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The Use of *Lactobacillus* probiotics in the Prevention of Antibiotic Associated *Clostridium Difficile* Diarrhea

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LIST OF ABBREVIATIONS

AAD	Antibiotic-associated diarrhea
CDAD	*Clostrium difficile* associated diarrhea
C difficile	*Clostridium difficile*
CrI	Credible interval (Bayesian)
MUHC	McGill University Health Centre
RCT	Randomized controlled trial
RR	Relative risk
TAU	Technology Assessment Unit

PRINCIPAL MESSAGES

The TAU reviewed the use of probiotics in the prevention and treatment of *Clostridium difficile* associated diarrhea (CDAD) in 2005 and again in 2009. The publication of additional trials prompted a further update and a meta-analysis of *Lactobacillus* probiotics in the prevention of CDAD.

A Bayesian meta-analysis of *Lactobacillus*-based probiotics for the prevention of CDAD associated with the use of antibiotics showed a pooled risk ratio with treatment of 0.17 or 83% reduction in risk of CDAD (95% confidence interval 0.04, 0.42). The number of cases of CDAD across studies was relatively small (most studies having been designed to study all-cause antibiotic-associated diarrhea), the studies varied greatly in background incidence of CDAD, and only two were considered at low risk of bias.

Therefore, although there is suggestive evidence that probiotics based on *Lactobacillus* may be effective in the prevention of CDAD, the evidence is not strong enough to be the basis for a general policy change. Accordingly, we cannot presently recommend routine use of probiotic *Lactobacillus* in the prevention of CDAD in hospitalized patients receiving antibiotics.

EXECUTIVE SUMMARY

Background

Clostridium difficile diarrhea (CDAD) is the most common nosocomial diarrhea, prolonging hospitalization and for some patients leading to colectomy or death. It is strongly associated with antibiotic use, and has been attributed to perturbation of the normal intestinal biota; for this reason there has been an interest in the effectiveness of live cultures (probiotics) in preventing CDAD. The TAU reviewed the use of probiotics in CDAD in 2005 and again in 2009 and on both instances could not recommend their use. The publication of additional trials prompted a further update.

Method

We conducted a systematic search and literature review of articles on efficacy of probiotics in prevention of CDAD published up to June 17, 2011, in EMBASE (Ovid), PubMed, Cochrane, DARE, and INAHTA. We identified a number of comparable studies of *Lactobacillus* (LB) probiotics for prevention of CDAD, allowing us to consider a meta-analysis. To allow for the modeling of heterogeneity between studies, we used a Bayesian hierarchical model to estimate the pooled relative risk (RR) of CDAD associated with probiotics, with a low information (vague) prior distribution. We performed subgroup analyses to examine the effect of risk of bias, the background incidence of CDAD in placebo-group subjects, and the source of funding. To test the strength of the evidence, we carried out a Bayesian credibility analysis. We also reviewed the evidence on safety of probiotic use.

Results

We found 11 RCTs (3 new since our last review in 2009) on the use of LB in prevention of CDAD, of which 7 met the inclusion criteria for the meta-analysis. Among the excluded studies, one study did not include *C difficile* testing, one tested for colonization rather than infection, one tested at defined time-points rather than for AAD, and one did not report number of patients tested for *C difficile*. Included studies varied in patient risk in contacting CDAD (as indicated by proportion of patients affected in the placebo group), probiotic strain and dose, and duration of follow-up. Trials ranged in size from 34 to 437 patients.

After adjustment for incomplete testing for *C difficile*, CDAD was observed in 17/595 patients in the LB group and 53/507 patients in the placebo group. The median pooled RR in the Bayesian analysis, using a vague prior, was estimated to be 0.17 (95% credible interval CrI 0.04, 0.42), representing an 83% reduction in risk. The between-study standard deviation in the log RR (median 0.59, 95% (CI) 0.06, 2.49) was relatively

high, indicating considerable statistical heterogeneity. The predicted RR in a future study was 0.18 (95% CrI: 0.01, 1.52).

Median RRs ranged from 0.13 to 0.23 across sub-group analyses, with the 95% credible interval crossing 1 only in those instances where the subgroup was restricted to only two or three studies. Bayesian credibility analysis (a method of assessing the credibility of a conclusion by calculating how much contradictory information is needed to change it) showed that a reasonably sceptical prior distribution centred on RR=1 and ranging from 0.5 to 2, would be sufficient to tip the pooled RR in the direction of no statistically significant beneficial effect. According to this prior, RRs less than 0.5 are extremely unlikely. It is equivalent to information from a balanced RCT resulting in just 14 CDAD cases in each arm, in other words, relatively weak evidence.

Safety

In general, the safety profile for probiotics in the RCTs involving *Lactobacillus* species was benign. There were no reports of deaths or infections attributed to the probiotic. A few cases of fever occurred in both treatment groups. In most studies, the entrance criteria specifically excluded those at increased risk of infection, eg, patients with immunosuppression, cardiac abnormalities, or intestinal disease. A published systematic review of probiotics in patients receiving nutritional support identified a small number of case reports of systemic infection with probiotic organisms in seriously ill patients and two adult trials (in transplant and pancreatitis) with an excess of adverse safety events in the probiotics group.

CONCLUSIONS

Efficacy

- In the 7 studies included in the present meta-analysis, the administration of *Lactobacillus* was associated with an average reduction in the relative risk of *C difficile* of 83% (median pooled RR=0.17 (95% credible interval (CrI) 0.04, 0.42)).
- The number of outcomes in the database was relatively small (17 in the probiotics group and 53 in the placebo group), there was considerable statistical heterogeneity in the RR between studies as well as heterogeneity in the background incidence of CDAD, raising concerns about the generalizability of the median pooled RR to individual studies.
- Bayesian credibility analysis, which tested the robustness of the findings to prior information, showed that even a relatively weak sceptical prior for the risk ratio produced a posterior distribution for RR that included 1.
- None of the RCTs reported so far have examined outcomes that actually impact hospital costs, e.g., length of stay, among CDAD patients.

- The results of the RCTs conducted so far constitutes suggestive evidence that probiotics based on *Lactobacillus* may be effective in the prevention of CDAD. However, for the reasons stated, the level of evidence is not yet strong enough to determine policy.

Safety

- For patient populations such as those studied in the included randomized controlled trials, in which severely debilitated and immunocompromised patients have been excluded, probiotic therapy appears to be without risk of significant side-effects. However, there have been some case reports of serious side effects in seriously ill patients.

<u>RECOMMENDATIONS</u>

Although there is suggestive evidence that probiotics based on *Lactobacillus* may be effective in the prevention of CDAD, the evidence is not strong enough to be the basis for a general policy change. Accordingly, we cannot presently recommend routine use of probiotic *Lactobacillus* in the prevention of CDAD in hospitalized patients receiving antibiotics.

SOMMAIRE

Contexte

La diarrhée au *Clostridium difficile* (DCD) est l'infection nosocomiale la plus commune prolongeant l'hospitalisation des patients et pour certains, menant à une colectomie ou la mort. Cette infection est fortement reliée à l'utilisation d'antibiotiques et peut être expliquée par une perturbation de la flore intestinale; c'est pour cette raison qu'il y a eu un intérêt particulier en regard de l'efficacité des cultures vivantes (probiotiques) dans la prévention de la DCD. L'Unité d'évaluation des technologies (« Technology Assessment Unit ») révisa l'utilisation des probiotiques lors de DCD en 2005 et à nouveau en 2009, et ne put recommander leur utilisation suite à ces deux études. La publication de nouvelles recherches nous incita ainsi à faire une mise à jour de ce sujet.

Méthodologie

Une recherche systématique ainsi qu'une revue de la littérature furent menées concernant les articles portant sur l'efficacité des probiotiques dans la prévention de la DCD et publiées jusqu'au 17 juin 2011 dans EMBASE (Ovid), PubMed, Cochrane, DARE et INAHTA. Nous avons identifié un nombre suffisant d'études portant sur l'utilisation des probiotiques *Lactobacillus* (LB) dans la prévention de la DCD nous permettant de considérer une méta-analyse. Pour réaliser la modélisation de l'hétérogénéité entre les diverses études, nous avons choisi un modèle hiérarchique bayésien pour estimer le risque relatif sommatif (RR) de la DCD associée aux probiotiques à partir d'une distribution préalable faible quant à l'information. Nous avons mené des analyses de sous-groupes pour évaluer les risques de biais, l'incidence en arrière-plan de DCD chez le groupe placebo des patients ainsi que les sources de financement. Pour évaluer la force des évidences, nous avons réalisé une analyse de crédibilité bayésienne. Enfin, nous avons revu les évidences sur l'innocuité concernant l'utilisation des probiotiques.

Résultats

Nous avons identifié 11 études randomisées (dont 3 nouvelles depuis notre dernière recherche menée en 2009) sur l'utilisation du LB dans la prévention de la DCD, dont 7 études rencontraient les critères d'inclusion requis pour une méta-analyse. Parmi les études qui furent exclues, une étude ne comportait pas de test pour le *C difficile*, une seconde examinait la colonisation plutôt que l'infection, une autre présentait des résultats à des moments donnés plutôt que d'évaluer la présence de la diarrhée associée à la prise d'antibiotiques, et la dernière ne mentionnait pas le nombre de patients affectés par le *C difficile*. Les études retenues rapportaient des valeurs

différentes concernant le risque d'un patient de contracter la DCD (tel qu'indiqué par la proportion des patients affectés dans le groupe placebo), la souche probiotique, la dose de même que la durée de la période de suivi. Le nombre de patients considérés variait de 34 à 437.

Après avoir réviser les résultats pour évaluation incomplète du *C difficile*, la DCD fut observée chez 17/595 patients dans le groupe LB et chez 53/507 patients dans le groupe placebo. Le RR sommatif de l'analyse bayésienne dans un contexte d'information peu précis fut évalué à 0,17 (95% CI = 0,04-0,42), signifiant une réduction du risque de 83%. La déviation standard du log RR de 0,59 entre les études (95% CI = 0,06-2,49) était relativement élevée, soulignant une hétérogénéité statistique considérable. Le RR prédit chez une étude subséquente était de 0,18 (95% CI = 0,01-1,52).

Les RR sommatifs des analyses des sous-groupes s'échelonnaient de 0,13 à 0,23 où l'intervalle CI de 95% traversait la valeur « 1 » uniquement lorsque les sous-groupes ne comportaient que deux ou trois études. L'analyse de crédibilité bayésienne (une méthode pour évaluer la crédibilité d'une conclusion en calculant l'importance de l'information contradictoire nécessaire pour la renverser) montra qu'un niveau raisonnable de scepticisme lié à une distribution préalable centrée sur un RR=1 et l'échelonnant de 0,5 à 2 serait suffisant pour faire pencher l'extrémité du RR sommatif dans une direction associée à aucun effet bénéfique statistiquement significatif. Ainsi, selon cette distribution, les RR inférieurs à 0,5 sont très peu probables. Ceci est équivalent à l'information provenant d'une étude randomisée équilibrée comprenant 14 patients avec DCD dans chaque branche; en d'autres termes, l'évidence est relativement faible.

Innocuité

De façon générale, la prise de probiotiques dans les études randomisées impliquant des *Lactobacillus* comportait peu de complications. Il n'y eu aucun décès ou aucune infections attribués aux probiotiques. Par contre, quelques cas de fièvre sont survenus dans les deux groupes de patients traités. Dans la plupart des études, les critères de sélection excluaient les patients avec un risque élevé d'infection, tels les patients immunosupprimés, les patients avec anomalies cardiaques ou avec une maladie intestinale. Une revue systématique portant sur les probiotiques chez les patients recevant un support nutritionnel identifia un faible nombre d'études de cas d'infections systémiques avec des organismes probiotiques chez les patients gravement malades ainsi que deux essais cliniques chez les adultes (transplantés ou souffrant de pancréatite) où l'on souligna plusieurs complications dans le groupe recevant des probiotiques.

CONCLUSIONS

Efficacité clinique

- Parmi les 7 études comprises dans la présente méta-analyse, l'administration de *Lactobacillus* était associée à une diminution moyenne du risque relatif de C difficile de 83% (RR sommatif = 0.17 (95% intervalle de crédibilité ICr = 0.04-0.42)).

- Le nombre d'évènements considérés dans la base de données était relativement faible (17 dans le groupe probiotique et 53 dans le groupe placebo). De même, il y avait une hétérogénéité statistique considérable du RR parmi les études ainsi qu'une hétérogénéité des incidences de DCD en arrière-plan, soulevant un questionnement quant à la généralisation du RR sommatif appliqué aux études individuelles.

- L'analyse de crédibilité bayésienne, qui évalue la robustesse des résultats par rapport à l'information préalable, montra qu'un niveau de scepticisme faible avant le calcul du risque produit une distribution ultérieure du RR qui inclut la valeur « 1 ».

- Aucune des études randomisées rapportées à ce jour n'a considéré les évènements qui affectent les coûts hospitaliers (par exemple, la durée de séjour) chez les patients avec DCD.

- À ce jour, les résultats des études randomisées fournissent des preuves à l'effet que les probiotiques basées sur le *Lactobacillus* peuvent être efficaces pour prévenir la DCD. Cependant, pour les raisons mentionnées précédemment, l'on ne peut s'appuyer sur ce faible niveau d'évidence pour rédiger une politique générale.

Innocuité

- Chez les patients considérés dans les études randomisées, ce qui exclut les patients gravement affaiblis et immunocompromis, le traitement impliquant les probiotiques semble sans risque d'effets secondaires. Cependant, certaines études de cas ont rapporté des effets secondaires importants chez les patients gravement malades.

RECOMMANDATIONS

Malgré certaines preuves nous suggérant que les probiotiques basés sur le *Lactobacillus* peuvent être efficaces dans la prévention de la DCD, ces preuves ne sont pas assez fortes pour supporter un changement de politique générale. Par conséquent, nous ne pouvons recommander actuellement l'utilisation du probiotique *Lactobacillus* dans la prévention de la DCD sur une base routinière, chez les patients hospitalisés recevant des antibiotiques.

The Use of *Lactobacillus* probiotics in the Prevention of Antibiotic Associated *Clostridium Difficile* Diarrhea

1. BACKGROUND

Clostridium difficile-associated diarrhea (CDAD) is the most common form of nosocomial diarrhea[1], is strongly associated with antibiotic use[1], and has been estimated to increase hospital stay for adult in-patients[1].The incidence of hospital acquired CDAD has changed over time, with recent outbreaks in Quebec over the period 2003-2005. In March 2004 it reached a winter peak in Quebec of 20.7 cases per 1000 discharges (data from 83 hospitals), subsequently declining to 9 cases per 1000 discharges in January 2006[2]. During that time, greater morbidity and mortality were observed, attributed to a new, more virulent strain of *C difficile*[3]; in 12 hospitals monitoring the outbreak, the overall mean incidence was 22.5 per 1000 admissions and the 30-day attributable mortality rate was 6.9%[3]. The most recent surveillance data, for December 2009 to March 2010, gives an incidence of 6.9 per 10 000 person-days of hospitalization[4].

A probiotic is a live microorganism or a mixture of various bacteria administered to improve the microbial balance in the host GI system. In 2005[5] and again in 2009[6], the use of probiotics in the prevention and treatment of CDAD in adults was evaluated by the Technology Assessment Unit (TAU) of McGill University Health Centre (MUHC). In 2005, there was little evidence relating to the use of probiotics for the prevention and treatment of CDAD, and its use was not recommended[5]. This conclusion was unchanged after the review of 2009[6]. However, new evidence published on prevention of CDAD prompted a third update, to evaluate if sufficient evidence has accrued to alter our previous recommendation.

2. OBJECTIVES

To evaluate the effectiveness of *Lactobacillus*-based probiotics for the prevention of CDAD associated with administration of antibiotics.

3. METHODS

3.1. Literature search

We updated the literature search reported in 2009[6], using the same search methodology as described in our previous reports in 2005[5] and 2009[6], but restricting the search period to the beginning or mid-2009, as the database permitted. We also added a search of EMBASE from 2005-2011 (Week 24), updating the published systematic review of Dendukuri et al, 2005[7]. We limited our search to randomized controlled trials and meta-analyses on the use of probiotics (any strain) for the prevention of AAD in which CDAD was a primary or secondary outcome. Language of publication was limited to English or French, and the literature search ended on June 17, 2011. Literature search and data extraction were carried out independently by two authors (AS, XX).

A supplementary grey literature search using the same terms was conducted with the assistance of a medical librarian (GB) in an attempt to identify studies not published in the indexed peer reviewed literature and unpublished studies. The search included conference abstracts (ProceedingsFirst), theses (ProQuest Dissertations and Theses, and Theses Canada), and clinical trial registries (ClinicalTrials.gov, Current Controlled Trials, CenterWatch Clinical Trials Listing Services, and WHO Clinical Trials Registry).

3.2. Study selection

Previous TAU reports on the subject of probiotics[5, 6] did not include a meta-analysis due to substantial heterogeneity between studies in terms of the type and dose of probiotic, as well as the patient population. With the availability of newer studies on prevention of AAD and/or CDAD in adult inpatients by *Lactobacillus* species, we concluded that there were a number of comparable studies, justifying the use of meta-analysis to summarize their results. We therefore refined our objective to assess the evidence of the use of *Lactobacillus*-based probiotics for the prevention of CDAD in hospital in-patients. Studies were selected for inclusion if they met the following criteria.

- The study was described as a double-blinded randomized controlled trial.
- Study subjects were exclusively or predominantly adult inpatients receiving antibiotics of any kind.
- The active treatment was a probiotic that included *Lactobacillus* species at any dose.
- The study measured CDAD as an outcome, and used a general case definition of diarrhea with a positive laboratory measurement of *C difficile* culture and/or toxin.
- The report contained sufficient information about the number of patients tested for CDAD to allow for adjustment for incomplete testing for *C difficile*.

We included double-blinded RCTs with incomplete testing for *C difficile* in subjects with AAD with the rationale that if treatment blinding was adequately maintained, then testing for *C difficile* should not depend upon treatment status, and therefore the likelihood of detection should not depend on treatment status. We also reasoned that dose differences may be less relevant beyond some threshold dose[8-10], however, that dose is yet to be determined.

For each included study, we assessed the risk of four biases identified by the Cochrane collaboration[11]:

- Selection bias (adequate randomization technique, allocation concealment),
- Treatment bias (adequacy of blinding),
- Attrition bias (equal follow-up, withdrawals) and
- Detection bias (blinded and systematic assessment of CDAD).

If the article reporting the study did not provide the information for us to ascertain the risk of bias, then we assumed that there was a potential risk of bias. Based on the risk assessment, studies were classified as:

A. No evidence of bias,

B. Possibility of one source of risk of bias,

C. Possibility of two or more sources of bias.

Risk of bias was assessed independently by all three authors. Discrepancies were resolved by discussion to arrive at a consensus.

3.3. Descriptive statistical analysis

A L'Abbé plot was used to explore the relationship between the treatment effect and the background incidence of CDAD (as measured by the proportion with CDAD in the placebo group of each RCT). This is a plot of the proportion with CDAD in the placebo group vs. the proportion with CDAD in the *Lactobacillus* probiotics group in each study included in the meta-analysis. We also used descriptive graphs to study how the sensitivity and specificity of the diagnostic test for CDAD impacted the observed risk ratio. Plots were prepared using the R statistical software package[12].

3.4. Meta-analysis

For each study, we estimated the risk ratio (RR) of CDAD, comparing the *Lactobacillus* treatment group with placebo. In Gao et al[13], which used two doses, we combined all patients treated with *Lactobacillus* into one group. In trials with incomplete testing for *C Difficile* we assumed that untested patients had the same risk of having CDAD as the tested patients within each of the placebo and treatment group(s). The adjusted risk of

CDAD cases was estimated as the product of (i) the risk of AAD, and (ii) the risk of CDAD among tested AAD cases.

For the meta-analysis we used a Bayesian hierarchical model to estimate the pooled RR and its 95% credible interval (CrI), including imputation as described. We used low information prior distributions over the unknown parameters (the pooled log risk ratio and the variance between log risk ratios in the different studies) so as to allow the observed data to dominate the final results. The meta-analysis was carried out using WinBUGS Version 1.4.3 for Windows[14]. For comparison, we also carried out a classical frequentist meta-analysis using the method described by Dersimonian and Laird[15]. This analysis was implemented using the rmeta[16] package in the R statistical software package.

3.5.   Sub-group analyses

We repeated the meta-analysis within sub-groups to examine the robustness of the pooled estimates to: (i) baseline CDAD risk, (ii) source of funding, (iii) risk of bias.

- We divided studies into two groups according to the background incidence rate of CDAD, assuming that this was captured by the incidence in the placebo arm. Based on discussions with local infection control experts, we defined the high incidence group as one with an incidence ≥6%.

- We divided studies into two groups according to the source of funding: no industry support versus support from probiotics companies. We did a separate subgroup analysis of the 3 studies that were sponsored by the probiotics company Bio-K+, which used the same strain of *Lactobacillus*. It has been pointed out[17] that the effectiveness of a probiotic preparation may be highly influenced by the strain.

- We repeated the meta-analysis with studies divided into those of low risk of bias (A) and those of moderate or high risk of bias (B or C).

3.6.   Identifying the critical prior distribution

Clinical trial data do not reflect the totality of the evidence around probiotics, and some reviewers have expressed scepticism of the potential efficacy on the basis of their understanding of biological mechanisms, strain variation, and assay of the probiotics products themselves[18, 19]. To assess the strength of our results in the presence of skepticism, we used an indirect approach called Bayesian credibility analysis[20]. This approach allows us to determine the critical sceptical prior distribution (centred over a pooled risk ratio of 1) that would be sufficiently influential as to change the results of our meta-analysis from statistically significant to non-significant (i.e. including RR=1 in the 95% posterior credible interval). If the critical prior distribution covers a very narrow

range, it would suggest that one would need to be extremely sceptical to doubt the observed results. If, on the other hand, the critical prior covers a wide range of plausible RRs, it would suggest that the observed evidence is weak, and a weakly sceptical prior is sufficient to make the pooled RR non-significant.

Further, using the method described by Spiegelhalter[21], we also expressed this sceptical prior distribution in terms of the number of CDAD cases obtained in a balanced, null trial.

4.   RESULTS

4.1.   Literature review: randomized controlled trials

Our previous report identified 8 RCTs that compared a *Lactobacillus* probiotic preparation with placebo for the prevention of AAD and/or CDAD in hospitalized adults receiving antibiotics. We identified 3 additional RCTs involving *Lactobacillus* [13, 22, 23], bringing the total to 11[13, 22-31].

Seven studies met our inclusion criteria for the meta-analysis. Four were excluded, for the following reasons: no testing for *C difficile*[26]; numbers tested for *C difficile* was not available (either in report or through contact with authors)[25]; outcome of interest was colonization with *C difficile*, not CDAD[22]; stool samples were to be obtained at baseline and study end, and during diarrhea, but compliance was low and the report did not make clear how many patients had been tested during diarrhea[30].

Included trials ranged in size from 34 to 437 patients, and were conducted in Canada[23, 29], the United States[24, 31], the UK[27, 28], and China[13]. Patients received *Lactobacillus* species as single preparations or in combination. Doses of *Lactobacillus*-containing preparations were generally reported in colony forming units (cfu), and ranged from <20 to 100 billion cfu. The lowest doses came from commercial yogurts, and the highest from capsules. None of the trials described additional interventions, eg, measures in infection control, cleaning protocols, or antibiotic stewardship. A variety of definitions of diarrhea were used, allowing for one to three liquid stools, over one to three days. Details of the design in these studies are summarized in Table 2, including definition of diarrhea, probiotic and dose, length of treatment and length of follow-up.

All trials reported the number of patients who experienced AAD and CDAD in each group. None of the studies reported the rate of CDAD (i.e. the person-time with CDAD out of the total person-time), thus it is unknown if fewer CDAD cases in the probiotics group corresponds to reduced burden to a hospital in terms of reduced length of stay. Efficacy results (proportion with AAD and CDAD) appear in Table 3, risk of bias in Table 4, and safety results in Table 5.

Search of non-peer-reviewed materials identified three studies that had been registered but not reported[32-34] (350 patients in total), and three ongoing trials using *Lactobacillus* probiotics with the prevention of CDAD as a primary or secondary endpoint[35-37]. Two of the unreported studies involved Bio-K+[32, 33], one of which was suspended for lack of recruitment[32]. The entry for one additional ongoing study indicated CDAD as an endpoint but did not specify the constituents of their probiotic drink[38].

4.2. Meta-analysis

Three RCTs[23, 29, 31] did not report results of testing for *C difficile* in all subjects with AAD, requiring us to estimate the adjusted number of CDAD cases in both arms. RRs calculated using adjusted CDAD cases were slightly larger than those calculated using unadjusted CDAD cases (Adjusted versus unadjusted RRs: Beausoleil 0.20 versus 0.14; Safdar 0.15 versus 0.13; Dylewski 0.19 versus 0.16).

4.2.1. *Results of descriptive statistical analyses*

The L'Abbé plot[39] in Figure 1 shows that most studies found a beneficial effect of probiotics on CDAD. There was considerable heterogeneity in the background incidence of CDAD ranging from 0 to 24%. The treatment effect was greater in studies with a higher background incidence of CDAD, and the risk of AAD was also higher in these studies.

Figure 1 L'Abbé plot illustrating estimates of risk of CDAD and AAD in *Lactobacillus* probiotics and placebo groups in individual trials included in the meta-analysis
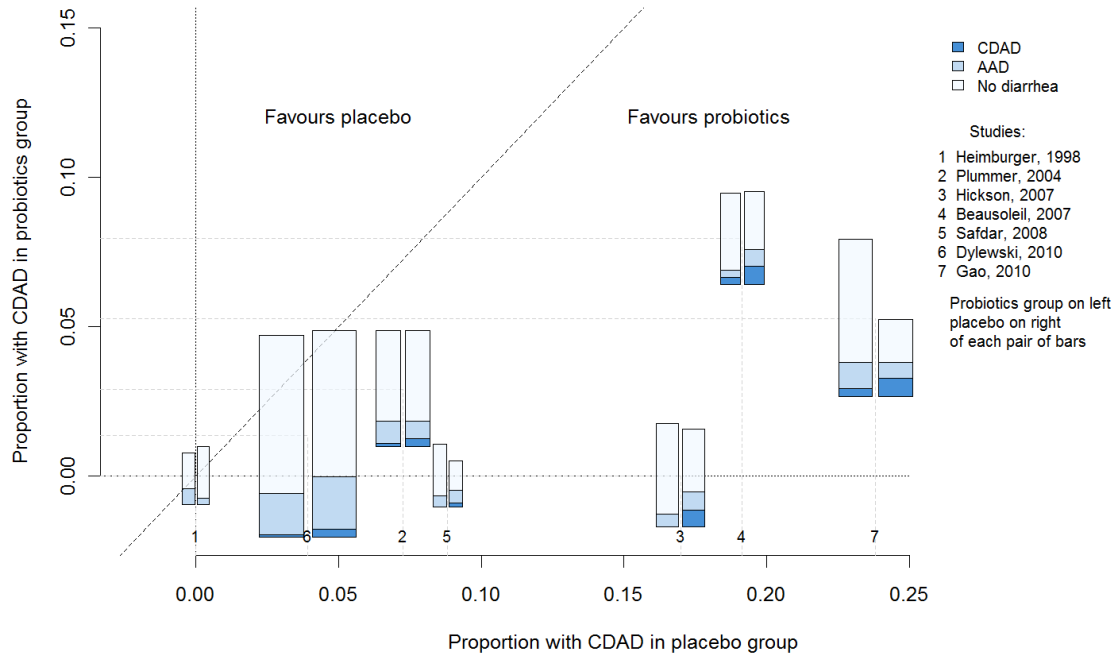
Each study is represented by a two-column stacked bar plot. Within the stacked bar plot, the bar on the right represents the placebo group and the bar on the left, the probiotics group. The area of the stacked bar plot is proportional to the total number of subjects in the study. Individual bars (dark to light) summarize the numbers with CDAD (diarrhea with a positive *C difficile* test), AAD only, and no diarrhea, respectively. The treatment effect (roughly proportional to the distance from the diagonal) was greater in studies with a higher background incidence of CDAD

4.2.2. Results of meta-analysis

After adjustment for incomplete testing for *C difficile*, an estimated 17 out of 595 patients in the *Lactobacillus* group and 53 out of 507 patients in the placebo group had the outcome of CDAD, respectively. A forest plot summarizing the results of the meta-analysis is presented in Figure 2. The RRs of *Lactobacillus* versus placebo for individual studies ranged from 0.05 to 1. The median pooled RR was estimated to be 0.17 (95% credible interval (CrI): 0.04, 0.42), indicating a statistically significant association on average between *Lactobacillus* and a lower risk of CDAD for inpatients, and an 83% risk reduction relative to placebo. The between-study standard deviation in the log risk ratio (Median: 0.59 (95% CrI: 0.06, 2.49)) was relatively high, indicating considerable statistical heterogeneity between studies. The predicted RR in a new trial was estimated

to be 0.18 (95% CrI: 0.01, 1.52), the wide CrI also reflecting the heterogeneity, and the resulting predictive uncertainty[40].

The analysis was repeated without adjustment for incomplete testing, assuming that untested patients did not have CDAD. There was minimal change in the RR, which was estimated to be 0.16 (95% CrI 0.03, 0.36).

Figure 2   Forest plot of effect of *Lactobacillus* on prevention of CDAD (Bayesian analysis)
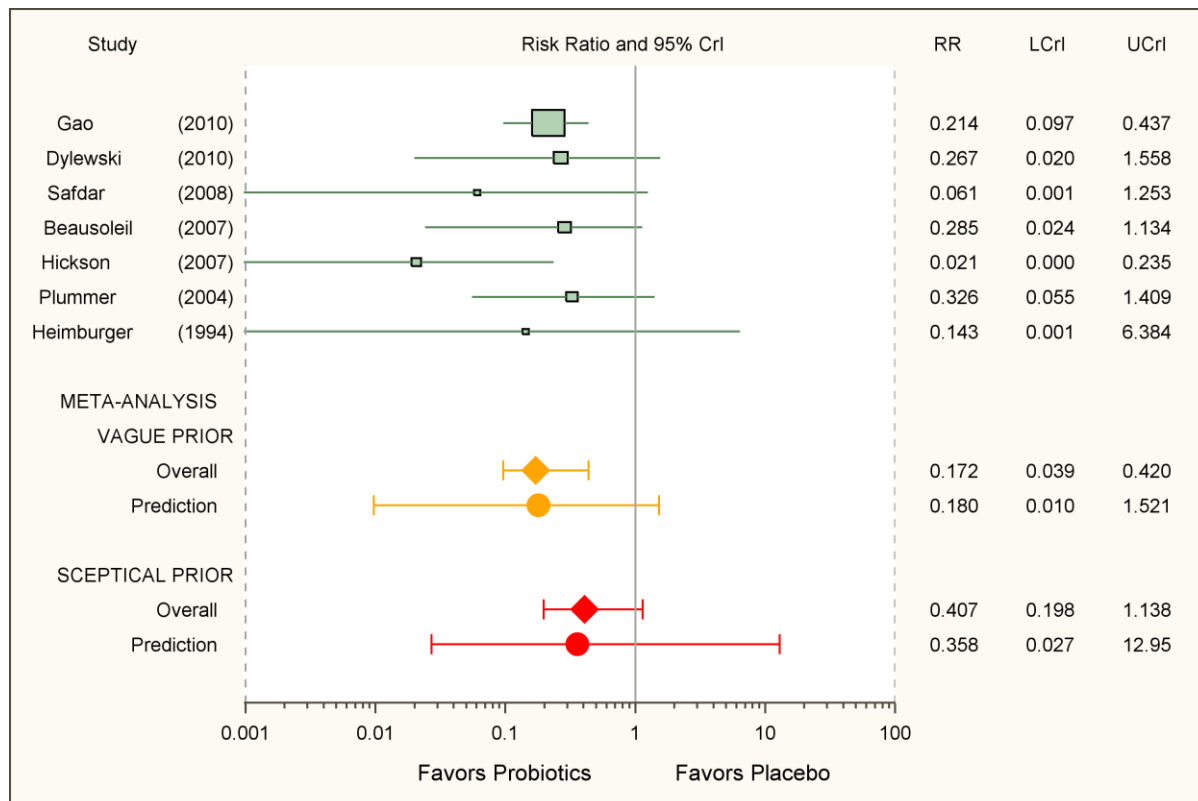
In order from the top, the plot shows the median RRs and 95% credible intervals estimated using the individual studies, the pooled RR and 95% credible interval estimated using a Bayesian meta-analysis with a vague (non-informative prior), and the predicted median RR and 95% credible for the next study, based on the Bayesian meta-analysis. The final two lines are the pooled and predicted RR and credible interval from a Bayesian meta-analysis with a moderately sceptical prior.

The estimate of the between study standard deviation from the frequentist meta-analysis was 0, and the resulting fixed effects pooled RR was 0.28 (95% confidence interval 0.17, 0.46). Though this result also implies that the average RR across studies is statistically significant, it clearly underestimates the heterogeneity between studies. We therefore preferred to use the results from Bayesian meta-analysis as it takes into account the complete uncertainty in all parameters including the between study variance[21].

4.2.3. Sub-group analyses of meta-analysis

Results of meta-analyses within sub-groups are shown in Table 1. Median RRs in all scenarios range from 0.13 to 0.23, close to the pooled estimate of 0.16 based on all 7 included studies. As expected, a number of the 95% credible intervals are very wide, and include RR=1, due to the small number of studies within sub-groups.

Table 1   Results of subgroup meta-analysis of Bayesian primary analysis

Study characteristic	RR Median (95% CrI)
Incidence of CDAD in Placebo group	
Less than or equal to 5% (2 studies, Dylewski[23] and Heimburger[24])	0.20 (0.1, 3.38)
Greater than 5% (5 studies)	0.16 (0.03, 0.51)
Source of funding	
No support from probiotics companies (2 studies, Safdar[31], Plummer[27])	0.20 (0.01, 2.43)
Support from probiotics companies (5 studies)	0.16 (0.03, 0.57)
Support from BioK+ (3 studies, Gao[13], Dylewski[23], Beausoleil[29])	0.23 (0.05, 1.15)
Study quality	
A (2 studies, Gao[13], Hickson[28])	0.13 (0.01, 1.42)
B or C (5 studies)	0.20 (0.03, 0.75)

Abbreviations: RR=Risk ratio, defined as the proportion of subjects with CDAD in the probiotics group divided by the proportion of subjects with CDAD in the placebo group

4.2.4. Bayesian credibility analysis

We determined that the critical sceptical prior, centred at RR=1, ranges from RR=0.5 to RR=2.0. It reflects the belief of a sceptic who considers that the true treatment effect is unlikely to be less than RR=0.5 or greater than RR=2, consistent with the belief that new treatments are unlikely to be associated with very strong effects. Figure 2 illustrates the impact of this sceptical prior on the pooled and predicted RR and their 95% credible intervals.

We can also think of this prior as being equivalent to information obtained from an RCT with equal numbers of subjects in both arms and 14 CDAD cases in each of the *Lactobacillus* and placebo arms. In other words, a prior distribution that is as informative as a null trial with a relatively small number of cases would be sufficient to move the pooled RR from the meta-analysis in the direction of 'no evidence of a beneficial effect of probiotics'.

4.3. Safety

The safety profile for *Lactobacillus* in the 8 RCTs that reported adverse events was benign (Table 5). The events were relatively mild (abdominal pain, bloating, fever, nausea, loose stools, etc) and might also arise as a result of AAD or CDAD). These events were reported with the same or higher frequency in the placebo groups as in those receiving probiotics. In most studies, the entrance criteria specifically excluded individuals at increased risk of infection, eg, patients with immunosuppression, cardiac abnormalities, or intestinal disease.

5.   DISCUSSION

Our meta-analysis suggested that probiotics containing *Lactobacillus* species (*L acidophilus, L bulgaricus, or L casei*) have a statistically significant preventative effect on CDAD with a pooled relative risk reduction of 83%. However, the wide credible interval around the predicted benefit in a future study reflects considerable heterogeneity in the studies done so far. Though our sub-group analyses did not identify any systematic source of heterogeneity, it should be noted that these analyses were under-powered due to the small number of studies. A credibility analysis also demonstrated that the evidence accrued so far is weak and can change quite easily with the use of a weak sceptical prior distribution. It should also be noted that none of the RCTs measured outcomes that actually affect hospital costs, such as the length of stay or number of colectomies, among their patients with CDAD. These issues remain to be answered in ongoing and future studies.

Below we discuss in detail the possible impact of common biases and sources of heterogeneity on our results.

5.1. Risk of bias

Risk of publication bias: The funnel plot for the meta-analysis (not shown) did not suggest publication bias, but funnel plots are relatively insensitive, particularly in small meta-analytic datasets, and the detection is complicated by the presence of heterogeneity[41]. A search of unpublished sources, including Clinical Trial registries, found three additional studies which had been registered but not yet reported (one of which had been suspended for lack of recruitment), and four studies of interest that are ongoing. The unreported trials represent an additional 350 patients, however the lack of information and their pilot design do not allow a definitive conclusion as to whether they might have been included and might have influenced the analysis. The ongoing trials represent a further 4800 patients, including a large study of nearly 3000 patients[37].

Possible inadequacy of blinding: Though all studies described their attempts to make the probiotic and placebo similar in appearance, texture and taste, none of them explicitly assessed the success of blinding during the study. Hickson et al[28] acknowledged that the probiotic drink they used and corresponding placebo were non-identical in packaging and appearance. They did, however, state that outcome assessment was blinded. In the other studies (Table 4), the blinding status of the outcome assessor was not described, although in those studies that used pills or capsules, allocation concealment and double-blinding (and the absence of unmasking side effects) might reasonably be expected to lead to blinded outcome assessment. Inadequate blinding is therefore not expected to be a significant source of bias.

Incomplete measurement of outcomes: Two of the studies (Hickson, 2007[28]; Heimburger, 1994[24]) had >15% missing outcomes. In Hickson[28], 4% patients withdrew, and 12% could not be contacted. In Heimburger, reasons for non-completion included, discontinuation of enteral feeding and protocol violations; none of the non-completers experienced diarrhea. Three[23, 29, 31] studies reported that some patients with AAD included in the analysis were not tested for *C difficile*. Given the methods described for allocation concealment and blinding, we would not expect a systematic difference between treatment groups in selection of patients for testing for *C difficile*. In our meta-analysis, we assumed that missing tests had the same probability of being positive as those measured. However, if testing depended upon severity of diarrhea, we may be overestimating the number of positive tests in the missing results. Nevertheless, our adjustment produced only slight changes to the risk ratios measuring the beneficial use of probiotics.

Imperfect sensitivity/specificity of the tests used: There was considerable variation across studies in methods used to measure CDAD. Two reports[13, 23] indicated that screening for CDAD involved both toxin A and toxin B, and the remainder indicated that testing was for *C difficile* toxin. Stool culture followed by assay for presence of toxin is the gold standard for detection, but is too slow for clinical purposes. Cytotoxin detection alone has a sensitivity of 67-100%[18]. Enzyme immunoassay (EIA) for toxin A and/or B has sensitivity 63-94% and specificity 75-100%[18]. The use of a less sensitive test would have led to underdetection of cases, which if the blind was maintained, should not be related to treatment.

The use of a less specific test would have led to a mixture of CDAD and AAD being identified as CDAD. As probiotics apparently prevent AAD (RR ~0.5)[8, 9, 17], inclusion of cases of AAD identified as CDAD might result in an apparent protective effect for CDAD, even in the absence of an effect for CDAD. Figure 3 illustrates the effect of variations in sensitivity and specificity on the observed RR, with the following assumptions: true RR of CDAD is 1, while the RR for AAD would be 0.5. Incidence of AAD was assumed to be 31.5% and of CDAD, 10.5% (the pooled placebo group

incidences from our group of studies). However, even at a plausible lower limit of measured specificity and sensitivity, the smallest observable RR would be between 0.5 and 0.6, still considerably higher than the estimated RR in the meta-analysis (RR=0.17).

Figure 3   Effect of CDAD test sensitivity/specificity on observed risk ratio for CDAD prevention assuming true risk ratio is 1
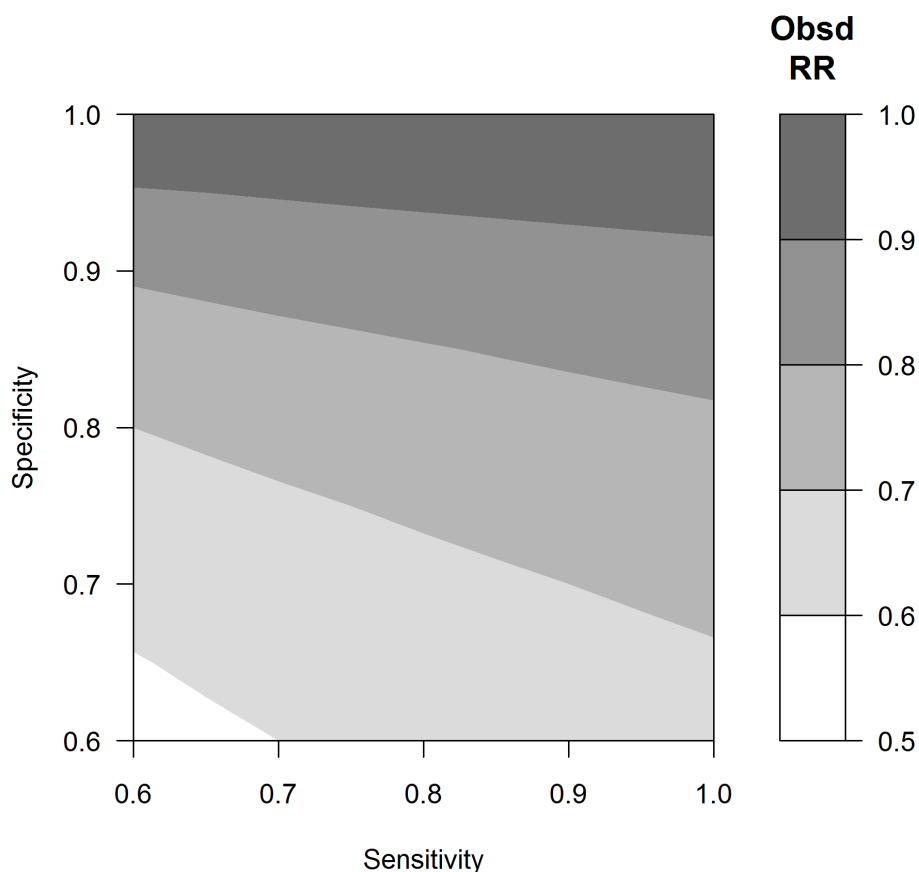
The graph depicts the relation between the observed RR of CDAD and the sensitivity and specificity of the diagnostic test, assuming RR for AAD is 1, true RR for CDAD is 0.5, prevalence of AAD is 31.5% and prevalence of CDAD is 10.5%. Each shaded segment corresponds to a different range for the observed RR. For example, when both sensitivity and specificity were as low as 70%, the observed RR of CDAD lies between 0.6 to 0.7.

Source of funding: Industry funding has been associated with the finding of favorable results in trials[42, 43]. Five[13, 23, 24, 28, 29] out of 7 studies were funded by probiotics companies. One[31] study stated that the company provided probiotics and placebo, but did not mention any direct financial contribution, and one study[27] did not report the source of funding. The subgroup analyses of studies with and without detailed funding does not suggest that funding source substantially influenced outcomes (Table 1), but

the numbers of studies in the groups were small, and funding was not clearly reported in all studies. Had we required an explicit statement of no industry support, none of the studies would have qualified.

5.2. Sources of heterogeneity

Variation in patient risk of contracting CDAD: Across all the studies the proportion of placebo patients who developed AAD ranged from 11 to 44%, while the proportion of patients with AAD who tested positive for C *difficile* ranged from 0 to 54%, and the proportion of all patients in the placebo group who tested positive for CDAD ranged from 0 to 24%. The study that contributed the largest number of cases to the analysis had a background incidence of 24%. This study restricted eligibility to patients receiving high risk antibiotic, and was conducted in China, where according to some reports, antibiotics are used more liberally, possibly contributing to a higher background infection rate[44]. We cannot assess the effect of variations in non-pharmaceutical interventions directed at reducing CDAD risk (eg, cleaning regimens, cohorting of affected patients, antibiotic stewardship) on any measured effect of probiotics.

Possible variation of local *C difficile* strains: The reported studies were conducted in a number of countries. Comparison of strains across countries is hampered by changing surveillance strategies, varying case definitions, multiple assay types and classifications, and incomplete testing within studies. Most of the attention has been focused on the NAP1/027 strain, which in Europe and America has been associated with rising incidence and an increased risk of severe, complicated disease with associated mortality[3, 45], although whether it is entirely responsible for the apparent increase in incidence and severity remains to be determined. In Asia, NAP1/027 has so far been relatively uncommon: Strains isolated at another Shanghai hospital over the period that the study by Gao et al[13] was conducted did not include the NAP1/027 strain[46, 47], but instead included a toxin-deficient strain that was common in Asia but not in the rest of the world. It is possible that variation in local strains has the potential to affect any observed response to probiotics. However, this is speculative; we do not have the data to confirm or refute it.

Variation in type and duration of antibiotic: Antibiotic use varied across studies, with one study restricting eligibility to those patients receiving antibiotics expected to carry a high risk of CDAD[13]. The proportion of patients on beta-lactams (penicillins and cephalosporins) ranged from 53.9% to 77.6%, those on macrolides ranged from 16.4% to 58.4%, and those on quinolones from 31.6% to 59.6%. Most patients received more than one antibiotic. Not all studies reported the duration of antibiotic treatment within the study; those that did reported durations of 8.2 to 12.5 days within treatment groups[13, 23, 25, 29]. We do not have the data or sufficient number of studies to incorporate antibiotic treatment (type and/or duration) as a variable in our analysis.

Variation in probiotic species, dose, and encapsulation: We selected only studies that included *Lactobacillus* species, alone or in probiotic mixtures. Still, as is common with studies involving complementary medicines, there was wide variation in dose and form in which the probiotics were given (Table 2). The reported daily dose of *Lactobacillus* species ranged from <20 to 100 colony forming units (cfu), with more recent studies tending to have higher cfu. Only a minority of studies reported assay results[28] or assessed compliance[25, 28]. Probiotic bacteria have the potential to colonize the gut, and therefore we might expect there to be less dose sensitivity above the minimum dose for innoculation. We do not, however, have evidence to indicate that the minimum dose was reached: Gao et al 2010[13] observed a difference in response between 50 and 100 billion cfu, which were the highest doses tested. We do not have the data or sufficient number of studies to examine the influence of dose-variation.

Duration of dosing and follow-up: Duration of dosing of probiotic and follow-up of patients was another significant source of variation in study design (Table 2). Not all studies reported the duration of probiotic treatment; in those that did, duration ranged from 7.3 to 24.5 days within treatment groups[23, 29, 31]. Follow-up ranged from 0 to 4 weeks. Too short a duration of probiotic risked an underestimate of any effect, and too short a follow-up risked missed detection of CDAD, since onset of symptoms can be delayed. Over half the cases reported by Beausoleil et al[29] occurred after hospital discharge, and a secondary analysis by Hickson et al[28] showed that the prevention they observed was confined to the period *after* antibiotic treatment. In those studies where follow-up did not extend beyond treatment, additional cases may have been unrecorded, but we cannot assess the likely effect on the analysis.

5.3. Generalizability of studies

In all studies where data from screening was reported, only a minority of potential subjects who were screened entered the study, with most of the exclusions arising from failure to meet the entrance criteria. Gao et al[13], for example, screened 1120 patients over 3 months, and recruited 255, while Dylewski et al[23] screened 2151 patients over 18 months and recruited 237. Studies generally excluded patients who had started antibiotics more than 24-72 days prior to probiotic (Heimburger et al[24] was an exception) or who had had a recent course of antibiotics, although criteria varied. They also excluded those who had preexisting diarrhea or gastrointestinal disease, or recent CDAD. For safety reasons, patients who had immunosuppression or damaged or artificial heart valves were also excluded.

5.4. Safety

When used in populations comparable to those in the eight studies reviewed above, probiotic therapy appears to be benign. From a regulatory perspective they are

"generally regarded as safe"[48, 49], although this designation is predicated on their use in a healthy population[41]. In a review of probiotics safety, Snydman found no epidemiologic evidence to suggest an increased risk with consumption of probiotics[48]. However, there are reports of rare, more serious side-effects observed in certain debilitated and immunosuppressed individuals.

Whelan et al[50] conducted a recent systematic review of all evidence on the safety of probiotics in adult and pediatric patients receiving nutritional support, identifying 53 trials in which 4131 adult and pediatric patients received probiotics. In 50 trials, probiotics were associated either with no effect or a positive effect on outcomes related to safety (eg, mortality and infections), while an increased complication rate was observed for probiotics in specific adult patient groups (liver transplant and severe pancreatitis), and one pediatric study. Whelan also identified 5 case reports of bacteremia from *Lactobacillus rhamnosus* and 27 of fungemia from *Saccharomyces boulardii*. Our non-systematic search retrieved several more case reports of *Lactobacillus* invasive infection in patients not receiving nutritional support that the authors linked to ingestion of *Lactobacillus* probiotics[51-54], again in people with immunological and barrier compromise. In part because of the risk of invasive infections, the 2010 clinical practice guideline for *Clostridium difficile* infection in adults by the Society for Healthcare Epidemiology or America and the Infectious Diseases Society of America did not recommend the routine use of probiotics in prevention of CDAD[18]. In his review Snydman notes that *Lactobacillus* is a normal gut inhabitant and bloodstream infection in the absence of probiotics has also been documented[48] as a complication of serious illness. He advocates appropriate safety monitoring in clinical trials of *Lactobacillus*, and population-based monitoring for reports of infection. The available evidence therefore supports the safety of probiotics for the treatment of non-severely immunocompromised adults

5.5.   Health technology assessments and systematic reviews

In a non-systematic search for health technology assessments and systematic reviews, 6 reviews were retrieved that considered *Lactobacillus* probiotics in the prevention of CDAD (Table 6). The selection of studies differed across reviews, but 4 of 6 have not recommended the routine use of *Lactobacillus* for the prevention of CDAD, on the grounds of insufficient data. Those reviews that did recommend the use of probiotics did not consider *Lactobacillus* separately.

6. CONCLUSIONS

Efficacy

- In the 7 studies included in the present meta-analysis, the administration of *Lactobacillus* was associated with an average reduction in the relative risk of C difficile of 83% (median pooled RR=0.17 (95% credible interval (CrI) 0.04, 0.42)).
- The number of outcomes in the database was relatively small (17 in the probiotics group and 53 in the placebo group), there was considerable statistical heterogeneity in the RR between studies as well as heterogeneity in the background incidence of CDAD, raising concerns about the generalizability of the median pooled RR to individual studies.
- Bayesian credibility analysis, which tested the robustness of the findings to prior information, showed that even a relatively weak sceptical prior for the risk ratio produced a posterior distribution for RR that included 1.
- None of the RCTs reported so far have examined outcomes that actually impact hospital costs, e.g. length of stay, among CDAD patients.
- The results of the RCTs conducted so far constitutes suggestive evidence that probiotics based on *Lactobacillus* may be effective in the prevention of CDAD. However, for the reasons stated, the level of evidence is not yet strong enough to determine policy.

Safety

- For patient populations such as those studied in the included randomized controlled trials, in which severely debilitated and immunocompromised patients have been excluded, probiotic therapy appears to be without risk of significant side-effects. However, there have been some case reports of serious side effects in seriously ill patients.

7. RECOMMENDATIONS

- **Although there is suggestive evidence that probiotics based on *Lactobacillus* may be effective in the prevention of CDAD, the evidence is not strong enough to be the basis for a general policy change. Accordingly, we cannot presently recommend routine use of probiotic *Lactobacillus* in the prevention of CDAD in hospitalized patients receiving antibiotics.**

TABLES

Table 2   Summary of design and risk of bias in randomized controlled trials of probiotics in the prevention of CDAD

Author (year)	Definition of diarrhea	Sample size Pro; Pla	Age (SD) Pro; Pla	Probiotics[a]	Treatment duration (days)	Follow-up (days)	Risk of bias[b]
Trials included in meta-analysis							
Gao[13] (2010)	Three or more liquid stools in a 24-hour period.	Pro-2: 86, Pro-1: 85, Pla: 84	60 (6); 60 (6); 60 (6)	LA and LC (Capsule Pro-1 L 50; Pro-2 L 100)	Antibiotics[c] + 5	21	A
Dylewski[23] (2010)	One or more episodes of unformed or liquid stool in a 24-hour period.	216; 221	59.5 (18.1); 58.1 (19.1)	LA and LC (Milk; L 50)	Antibiotics[c] + 5	21	C
Safdar[31] (2008)[d]	Either watery or liquid stools for 2 or more consecutive days.	23; 17	66.6 (14.5); 72.5 (11)	LA (Capsule; L 60)	Pro: 22.8(9.4) Pla: 24.5(4.8)	0	B

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[a] Dose of probiotics is given in the form of colony-forming units unless specified otherwise. Where two figures are given, the first represents the total dose of *Lactobacillus* species (L), and the second the dose of other species (O). Abbreviations: N=number; Pro= probiotics; Pla= placebo; comm=commercial; N.A.= not applicable; SD=standard deviation; B=*Bifidobacterium*; SB=*Saccharomyces boulardii*; LA= *Lactobacillus acidophilus*; LC= *Lactobacillus casei*; LB= *Lactobacillus bulgaricus*; LR=*Lactobacillus rhamnosus*; LP=*Lactobacillus plantarum*; ST= *Streptococcus thermophilus*; BC= *B. clausii*; CB= *Clostridium butyricum*.

[b] We assessed risk of bias of RCTs included in the meta-analysis according to the Cochrane criteria, mainly focusing on selection bias, performance bias, and attrition bias [11]. RCT quality was categorized into 3 levels, A (low), B (moderate) and C (high). Details are shown in Table 4.

[c] Probiotics were given for the duration of antibiotic therapy (plus an additional number of days, if indicated)

[d] Study was included in our previous report .

Author (year)	Definition of diarrhea	Sample size Pro; Pla	Age (SD) Pro; Pla	Probiotics[a]	Treatment duration (days)	Follow-up (days)	Risk of bias[b]
Beausoleil[29] (2007)[d]	Three or more liquid stools in a 24-hour period.	44; 45	68.8 (14.5); 72.9 (13.4)	LA and LC (Milk; L 50)	Antibiotics[c]	21	C
Hickson[28] (2007)[d]	More than 2 liquid stools a day in excess of normal for 3 or more days.	69; 66	73.7 (11.1); 73.9 (10.5)	LC, LB, ST (yogurt; L 22, O 20)	Antibiotics[c] + 7	28	A
Plummer[27] (2004)[d]	N.A.	Elderly	Elder	LA and B (Capsule, total 20)	20	0	C
Heimburger[24] (1994)[d]	The excretion of > 200g of stool in any 24-hour period.	16; 18	Adult	LA, LB (Granules, dose not given)	≥5	0	C
Trials not included in meta-analysis							
Lonnermark[22] (2010)	At least 3 loose or watery stools per 24 hours for at least 2 consecutive days.	80; 83	47; 43	LP (Milk; L 10)	Antibiotics[c] + 7	7	
Wenus[30] (2008)[d]	At least three fluid stools/day for at least 2 days.	46; 41	58.8 (16.5); 56.2 (18.7)	LR, LA, B (Milk; L 27.5, O 25)	14	0	
Can[55] (2006)[d]	N.A.	73; 78	(25-50); (25-50)[e]	SB (N.A., 0, N.A.)	Antibiotics[c]	28	
Beniwal[26] (2003)[d]	2 or more loose stools per day, representing a	105; 97	69.5 (20-94);	LA, LB, ST (comm yogurt, total 2.3)	8	0	

[e] Mean (range).

Author (year)	Definition of diarrhea	Sample size Pro; Pla	Age (SD) Pro; Pla	Probiotics[a]	Treatment duration (days)	Follow-up (days)	Risk of bias[b]
	change from the prior bowel pattern.		70.5(19-92)[e]				
Thomas[25] (2001)[d]	Either watery or liquid stools for 2 or more consecutive days, or 3 or more bowel more than normal pattern.	133; 134	57.2 (18); 54.4 (17.4)	LA (Capsule, L 20)	14	7	
Lewis[56] (1998)[d]	At least 3 loose stools per day.	33; 36	75 (71, 81)[f]; 77 (70, 85)[f]	SB (Capsule, 226 mg/day)	Antibiotics[c]	0	
McFarland[57] (1995)[d]	At least 3 loose stools per day for at least 2 consecutive days.	97; 96	40.7 (16.0); 42.3 (17.7)	SB (Capsule, 1 g/day)	Antibiotics[c] + 3, up to 28	42	

[f] Mean (interquartile) range.

Table 3   Summary of efficacy and risk of bias in randomized controlled trials of probiotics on AAD and CDAD

Author (year)	AAD no./total no. (%)		P-value	CDAD[a] no./total no.[b] (%)		P-value	Risk of bias[c]
	Pro	Pla		Pro	Pla		
Trials included in meta-analysis							
Gao[13] (2010)	13/86 (15.5); 24/85 (28.2)	37/84 (44.1)	<0.05	1/86 (1.2); 8/85 (9.4)	20/84 (23.8)	<0.05	A
Dylewski[23] (2010)	47/216 (21.8)	65/221 (29.4)	>0.05	1/216 (0.5)	4/221 (1.8)	>0.05	C
Safdar[31] (2008)[d]	4/23 (17)	6/16 (37)	>0.05	0/3 (0)	1 /4 (25)	>0.05	B
Beausoleil[29] (2007)[d]	7/44 (16)	16/45 (36)	<0.05	1/2 (50)	7/13 (53.8)	>0.05	C
Hickson[28] (2007)[d]	7/57 (12)	19/56 (34)	<0.05	0/56 (0)	9/53 (17)	<0.05	A
Plummer[27] (2004)[d]	15/69 (22)	15/69 (22)	>0.05	2/15 (13)	5/15 (33)	>0.05	C
Heimburger[24] (1994)[d]	5/16 (31)	2/18 (11)	>0.05	0/5 (0)	0/2 (0)	--	C
Trials not included in meta-analysis							
Lonnermark[22] (2010)	6/80 (7.5)	5/83 (6.0)	>0.05	1[e]		--	
Wenus[30] (2008) [d]	2/34 (6)	8/29 (28)	<0.05	0/34 (0)	1/29 (3.4)	--	

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[a] Definition of CDAD: Diarrhea was present and *C. difficile* toxin was positive in stool samples.

[b] Denominator is the number of patients with AAD who were tested for CDAD. Values are prior to adjustment for missing data.

[c] We assessed risk of bias RCTs according to the Cochrane criteria, mainly focusing on selection bias, performance bias, and attrition bias [11]. RCT quality was categorized into 3 levels, A (low), B (moderate) and C (high). Details are shown in Table 4.

[d] Study was included in previous report.

[e] Four subjects were reported as tested for *C difficile* at inclusion, but group was only identified for the single positive finding. Otherwise testing was done at inclusion and end of study.

Author (year)	AAD no./total no. (%)		P-value	CDAD[a] no./total no.[b] (%)		P-value	Risk of bias[c]
	Pro	Pla		Pro	Pla		
Can[58] (2006) [d]	1/73 (1.4)	7/78 (9)	<0.05	0/73 (0)[f]	2/78 (2.6)[f]	>0.05	
Beniwal[26] (2003) [d]	13/105 (12)	23/97 (24)	<0.05	--	--	--	
Thomas[25] (2001)[d]	39/133 (29)	40/134 (30)	>0.05	2/133 (1.5)	3/134 (2.2)	>0.05	
Lewis[56] (1998) [d]	7/33	5/36	>0.05	In both groups, 4 cases in total.		--	
McFarland[57] (1995) [d]	7/97 (7)	14/96 (15)	<0.05	3/10 (30)	4/14 (29)	>0.05	

Abbreviations: no=number; Pro= probiotics; Pla= placebo; N.A.= not applicable.

[f] Only *Clostridium difficile* toxin A was assayed in this study [55]. Information of toxin B was not reported.

Table 4   Assessment of risk of bias for studies included in the meta-analysis

| Author | Selection bias | | Treatment bias | Attrition bias | Detection bias | | Final Rating |
	Randomization Technique	Allocation Concealment	Adequacy of blinding	Equal follow-up	Blinded CDAD assessment	Systematic CDAD assessment	
Gao, 2010	Yes	Yes	Yes	Yes	NR	Yes	A[a]
Dylewski, 2010	NR	NR	Yes	Yes	NR	No	C
Safdar, 2008	Yes	Yes	Yes	Yes	NR	No	B[a]
Beausoleil, 2007	NR	NR	Yes	Yes	NR	No	C
Hickson, 2007	Yes	NR	Yes	Yes	Yes	Yes	A
Plummer, 2004	NR	NR	Yes	Yes	NR	Yes	C
Heimberger, 1994	NR	NR	Yes	Yes	NR	Yes	C

NR, not reported

[a] For the purposes of the final rating, studies with adequate randomization and allocation concealment were accepted as having adequate blinding of CDAD assessment.

Table 5   Summary of adverse effects (AEs) in randomized controlled trials of probiotics on AAD and CDAD

Author (year)	Adverse effects	
	Probiotics	**Placebo**
Trials included in meta-analysis		
Gao[13] (2010)	Abdominal pain: 11/86 (12.8%); 21/85 (24.7%); Abdominal distention 8/86 (9.3%); 18/85 (21.2%); Loose stool: 27/86 (31.4%); 38/85 (44.7%); Constipation: 7/86 (8.1%); 10/85 (11.8%); Fever, 1/86 (1.1%); 0/85. No mortality.	Abdominal pain: 34/84 (40.5%); Abdominal distention: 30/84 (37.5%); Loose stool: 49/84 (58.3%); Constipation: 12/84 (14.3%): Fever, 1/84 (1.2%); Hematochezia, 1/84 (1.2%). No mortality.
Dylewski[23] (2010)	All non-serious AEs: 72/216 (33%); Bloating: 47/216 (21.8%); Painful cramps: 32/216 (14.8%); Bloody stool: 4/216 (1.9%).	All non-serious AEs: 76/216 (34%); Bloating: 44/221 (19.9%); Painful cramps: 35/221 (15.8%); Boody stool: 9/221 (4.1%).
Safdar[31] (2008)[13]	Fever: 2/23(9%); Nausea: 0/23 (0%).	Fever: 2/16(12%); Nausea: 3/16 (19%).
Beausoleil[29] (2007)[d]	Withdrawal due AE: 4 (9.1%) patients. 21/44 (48%) patients experienced softened stools, taste disorder, abdominal cramping, etc. 3 deaths not related with probiotics.	Withdrawal due AE: 9 (20%) patients. 20/45 (44%) patients experienced softened stools, taste disorder, abdominal cramping, etc. No deaths.
Hickson[28] (2007)[d]	No related AEs	No related AEs
Plummer[27] (2004)[d]	Not reported	Not reported
Heimburger[24] (1994)[d]	Not reported	Not reported
Trials not included in meta-analysis		
Lonnermark[22] (2010)	Loose stools: 46/80 (57%).	Loose stools: 59/83 (71%)
Wenus[30] (2008)[d]	Not reported	Not reported
Can[58] (2006)[d]	Not reported	Not reported

[13] Study was included in previous report.

Author (year)	Adverse effects	
	Probiotics	**Placebo**
Beniwal[26] (2003)[d]	Bloating: 6/102 (6%)	Bloating: 8/97 (8%)
Thomas[25] (2001)[d]	No difference in nausea or abdominal cramping. Gas or bloating: (28%).	No difference in nausea or abdomina cramping. Gas or bloating: (39%).
Lewis[56] (1998)[d]	No side effects contributable to probiotics.	
McFarland[57] (1995)[d]	No significant adverse reactions. Fever: 0.; intestinal gas: 0.	No significant adverse reactions. Fever: 5 (5%); intestinal gas: 7 (7%).

Abbreviations: AE, adverse event

Table 6   Systematic reviews or health technology assessments that assess the effect of *Lactobacillus* probiotics on CDAD in adults

Reference	Studies[a]	Conclusion
Avadhani et al, 2011[59]	CDAD/CDI primary outcome: Plummer, 2004. CDAD/CDI secondary outcome: McFarland, 1995; Lewis, 1998; Thomas, 2001; Beniwal, 2003; Can, 2006; Hickson, 2007; Wenus, 2008.	"This meta-analysis supports that probiotics are efficacious in preventing AAD and CDAD among hospitalized adults."
Bussières et al, 2010[60]	CDAD/CDI primary outcome: Plummer, 2004. CDAD/CDI secondary outcome: Surawicz, 1989; McFarland, 1995; Thomas, 2001; Beausoleil 2007; Hickson, 2007; Gao, 2010; Lonnermark, 2010.	"L'hétérogénéité et la faible qualité des ECR disponibles ne permettent pas de se prononcer sur l'efficacité des probiotiques pour prévenir les DAA et les DACD chez les adultes. Les résultats selon l'espèce de probiotique ne sont pas cohérents d'une étude à l'autre. Le niveau de preuve est par conséquent indéterminé."
Hsu et al, 2010[61]	CDAD/CDI primary outcome: Plummer, 2004. CDAD/CDI secondary outcome: Surawicz, 1989; McFarland, 1995; Lewis, 1998; Thomas, 2001; Can, 2006; Hickson, 2007.	"There is insufficient evidence to make a recommendation for or against the use of probiotics."
Scheike et al, 2006[62]	CDAD/CDI primary outcome: Plummer, 2004. CDAD/CDI secondary outcome (adults and children): Surawicz, 1989; McFarland, 1995; Lewis, 1998; Arvola, 1999 (children); Thomas, 2001; Kotowska, 2005 (children).	"Probiotics appear to be effective in the prevention of AAD including *C difficile* and have little or no harmful effects."
McFarland, 2006[9]	Primary prevention, primary outcome: Plummer 2003. Recurrent CDAD/CDI: McFarland, 1994; Surawicz 2000; Pochapin 2000, Wullt 2003, Lawrence 2005.	"... three types of probiotics (*Saccharomyces boulardii, Lactobacillus rhamnosus GG,* and probiotic mixtures) significantly reduced the development of antibiotic-associated diarrhea. Only *S. boulardii* was effective for CDAD."
Dendukuri et al, 2005[7]	CDAD/CDI primary outcome: Prevention: Plummer, 2004. CDAD/CDI secondary outcome: Surawicz, 1989; McFarland, 1995; Lewis, 1998; Thomas, 2001	"Studies conducted to date provide insufficient evidence for the routine clinical use of probiotics to prevent or treat CDAD."

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APPENDICES

Appendix 1 Detailed description of search terms

(From previous reports)

PubMed: Mid 2005 to June 17, 2011

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

AND (Clostridium OR difficile OR diarrhea OR antibiotic-associated)

AND (patients)

(Search was repeated without the last term; no additional relevant articles were found)

EMBASE (Ovid Excerpta Medica): 2005 to 2011 Week 24

(Probiotic or Probiotics or Lactobacillus or lactic-acid or acidophilus or casei or bulgaricus or plantarum or rhamnosus or yeast or Saccharomyces or boulardii or cervisiae or Bifidobacterium or bifidum or SF68).mp

AND (Clostridium or difficile or diarrhea or antibiotic-associated).mp

AND (patients or subjects).mp

(Search was repeated without the last term; no additional relevant articles were found)