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Review Article

Saccharomyces boulardii CNCM I-745 for Prevention of Antibiotic-Associated Diarrhea and *Clostridioides difficile* in China: Systematic Review and Meta-Analysis

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Abstract

Probiotics are commonly used for patients receiving antibiotics to prevent antibiotic-associated diarrhea (AAD). Randomized, controlled trials (RCTs) conducted in China are often not included in probiotic reviews and meta-analyses due to the difficulties in accessing Chinese journals and translating the Chinese language. Our aim is to evaluate the efficacy and safety of *S. boulardii* CNCM I-745 in AAD prevention trials done in China. A literature search was conducted using PubMed, Google Scholar and Chinese databases (CNKI, CMB) (from inception to June 2023) for RCTs assessing *S. boulardii* CNCM I-745 for the prevention of AAD and *Clostridioides difficile* infections (CDI). Inclusion criteria: RCT done in China, new prescription of antibiotic, randomized to *S. boulardii* and control groups. Random-effect or fixed-effects models were used depending upon the degree of heterogeneity and the risk of bias for each study was determined. This review was registered with Prospero (PROSPERO CRD 429831). The literature search found 361 articles, which were screened and 46 (8,201 participants) were included in the analysis. *S. boulardii* CNCM I-745 significantly reduced the incidence of AAD (RR=0.43, 95% C.I. 0.40,0.48, $P < 0.0001$) and CDI (RR=0.30, 95% C.I. 0.10, 0.87, $P = 0.03$). *S. boulardii* CNCM I-745 had similar degrees of efficacy for both the prevention of AAD in trials done in China and for trials done in other countries. *Saccharomyces boulardii* CNCM I-745 was well tolerated and effective in trials in China for both the prevention of AAD and CDI.

Keywords: Antibiotics; Diarrhea; Saccharomyces boulardii; Probiotics; Clostridioides difficile

Introduction

Antibiotic-associated diarrhea (AAD) is a common complication of antibiotic use resulting from the disruption of the normally protective intestinal microbiome. While some factors that disrupt the intestinal microbiome are well-known (such as antibiotic exposure, advanced age, diet, and health status) [1], other factors relating to geography and ethnicity are less well documented, but may have important implications for microbiome-based clinical therapies [2]. The World Health Organization (WHO) defines acute diarrhea as ≥3 loose or liquid stools per day for ≥3 days and lasting <14 days [3]. Antibiotic-associated diarrhea is defined as diarrhea occurring while on antibiotics or within 8 weeks of antibiotics [1]. The prevalence of AAD in patients receiving antibiotics can be quite high, ranging from 5-37% in adults and may be higher in children (11-40%) [1]. The onset of AAD typically occurs during antibiotic administration, but delayed onset AAD may occur in 10-20% for up to 8 weeks after antibiotics have been discontinued [1]. While the severity of AAD is typically mild-moderate in severity, 16% of cases are more severe, requiring further antibiotic treatments and 10-35% may be due to *Clostridioides difficile* infection (CDI), which can cause healthcare-associated outbreaks. Consequences of AAD include dehydration (especially in young children), higher mortality and morbidity, increased lengths-of-stays for inpatients (4-24 days), higher risk of colectomy (1-9%), higher healthcare costs and 25-28% of those with CDI develop recurrent form of the infection [1,4].

China is the second largest consumer of antibiotics, most commonly azithromycin, clindamycin and erythromycin [5,6,7]. However, only 25-39% of antibiotics prescribed in China are given appropriately [5]. The prevalence of AAD in China ranges from 14-35%, depending upon age, hospitalization status and co-morbidities [4,8]. Efforts prevent AAD include antibiotic stewardship programs and the adjunctive use of probiotics during antibiotic use [9,10]. A survey of 138 pediatric practitioners in China found 31% gave probiotics along with antibiotics, mainly due to concerns about antibiotic resistance or development of AAD [6].

Specific types of probiotics have been recommended in clinical guidelines for the prevention of AAD, but not all probiotic strains have shown efficacy [10,11,12]. Guidelines for probiotic use to prevent AAD in children focused on Asia-Pacific populations had strong recommendations for *S. boulardii* CNCM I-745 and *L. rhamnosus* GG [13]. These two stains were also recommended for the prevention of AAD in adults in the Asia-Pacific region [14]. Indeed, the efficacy for probiotics is strain-specific and each strain or multi-strained blend must be assessed separately to determine efficacy for AAD prevention [15]. Prior meta-analyses have found *Saccharomyces boulardii* CNCM I-745 and *Lactobacillus rhamnosus* GG to have the strongest evidence for the prevention of AAD, but often have not included RCTs published in non-English languages, due to translation problems and limited access to Chinese journals [16,17,18,19,20].

Saccharomyces boulardii CNCM I-745 is a strain of probiotic that has a long history of use and has demonstrated significant efficacy and safety for a variety of diseases ranging from prevention of AAD and CDI, treatment of pediatric acute gastroenteritis, to reduction of side-effects of *H. pylori* eradication therapies [17,20,21,22,23]. *S. boulardii* CNCM I-745 reaches high levels in the intestine within 2-3 days, is cleared from the colon within 5 days and can be given concurrently with antibiotics, as the yeast is not susceptible to antibiotics [24]. The ability of *S. boulardii* CNCM I-745 to be therapeutic is due to multiple mechanisms-of-action: direct inhibition of pathogen growth or destruction of pathogenic toxins, interference with pathogen/toxin attachment on enteric cell surfaces, improving intestinal cell health, reduction of water secretion, immune system regulation and restoring the normally protective microbiome barrier layer [14,21,25]. *S. boulardii* CNCM I-745 has a remarkable safety profile in that this strain has been given to a wide variety of patient types (children, adults and the elderly, hospitalized inpatients and outpatients, patients with acute and chronic conditions) and data gathered since its introduction in Europe in the 1950s has shown few adverse reactions, most were thirst and constipation [22]. Genetic transfer of antibiotic-resistant genes has not been observed with this strain [26]. In rare cases (1/5.6 million users), immunocompromised patients or inpatients with central catheters have developed fungemia [23].

Our aim in this study is to determine its efficacy and safety of *Saccharomyces boulardii* CNCM I-745 for the prevention of AAD and CDI in trials done in China using a comprehensive literature search to uncover trials not previously found in non-Chinese databases.

Materials and Methods

Literature search

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed [27]. The PRISMA checklist is provided (Supplementary Table S1). Recent recommendations for reporting probiotic meta-analyses were also followed [28]. The project and protocol were prospectively registered with PROSPERO database of systematic reviews [www.crd.york.ac.uk/PROSPERO/] on May 25, 2023 (CRD 429831).

The following databases were searched [PubMed, Google Scholar, and Chinese databases (Chinese Biomedical Literature (CBM) and Chinese National Knowledge Infrastructure (CNKI)] from database inception to June 2023 to identify randomized controlled trials (RCTs) of probiotic use (limited to *Saccharomyces boulardii* CNCM I-745) to prevent antibiotic-associated diarrhea (AAD), including *Clostridioides difficile* infections (CDI) using randomized controlled trials done in China. The search terms were used: (antibiotic-associated diarrhea OR *C. difficile* AND *Saccharomyces boulardii* AND China). No language restrictions were imposed and publications in Chinese were translated into English.

Secondary searches of grey literature included reference lists, authors, reviews, meeting abstracts websites and clinicaltrials.gov for unpublished trials. A recursive search was also performed, using the bibliographies of all obtained articles.

Study selection

Inclusion criteria included: randomized, controlled clinical trials (RCTs) in children or adults receiving new prescription of antibiotics, comparison of *S. boulardii* versus control interventions and published in peer-reviewed journals. Only formulations of *S. boulardii* CNCM I-745 fulfilling the standard definition (must be living microbe, of adequate dose and conferring a health effect on the host) were included [29]. This definition excludes dead or heat-killed microbes and prebiotics. As bacterial and fungal taxonomies shift over time, the most current strain designations are presented in this review and strain identification was confirmed with the original authors or the manufacturer whenever possible. Trials with the primary outcome of prevention of AAD or CDI were included. Studies were included only if performed in China.

Exclusion criteria included: study not done in China, non-human studies, early phase 1 or 2 safety or mechanism of action studies, no control group, probiotic or AAD not well-described, clinical trials for the treatment of existing diarrhea, primary outcome was for the eradication of *H. pylori*, other probiotic strain(s) contained in the probiotic formulation, non-China setting, reviews and duplicate reports. Cross-over trials were excluded due to the potential for effect carry-over after short wash-out periods used in these trials and direct probiotic-probiotic studies were excluded if no non-probiotic control group was included.

Data extraction

The literature was searched independently by two co-authors (LM, LT). Data from all RCTs were extracted using a standardized data extraction form (Supplementary data, Form S1) initially completed by one co-author and then each study was independently scored by the other co-author following the standard methods for systematic reviews and meta-analysis [27,30]. Any disagreements were discussed until consensus was reached. The data extracted included PICOS data: (1) population (neonate, pediatric or adults, age range and country), (2) intervention (type of probiotic or controls used, daily doses, formulation, duration and follow-up times), (3) comparisons (type of control group either placebo or open, unblinded), (4) Primary outcomes (incidence of AAD or CDI), (5) secondary outcomes (stool frequency, length of hospitalization, cost data, safety data and clinical characterization of AAD). In addition, data on potential confounding factors were collected: type of antibiotic given, type of control, study quality, setting (inpatient or outpatient), dose and duration of probiotic. For missing data not reported in the published article, we attempted to contact the author or co-authors to obtain the missing data.

Intervention

The intervention was the yeast probiotic strain *Saccharomyces boulardii* CNCM I-745 (Yihuo ™ or Bioflor™, Biocodex, France). If the strain designation was not reported in the published study, the strain was verified with the manufacturer or website. The intervention may be given in oral administration as capsules or sachets. The duration of the intervention was typically given for the duration of the antibiotic(s), but may have been continued after the antibiotics are discontinued.

Outcomes

The primary outcomes include the efficacy for the reduction of AAD incidence and the reduction of CDI incidence. The outcome measures included: the number of patients developing AAD (diarrhea defined as at least two loose/watery stools in young children or ≥3 loose/watery stools in older children and adults for 48 hours, not caused by rotavirus, with an onset while on antibiotics or within 8 weeks of antibiotic cessation or CDI (AAD with positive *C. difficile* toxin result). Secondary outcomes included: daily stool frequency at the end of the intervention, length of hospitalization, clinical presentation of AAD (measured by duration of AAD, severity of AAD symptoms and treatment effectiveness rating), costs of medical care and frequency of adverse events. Severe AAD was defined differently in the RCTs, but typically included "diarrhea with electrolyte disorders, severe dehydration, acidosis or other symptoms of toxicity". Outcomes may be documented by patients/parents using daily diaries or by healthcare providers if hospitalized.

Risk of bias

Each included RCT was reviewed for quality and risk of bias and scored independently by both co-authors using standard methods [31]. The risk of bias (RoB) assessed with the RoB 2.0 tool and was graded (high, some concerns, or low) for each of five domains of bias (randomization process, deviations from intended interventions, missing outcome data, measurement of outcome, selection of reported result) and a summary table and figure of bias was generated [32].

Statistical Analysis

As recommended by experts, inclusion of studies in our meta-analysis also required at least two RCTs using a common outcome measure [28]. Statistical analysis and generation of forest plots of pooled summary estimates was performed using Stata software version 16 (Stata Corporation, College Station, Texas) with meta-analysis modules [33]. Dichotomous outcomes were assessed using relative risks (RR) and 95% confidence intervals (C.I.) and continuous outcomes were assessed using standardized mean difference (SMD) and 95% C.I. using standard methods [31]. The significance level was set at P-value < 0.05. Heterogeneity across trials was evaluated using the I^2 statistic, 0% indicating none and ≥50% indicating a high degree of heterogeneity across the trials [33]. Random-effects models were used for the meta-analysis if significant heterogeneity was found (I^2>50%), otherwise fixed-effects models were used. Publication bias was assessed using funnel plots and the Egger's test [33]. Subgroup analysis was used to explore sources of heterogeneity and was assessed with the Cochrane Q test [31]. *A priori* subgroup analyses based on factors that might influence the magnitude of efficacy estimates were planned for the following: (a) age, (b) daily dose *S. boulardii*, (c) risk of bias, (d) indication for antibiotic use (upper respiratory disease, gastrointestinal, etc.), (e) type of patient (inpatient/outpatients), (f) time of intervention initiation from onset of diarrhea, (g) rural versus urban setting, (h) route antibiotic given (IV or oral) and (i) extent of blinding. Trials were pooled in the meta-analysis if a common outcome was used and there were at least two trials within each sub-group. Sequential sensitivity analysis was done to explore the extent outcomes were dependent upon a particular trial.

Results

The literature search resulted in 361 articles that were initially screened and 233 were excluded during the initial screening (Figure 1): duplicate publications (n=88), reviews (n=73), cross-over studies (n=23), pre-clinical studies (n=18) or other miscellaneous reasons (n=31). Full articles (n=128) were reviewed and 82 were excluded: treatment of existing AAD (n=18), *H. pylori* eradication trials (n=17), no non-probiotic controls (n=16), unclear AAD definition (n=14), direct probiotic to probiotic studies (n=12), probiotic tested was other than *S. boulardii* (n=2), treatment of rotaviral diarrhea (n=2) and unclear outcome data (n=1). A total of 46 RCTs published 2011-2022 were included (8,201 participants) [34-79]. Only four RCTs were found in commonly used non-Chinese databases [54,59,60,64] and the other 42 trials were found using CNKI/CMI databases. A funnel plot (Supplementary Figure S1) showed significant publication bias for the included studies Egger's test: t= -3.15, P = 0.003) due to a lack of RCTs showing no significant efficacy of *S. boulardii* CNCM I-745.

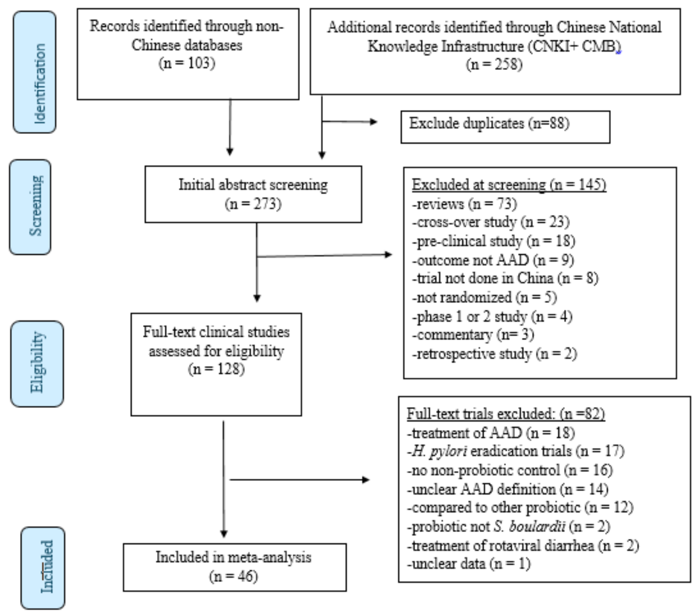

Figure 1: PRISMA study inclusion flow-chart.

Study participant characteristics

There was a mean of 178 + 125 enrolled subjects/trial (range 46-552), as shown in (Table 1). Most trials (n=44, 96%) were in pediatric subjects (aged neonates to 16 years old) and two trials were in elderly (>65 years old) patients [44,72]. All patients were hospitalized.

The types of antibiotics used varied, with most RCTs (n=23, 50%) using different types of antibiotics while four RCTs enrolled patients given only a single type of antibiotic (9%), but the types of antibiotics were not reported in 19 (41%) trials (Table 1). Most of the antibiotics were given for upper respiratory infections (38, 83%) or mixed types of infections (5, 11%) or not reported (n=3, 6%). The duration of antibiotics ranged from 5 to 21 days and most (26, 56.5%) were given only intravenously while a few RCTs (n=3, 6.5%) enrolled patients given either IV or oral antibiotics. The antibiotic route was not reported in 17 (37%) of the RCTs.

Ref.	Year	No.	Age range of inclusion	Type of antibiotic(s) given (route)	Antibiotic indication	Type of control	Dose (mg/day)[1] for *S. boulardii*	Duration given (days)[2]	Follow up (days)	Attrition (%)
Bai SX et al. [34]	2012	120	6 mon-3 y	C,M,Ot (nr)	RTI	Open	500	On abx	5	5
Cao TR [35]	2013	70	6 mon- 2 y	nr (IV)	RTI	Open	500	On abx	14	5.7
Chen JW et al. [36]	2013	236	6 mon- 14 y	nr (nr)	RTI	Open	250	On abx	0	0
Chen JB [37]	2013	120	6 mon- 3 y	C,M,P (IV)	RTI	Open	500	On abx	0	0
Chen Juan [38]	2013	64	2 mon- 3.5 y	C,M,P (IV)	RTI	Open	250/500	On abx	0	0
Chen W et al. [39]	2012	100	1 y- 14 y	nr (IV)	RTI	Open	500	On abx	0	0
Chen YY [40]	2020	94	5 mon- 3 y	Ot (IV)	RTI	Open	500	5	0	0
Dong G [41]	2015	80	5 mon-12 y	C,P (IV)	RTI	Open	500	7	0	0
Dong X et al. [42]	2014	62	2 mon- 3 y	C, M (IV)	RTI	Open	500	On abx	0	0
Feng X et al. [43]	2015	58	7 mon- 3 y	C, M, P (nr)	RTI	Open	250/500	LOS	0	0
Guo T et al. [44]	2018	127	>65 yrs	C, E, L (IV)	RTI	Placebo	500	On abx +7	28	0
Huang MH [45]	2018	84	2 mon- 3 y	C,M (IV)	RTI	Open	250	7	0	0
Huang S et al. [46]	2017	66	1 y- 3 y	C,M,Ot (Or/IV)	RTI	Open	300	On abx	0	0
Li B et al. [47]	2011	46	5 mon- 7 y	nr (IV)	RTI	Open	500	On abx	0	0
Li JM et al. [48]	2014	210[3]	1 mon- 3 y	C, M, P (IV)	RTI	Open	250/500	5	0	0
Li YY et al. [49]	2021	298	<15 d	nr (IV)	Mixed	Open	250	14	14	0
Li YF [50]	2013	237	<6 mon- 1 y	nr (IV)	RTI	Open	500	On abx	0	0
Lin X et al. [51]	2018	168	1 mon- 3 yrs	C (nr)	RTI	Open	250/500/1000	7-10	0	0
Lou QY et al. [52]	2020	499	1 mon- 6 y	C, M, P (nr)	RTI	Open	250/500	7-8	14	25.8
Peng XH [53]	2018	276	2 mon- 3 y	nr (nr)	RTI	Open	250	5 + 5	0	0

Shan LS et al. [54]	2013	333	6 mon- 14 y	A, C, Ot (IV)	RTI	Open	500	14	14	15
Shan LS et al. [55]	2014	468	6 mon- 14 y	C, Ot (IV)	RTI	Open	500	8	6	0
Song YH et al. [56]	2012	60	6 mon- 14 y	Nr (nr)	RTI	Open	500	On abx	0	0
Tan G et al. [57]	2022	150	25 mon-3 y	nr (IV)	RTI	Open	250	7	0	0
Tang M [58]	2014	146	< 14 d	C,P (nr)	RTI	Open	250	On abx	0	0
Tao Z et al. [59]	2016	120	<16 y	C (IV)	RTI	Open	250/500	On abx	0	0
Wan CM et al. [60]	2017	408	1 mon- 3 y	Mixed (Or/IV)	RTI/mixed	Open	250	On abx	14	0
Wang LH[61]	2014	100	9 mon- 7 y	nr (nr)	RTI	Open	500	On abx	0	0
Wang XY[62]	2013	126	1 y- 3 y	nr (IV)	RTI	Open	250	On abx	0	0
Wang ZZ [63]	2013	129	< 2 y	nr (IV)	RTI	Placebo	500	On abx +3	53	17
Wu XY et al. [64]	2015	180	6 mon- 8 y	C, M, P (IV)	RTI	Open	125/250/500	7	0	0
Wu YX [65]	2012	68	6 mon – 3 y	C,Ot (IV)	RTI	Open	250	On abx	0	0
Xiao J [66]	2018	92	6 mon-5 y	C (nr)	RTI	Open	250/500	On abx	7	0
Xu H [67]	2013	51	2 mon- 3 y	nr (IV)	RTI	Open	250/500	On abx	0	0
Xu LF et al. [68]	2015	552	3 mon– 14 y	C, M, P, Ot (Or/IV)	RTI or UTI	Open	500	7	0	13
Yang X [69]	2015	200	1 mon- 13 y	nr (nr)	nr	Open	250/500	On abx	14	0
Yao TX et al. [70]	2014	240	1 mon- 3 y	nr (nr)	Mixed	Open	500	On abx	0	0
Yu M [71]	2014	271[4]	6 mon- 3 y	nr(IV)	RTI	Open	250/500	On abx	0	0
Zhang DM et al. [72]	2017	163	>65 y	C, Ot (nr)	nr	Open	1000	21	63	0
Zhang J et al. [73]	2013	312	3 mon-3 y	nr (nr)	Mixed	Open	250/500	On abx	0	0
Zhang WX [74]	2013	220	1 mon- 8 y	Mixed (nr)	nr	Open	250/500	On abx	0	0

Zhang Y et al. [75]	2018	326	6 mon–12 y	A, C, Ot (IV)	RTI	Open	250/500	5	0	0
Zhao G [76]	2013	96	5 mon-3 y	nr (IV)	RTI	Open	250/500	On abx	0	0
Zhao GD[77]	2018	89	1 y- 10 y	nr (IV)	RTI	Open	250/500	On abx	0	0
Zheng SQ [78]	2013	186	10 – 16 mon	Broad-spect (nr)	RTI	Open	250	On abx	0	0
Zhu HY [79]	2017	100	1.5 y- 2 y	nr (nr)	RTI	Open	250	On abx	0	0

Table 1: Study population characteristics of 46 included trials for the prevention of AAD. [1]Dose/day may vary by age of patient; [2] On abx + days extended post-antibiotics; [3]Li J (treatment AAD arm excluded); [4]Yu M (two study arms excluded, on for treatment of AAD by S. boulardii and another with probiotic blend). A, ampicillin; C, cephalosporin; d, days; E, ertapenum; IV, intravenous route; L, levofloxacin; LOS, during length of stay in hospital; M, macrolide; mon, months; nr, not reported in paper; No., number enrolled; OM, otitis media; On abx, given while on antibiotics; Or, oral route; Ot, other types of antibiotics; P, penicillin; Ref, reference; RTI, upper or lower respiratory tract infection; UTI, urinary tract infection; wk, weeks; y, years old.

Intervention/control characteristics

Most trials (n=44, 96%) used open controls, while two (4%) RCTs used a blinded placebo control, as shown in Table 1 [44,63]. The most common formulation for *S. boulardii* was as a powder (n=41 trials, 89%) or in capsules (n=1, 2%) [72], but was not reported in four trials. Many RCTs used age-dependent daily doses for *S. boulardii* (n=16, 35%, ranging from 150 mg to 1 g/d), while others gave a fixed daily dose, regardless of age: 250 mg/d (n=11, 24%), 300 mg/d (n=1, 2%), 500 mg/d (n=17, 37%) or 1000 mg/d (n=1, 2%). Most trials continued the intervention for the duration of the antibiotic (n= 42, 91%), three (6%) continued the intervention after antibiotics were discontinued [44,53,63] and one RCT continued for the length of the hospitalization [43]. Only 12/46 (26%) of the RCTs followed subjects post-intervention for delayed-onset AAD (follow-up times ranged from 5-63 days), while most RCTs (n=34, 74%) did not follow subjects after antibiotics were discontinued. No loss to follow-up (attrition) was reported for 40 (87%) of the trials, while six (13%) trials reported 5-26% attrition [34,35,52,54,63,68]. In 31 (67.4%) of the trials, the interventions were started within 24 hours of the antibiotic, but the initiation time was not reported in 15 (32.6%) trials.

Primary outcome: prevention AAD

Efficacy to prevent AAD was reported all included trials (n=46), as shown in (Table 2). However, the reported definitions for AAD varied (Supplementary Table S2) with 5 (11%) not reporting how AAD was defined. Most RCTs (n=41, 89%) did report their definition of AAD in their papers, typically representing a change in stool consistency, increased frequency of watery/loose stools for longer than one day, which was associated with antibiotic use or shortly afterwards. The incidence of AAD ranged from 3/100 to 38/100 (mean 15.0 ± 7.2/100) in *S. boulardii* groups compared to 9/100 to 56/100 (mean 35.9 ± 10.7/100) in controls. The pooled RR for AAD was significantly lower for *S. boulardii* compared to controls (RR= 0.43, 95% C.I. 0.40, 0.48, P<0.0001, I^2=5.5%), as shown in (Figure 2).

Ref.	**Year**	**AAD in *S. boulardii*, number (%)**	**AAD in controls, number (%)**	**P-value**	**CDI in *S. boulardi*i, number (%)**	**CDI in control, number (%)**	**% AE in *S. boulardii***	**% AE in controls**
Bai SX et al [34]	2012	20/60 (33.3)	32/60 (53.3)	<0.01	nr	nr	0, state	0, state
Cao TR [35]	2013	4/34 (11.8)	12/32 (37.5)	<0.05	nr	nr	0, state	0, state
Chen JW et al [36]	2013	23/121 (19.0)	43/115 (37.4)	<0.05	nr	nr	0, state	0, state
Chen JB [37]	2013	4/60 (6.7)	14/60 (23.2)	<0.05	nr	nr	nr	nr
Chen Juan [38]	2013	6/32 (18.8)	18/32 (56.2)	<0.05	nr	nr	0, state	0, state
Chen W et al [39]	2012	7/50 (14.0)	18/50 (36.0)	<0.05	nr	nr	nr	nr
Chen YY [40]	2020	5/47 (10.6)	14/47 (29.8)	0.001	nr	nr	nr	nr
Dong G [41]	2015	4/40 (10)	13/40 (32.5)	<0.05	nr	nr	nr	nr
Dong X et al [42]	2014	5/31 (16.1)	11/31 (35.5)	<0.01	nr	nr	0, state	0, state
Feng X et al [43]	2015	8/29 (27.6)	15/29 (51.7)	<0.05	nr	nr	nr	nr
Guo T et al [44]	2018	7/63 (11.1)	17/64 (26.6)	0.04	nr	nr	19	11
Huang MH [45]	2018	7/ 42 (16.7)	15/42 (35.7)	<0.05	nr	nr	0, state	0, state
Huang S et al [46]	2017	3/33 (9.1)	9/33 (33.0)	<0.05	nr	nr	nr	nr
Li B et al [47]	2011	3/23 (13.0)	9/23 (34.6)	< 0.05	nr	nr	nr	nr
Li JM et al [48]	2014	26/150 (17.3)	25/60 (41.7)	<0.001	nr	nr	nr	nr
Li YY et al [49]	2021	17/148 (11.4)	44/150 (29.3)	<0.001	nr	nr	0, state	0, state
Li YF [50]	2013	21/120 (17.5)	43/117 (36.7)	<0.05	nr	nr	0, state	0, state
Lin X et al [51]	2018	17/90 (18.9)	34/78 (43.6)	<0.01	nr	nr	nr	nr
Lou QY et al [52]	2020	40/182 (22.0)	55/164 (33.5)	0.02	nr	nr	0, state	0, state
Peng XH [53]	2018	21/140 (15.0)	64/136 (47.1)	<0.05	nr	nr	0, state	0, state
Shan LS et al [54]	2013	6/139 (4.3)	28/144 (19.4)	<0.001	1/139 (0.7)	8/144 (5.6)	0, state	0, state
Shan LS et al [55]	2014	19/233 (8.2)	63/235 (26.8)	<0.05	2/233 (0.8)	11/235 (4.7)	0, state	0, state
Song YH et al [56]	2012	3/30 (10.0)	10/30 (33.3)	0.03	nr	nr	nr	nr

Tan G et al [57]	2022	9/75 (12)	26/75 (34.7)	<0.001	nr	nr	nr	nr
Tang M [58]	2014	15/73 (20.6)	34/73 (46.6)	<0.05	nr	nr	3	4
Tao Z et al [59]	2016	13/60 (21.7)	23/60 (38.3)	<0.05	nr	nr	0, state	0, state
Wan CM et al [60]	2017	27/213 (12.7)	89/195 (45.6)	<0.001	nr	nr	0, state	0, state
Wang LH [61]	2014	19/50 (38.0)	28/50 (56.0)	<0.05	nr	nr	nr	nr
Wang XY [62]	2013	9/60 (15.0)	20/66 (30.3)	<0.05	nr	nr	nr	nr
Wang ZZ [63]	2013	9/56 (16.1)	20/51 (39.2)	<0.05	nr	nr	nr	nr
Wu XY et al [64]	2015	21/90 (23.3)	30/90 (33.3)	<0.05	nr	nr	0, state	0, state
Wu YX [65]	2012	6/34 (17.6)	16/34 (47.0)	<0.05	nr	nr	nr	nr
Xiao J [66]	2018	3/46 (6.5)	12/46 (26.1)	< 0.05	nr	nr	0, state	0, state
Xu H [67]	2013	4/21 (19.1)	14/30 (46.7)	0.04	nr	nr	0, state	0, state
Xu LF et al [68]	2015	8/160 (5.0)	49/240 (20.4)	0.007	nr	nr	0, state	0, state
Yang X [69]	2015	17/100 (17.0)	51/100 (51.0)	<0.05	nr	nr	0, state	0, state
Yao TX et al [70]	2014	4/120 (3.3)	11/120 (9.2)	<0.05	nr	nr	nr	nr
Yu M [71]	2014	12/150 (8.0)	23/121 (19.0)	<0.05	nr	nr	0, state	0, state
Zhang DM et al [72]	2017	12/81 (14.8)	23/82 (28.0)	<0.05	3/81 (3.7)	4/82 (4.9)	nr	nr
Zhang J et al [73]	2013	11/150 (7.3)	38/162 (23.5)	<0.05	nr	nr	nr	nr
Zhang WX [74]	2013	8/110 (7.3)	29/110 (26.4)	<0.01	nr	nr	nr	nr
Zhang Y et al [75]	2018	38/171 (22.2)	53/155 (34.2)	<0.05	nr	nr	nr	nr
Zhao G [76]	2013	6/50 (12.0)	17/46 (37.0)	<0.05	nr	nr	nr	nr
Zhao GD [77]	2018	9/45 (20.0)	23/44 (52.3)	<0.05	nr	nr	0, state	0, state
Zheng SQ [78]	2013	21/100 (21.0)	38/86 (44.2)	<0.01	nr	nr	nr	nr
Zhu HY [79]	2017	4/50 (8.0)	13/50 (26.0)	<0.05	nr	nr	nr	nr

Table 2: Prevention of antibiotic-associated diarrhea (AAD), *C. difficile* infection or adverse events in 46 included trials. AAD, antibiotic-associated diarrhea, excluding rotaviral diarrhea; AE, adverse event; CDI, *Clostridioides difficile* infection; nr, not reported; Ref, reference; S., *Saccharomyces*; state, only statement of 'No adverse events noted'.

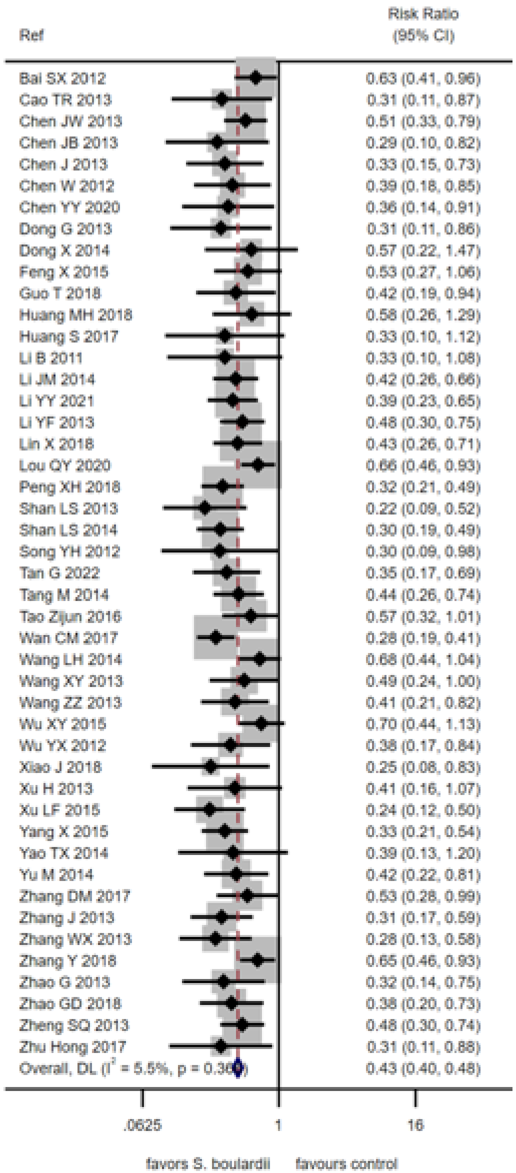

Figure 2: Forest plot of efficacy for prevention of AAD: *S. boulardii* compared to controls. DL, Der Simonian-Laird.

Primary outcome: **Efficacy to prevent CDI**

Only three trials reported CDI as an outcome (Table 2) in their trials [54,55,72]. As shown in (Figure 3), the risk for CDI was significantly reduced for *S. boulardii* CNCM I-745 compared to controls (RR= 0.30, 95% C.I. 0.10, 0.87, P = 0.03, I^2=22.9%).

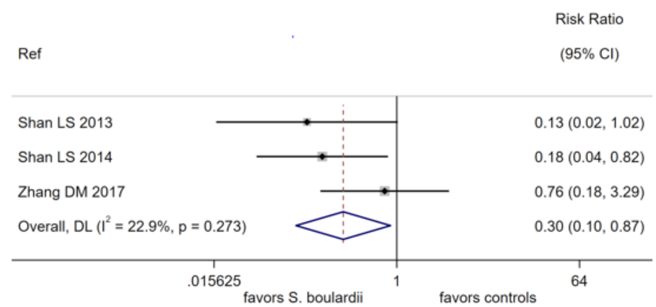

Figure 3: Forest plot of efficacy for prevention of CDI: *S. boulardii* compared to controls. DL= Der Simonian-Laird.

Secondary outcomes

Several secondary outcomes had sufficient data for analysis: daily stool frequency at study end, clinical characterization of AAD (duration, severe AAD, effectiveness rating) and safety. Other *a priori* secondary outcomes were infrequently reported (rural versus urban and cost-effectiveness) and could not be analyzed.

Stool frequency

Only 16/46 (35%) reported the daily stool frequency during the intervention/control phase and 30 (65%) did not report this outcome measure (Table 3). Those given *S. boulardii* had significantly fewer mean stools/day compared to the controls (Figure 4), SMD: --1.25 stools/day, 95% C.I. -1.35, -1.16, P< 0.0001, I^2=96%).

Ref.	**Year**	**Number of stools/day at end of study**						**Duration of AAD (days)**					
		No. Sb	**mean stools/d in Sb**	**Std dev in Sb**	**No. control**	**mean stools/d in control**	**Std dev in controls**	**# Sb**	**mean days AAD Sb**	**Std dev Sb**	**# controls**	**mean AAD days control**	**Std Dev controls**
Bai SX et al [34]	2012	60	1.8	0.5	60	3.2	0.9	19	3.2	1.0	27	5.1	1.8
Cao TR [35]	2013	nr	nr	nr	nr	nr	nr	4	2.3	0.13	12	4.6	0.4
Chen JW et al [36]	2013	nr	nr	nr	nr	nr	nr	23	3.1	0.7	43	4.6	0.94
Chen JB [37]	2013	60	3.1	0.8	60	3.9	1.1	4	2.0	1.5	14	4.0	2.5
Chen Juan [38]	2013	nr	nr	nr	nr	nr	nr	6	2.4	1.3	18	4.3	1.8
Chen W et al [39]	2012	50	3.1	0.7	50	3.8	1.1	7	2.8	1.2	18	4.0	2.1
Chen YY [40]	2020	47	4.2	0.3	47	5.1	0.56	5	3.1	0.52	14	4.2	0.74
Dong G [41]	2015	nr	nr	nr	nr	nr	nr	4	0.3	1.2	13	5.4	3.1
Dong X et al [42]	2014	nr	nr	nr	nr	nr	nr	5	3.1	0.43	11	4.23	1.26
Feng X et al [43]	2015	29	1.7	0.4	29	3.4	0.8	8	3.1	0.9	15	5.2	1.7
Guo T et al [44]	2018	63	2.69	1.2	64	4.3	1.6	nr	nr	nr	nr	nr	nr
Huang MH [45]	2018	nr	nr	nr	nr	nr	nr	7	3.11	0.42	15	4.22	1.25
Huang S et al [46]	2017	nr	nr	nr	nr	nr	nr	3	1.6	0.7	9	3.9	0.7
Li B et al [47]	2011	23	3.0	0.7	23	3.8	1.1	3	2.7	1.3	9	4.1	2.1
Li JM et al [48]	2014	nr	nr	nr	nr	nr	nr	26	3.35	0.9	25	4.64	1.08
Li YY et al [49]	2021	148	2.11		150	3.15	1.27	17	3.15	1.28	44	4.75	2.16
Li YF [50]	2013	nr	nr	nr	nr	nr	nr	21	3.1	1.2	43	4.3	1.5
Lin X et al [51]	2018	90	3.68	1.1	78	5.38	1.5	17	3.73	1.33	34	4.84	1.32
Lou QY et al [52]	2020	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Peng XH [53]	2018	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr

Shan LS et al [54]	2013	nr	nr	nr	nr	nr	nr	6	2.3	0.9	28	8.97	1.1
Shan LS et al [55]	2014	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Song YH et al [56]	2012	nr	nr	nr	nr	nr	nr	3	2.7	0.3	10	4.4	0.93
Tan G et al [57]	2022	75	2.83		75	5.41	0.36	9	2.4	1.2	26	5.1	1.7
Tang M [58]	2014	73	5.3	1.5	73	8.2	1.4	15	3.4	1.3	34	5.0	1.7
Tao Z et al [59]	2016	60	1.13		60	2.32	0.43	13	3.78	1.34	23	4.67	1.47
Wan CM et al [60]	2017	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Wang LH [61]	2014	nr	nr	nr	nr	nr	nr	19	2.45	0.9	28	4.2	0.9
Wang XY [62]	2013	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Wang ZZ [63]	2013	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Wu XY et al [64]	2015	nr	nr	nr	nr	nr	nr	21	3.19	0.68	30	5.24	0.79
Wu YX [65]	2012	nr	nr	nr	nr	nr	nr	6	2.7	1.3	16	4.1	1.8
Xiao J [66]	2018	46	6.4	1.3	46	4.3	1.24	3	3.0	0.7	12	4.56	1.19
Xu H [67]	2013	nr	nr	nr	nr	nr	nr	4	1.5	0.4	14	4.68	0.87
Xu LF et al [68]	2015	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Yang X [69]	2015	nr	nr	nr	nr	nr	nr	17	4.47	1.01	51	5.1	1.19
Yao TX et al [70]	2014	120	3.01	0.7	120	3.8	1.3	4	2.7	1.3	11	4.1	2.1
Yu M [71]	2014	nr	nr	nr	nr	nr	nr	12	3.4	1.3	23	7.0	1.8
Zhang DM et al [72]	2017	81	4.3	1.7	82	6.9	2.0	12	3.0	1.1	23	5.7	1.8
Zhang J et al [73]	2013	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Zhang WX [74]	2013	nr	nr	nr	nr	nr	nr	8	1.2	1.1	29	3.5	2.8
Zhang Y et al [75]	2018	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr

Zhao G [76]	2013	nr	nr	nr	nr	nr	nr	6	2.8	1.1	17	4.3	0.9
Zhao GD [77]	2018	nr	nr	nr	nr	nr	nr	9	2.85	1.3	23	4.64	1.52
Zheng SQ [78]	2013	nr	nr	nr	nr	nr	nr	21	3.26	1.05	38	5.13	1.86
Zhu HY [79]	2017	50	2.11	0.6	50	3.94	1.02	4	2.13	0.47	13	5.2	1.32

Table 3: Efficacy of *S. boulardii* on prevention of secondary AAD: stool frequency and duration of AAD, and stool frequency/day, AAD, antibiotic-associated diarrhea; na, not applicable; nr, not reported; Ref, reference; Sb, *Saccharomyces boulardii* CNCM I-745, Std dev, standard deviation.

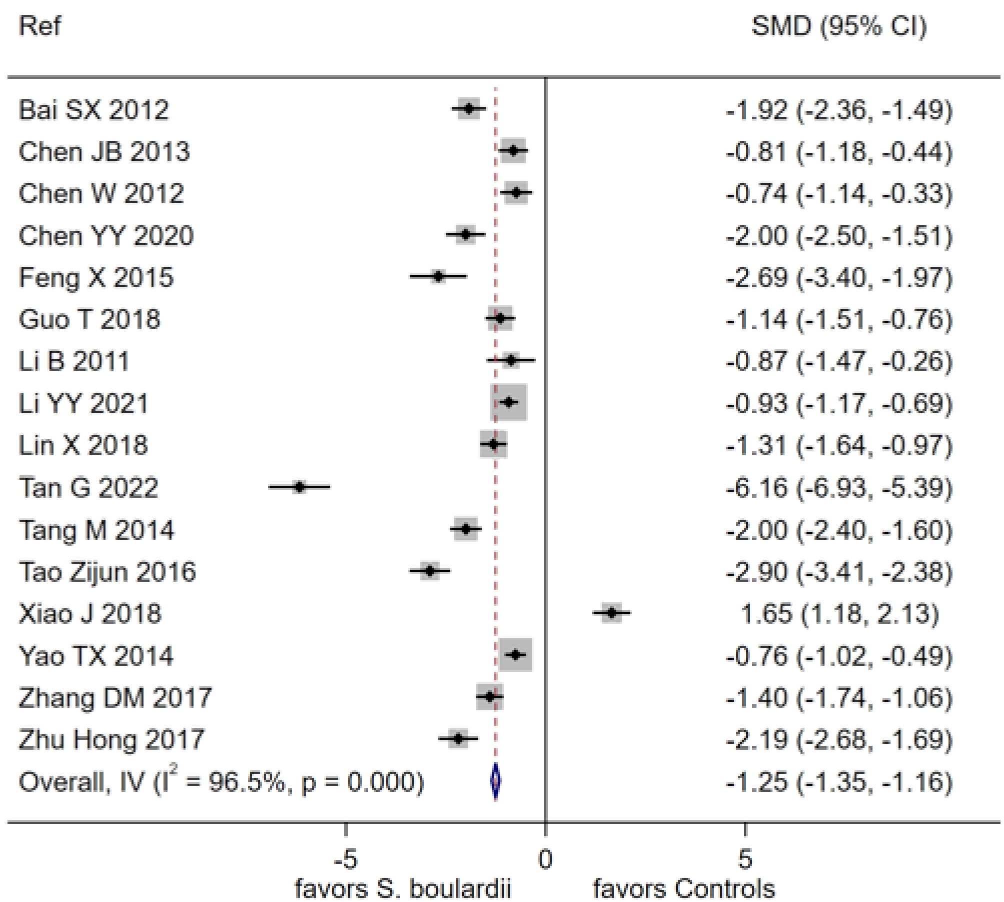

Figure 4: Forest plot of efficacy for stool frequency/day: *S. boulardii* compared to controls. SMD, standardized mean difference; IV, Inverse variance.

Clinical characteristics of AAD

Three measures were used to determine if *S. boulardii* influenced the severity of AAD (duration of AAD, frequency of severe AAD and effectiveness rating). Data was collected from 232 cases of AAD in *S. boulardii* treated patients and 454 cases of AAD in the controls.

Clinical characterization of AAD: duration of AAD. The duration of AAD (Table 3) was reported in 36 (78.3%) of the 46 prevention trials, while 10 (22%) of the RCTs did not report this outcome. Mean duration of AAD ranged from 0.3 to 5 days in the *S. boulardii* subjects and ranged 4 to 9 days in the controls. The reduction of the days of diarrhea was significantly reduced for those given *S. boulardii* compared to the controls (SMD: -1.29 days, 95% C.I. -1.43, -1.15 P<0.001, I^2=68.8%), as shown in (Figure 5).

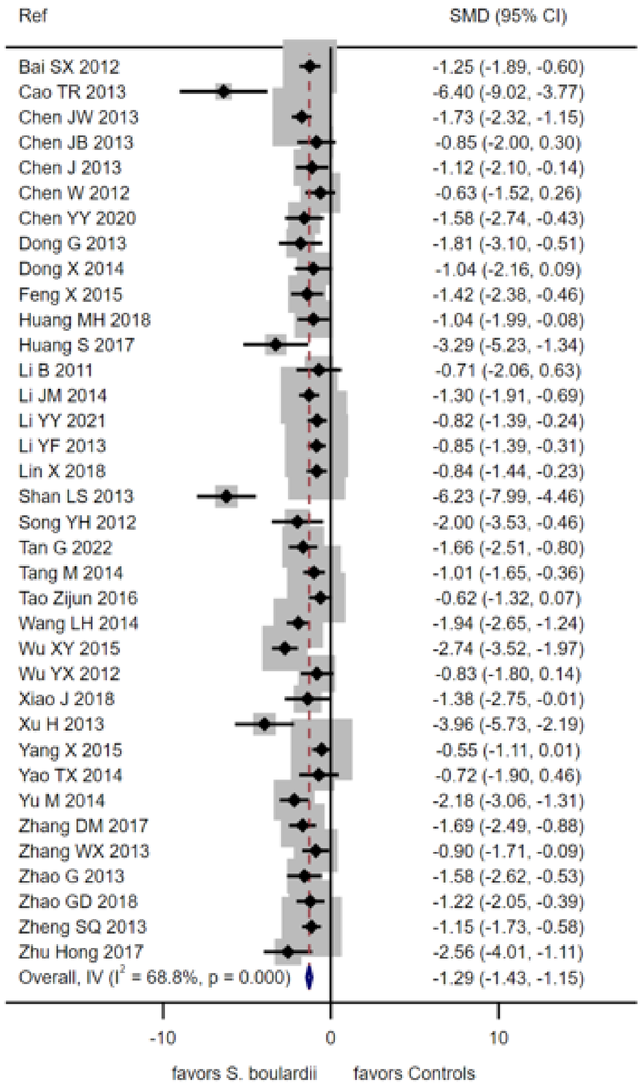

Figure 5: Forest plot of efficacy for reduction of AAD duration in days: *S. boulardii* compared to controls. SMD, standardized mean difference; IV, inverse variance.

*Clinical characterization of AAD***:** *Incidence of severe AAD*. Only 17 (37%) of the RCTs reported incidence of severe AAD (Table 4) [36,38, 40, 42, 45, 46, 48, 50, 59, 61, 63, 65, 69,71,76,77,79], while 29 (63%) did not assess the severity of AAD. Of the 17 RCTs that analyzed the severity of AAD, most (70%) reported a definition of 'severe' AAD (Supplementary Table S2). Treatment with *S. boulardii* did not significantly result in less severe cases of AAD, as shown in (Supplementary Figure S2) (RR=1.00, 95% C.I. 0.57, 1.76, P = 1.0).

Ref.	Year	Number with severe AAD n/N (%)		Rated effective for AAD n/N (%)	
		in *S. boulardii*	in controls	in *S. boulardii*	in controls
Bai SX et al [34]	2012	nr	nr	19/20 (95)	23/32 (71.9)
Cao TR [35]	2013	nr	nr	nr	nr
Chen JW et al [36]	2013	3/23 (13.0)	12/43 (27.9)	22/23 (95.6)	28/43 (65.1)
Chen JB [37]	2013	nr	nr	nr	nr
Chen Juan [38]	2013	1/6 (16.7)	3/18 (16.7)	nr	nr
Chen W et al [39]	2012	nr	nr	nr	nr
Chen YY [40]	2020	0/5 (0)	1/14 (7)	nr	nr
Dong G [41]	2015	nr	nr	nr	nr
Dong X et al [42]	2014	1/5 (20)	4/11 (36.4)	nr	nr
Feng X et al [43]	2015	nr	nr	nr	nr
Guo T et al [44]	2018	nr	nr	nr	nr
Huang MH [45]	2018	1/7 (14.3)	5/15 (33.3)	7/7 (100)	11/15 (73.3)
Huang S et al [46]	2017	0/3 (0)	2/9 (22.2)	3/3 (100)	8/9 (88.8)
Li B et al [47]	2011	nr	nr	nr	nr
Li JM et al [48]	2014	4/26 (15.4)	8/25 (32.0)	25/26 (96.1)	17/25 (68.0)
Li YY et al [49]	2021	nr	nr	nr	nr
Li YF [50]	2013	4/21 (19)	13/43 (30.2)	20/21 (95)	30/43 (69.8)
Lin X et al [51]	2018	nr	nr	nr	nr
Lou QY et al [52]	2020	nr	nr	nr	nr
Peng XH [53]	2018	nr	nr	nr	nr
Shan LS et al [54]	2013	nr	nr	nr	nr
Shan LS et al [55]	2014	nr	nr	nr	nr
Song YH et al [56]	2012	nr	nr	nr	nr
Tan G et al [57]	2022	nr	nr	nr	nr
Tang M [58]	2014	nr	nr	nr	nr
Tao Z et al [59]	2016	3/13 (23)	7/23 (30.4)	12/13 (92)	14/23 (60.9)
Wan CM et al [60]	2017	nr	nr	nr	nr
Wang LH [61]	2014	2/19 (10.5)	10/28 (35.7)	17/19 (89.5)	16/28 (57.1)
Wang XY [62]	2013	nr	nr	nr	nr
Wang ZZ [63]	2013	2/9 (22)	9/20 (45)	nr	nr
Wu XY et al [64]	2015	nr	nr	20/21 (95.2)	22/30 (73.3)
Wu YX [65]	2012	1/6 (16.7)	6/16 (37.5)	nr	nr

Xiao J [66]	2018	nr	nr	nr	nr
Xu H [67]	2013	nr	nr	nr	nr
Xu LF et al [68]	2015	nr	nr	nr	nr
Yang X [69]	2015	1/17 (5.9)	15/51 (29.4)	nr	nr
Yao TX et al [70]	2014	nr	nr	nr	nr
Yu M [71]	2014	1/12 (8.0)	2/23 (11.5)	nr	nr
Zhang DM et al [72]	2017	nr	nr	nr	nr
Zhang J et al [73]	2013	nr	nr	nr	nr
Zhang WX [74]	2013	nr	nr	nr	nr
Zhang Y et al [75]	2018	nr	nr	nr	nr
Zhao G [76]	2013	0/6 (0)	2/17 (11.7)	nr	nr
Zhao GD [77]	2018	0/9 (0)	0/23 (0)	8/9 (88.9)	15/23 (65)
Zheng SQ [78]	2013	nr	nr	nr	nr
Zhu HY [79]	2017	0/4 (0)	2/13 (15.4)	nr	nr

Table 4: Clinical characteristics of AAD in 232 cases of AAD in *S. boulardii* and 454 cases of AAD in controls; %, percentage; AAD, antibiotic-associated diarrhea; n, number in numerator; N, total number in denominator, nr, not reported.

Clinical characterization of AAD: effectiveness rating. Only 10 (22%) of the RCTs used an effectiveness rating for AAD, while most (n=36, 78%) did not report this outcome (Table 4). The range of AAD rated "effectively prevented" was higher in *S. boulardii* (89%-100%) compared with the controls (57%-89%) but was not significantly different when analyzed (RR=1.00, 95% C.I. 0.93, 1.08, I^2=82.9%, P=1.00, as shown in (Supplementary Figure S3).

Length of hospitalization

Four RCTs found shorter length-of-stays when *S. boulardii* was given compared to controls (SMD: -0.75 days, 95% C.I. -0.89, -0.61, P <0.0001) [42,49,52,53], as shown in (Supplementary Figure S4).

Safety

Many (23/46, 50%) of the RCTs provided data on adverse reactions or tolerance (Table 2), while no data on safety was provided in 23 (50%) of the RCTs. Twenty-one RCTs only provided a statement that the "probiotic was well-tolerated" or "no adverse events were noted", while two RCTs provided more detailed data on mild adverse reactions [44,58]. Guo *et al* reported abdominal pain (19% in *S. boulardii* group and 11% in controls, P = 0.22), abdominal distension (16% and 11%, respectively, P = 0.42) and nausea (13% and 8%, respectively, P = 0.40). Tang *et al*. noted only mild allergic reactions in two (3%) of those given *S. boulardii* and in 3 (4%) of controls (P>0.05) [58]. No serious adverse events were reported in any of the trials.

Sub-groups

Age

As the enrollment age ranges were not mutually exclusive, only three age sub-groups could be analyzed: neonates (under 14 days old), pediatric (1 month to 16 years old) and elderly (>65 years old). As shown in (Supplementary Figure S5), *S. boulardii* significantly prevented AAD in all three age groups, with no significant differences by age groups (X^2=0.8, P = 0.67).

Daily dose

The effect of *S. boulardii* dose was explored, which reduced the degree of heterogeneity and three dose groups had significant efficacy as shown in (Supplementary Figure S6). AAD risk was significantly reduced for 250 mg/d dose group (RR=0.36, 95% C.I. 0.33, 0.45, P < 0.001, I^2=0%) and in the 500 mg/d group (RR= 0.39, 95% C.I. 0.33, 0.47, P < 0.001, I^2 =8.9%) and when doses were given according to age groups in children (RR= 0.46, 95% C.I. 0.41, 0.53, P < 0.001, I^2 =20.9%). Two other doses could not be analyzed as there was only one RCT for each dose: 300 mg/d [46] and 1000 mg/d [72]. There was no significant difference by dose sub-group (X^2=2.52, p=0.64).

Study Quality

All the RCTs were assessed for risk of bias (Supplementary Table S3). Of the 46 RCTs, two (4.3%) were considered an overall low risk of bias (Supplementary Figure S7), 84.8% had 'some concerns' about bias and five (10.9%) were considered high risk bias (when more than one bias domain was rated as high risk). The only individual domain in the 46 RCTs commonly rated high-risk was for the lack of double-blinded design (96%). Other individual domains were rated as low risk. When five high risk trials for prevention of AAD were excluded [42,43,49,52,57], the reduction of AAD risk was similar (RR=0.42, 95% CI 0.38, 0.46, P<0.001, I^2 =0.5%), as shown in (Supplementary Figure S8).

Other sub-groups

Some sub-groups could not be analyzed as the groups had similar characteristics or were infrequently reported. For example, respiratory tract infections were the most common indication for antibiotics (94% trials), most trials were in children (96%), 96% had open controls and all patients were hospitalized. Some data was not reported in sufficient numbers of trials (for example, 33% of the trials did not report intervention initiation time and only four trials reported the length of hospitalization (Supplementary Table S4). None of the trials reported if the hospital patients were from urban or rural healthcare facilities and none reported cost or cost-saving data.

Discussion

Our meta-analysis found *S. boulardii* CNCM I-745 was significantly effective for the prevention of AAD and the prevention of CDI in trials done in China and was well-tolerated. *S. boulardii* CNCM I-745 effectively reduced AAD in hospitalized children and adults receiving the types of antibiotics typically prescribed for respiratory tract infections in China (cephalosporins, penicillins, macrolides, etc.), which are well known risk factors for AAD and CDI [80]. *S. boulardii* CNCM I-745 was shown to not only significantly reduce the incidence of AAD and CDI, but also reduce the duration of AAD and the daily stool frequency. *S. boulardii* was well tolerated in the included trials and safety has been thoroughly documented in patients receiving antibiotics in comprehensive meta-analyses and reviews [17,21,22]. Antibiotic resistance is another safety concern associated with antibiotic use and one study reported a reduction in the antibiotic resistance gene load when *H. pylori* eradication therapy was combined with *S. boulardii* CNCM I-745 [81]. Healthcare providers and policy makers should be aware of the high rate of AAD in their patients treated with antibiotics in China and strive to implement strategies to reduce AAD.

As the intestinal microbiome is influenced by ethnicity and geography, the question often arises if probiotic efficacy is also affected by these factors [2]. The effects of differences in diet, genetic make-up, nutritional status, healthcare access and living conditions are not commonly explored in probiotic reviews. One method of controlling for these confounding factors is to limit the meta-analysis to one country or to assess the efficacy using subgroups of different geographic regions [17,82]. We showed *S. boulardii* significantly reduced the risk of AAD (RR=0.43) in China, which was similar to studies done in other countries and in different populations. In a meta-analysis, Szajewska *et al*. [17] reported *S. boulardii* significantly reduced the risk of AAD regardless of where the trial was conducted: trials done in Europe (RR=0.46) or in USA (RR=0.46), as shown in (Supplementary Figure S9). Guo *et al*. [19] reviewed different types of probiotics for the prevention of AAD in children and pooled data from nine RCTs using *S. boulardii*. These nine trials were done in different countries (China, France, Turkey, India and Poland) but were not assessed by country sub-groups but nevertheless found a significant reduction of AAD (RR=0.36, 95% C.I. 0.24, 0.54, P<0.0001) for the nine trials. In a network meta-analysis with 10 different probiotic types, Cai *et al*. [18] reported a significant reduction in AAD from 11 RCTs using *S. boulardii* (RR=0.41, 95% C.I. 0.29, 0.57, P<0.05) and a significant reduction in CDI from three RCTs (RR= 0.35, 95% C.I. 0.15, 0.85, P<0.05), but did not report in which countries the trials were conducted.

Study Strengths

Use of Chinese databases (CNKI and CBM) for the literature search revealed 42 trials that would have been undetected if only the most common literature databases (PubMed, Google Scholar, etc.) had been used. We also followed recently published guidelines for reporting meta-analyses involving probiotics [28]. We used rigorous inclusion criteria to include trials that adequately described the probiotic intervention and outcome measures. Sub-group analysis was done to examine factors that might influence the estimates of efficacy. As geographic region factors might influence the efficacy of a probiotic intervention, one strength was that we limited trials done in one country (China), although we acknowledge vast differences in regions within China exist. Another strength was that we limited our meta-analysis to one specific type of probiotic (*S. boulardii* CNCM I-745), as the efficacy of probiotics has been shown to be both strain-specific and disease-specific [15].

Study limitations

Geographic factors in China that might affect probiotic efficacy were not documented in the included trials (such as differences in diet, health status, urban/rural, etc.). Future trials may benefit in exploring these factors. Significant heterogeneity was observed in some outcomes and sub-group analyses failed to explain the possible sources of the heterogeneity. Another limitation is that some trials failed to report specific confounder data, so that not all proposed sub-group analyses could be performed. Several meta-analyses found the efficacy of a probiotic is better when the probiotic is started within 48 hours of the antibiotic [83,84], and while many RCTs in China reported the intervention was started at the same time as the antibiotics, the specific times were rarely stated. We excluded 17 trials with a primary outcome of *H. pylori* eradication, but *S. boulardii* has been shown to significantly reduce AAD in patients treated with antibiotics for *H. pylori* infections [21]. In a meta-analysis by McFarland *et al*. [85], *S. boulardii* CNCM I-745 significantly reduced the risk of AAD associated with *H. pylori* eradication therapies in eight RCTs (RR=047, 95% C.I 0.37, 0.60). Even though the 46 trials in this meta-analysis were limited to those done in China, the robustness of the efficacy estimates from trials done in other countries may show the efficacy of *S. boulardii* CNCM I-745 may be generalized to other populations.

Future studies

To improve the quality of future RCTs, it is recommended to provide a complete description of the method of randomization, consider using a placebo control and to follow subjects post-antibiotics to detect late-onset cases of AAD. In addition, RCTs need to report safety data as this is an important clinical consideration when using probiotics. Most studies did not compare their study results with other trials using *S. boulardii* CNCM I-745 and this should be included in the discussion of future papers. This study shows the value of utilizing resource rich literature databases, especially in non-English language studies.

Conclusion

In summary, the results of this meta-analysis of 46 randomized, controlled trials in China demonstrated that *S. boulardii* CNCM I-745 was effective in both the prevention of AAD and CDI and was well tolerated. The use of Chinese databases for literature searches revealed an untapped resource to find important clinical trials and offers clinicians and policy-makers guidance for an effective strategy to prevent complications of antibiotic use.

Disclosures

Author contributions

Conceptualization: Lynne McFarland and Tong Li; Methodology: Lynne McFarland and Tong Li; Software: Lynne McFarland; Validation: Lynne McFarland and Tong Li; Formal analysis: Lynne McFarland; Resources: Lynne McFarland and Tong Li; Data curation: Lynne McFarland; Writing-original draft preparation: Lynne McFarland; Writing-review and editing: Lynne McFarland and Tong Li; Supervision: Lynne McFarland.

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Data Availability statement

Data is available in the Supplementary Materials.

Conflicts of Interest

Lynne McFarland is a member of the Biocodex Microbiota Foundation's advisory board) and a paid consultant for Biocodex. Tong Li declares no conflicts of interest.

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