Control and Epidemiology	None	None
European Society for Clinical Microbiology and Infectious Diseases	OCEBM Levels of Evidence (2008) Level 1a: Systematic review (with homogeneity) of randomised controlled trials Level 1b: Individual randomised controlled trial (with narrow confidence interval) Level 1c: Studies with the outcome 'All or none' Level 2a: Systematic review (with homogeneity) of cohort studies Level 2b: Individual cohort study (including low-quality randomised controlled trials; e.g., <80% follow-up) Level 2c: 'Outcomes' research; ecological studies Level 3a: Systematic review (with homogeneity) of case–control studies Level 3b: Individual case–control study Level 4: Case series (and poor quality cohort and case–control studies)	HICPAC categories for implementation IA: Strongly recommended for implementation and strongly supported by well-designed experimental, clinical or epidemiological studies IB: Strongly recommended for implementation and strongly supported by some experimental, clinical or epidemiological studies and a strong theoretical rationale IC: Required for implementation, as mandated by federal and/or state regulation or standard (may vary among different states/countries) II: Suggested for implementation and supported by suggestive clinical or epidemiological studies or a theoretical rationale Unresolved issue: Practices for which insufficient

	Level 5: Expert opinion without explicit critical appraisal, or based on physiology, bench research or 'first principles'	evidence exists or no consensus regarding efficacy exists (no recommendation)
Department of Health, Health Protection Agency	Own system; combined evidence and recommendation A: Strongly recommended and supported by systematic review of randomised controlled trials (RCTs) or individual RCTs B: Strongly recommended and supported by non-RCT studies and/or by clinical governance reports and/or the Code C: Recommended and supported by group consensus and/or strong theoretical rationale	
Infectious Diseases Society of America, Society for Healthcare Epidemiology of America	Modified GRADE I. High: Highly confident that the true effect lies close to that of the estimated size and direction of the effect. Evidence is rated as high quality when there is a wide range of studies with no major limitations, there is little variation between studies, and the summary estimate has a narrow confidence interval. II. Moderate The true effect is likely to be close to the estimated size and direction of the effect, but there is a possibility that it is substantially different. Evidence is rated as moderate quality when there are only a few studies and some have limitations but not major flaws, there is some variation between studies, or the confidence interval of the summary estimate is wide. III. Low The true effect may be substantially different from the estimated size and direction of the effect. Evidence is rated as low quality when supporting studies have major flaws, there is important variation between studies, the confidence interval of the summary estimate is very wide, or there are no rigorous studies, only expert consensus.	Own system (1) Basic practices: should be adopted by all acute care hospitals; potential to impact HAI risk clearly outweighs the potential for undesirable effects (2) Special approaches: can be considered for use in locations and/or populations within hospitals when HAIs are not controlled by use of basic practices; the intervention is likely to reduce HAI risk but where there is concern about the risks for undesirable outcomes, where the quality of evidence is low, or where evidence supports the impact of the intervention in select settings (eg, during outbreaks) or for select patient populations (3) Approaches that should not be considered a routine part of CDI prevention

Table 4S. Rating evidence using the OCEBM system.					
Question	**Step 1 (Level 1*)**	**Step 2 (Level 2*)**	**Step 3 (Level 3*)**	**Step 4 (Level 4*)**	**Step 5 (Level 5*)**
How common is the problem?	Local and current random sample surveys (or censuses)	Systematic review of surveys that allow matching to local circumstances**	Local non-random sample**	Case-series**	n/a
Is this diagnostic or monitoring test accurate? (Diagnosis)	Systematic review of cross sectional studies with consistently applied reference standard and blinding	Individual cross sectional studies with consistently applied reference standard and blinding	Non-consecutive studies, or studies without consistently applied reference standards**	Case-control studies, or "poor or non-independent reference standard**	Mechanism-based reasoning
What will happen if we do not add a therapy? (Prognosis)	Systematic review of inception cohort studies	Inception cohort studies	Cohort study or control arm of randomized trial*	Case-series or casecontrol studies, or poor quality prognostic cohort study**	n/a
Does this intervention help? (Treatment Benefits)	Systematic review of randomized trials or n-of-1 trials	Randomized trial or observational study with dramatic effect	Non-randomized controlled cohort/follow-up study**	Case-series, case-control studies, or historically controlled studies**	Mechanism-based reasoning
What are the COMMON harms? (Treatment Harms)	Systematic review of randomized trials, systematic review of nested case-control studies, nof-1 trial with the	Individual randomized trial or (exceptionally) observational study with dramatic effect	Non-randomized controlled cohort/follow-up study (post-marketing surveillance) provided there are	Case-series, case-control, or historically controlled studies**	Mechanism-based reasoning

	patient you are raising the question about, or observational study with dramatic effect		sufficient numbers to rule out a common harm. (For long-term harms the duration of follow-up must be sufficient.)**		
What are the RARE harms? (Treatment Harms)	Systematic review of randomized trials or n-of-1 trial	Randomized trial or (exceptionally) observational study with dramatic effect			
Is this (early detection) test worthwhile? (Screening)	Systematic review of randomized trials	Randomized tria	Non - randomized controlled cohort/follow-up study**	Case-series, case-control, or historically controlled studies**	Mechanism-based reasoning

* Level may be graded down on the basis of study quality, imprecision, indirectness (study PICO does not match questions PICO), because of inconsistency between studies, or because the absolute effect size is very small; Level may be graded up if there is a large or very large effect size.

** As always, a systematic review is generally better than an individual study.

Table 5S. Hierarchy of Infection Prevention and Control Research.	
Study design	**Level**
Systematic review of RCTs	1
Systematic review of observational studies (all kinds)	2
RCT (including cluster RCT)	2
ITS, control group	2
Non-systematic review	3 or 4
Non-randomized cross-over control	3
Before after, control group	3
ITS, historical control	3
Before after study, historical control	4
Case control study; must be related to recommendation	4
Diagnosis or prevalence study; must be related to recommendation	4
Case review	4
RCT or ITS with control, but with a surrogate outcome	4
Ecological study (e.g. bacterial sampling); studies that do not have CDI outcome as result (i.e. make recommendations based on indirect evidence), regardless of the design or quality of the study	5
Not relevant, e.g. study does not involve CDI or prevention of CDI even indirectly	5

Acronyms: ITS = Interrupted time series; RCT = Randomized controlled trial.

Notes: A study conducted during an outbreak will be downgraded one level, but not lower than 4. An observational study with a large effect will be upgraded one level, but not if it is conducted during an outbreak or if it's a before-after study.

Table 6S. Limitations and actions to improve guideline quality.		
Guideline	**Key limitations**	**Actions to improve next update**
All guidelines	Guideline authors' contributions to the guideline are not discussed	Outline the role of each author in the guideline development panel
	No views and preferences sought of target population	Engage with patient advocacy groups
	Limited or no systematic search for evidence, and selection criteria for studies (except Vonberg et al 2009)	Conduct a formal systematic review to find all available evidence
	Limited or no description of strengths and limitations of evidence body and formal method of assigning strengths of recommendations	Adopt systematic method of guideline development, preferably GRADE
	Limited discussion of health benefits, side effects, and risks of recommendations	Present details of discussions regarding benefits and harms during development of recommendations
	The link between evidence and recommendations is not explicit	Be transparent about the quality of evidence used to support recommendations, and discuss the authors' confidence regarding the potential impact that future research may have on recommendation; limit drawing conclusions about the effectiveness of single strategies from studies that implemented bundle strategies
	No procedure for updating the guideline (except for Dubberke et al 2014)	Define criteria for updating guidelines, such as number of years of if large studies are published that may change current recommendations
	Guidelines have a limited discussion on how to disseminate the guideline, and do not discuss potential barriers to its implementation	Obtain feedback from key stakeholders
	Limited discussion of resource implications of implementing guidelines	Conduct cost effectiveness analysis; if resources are limited, discuss previously conducted cost effectiveness analyses on relevant recommendations
AJG 2013	Guideline was not peer reviewed prior to publication	See Hawkey 2008
	No advice or tools on how to put	Include an implementation section

	recommendations into practice	to the guideline, with tools such as checklists, how-to manuals, etc.
	No monitoring or auditing criteria for assessing the effect of the guideline have been described	Include a section on criteria to assess the implementation of guidelines, description of what and how often should be measured, etc.
APIC 2013	Target users of guideline are not clearly defined	Specify which recommendations apply to which users
	Key recommendations are not easily identifiable	Summarize key recommendations in a single, clearly specified table
	Views of funding body may have influenced the guideline	Be transparent about what influence the sponsor may have had on guideline development and reporting
ESCMID 2009	Limited monitoring or auditing criteria for assessing the effect of the guideline have been described	See Surawitz 2013
	The recent guideline, published in 2014, only updated the treatment section, and additional research has been published on the subject	See Hawkey 2008
HPA/DH 2008	Guideline was not peer reviewed prior to publication	Conduct formal peer review, including the description of reviewers, their suggestions, and how their advice was used (if at all) in further development
	None of the authors listed competing interests	For each author, list all potential financial and other conflicts of interest
	The recent guideline, published in 2013, only updated the treatment section, and additional research has been published on the subject	Include a review of prevention strategies to update recommendations
SHEA/IDSA 2014	See advice in “all guidelines”	

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PROBIOTICS FOR THE PREVENTION OF *CLOSTRIDIUM DIFFICILE*-INFECTION IN ADULTS AND CHILDREN: AN INDIVIDUAL PATIENT DATA META-ANALYSIS

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Keywords: probiotics, antibiotics, *Clostridium difficile,* individual patient data meta-analysis

Abstract

Background/Objectives: Antibiotics are the most commonly associated risk factor with *Clostridium difficile* infection (CDI). A recent systematic review and meta-analysis found that probiotics, taken concurrently with antibiotics, reduce CDI risk by 64%. We conducted an individual participant data meta-analysis (IPDMA) to examine the treatment effect given CDI risk factors.

Methods: We searched for randomized trials investigating probiotics (any species, any strain, any dose) compared to placebo, alternative prophylaxis, or no treatment control, for prevention of CDI. We used the results from a previously conducted comprehensive search of PubMed, EMBASE, CENTRAL, CINAHL, AMED, and ISI Web of Science (database inception until February 2013), as well as grey literature. In September 2013 we searched PubMed (January-September 2013) and ClinicalTrials.gov for additional studies. We contacted at least two authors of eligible studies inviting them to collaborate and share their data. The primary outcome was CDI, and the secondary outcome was serious adverse events (SAEs). Risk of bias of individual studies and evaluation of the overall certainty in the estimates of effect was conducted by one reviewer and checked by a second reviewer. We pooled IPD across trials using a generalized linear mixed model (GLMM), where study level was a random effect, and participant variables were fixed effects. We created an adjusted model controlling for age, sex, hospitalization status, and exposure to high risk antibiotics. Adjusted subgroup analyses were conducted on CDI control group event rate, single- versus multi-species probiotics, and probiotic dose. Sensitivity analyses were conducted to test the robustness of the effect estimate by comparing to aggregate data estimates, categorization of age groups, and fixed-

effects meta-analyses (generalized estimating equations [GEE]). Results were reported as odds ratios (OR) and associated 95% confidence intervals (CIs).

Results: We identified 32 potentially eligible trials, of which 15 agreed to share their data. One study is currently pending data transfer. Among 14 included studies (n=3,222 participants), probiotics reduced the odds of CDI (1.4% versus 4.0%; OR 0.27; 95% CI 0.17 to 0.45; p<0.0001). This effect was similar in the adjusted model of 10 studies (n=2,001) controlling for baseline covariates (1.7% versus 5.2%; OR 0.24; 95% CI 0.13 to 0.42; p<0.0001). None of the covariates were significantly associated with CDI. Control group event rate was not an interaction with group effects (p=0.09). We found a multi-species and dose response. Compared to no probiotics, multi-species probiotics (OR 0.14; 95% CI 0.06-0.32; p<0.0001) are more beneficial than single-species probiotics (OR 0.44; 95%CI 0.20-0.97; p=0.04) for reducing CDI. A one billion colony forming units/day increase in dose significantly reduced the odds of CDI (OR 0.97; 95%CI 0.96-0.98; p<0.0001). The IPDMA estimates were robust to all sensitivity analyses. Among 12 studies (n=2,063), probiotics did not affect the odds of SAEs (3.9% versus 3.1%; OR 1.31; 95% CI 0.80 to 2.12; p=0.28). None of the SAEs were reported to have been attributable to probiotics. This effect was similar in the adjusted model of 9 studies (n=1,867) controlling for baseline covariates (3.2% versus 3.1%; OR 0.24; 95% CI 0.13 to 0.42; p<0.0001). Age was significantly associated with SAEs (OR 1.07; 95% CI 1.04-1.10; p<0.0001). For both CDI and SAEs, estimates from obtained and not obtained studies were similar. The certainty in the estimates of effect of both outcomes was moderate, due to imprecision arising from low event rates.

Conclusions: In our preliminary analysis, probiotic prophylaxis was found to be a useful and safe infection prevention strategy for CDI, independent of participant age, sex, hospitalization

status, and exposure to high risk antibiotics. However, we will be conducting further analyses, including looking at additional confounders and the effect of missing participant outcome data with the addition of a new study (n=2,941).

Introduction

Clostridium difficile infection (CDI) is the leading cause of hospital-associated diarrhea, with an increasing incidence of community-acquired cases[1]. Globally, the incidence of CDI varies, with the majority of cases in higher income countries[2]. Surveillance data suggests the incidence density ranges between 2.45 to 7.5 per 10,000 patient days, or 9 to 80 per 10,000 patient admissions, with higher rates in outbreak settings[3-6]. An individual patients risk of CDI differs based on a number of patient factors[3,4,7,8]. The most commonly associated risk factor is antibiotic exposure, which is thought to disrupt the intestinal microflora, allowing *C. difficile* bacteria to proliferate[3]. Diarrhea is the most common presentation, however CDI may cause pseudomembranous colitis, toxic megacolon, and death[4,9]. Mortality ranges from 5-10%, though may be higher in outbreak settings[9]. The high rate of recurrence, affecting approximately 20% of treated patients, is a particular challenge in CDI management[10].

Probiotics -live microbial preparations that may provide benefit when taken in sufficient quantities - are a potential infection prevention strategy[11]. Although moderate quality evidence exists suggesting the safety and efficacy of probiotics for CDI prevention, a review of clinical practice guidelines (CPGs) on CDI prevention indicates that none recommend probiotics as a prevention strategy.[12-16] For example, a recent meta-analysis of 23 randomized controlled trials (RCTs) demonstrated a 64% decrease (95% CI 49-73%) in primary CDI rates with the administration of probiotics[17]. While the majority of trials (20/23) showed a benefit with probiotics, only 3/20 had statistically significant results. Reasons for not recommending probiotics have been cited as insufficient evidence[14,18], too much weight given to studies with high baseline CDI incidence[13], and concerns about safety[13,18]. Furthermore, the systematic

review conducted subgroup analysis to examine the effectiveness of probiotics on different participant populations, these could not be fully explored with aggregate data. Lastly, there is no guidance regarding the type and dose of probiotics on the overall efficacy.

Our objectives were to determine in an individual-patient meta-analysis whether adding probiotics to an antibiotic regime reduces the incidence of CDI compared to placebo, alternative prophylaxis or no treatment (standard care) among children and adults, when adjusting for age, length of hospitalization, type of antibiotics, length of antibiotic treatment, multi-species versus single-species probiotics, and probiotic dose.

Methods

Study and patient eligibility criteria

We conducted our search in two stages. First, we used the results from a comprehensive search strategy from a recently published systematic review on probiotics for the prevention of CDI and *C. difficile* incidence[17]. The search strategy for this review was conducted up until February 21, 2013. An example of the full electronic search strategy for EMBASE can be found in Appendix 1. Second, in September 2013 we searched PubMed (January-September 2013) and ClinicalTrials.gov for additional studies. Our study level inclusion criteria were children (0 to <18 years) or adults (≥18 years) who were administered antibiotics, and randomized to concomitantly receive probiotics (any dose, any species, any strain), compared to placebo, alternative prophylaxis, or no treatment (standard care) control, and that reported on CDI. There was no restriction on language or publication status.

Our primary outcome was CDI, defined as laboratory confirmation of *C. difficile* (e.g. cytotoxin assay, nucleic acid amplification, or toxigenic culture) with diarrhea, or presence of pseudomembranes on sigmoidoscopy/colonoscopy, or histological diagnosis of *C. difficile*, or diagnosis of toxic megacolon[19]. Of note, for studies included in the previous review that reported on *C. difficile* incidence, i.e. a positive test for *C. difficile* regardless of symptoms, we contacted them to clarify their eligibility[20]. Our secondary outcome was the incidence of serious adverse events (SAEs). We used author-reported SAEs, when available. If they were not reported in the study, we asked them for SAE data based on the National Institute of Health criteria, referred to as Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, to standardize terminology[21]. We considered all deaths as serious adverse events.

For each potentially eligible study, we contacted at least two authors, each on at least three occasions, by email and phone, between October 2013 and December 2014. If a response was obtained sooner, we ceased additional contact attempts. We discussed the eligibility of their study and asked whether they would share their anonymized individual participant data (IPD) and join the collaboration. We requested IPD that was de-identified and to include participants' allocated treatment, date of birth and admission date or age, admission and discharge dates or total length of hospital stay, CDI history, antibiotics given (type[s], duration of administration), probiotics given (specie, dose[s], and duration of administration), diarrhea diagnosis, CDI diagnosis, and SAEs. We also requested that authors include any information of missing participant outcome data. For one study, we received case report forms, which were extracted by one reviewer (LL) and checked by a second reviewer (LW)[22].

Quality assessment

Risk of bias was assessed for all included individual trials as described in the Cochrane Handbook for Systematic Reviews of Interventions[23]. Risk of bias factors assessed were sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, missing participant outcome data, selective outcome reporting, and other sources of bias (e.g. distribution of baseline characteristics, industry initiation and funding). All studies included in this review were included in the previous review, thus we used the previous assessment[17], with six modifications. First, the previous study considered all adverse events, whereas we are only considering SAEs. Thus, for SAEs, risk of bias due to inadequate blinding was considered low, as our primary outcome was considered an objective outcome for which lack of blinding was unlikely to have an effect[24]. Second, for studies where

new outcomes (i.e. CDI, SAE) became available after IPD requests, judgements for those outcome-specific domains were added. Third, if there was considerable discrepancy between published results and IPD that resulted in less data and was not resolved with study authors, we considered this at high risk of bias for incomplete outcome data. For example, one abstract reported 16 CDI cases, however in their IPD there were only two confirmed cases[25]. We did not exclude studies if their IPD was not consistent with their published data. Fourth, participants who had diarrhea but were not tested for CDI were considered to have missing participant outcome data. If these participants were not specified in the IPD, we considered this an unclear risk of bias for missing outcome data under 10%, and high for over 10%[26]. Fifth, if a study reported outcome data (e.g. SAEs) but it was not available in the IPD, it was considered a high risk of bias for selective outcome reporting, for the reasoning that "one or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis[23]." Furthermore, if a variable in the model (e.g. antibiotic use) was reported in published results but was not available in the IPD, since it would be excluded from the adjusted model, this was also considered a high risk for selective outcome reporting. Lastly, in one case, risk of bias assessment was done for an abstract and the updated study was published and used in our manuscript, thus risk of bias judgements were re-assessed based on the published paper. For the overall quality of evidence, we used the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach, which includes assessing methodological limitations of included studies, directness of evidence, heterogeneity, precision of effect estimates, and risk of publication bias[27]. Publication bias was evaluated in two ways: with a funnel plot of all potentially eligible studies, for which studies where IPD was obtained and not

obtained were compared by visual inspection for symmetry[28], and by comparing the estimates of the IPD meta-analysis and the aggregate data meta-analysis of studies for which IPD was not obtained. Risk of bias and GRADE assessments were completed by one reviewer (LL), and, for the purpose of manuscript preparation, an independent and duplicate process will follow.

Data verification, synthesis, and analysis

All datasets obtained from authors were compared with the published results and checked for the randomization sequence, data items of interest, and completeness. Discrepancies were discussed with study authors and corrected, when possible. For studies that stated no SAEs occurred, we confirmed this with authors.

We pooled IPD across trials, and analysed it through a generalized linear mixed model (GLMM). The first level was the patient and the second level was the study. We considered the study level to be a random effect, and the participant variables to be fixed effects. Based on the currently available literature on CDI risk factors and the variables available across datasets, we developed a model and adjusted for the following four patient variables: age (years)[8], sex, whether the patient was hospitalized, and whether the patient was on high risk antibiotics (3rd and 4th generation cephalosporins, lincosamides, and fluoroquinolones)[3] at any time during the trial. For the SAE outcome, only hospitalized participants had the outcome, thus this variable was removed from the adjusted model.

Subgroup analysis

Furthermore, we conducted three *a priori* subgroup analyses. First, we examined the effect of a low (<5%) control group event rate versus moderate to high ($\geq$5%) control group

event rate, as an estimate of baseline CDI incidence[13]. We used two approaches: we retained the group variable in the model and added the event rate variable, as well as adding the event rate variable and also an interaction term with group and event. Second, we compared no probiotics to multi-species probiotics and to single-species probiotics[29]. Third, we looked at probiotic dose, where participants in the control group had zero colony forming units (CFU) per day, and we examined the effect of increasing the dose by one billion CFU/day in the intervention group[30]. We planned to conduct a subgroup analysis for probiotic species, however we did not have sufficient data.

Sensitivity analysis

We conducted four *a priori* sensitivity analyses on our primary outcome, CDI. First, we compared the unadjusted analysis (14 studies, n=3,222) with the pooled estimate of effect based on aggregate data (14 studies, n=3,222). Second, we compared the complete case unadjusted analysis (10 studies, n=2,001) with the pooled estimate of effect based on aggregate data (10 studies, n=2,156). Third, for the complete case analysis of CDI (10 studies, n=2,001), we categorized age (infants 0 to <1, children 1 to <18, adults 18 to <65, and older adults 65+) to determine whether age groups are more predictive of CDI than an incremental increase in age. Fourth, for the primary complete case analysis, we accounted for clustering using a generalized estimation equations (GEE) model (GEDMOD procedure) approach, which assumes that both the patient and study levels are fixed effects. We also conducted a *post hoc* sensitivity analysis by removing infants under the age of one from the dataset and running the complete model (10 studies, n=1,932). We chose this sensitivity analysis because at this age *C. difficile* colonization is common and does not reflect an infection[31].

Handling missing patient data

For participants without a CDI outcome that had no reports of having diarrhea, we assumed that none of these patients had CDI. Participants who were reported to have had diarrhea but were not tested for CDI were considered as having missing participant outcome data. Eleven studies had participants with missing outcome data, ranging from 0.8%[25] to 34.8%[32]. In two additional studies, the number of participants with missing outcome data was unclear. Excluding the two aforementioned studies, data on missing outcomes, either with or without diarrhea, was not provided in 8 trials, totalling 214 participants (6.6%). All missing data, provided or not, amounted to 456 participants (14.2%). However, for most studies it was not specified in the IPD which patients had missing outcomes, both with and without diarrhea, and we could not conduct a true complete case analysis. Thus, for our primary analysis, we included all patients randomized, and assumed that all missing patients had no CDI or SAEs, as a conservative approach. We planned to conduct multiple imputation (MI) analysis to examine the effect of missing participant data on our effect estimate, however given the limited number of studies that specified which patients completed the trial, we will do this analysis when we receive the final data set from Allen et al[33].

Statistical analysis

Baseline data for all included participants were summarised as mean (SD) or median (first and third quartiles, Q1, Q3) for continuous variables and number (% of total) for categorical variables. For estimates of effect, the odds ratio (OR) and relative risk reduction (RRR), as well as their associated 95% confidence intervals (CIs), were reported. For pooled meta-analyses, heterogeneity was reported with the I^2 value, where an I^2 of 0-40% represented

low heterogeneity, 30-60% as moderate, 50-90 as substantial, and 75-100% as considerable[34]. We planned to calculate the intra-class correlation coefficient, to examine the correlation between the outcome variable (CDI) and group (control versus probiotics), however this was an unreliable estimate based on our data and thus was not reported. The level of statistical significance, α, was set at 0.05. We used ReviewManager version 5.3 (Copenhagen, Denmark) for aggregate data meta-analyses and funnel plots, IBM SPSS version 20 (Armonk, New York) and SAS/STAT 9.4 (Cary, North Carolina) for data cleaning and analysis, respectively, and Stata 13 (College Station, Texas) for graphing the IPDMA forest plots.

A protocol for this study was registered with the International Prospective Register of Systematic Reviews (PROSPERO 2015:CRD42015015701).

Results

IPD selection and IPD obtained

We identified and contacted the authors of 32 potentially eligible trials (Figure 1). We were not able to obtain IPD from 14 trials (no response, authors no longer had access to data, ethics approval not granted) and three trials were not eligible after further clarification. The details for exclusions are specified in Figure 1. One study (3.5%) is currently pending data transfer[33].

Study characteristics

We included 14 trials with 3,222 participants, and a total of 86 CDI events and 81 SAEs (Table 1). There were 7 formulations of probiotics given, with doses ranging from 10 to 900 billion colony forming units (CFUs) per day. Nine trials (64.3%) were conducted in hospitalized patients[22,25,35-40], two (14.3%) in non-hospitalized patients[41,42], and three trials included both inpatients and outpatients (21.4%)[32,43,44]. Two trials (14.3%) were conducted in children[43,44]. Among our 14 trials, patients ranged in age from less than six months to 99 years. Thirteen trials (92.9%) had approximately equal numbers of males and females. The proportion of patients on high risk antibiotics at any given time ranged from 0%[41,42] to 76.7%[38]. For the outcome CDI, two studies (14.3%) did not report patient level data on antibiotics taken[37,39], and two (14.3%) did not report age[25], thus these four studies were excluded from the adjusted CDI model (n=1,221 participants). For SAE, one study (7.1%) did not report IPD on antibiotics[37], two did not report age (14.2%)[25], one did not report IPD SAEs (7.1%), and one did not report antibiotics or IPD SAEs (7.1%), thus these five studies were excluded from the adjusted SAE model (n=793 participants). For SAE (n=9 studies), baseline characteristics were similar in the

treatment and control groups. The mean age was 50.2 (SD 26.6) for the treatment group and 48.5 (SD 27.5) for the control group (Table 2). Half (52%) of participants were male. Approximately three quarters of participants (73.0%) were hospitalized. Half of hospitalized patients had length of stay available, which was a median of 3 days (IQR 0-7 days) in the control group and 3.5 days (IQR 0-7.25 days) in the probiotics group. The most frequently prescribed antibiotics were from the betalactam +/- betalactamase inhibitor class (1323/3222 patients). Approximately one quarter of patients were on a high risk antibiotic at any time. The median number of antibiotics per patient was one (IQR 1-2), and the median length of treatment was 10 days (IQR 7-14). The median length of probiotics treatment was 14 days (IQR 11-17).

Risk of bias assessment within studies

For CDI, five studies were at high risk of bias for incomplete outcome data of greater than 10% for the CDI outcome because not all patients who had diarrhea were tested for CDI or no data on patients were provided[35,39,41,42] (Figure 2). For SAE, two studies were at high risk of bias because they reported on but did not provide IPD for SAEs (deaths)[35,39]. Four studies were at high risk of bias for selective outcome reporting, where two studies reported antibiotics use but did not have IPD[37,39], and two studies did not report age[35,42]. Four studies were at high risk of other bias due to potential conflict of interest due to industry funding[25,36,37]. There was no suspicion of publication bias for either outcome among the included studies, as well as in comparing studies for which IPD was obtained and studies for which it was not obtained (Figures 3 and 4).

Primary outcome: *Clostridium difficile* Infection

Of the 14 studies (n=3,222 participants) reporting on the incidence of CDI, probiotic prophylaxis reduced the odds of the outcome (OR 0.27; 95% CI 0.17 to 0.45; p<0.0001; Figure 5). Our effect estimate was marginally lower than the pooled estimate for the 10 studies (n=1,326) whose IPD was not obtained (OR 0.36; 95% CI 0.21 to 0.64; p=0.0004; Figure 6). The patient characteristics for the probiotics and control group among studies included in the adjusted model are reported in Table 3; data was missing for 5.2-7.6% of values for the variables. Of the 10 studies (n=2,001 participants) in the adjusted model, probiotics significantly reduced the incidence of CDI (OR 0.24; 95% CI 0.13 to 0.42; p<0.0001; Figure 6). Age, sex, hospitalized versus not hospitalized participants, and being on high risk antibiotics were not significantly associated with CDI. We graded the certainty in the effect estimate as moderate, downgraded for imprecision (Table 5).

Secondary outcome: Serious adverse events

Of the 12 studies (n=2,650 participants) reporting on the incidence of SAEs, the probiotics group and control groups had a similar odds of the outcome (OR 1.31; 95% CI 0.80 to 2.12; p=0.28; Figure 5). Our effect estimate was higher than the pooled estimate of the two trials whose IPD was not obtained (OR 0.97; 95% CI 0.25 to 3.73; p=0.97; Figure 7). None of the SAEs were deemed to be attributable to probiotics based on correspondence with investigators and co-authors. The patient characteristics for the probiotics and control group among studies included in the adjusted model are reported in Table 4. Data was missing for 5.4-8.5% of values for the variables. Of the 9 studies (n=1,857 participants) in the adjusted model, the probiotics group and control group had a similar risk of SAEs (OR 1.07; 95% CI 0.62 to 1.86; p=0.81; Figure

6). Age was significantly associated with SAEs (OR 1.07; 95% CI 1.04 to 1.10; p<0.0001; Figure 6), whereas being on high risk antibiotics and sex were not significantly associated. We graded the certainty in the effect estimate as moderate, downgraded for imprecision (Table 5).

Subgroup analyses

In the subgroup analyses, a control group event rate of higher than 5% was a significant predictor of CDI when adjusted for in addition to the primary adjusted model (OR 18.03; 95% CI 6.07 to 53.62; p<0.0001; Figure 5), however, we found no significant interaction with the treatment effect (p=0.09). Compared to no probiotics, both single-species probiotics (OR 0.44; 95%CI 0.20-0.97; p=0.04; Figure 5), and multi-species probiotics (OR 0.14; 95% CI 0.06-0.32; p<0.0001; Figure 5) significantly reduced the odds of CDI. Compared to no probiotics (dose=0 CFU/day), a one billion CFU/day increase in dose significantly reduced the odds of CDI (OR 0.97; 95%CI 0.96-0.98; p<0.0001; Figure 5).

Sensitivity analyses

When we treated age as a categorical rather than a continuous variable in multivariate analysis (four groups: infants, children, adults, and older adults), the effect of probiotics remained similar (OR 0.23; 95% CI 0.13 to 0.42; p<0.0001; Figure 5), and the youngest age group (aged under 1 year) was significantly associated with detection of *C. difficile* (OR 12.78; 95% CI 1.13-144.63; p=0.040). We conducted a *post hoc* analysis because true CDI is rare in infants <1 years of age[31]. Excluding infants, the effect estimate for probiotics was again similar (OR 0.25; 95% CI 0.13 to 0.45; p<0.001) and none of the age groups were associated with CDI.

We found a similar estimate of effect of probiotics on the odds of developing CDI when we conducted a random-effects aggregate data meta-analysis for the 10 studies included in the adjusted model (OR 0.27; 95% CI 0.15 to 0.49, $p<0.0001$, $I^2=4\%$), and when we used the GEE approach (OR 0.25; 95% CI 0.12 to 0.51; $p=0.0002$).

Discussion

Summary of evidence

Our IPD meta-analysis of 14 trials with data on 3,121 participants found that probiotics reduced the risk of CDI by 73% (95% CI 65% to 83%), which was slightly more beneficial than the estimate from a previous systematic review based on aggregate data. In our adjusted model, this effect was independent of participant age, sex, hospital admission status, and whether they received high-risk antibiotics. We also found that the risk of SAEs was not significant for the control and intervention groups. Age was, however, a significant predictor in the adjusted model. In our data, we found that for every year increase in age, SAE risk increased by 7% (95% CI 4% to 10%). Importantly, none of the SAEs were reported to be associated with probiotics. We graded the confidence in effect estimates for both outcomes as moderate, downgraded for imprecision due to a low number of events. We obtained approximately 41% of all available data, and the effect estimate of obtained studies was similar to studies that were not obtainable. However, this is a preliminary analysis, as we are in the process of obtaining an additional study, the largest trial to date, having randomized 2,971 participants, after which we will have 78% of all available data. Inclusion of this study will nearly double our sample size and increase events by a third; the authors did not find a benefit to using probiotics, which may change our current estimate of effect.

Our finding that probiotics do not influence SAEs was similar to what is reported in a previous comprehensive review of the literature[45]. None of the studies reported SAEs due to probiotic treatment. The generalizability of our findings is somewhat limited, however, since

only hospitalized patients had SAEs among our included studies, and all but one study[36] excluded immunocompromised patients from participating in the trial.

It has been suggested that probiotics have a benefit only in high incidence settings[46]. We were interested in obtaining hospital disease pressure estimates from trialists, as this was previously demonstrated to be a significant predictor of CDI[47], however this data was not available for any of our included trials. Our subgroup analysis looking at trials with control group event rates, which we chose as an approximation of baseline risk with the limited data we had available, suggested that while CDI incidence over 5% is highly associated with CDI, it does not interact with the overall group effect. Thus, it suggests that probiotics are still beneficial in low incidence settings.

The most common questions regarding implementing probiotics for infection prevention is which product to use, including species, a multi-species versus a single-species formulation, and dose. Given our limited data, we were unable to estimate the relative effectiveness of different probiotic types. For multi-species compared to single species probiotics, our data suggests that while they both reduce CDI compared to no probiotic, multi-species probiotics may have a more beneficial role than single-species probiotics, with risk reductions of 56% (95%CI 3-80%) and 86% (95% CI 67-94%), respectively. Our findings reflect those reported in a recent aggregate data meta-analysis of probiotics for CDI prevention (citation). Of interest, we also explored the impact of a linear dose-response finding that an increase in dose by 1 billion CFU/day reduces the odds of CDI by 3% (2-4%), compared to no probiotic, suggesting that a higher dose may be beneficial.

Strengths and limitations

Our study had several limitations. First, we were only able to obtain IPD from 14 of 29 trials. We included only two thirds of the patients in our adjusted model, however given that our estimate for the adjusted model was similar to the unadjusted model, we assume that the estimates of effect would have been similar if all studies were in the adjusted model. Further, one of the trials not included, the largest trial conducted to date (n=2,971 participants), did not find a reduction of CDI with probiotics supplementation. Ethics approval for IPD from this study was delayed, however we will incorporate this study in the upcoming manuscript. Second, we created a dichotomous variable for high risk antibiotics. A number of antibiotics have been associated with risk of CDI, and our decision was based on those most frequently associated. A more informative strategy would have been to look at each antibiotic group separately; however we did not have a sufficient number of CDI events to do so. Third, serious adverse events reporting was actively conducted in only one trial[40], thus we may have underestimated the total number of events in the trials. However, we do not anticipate that this would have affected one group over another as the previous aggregate data MA for SAE did not demonstrate that probiotics were associated with important harms. Fourth, we adjusted for a limited number of variables, and may have missed an important confounder. While we originally planned to adjust for length of hospital stay, specific types of antibiotics, and length of antibiotic exposure, we were limited to the information available in the original databases. We plan to conduct the updated analysis with these variables, as we will have sufficient IPD to do so. Fifth, we had a relatively high level of missing data (14.6%) which may have impacted our effect estimate. We felt that our approach of imputing no CDI outcome for all missing

participants was the most conservative approach. Though it may under-estimate the incidence of CDI, it is likely to be equal in both groups. We did not conduct further analyses on missing data, however we plan to conduct multiple imputation (MI) analysis for our more inclusive manuscript, after obtaining data from Allen *et al* (n=2941)[33]. Lastly, given that only 7/10 studies reported CDI events, it is possible that our subgroup analyses may be driven by study-level differences.

Our study has several strengths. First, we used a comprehensive search for trials and had a relatively high response rate from authors (84%). Second, relative to other IPDMAs, we obtained a large number of trials, and our results are based on a large sample size. In a recent review of IPDMAs with binary outcomes conducted in 2011, of 26 articles the median number of trials included was 12 (IQR 6-18), and 9 had fewer than 1000 patients[48]. Third, we used sophisticated statistical modeling to control for within study and between study heterogeneity. The recent review of binary data IPDMA's suggests that only 19 of 26 studies used a one-stage approach, and of those only 10 used random effects modeling[48]. Fourth, our primary analysis was robust to sensitivity analyses using different analytic methods, including aggregate data meta-analyses, fixed-effects meta-analyses, and categorization of age.

Conclusion

Probiotic prophylaxis is a useful and safe infection prevention strategy for CDI, which appears to have a large benefit regardless of participant age, sex, hospitalization status, and exposure to high risk antibiotics. These results are preliminary and once we obtain the IPD from

Allen *et al*[29], we will be conducting further analyses, including looking at additional confounders and the effect of missing participant outcome data.

Funding sources/sponsors

This study was unfunded.

Conflicts of interest

AC, BK, DM, EL, HS, LL, LT, LW, PP, and SH have no conflicts of interest related to this study.

BCJ has received an unrestricted grant from BioK+ Inc to conduct a non-interventional prospective observational study evaluating the incidence of antibiotic-associated diarrhea in children.

CPS has received an unrestricted research grant from Ferring Pharmaceuticals Ltd to cover the costs of conducting a randomized control on VSL#3 for the prevention of antibiotic and *Clostridium difficile*-associated diarrhea.

DW has served as a statistical consultant to some probiotics trials supported by Cultech, UK.

JS has worked in the past with Bio K+ but has had no interactions for the last 7 years.

MH has provided advice for Danone Ltd and received support to conduct two clinical trials, as well as honoraria to attend various conferences.

MM has received research support from Conagra to conduct 2 clinical trials evaluating the effectiveness of probiotics (LGG) to prevent *C. difficile* infection. MM has been an employee of bioMerieux since October 2012.

SJA has done research in probiotics supported by Cultech, UK, has been an invited guest at a Yakult Symposium, has received research funding from Yakult, UK and has received speakers fees from Astellas Pharma.

Figures

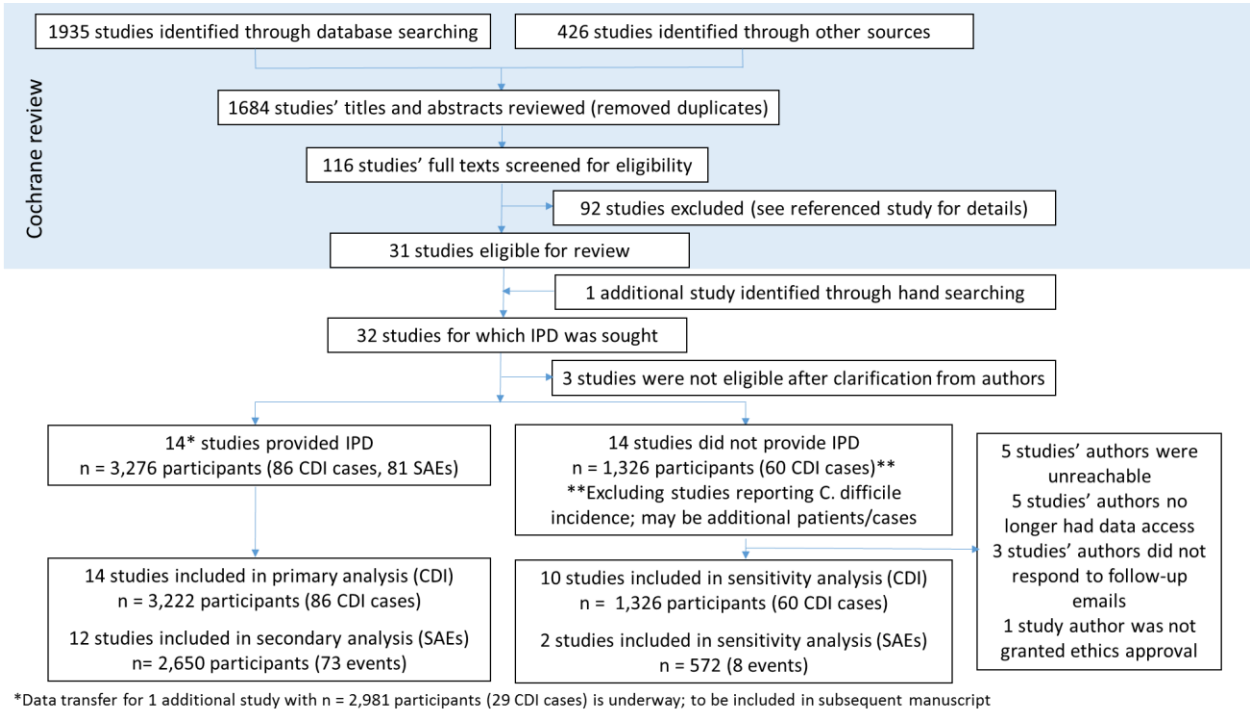

Figure 1. Study flow diagram.

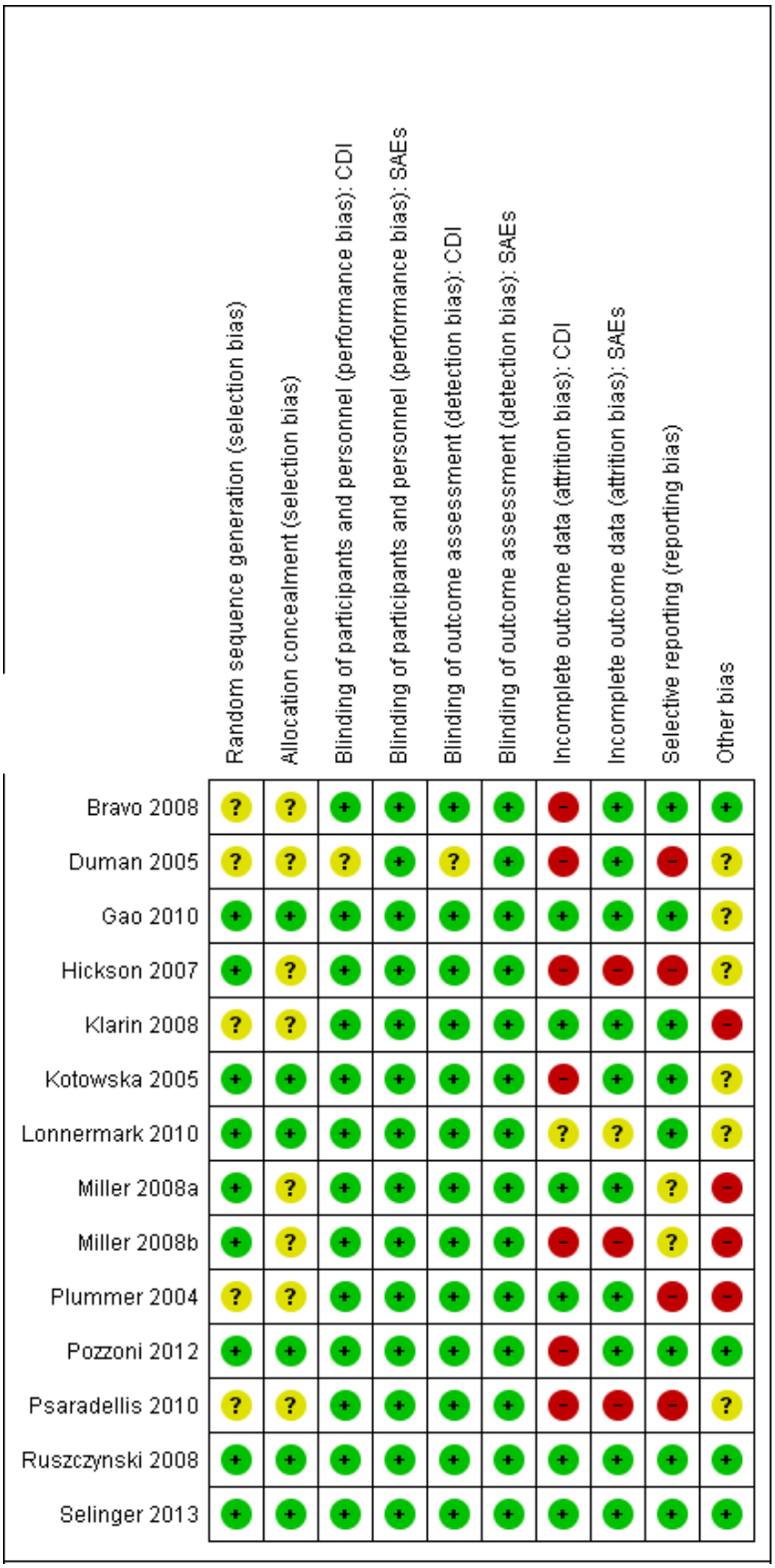

Figure 2. Risk of bias assessment for included studies.

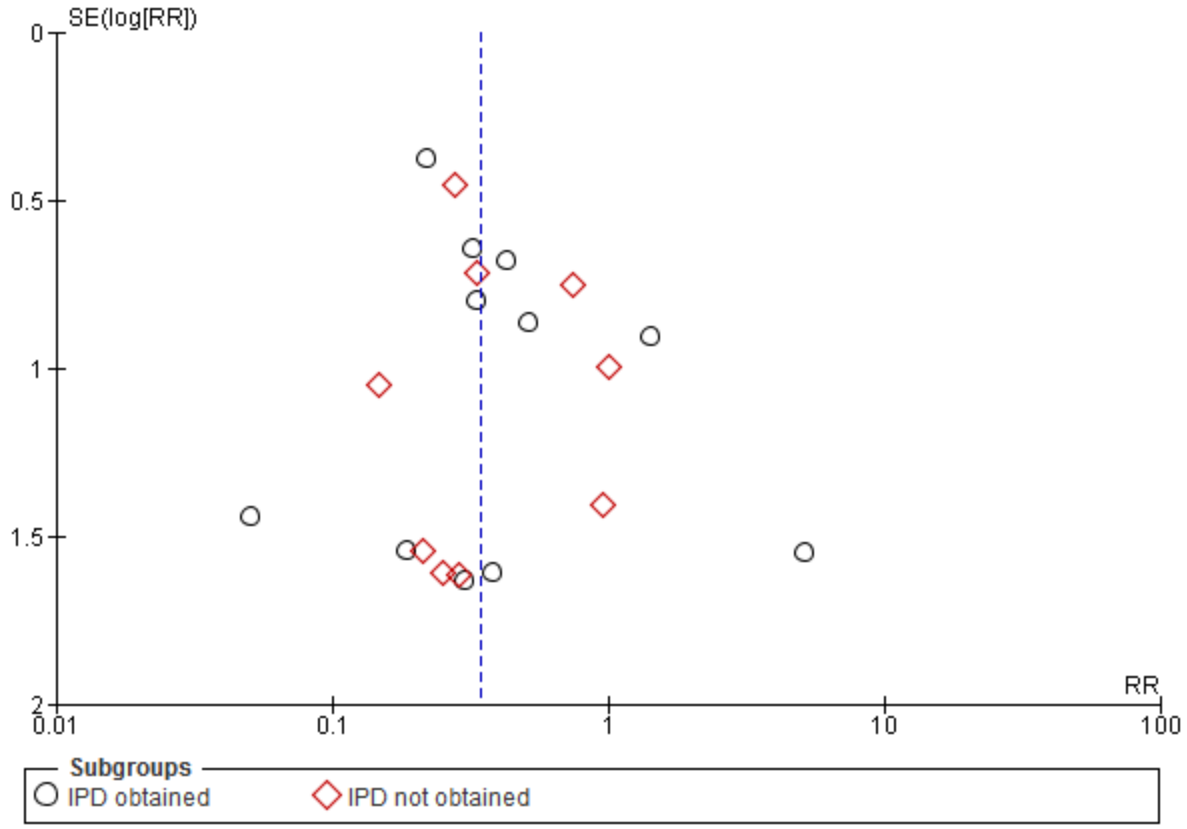

Figure 3. Funnel plot for studies, with effect estimates, that reported CDI, comparing studies obtained for IPDMA and not obtained.

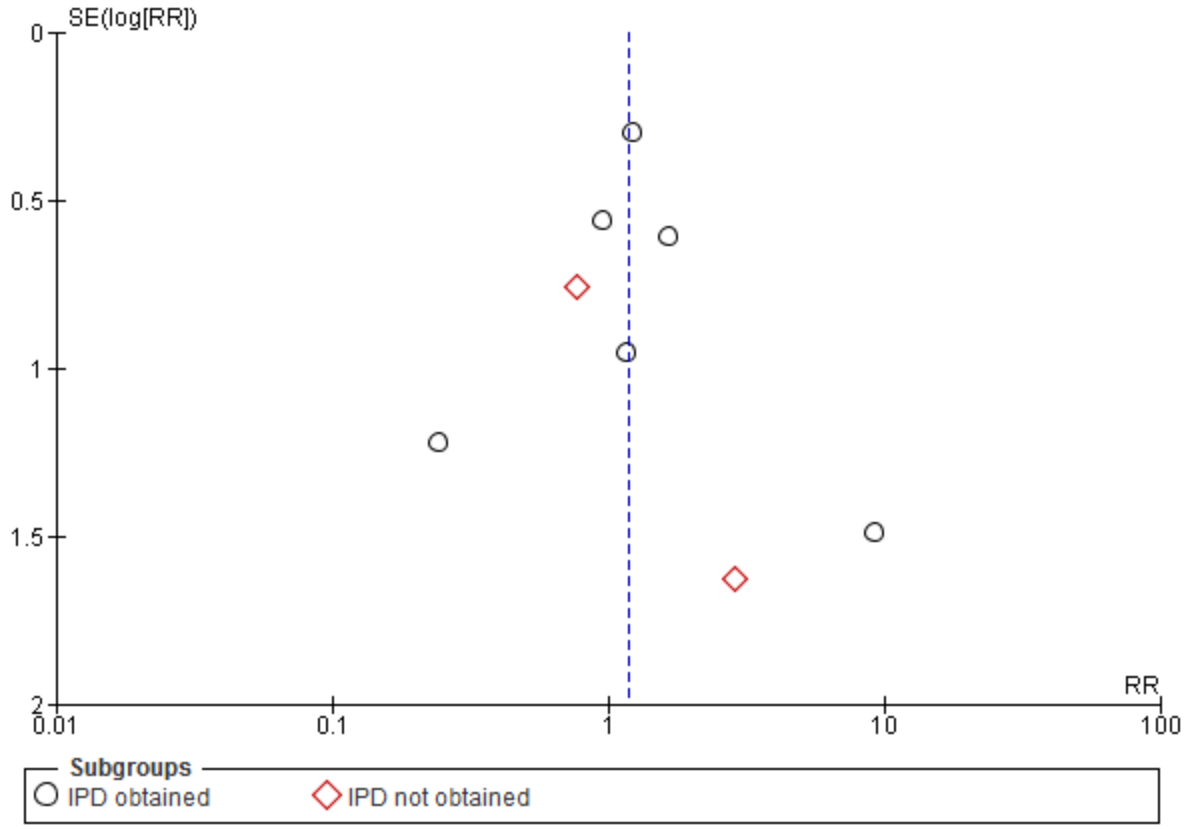

Figure 4. Funnel plot for studies, with effect estimates, that reported SAEs, comparing studies obtained for IPDMA and not obtained.

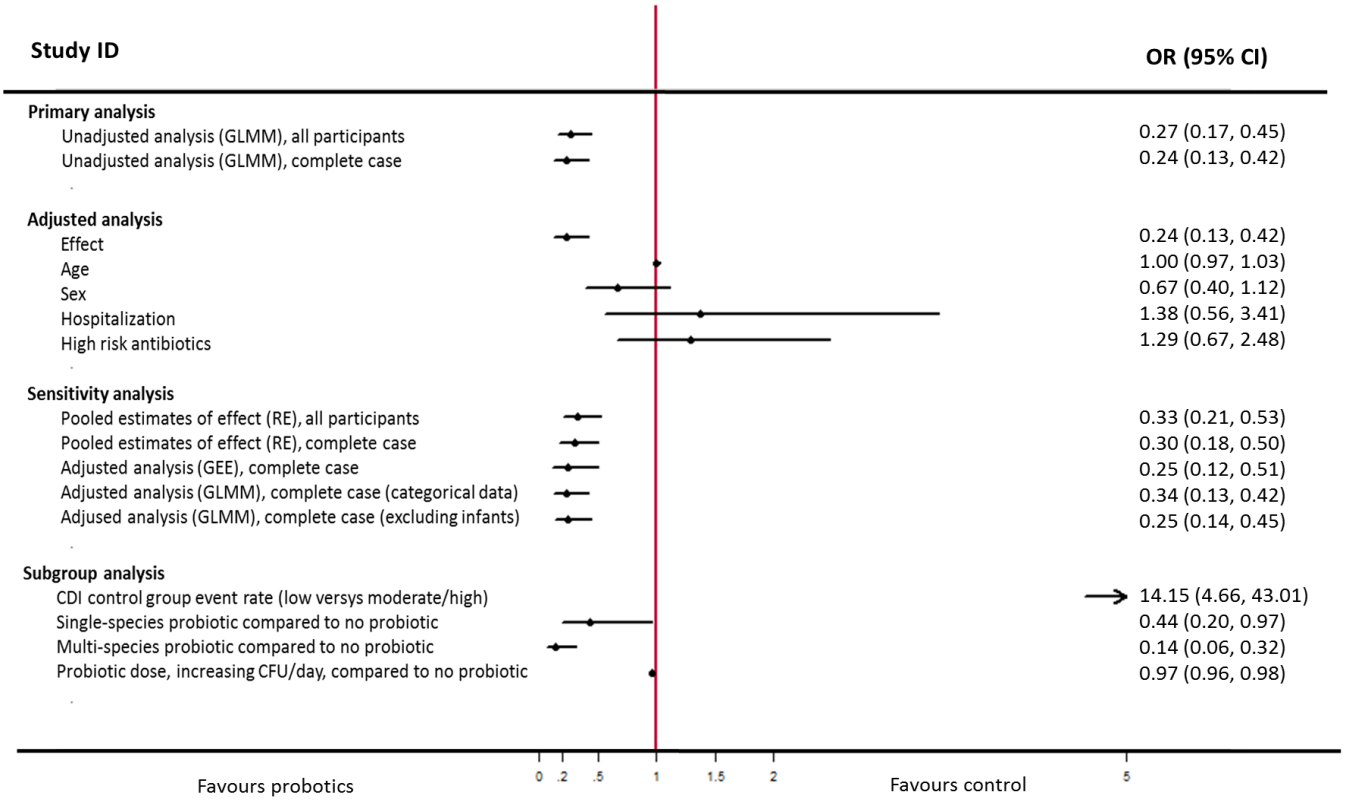

Figure 5. Forest plot for primary, adjusted, sensitivity and subgroup analysis of probiotics for CDI.

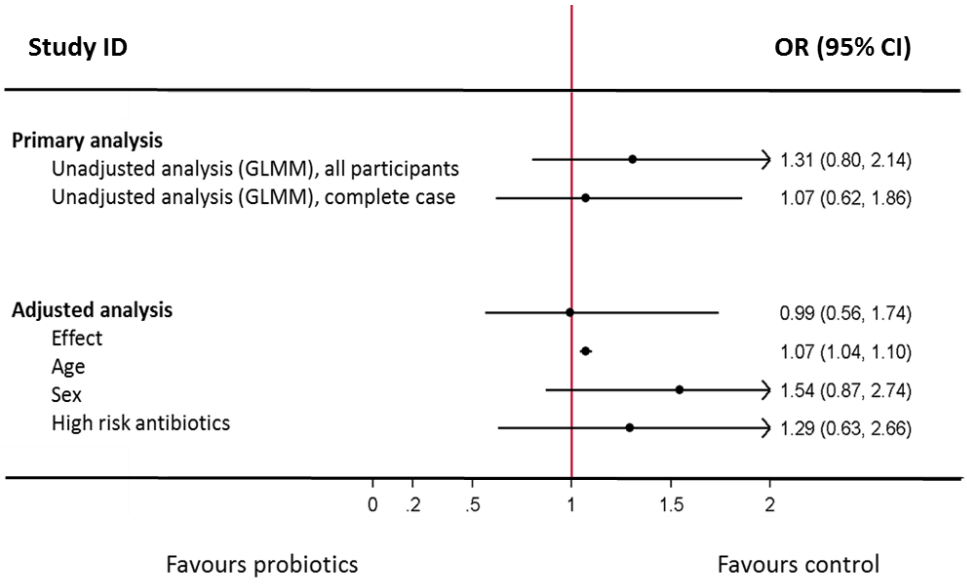

Figure 6. Forest plot for primary and adjusted analyses for SAEs.

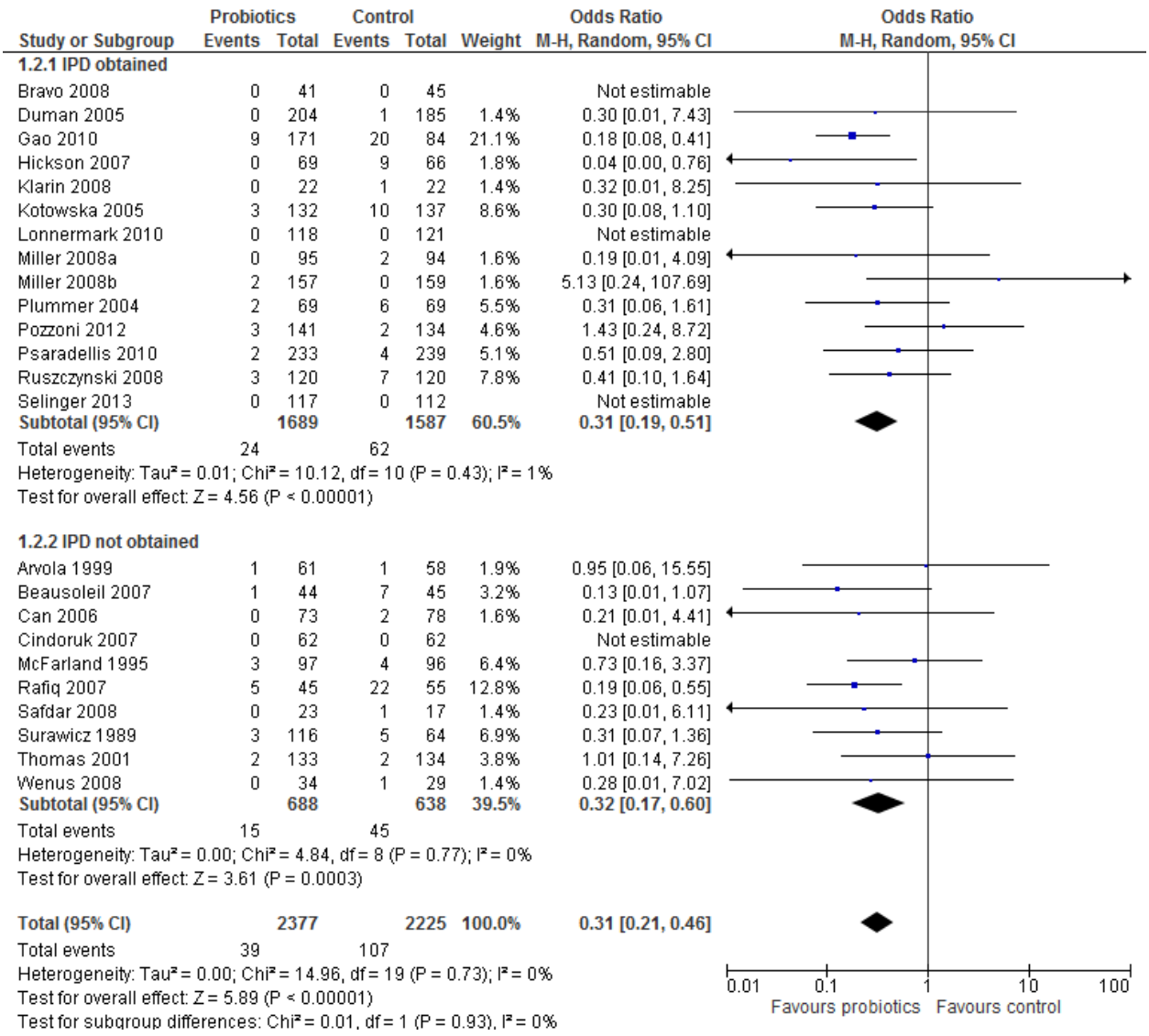

Figure 7. Pooled random effects meta-analysis for probiotics versus control on CDI, comparing studies obtained for IPDMA and not obtained.

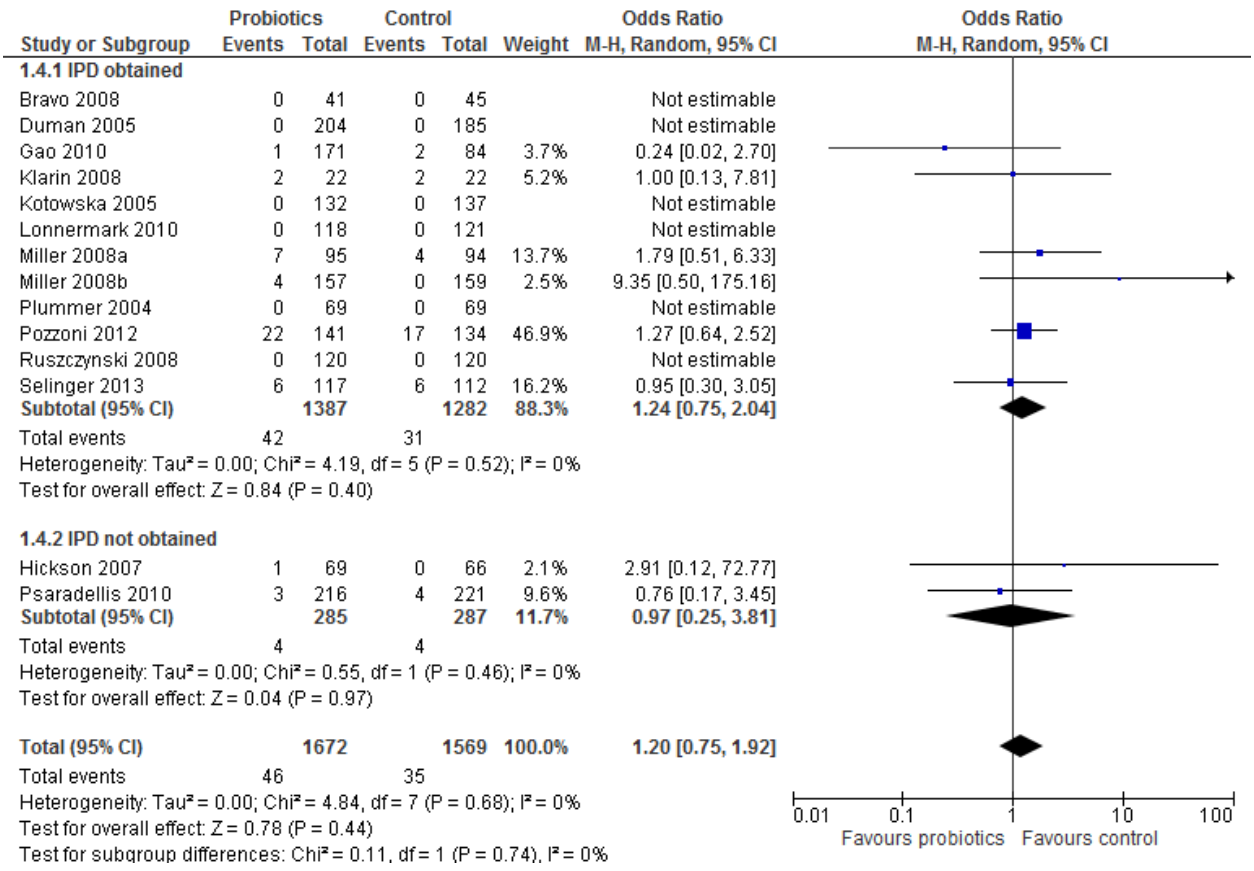

Figure 8. Pooled random effects meta-analysis for probiotics versus control on SAEs, comparing studies obtained for IPDMA and not obtained.

Tables

Table 1. Characteristics of all included studies

Study	Probiotic type	Dose (billion CFU/d)	Probiotics group n=1664			Control group n=1558			Inpatients (% n)	Age (mean, SD)	Sex (% male)	High risk* antibiotics (% n)
			n	CDI	SAE	n	CDI	SAE				
Bravo 2008	*S. boulardii*	10.2	41	0	0	45	0	0	0	50.4 (19.1)	23.3	0
Duman 2005	*S. boulardii*	10	204	0	0	185	1	0	0	45.2 (13.4)	51.3	0
Gao 2010	*L. acidophilus, L. casei, L. rhamnosus*	50, 100	171	9	1	84	20	2	100	59.6 (6.3)	51.8	29.2
Hickson 2007	*L. casei, L. bulgaris, S. thermophiles*	40.74	69	0	1	66	9	0	100	73.8 (10.7)	45.9	19.3
Klarin	*L. plantarum*	80	19	0	2	22	1	2	100	60.8 (17.1)	68.3	48.8
Kotowska 2005	*S. boulardii*	30	132	3	0	137	10	0	23.2	3.8 (1.7, 7.2) ‡	43.1	1.6
Lonnermark 2010	*L. plantarum*	100	118	0	0	121	0	0	54.6	47.7 (17.9)	44.2	24.5
Miller 1 2008	*L. rhamnosus*	20	94	0	7	88	2	4	100	-	50.0	62.1
Miller 2 2008	*L. rhamnosus*	60	153	2	4	156	0	0	100	-	47.5	23.0
Plummer 2004	*L. acidophilus, B. bifidum*	20	69	2	0	69	6	0	100	62.1 (19.0)	53.6	-
Pozzoni 2012	*S. boulardii*	10	141	3	22	134	2	17	100	79.2 (9.8)	49.8	76.7
Ruszczynski 2008	*L. rhamnosus*	20	120	3	0	120	7	0	54.2	3.6 (1.5, 6.6) ‡	54.0	9.6
Sampalis 2010	*L. acidophilus, L. casei, L. rhamnosus*	50	216	2	3	221	4	4	100	62.1 (17.0)	49.0	-
Selinger 2013	VSL#3†	900	116	0	6	111	0	6	100	57.3 (18.0)	52.9	11.0

*High risk antibiotics were considered 3rd and 4th gen cephalosporins, lincosamides, and fluoroquinolones. †B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. paracasei, L. bulgaricus, S. thermophiles. ‡Median and IQR. CFU = colony forming units, d = day.

Table 2. Characteristics of patients in total data set						
	Probiotics group (n=1664)			Control group (n=1558)		
	Valid sample	Missing	Measure	Valid sample	Missing	Measure
Age (median, IQR) years	1359	305	50.19 (26.55)	1258	300	48.47 (27.50)
0-1			68			84
2-17			176			168
18-64			644			571
65+			470			435
Sex (Male, %)	1518	146	747 (52.4)	1407	151	687 (52.6)
Hospitalized (n, %)	1610	53	1184 (73.5)	1509	48	1093 (72.4)
Length of hospital stay (median, IQR) days	754	858	3.5 (0-7.25)	652	857	3 (0-7)
Antibiotics class (at any time)	1327	337	1327	1219	338	1219
Aminoglycoside			30			51
Betalactam +/- Betalactamase inhibitor			689			634
Carbapenem*			15			19
Cephalosporin (1st gen)			219			180
Cephalosporin (2nd gen)			154			162
Cephalosporin (3rd gen)*			117			128
Cephalosporin (4th gen)*			1			3
Fluoroquinolone*			138			138
Glycopeptide			46			55
Lincosamide			79			52
Macrolide			317			303
Others			98			105
High risk* antibiotic at any time (n, %)			311 (23.4)			296 (24.3)
Number of antibiotics (median, IQR)			1 (1-2)			1 (1-2)
Antibiotic exposure (median, IQR) day	1201	463	10 (7-14)	1106	452	10 (7-14)
Probiotics exposure (median, IQR) day	1115	549	14 (12-17)	1022	536	14 (11-17)
***C. difficile* infection (n, %)**	1612	0	24 (1.49)	1509	0	62 (4.12)
Serious adverse events (n, %)	1088	524	42 (3.86)	975	534	31 (3.12)

*High risk antibiotics were considered 3rd and 4th gen cephalosporins, lincosamides, and fluoroquinolones.

Table 3. Characteristics of patients included in primary analysis of CDI (complete case)		
	Probiotics group (n=1052)‡	**Control group (n=949)‡**
Age (mean, SD) yearsδ	47.00 (27.73)	44.59 (28.88)
0-1	69	84
2-17	331	291
18-64	479	408
65+	173	166
Sex (Male, %)§	522 (49.6)	456 (48.1)
Hospitalized (yes, %)ѵ	650 (61.8)	556 (58.6)
High risk antibiotic at any time*	221 (21.0)	202 (21.3)
***C. difficile* infectionΦ**	18 (1.7)	49 (5.2)

*High risk antibiotics; 3rd and 4th gen cephalosporins, lincosamides, and fluoroquinolones.
†Miller 2008a and Miller 2008b excluded for not reporting age, Plummer 2004 and Psaradellis 2012 excluded for lack of antibiotics data.
‡78 missing in the probiotics group, 77 missing in the control group.
δ57 missing in the probiotics group, 54 missing in the control group.
§80 missing in probiotics group, 75 missing in control group.
ѵ54 missing in probiotics group, 49 missing in control group.
ΦOne missing in control group.

Table 4. Characteristics of patients included in primary analysis of SAEs (complete case)		
	Probiotics group (n=984)‡	**Control group (n=883)‡**
Age (mean, SD) years	42.40 (28.62)	45.15 (27.59)
Sex (Male, %) δ	493 (50.10)	424 (48.02)
Hospitalized (yes, %) §	582 (59.15)	490 (55.49)
High risk antibiotic at any time*	209 (21.24)	188 (21.29)
Serious adverse events	31 (3.15)	27 (3.06)

*High risk antibiotics; 3rd and 4th gen cephalosporins, lincosamides, and fluoroquinolones.
†Miller 2008a and Miller 2008b excluded for not reporting age, Plummer 2004 excluded for lack of antibiotics data, Psaradellis 2012 excluded for not reporting antibiotics data and IPD on SAEs, and Hickson excluded for not reporting IPD on SAEs.
‡69 missing in the probiotics group, 75 missing in the control group.
δ53 missing in probiotics group, 52 missing in control group.
§53 missing in probiotics group, 52 missing in control group.

Table 5. Probiotics for the prevention of *Clostridium difficile* associated diarrhea

Patient or population: Adults and children exposed to antibiotics
Settings: Inpatient and outpatient
Intervention: Probiotics

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE) Comments
	Assumed risk **Control**	Corresponding risk **Probiotics**			
***Clostridium difficile* associated diarrhea** Defined by: cytotoxin and/or culture	**Study population**		**OR 0.24** (0.13 to 0.42)	2,001 (10 studies)	⊕⊕⊕⊖ **moderate**[1]
	49 per 1000	**14 per 1000** (8 to 24)			
	Moderate				
	30 per 1000	**8 per 1000** (5 to 15)			
Serious adverse events Defined by: author reported and/or the individual participant data	**Study population**		**OR 1.07** (0.62 to 1.86)	1,857 (9 studies)	⊕⊕⊕⊖ **moderate**[1]
	28 per 1000	**30 per 1000** (18 to 51)			
	Moderate				
	0 per 1000	**0 per 1000** (0 to 0)			

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio.

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] We had a low number of total events (under 300), thus we rate down for imprecision.
The study authors had no other reasons for grading down the study.

Supplementary tables

Table S1. Example search strategy in EMBASE, conducted February 21st, 2013.

#	Searches
1	'probiotic agent'/exp OR 'probiotic agent' OR probio* OR 'dairy product':de OR 'yoghurt'/exp OR yoghurt OR 'yogurt'/exp OR yogurt OR 'kefir'/exp OR kefir OR 'fermented product'/exp OR 'fermented product'
2	'lactobacillus'/exp OR lactobacillus OR lactobacill* OR l AND acidophilus OR l AND casei OR l AND delbrueckii OR l AND helveticus OR l AND johnsonii OR l AND paracasei OR l AND plantarum OR l AND reuteri OR l AND rhamnosus OR l AND salivarius
3	saccharomyce*OR'streptococcus'/expORstreptococcus ANDthermophilusOR'clostridium'/ exp OR clostridiumANDbutyricum OR 'enterococcus'/exp OR enterococcus AND faecium OR 'antibiosis'/exp OR antibiosis OR biotherapeutic AND agent*
4	'bifidobacterium'/exp OR bifidobacterium OR bifidobacter* OR b AND animalis OR b AND bifidum OR b AND breve OR b AND infantis OR b AND lactis OR b AND longum
5	#1 OR #2 OR #3 OR #4
6	'anti-bacterial agents':de OR antimicrobial* OR antibiotic* OR 'antimicrobial'/exp OR antimicrobial OR 'anti microbial' OR antimycobacteri* OR antibacteri* OR bacteriocid* NEAR/1 agent*
7	'*Clostridium difficile* infection':de OR 'clostridium'/exp OR clostridium AND difficile OR c AND diff OR '*Clostridium difficile* associated' NEXT/1 diarrhea OR 'disease'/exp OR disease OR 'colitis'/exp OR colitis OR infections OR '*Clostridium difficile* toxin a'/ exp OR '*Clostridium difficile* toxin a' OR '*Clostridium difficile* toxin b'/exp OR '*Clostridium difficile* toxin b' OR 'diarrhea'/exp OR diarrhea OR diarrhea* OR diarrhoe* OR diarhe* OR diarhoe* OR dysenter* OR gastroenteritis* OR 'gastro'/exp OR gastro AND enteritis*
8	random* OR factorial* OR crossover* OR cross AND over* OR placebo* OR doubl* OR singl* NEXT/1 blind* OR assign* OR allocate* OR volunteer* OR 'crossover procedure'/exp OR 'crossover procedure' OR 'double blind procedure'/exp OR 'double blind procedure' OR 'randomized controlled trial'/exp OR 'randomized controlled trial' OR 'single blind procedure'/exp OR 'single blind procedure'
9	#9 #5 AND #6 AND #7 AND #8

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