

Probiotic supplementation in children with cystic fibrosis— a systematic review

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Abstract Probiotics may benefit in cystic fibrosis (CF) as gut dysbiosis is associated with gastrointestinal symptoms and exacerbation of respiratory symptoms in CF. We conducted a systematic review of randomized controlled trials (RCTs) and non-RCTs of probiotic supplementation in children with CF, using the Cochrane methodology, preferred reporting items for systematic reviews (PRISMA) statement, and meta-analysis of observational studies in epidemiology (MOOSE) guidelines. Primary outcomes were pulmonary exacerbations, duration of hospitalization and antibiotics, and all-cause mortality. Secondary outcomes included gastrointestinal symptoms, markers of gut inflammation, and intestinal microbial balance. A total of nine studies (RCTs, 6, non-RCTs, 3; $N = 275$) with some methodological weaknesses were included in the review. The pooled estimate showed significant reduction in the rate of pulmonary

exacerbation (fixed effects model, two parallel group RCTs and one cross-over trial: relative risk (RR) 0.25, (95 % confidence interval (95 % CI) 0.15,0.41); $p < 0.00001$; level of evidence: low) and decrease in fecal calprotectin (FCLP) levels (fixed effect model, three RCTs: mean difference (MD) -16.71 , 95 % CI $-27.30, -6.13$); $p = 0.002$; level of evidence: low) after probiotic supplementation. Probiotic supplementation significantly improved gastrointestinal symptoms (one RCT, one non-RCT) and gut microbial balance (decreased *Proteobacteria*, increased *Firmicutes*, and *Bacteroides* in one RCT, one non-RCT).

Conclusion: Limited low-quality evidence exists on the effects of probiotics in children with CF. Well-designed adequately powered RCTs assessing clinically meaningful outcomes are required to study this important issue.

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What is Known:

- Gut dysbiosis is frequent in children with cystic fibrosis due to frequent exposure to pathogens and antibiotics.
- Probiotics decrease gut dysbiosis and improve gut maturity and function.

What is New:

- This comprehensive systematic review shows that current evidence on the safety and efficacy of probiotics in children with cystic fibrosis is limited and of low quality.
- Well-designed and adequately powered trials assessing clinically important outcomes are required considering the health burden of cystic fibrosis and the potential benefits of probiotics.

Keywords Cystic fibrosis · Fecal calprotectin · Gut dysbiosis · Probiotics · Pulmonary exacerbation

Abbreviations

BMI Body mass index
CF Cystic fibrosis
FCLP Fecal calprotectin

FEV	Forced expiratory volume
GI	Gastrointestinal
LGG	<i>Lactobacillus GG</i>
RCT	Randomized controlled trial
RNO	Rectal nitric oxide
ORS	Oral rehydration solution

Background

Cystic fibrosis (CF) is a lethal hereditary disorder leading to respiratory infections and gastrointestinal inflammation with a possible association with intestinal dysbiosis [19]. In acute pulmonary exacerbations in CF, there is an increase in pathogens (e.g., *Enterobacteriaceae*) and decrease in beneficial microbes (e.g., *Bifidobacteria*) in the gut [19, 20, 36, 56]. The fecal-oral transmission of increased gut pathogens could explain these exacerbations in CF [28, 36]. Because of recurrent bronchopulmonary infections, patients with CF are treated with repeated courses of antibiotics thereby disrupting the gut microbial balance and innate immune mediators [34]. Increased gut colonization by *Fusobacterium* and *Bacteroides* also predisposes to gut infection and inflammation in CF [48, 57]. Immune-mediated intestinal inflammation in CF has been reported in several studies [12, 60]. Fecal calprotectin (FCLP) and rectal nitric oxide (RNO) are inflammatory markers that are elevated in CF and other inflammatory bowel diseases [5, 62, 72]. Since endoscopy and biopsy which are the gold standards to diagnose intestinal inflammation are invasive and expensive, non-invasive markers of intestinal inflammation such as FCLP and RNO are increasingly being used to detect intestinal inflammation in CF [5, 8, 9]. Normal concentrations of FCLP range between 0 and 50 $\mu\text{g/g}$ of wet stools; values between 50 and 100 $\mu\text{g/g}$ are intermediate and need to be followed up, whereas values over 100 $\mu\text{g/g}$ indicate intestinal inflammation [22, 51]. Similarly, RNO can be easily measured non-invasively and that nitric oxide (NO) increase is observed in active intestinal inflammation [10].

Probiotics are live microbial agents which when ingested in an adequate amount, provide health benefits to the host [39]. Probiotics decrease gut microbial imbalance [59] and result in more stable and diverse flora by various mechanisms including modulation of gut immunity, competition with gut microorganisms for nutrients, and production of growth substrates/inhibitors [42, 54, 55, 59, 65]. The ability to reduce intestinal dysbiosis is one of the most important mechanisms of benefits of probiotics in inflammatory bowel diseases [1, 37]. Probiotics have direct antimicrobial [50], immune modulatory [2, 50], and anti-inflammatory effects [47, 66] and enhance gut maturity and function [2]. Both FCLP and RNO production decrease after probiotic supplementation [5, 7]. Pulmonary exacerbations (PE) in CF are associated with immune-mediated lung damage [14]. Given the health burden

of the disease, and the potential benefits of probiotics [2, 4, 41, 47, 50, 53, 66], we aimed to conduct a systematic review of studies assessing the effects of probiotic supplementation in children with CF.

Methods and participants

We followed the Cochrane methodology [35], the preferred reporting items for systematic reviews (PRISMA) statement [46], and the meta-analysis of observational studies in epidemiology (MOOSE) guidelines [61] for conducting and reporting this systematic review. Ethics approval was not required.

Eligibility criteria

Types of studies Randomized controlled trial (RCT) as well as non-RCT studies were eligible for inclusion in the review.

Participants Participants comprised children under 18 years with CF.

Type of interventions Oral supplementation was given with any probiotic supplementation (with or without prebiotic) in any form and dose and continued for minimum 2 weeks in children with CF. Control intervention was either placebo or standard treatment without probiotic supplementation.

Primary outcomes Primary outcomes include the following: (1) pulmonary exacerbation as defined by the CF foundation criteria [13, 67], which include at least one major criterion or two minor signs/symptoms with fulfillment of symptom duration. Major criteria include decrease in forced expiratory volume in 1 second (FEV1) of $\geq 10\%$ from best baseline within past 6 months, unresponsive to albuterol, oxygen saturation $< 90\%$ on room air or $\geq 5\%$ decline from previous baseline, new lobar infiltrates or atelectasis on chest radiograph and hemoptysis. Minor signs/symptoms include increased work of breathing or respiratory rate, new or increased adventitious sounds on lung exam, and weight loss CF. Control intervention was either placebo or standard treatment without probiotic supplement in months, increased cough, decreased exercise tolerance or level of activity, and increased chest congestion or change in sputum activity. (2) Duration of hospitalization; (3) duration of antibiotic treatment (oral/intravenous); and (4) all-cause mortality.

Secondary outcomes Secondary outcomes include the following: (1) GI symptoms/signs including diarrhea, vomiting, constipation, reduced appetite, abdominal distension, flatulence, abdominal pain, jaundice, pale colored stools, steatorrhea, gastroesophageal reflux (heart burn), and infection [3];

(2) GI inflammation demonstrated by mean FCLP >50 µg/g and RNO >5 µmol/L; (3) plasma/blood cytokines (interleukin (IL)-6, IL-8, tumor necrosis factor (TNF α) as systemic inflammatory markers; (4) intestinal microbial balance assessed by fecal meta-genomic studies; and (5) change in weight/height/body mass index (BMI).

Search strategy We searched the Cochrane central register of controlled trials (CENTRAL), PubMed, EMBASE, CINAHL databases, proceedings of PAS/SPR and Pediatric Pulmonology conferences, and Psyc INFOS for studies reported from the earliest available online year of indexing until December 2015 using the keywords/MeSH terms (*Lactobacillus* or *Bifidobacterium* or *Saccharomyces* or probiotic agent or probiotics or prebiotics or synbiotics) and cystic fibrosis. No restrictions were applied on study design or language. References of the obtained studies were reviewed to identify additional studies. The international trial registries and Australian Clinical Trials Registry were checked for ongoing/registered trials in this area. Google scholar was searched for articles that might not have been cited in the standard medical databases. Reviewers conducted the literature search independently. Conclusions regarding quality and strength of evidence were based on the grading of recommendations, assessment, development, and evaluation (GRADE) system [33]. Search strategy is summarized in Fig. 1.

Study selection Reviewers AA and HB independently assessed eligibility for selection of all studies identified using the prespecified search strategy. Any disagreements were resolved by discussion among all reviewers.

Data extraction Reviewers AA and HB independently completed a prespecified data extraction form for all included studies. For dichotomous outcomes, the number of patients with the event and the number of patients analyzed in each treatment group of each study were entered into the form. For continuous outcomes, we planned to enter the mean and standard deviations (SD). Information about study design and outcomes was verified by all reviewers. Any disagreements were discussed until consensus was achieved. We contacted the investigators for clarification and/or additional data for analysis.

Assessment of risk of bias We used the Cochrane Neonatal Review Group guidelines to assess the methodological quality of the included RCTs [35]. Additional information was requested from the trial authors to clarify methodology and results if necessary. For each trial, information was sought regarding the method of randomization, allocation concealment and blinding, and reporting of all outcomes of all children enrolled in the trial. Reviewers AA and HB separately

assessed each study. Any disagreement was resolved by a group discussion.

The quantitative scoring tool, Newcastle-Ottawa scale (NOS), proposed by Cochrane Collaboration, was adopted for evaluating the methodological quality of the included non-RCTs [71]. The NOS contains three major domains: selection of subjects, comparability between groups, and the outcome measures. The maximum score for each area is 4, 2, and 3 points, respectively. A total score of 3 or lower indicates low methodological quality.

Assessment of publication bias This was planned to be assessed by a funnel plot [35].

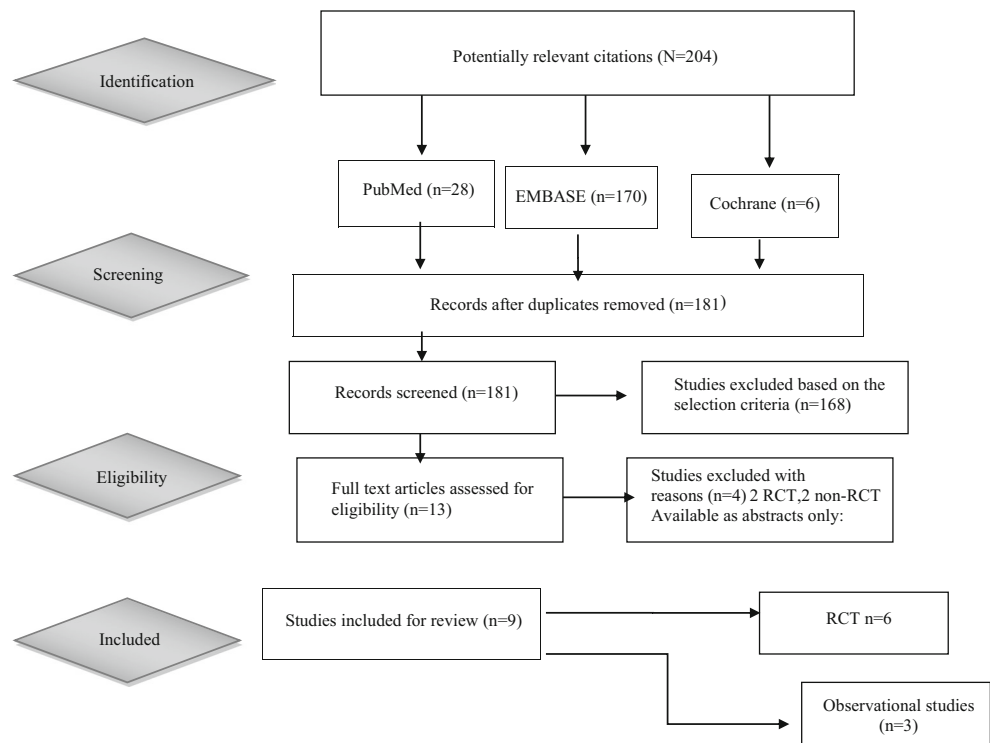
Data synthesis The assessment of risk of bias and heterogeneity in the included studies, data extraction and synthesis, and pooling of treatment effects was planned according to the standard Cochrane methodology [35]. Meta-analysis was planned if pooling of data was possible and justified. We planned to calculate the I^2 statistic for each analysis to quantify inconsistency across studies and describe the percentage of variability in effect estimates that may be due to heterogeneity than due to sampling error. A narrative synthesis was planned if meta-analysis was not possible due to significant heterogeneity in included studies and/or non-availability of the outcome measures in the desired form.

Results

The literature search retrieved 204 potential relevant citations (Fig. 1). After carefully reviewing the abstracts, 23 duplicate studies were excluded. A total of 168 studies were excluded due to non-fulfillment of the inclusion criteria. We considered 13 studies including 4 RCTs, 4 cross-over trials, and 5 non-RCTs for inclusion in the review. Among them, two RCTs and two non-RCTs were excluded since only abstracts were available and the full texts could not be obtained after a detailed search and trying to contact the authors. Finally, nine studies including four RCTs, two cross-over trials, and three non-RCTs were included in the review. The characteristics of the included studies are described in Tables 1 and 2. These studies enrolled 275 children (RCTs, 235; non-RCTs, 40) and had significant variation in patient age group, probiotic type, dose and duration, and outcome measures (Tables 1 and 2). The characteristics of the four excluded studies available only as abstracts are described in Table 3.

Details of included studies

Bruzzese [7]: This double-blinded RCT recruited 22 study participants (10 in probiotic group and 12 in placebo group) who received either probiotic supplementation or placebo for

Fig. 1 Flow chart showing study selection process

1 month. The primary outcome measured was intestinal inflammation defined as increase in FCLP (0–50 $\mu\text{g/g}$ as normal, 50–100 $\mu\text{g/g}$ as intermediate, and >100 $\mu\text{g/g}$ abnormal) and RNO levels (>2.6 $\mu\text{mol/L}$). Sixty-three percent of children with CF had increased FCLP (>100 $\mu\text{g/g}$). Mean FCLP in probiotic group after treatment with probiotics decreased significantly ($p < 0.05$), while mean FCLP in the placebo group after treatment with probiotics did not show significant change ($p = 0.3$). The correlation between intestinal inflammatory markers and the richness of intestinal microbiota in children with CF was evaluated. Mean difference in the *Bacteroides* count before and after intervention in the probiotic group was 4.84×10^{10} and in the placebo group was 1.37×10^{10} . Mean difference in the *Faecalibacterium prausnitzii* count before and after intervention in the probiotic group was 2.46×10^{10} and in the placebo group was 0.48×10^{10} . Mean difference in the *Eubacterium rectale* count before and after intervention in the probiotic group was 0.59×10^{10} and in the placebo group was 5.62×10^{10} . Probiotic supplementation reduced FCLP and RNO levels significantly and showed a trend towards restoring intestinal microbiota composition though it was not statistically significant.

Campo [16]: This was a double-blinded cross-over trial which randomized 30 children with CF to receive probiotic supplementation in period 1 followed by placebo in period 2 or vice versa for 6 months in each period. Primary outcomes measured were FCLP, GI discomfort, inflammatory parameters such as IL-8, IL-6, and TNF- α , and gut microbial balance.

The mean FCLP after probiotic consumption decreased significantly. The study found statistically significant improvement in the G1QL1 scoring for GI comfort ($p = 0.003$) and improved gut microbial balance. The inflammatory parameters such as IL-8, TNF- α , and IL-6 did not show any significant variations (4 vs. 3.6 pg/mL for IL-8, 3.3 vs. 2.7 pg/mL for TNF- α , and 2.3 vs. 2.4 pg/mL for IL-6).

Di Nardo [18]: This double-blinded RCT randomized 61 study participants to receive either probiotic supplementation or placebo for 6 months. The primary outcomes measured were pulmonary exacerbations, hospital admissions, GI, and upper respiratory tract infections (URTI). The study found significant reduction in the rate of pulmonary exacerbation in the probiotic group (number needed to treat, 3 (95 % confidence interval (95 % CI), 2–7)). The number of URTI significantly reduced in the probiotic group (number needed to treat, 6 (95 % CI, 3–102)). There was no significant difference in the number and mean duration of hospitalization between the two groups, GI infection and inflammatory markers. There was no difference detected in the qualitative and quantitative analyses of bacteria in the sputum.

Fallahi [23]: This double-blinded RCT enrolled 47 study participants (24 in probiotic group, 23 in placebo) to receive probiotic supplementation or placebo for 4 weeks. The primary outcome measured was intestinal inflammation defined as FCLP >50 $\mu\text{g/g}$. The study found 65.9 % of the CF patients with intestinal inflammation at baseline. Mean FCLP showed a significant decrease after treatment with probiotic supplementation ($p = 0.031$) compared with the placebo. The study

Table 1 Characteristics of included randomized controlled trials

Study ID (sample size)	Age group	Intervention vs. comparison	Outcomes	Key results
Bruzzese [7]	2–9 years	<i>Lactobacillus rhamnosus</i> GG (6×10^9 CFU) vs. placebo	Primary outcome: intestinal inflammation (FCLP, RNO levels). Secondary outcome: composition of intestinal microbiota	Mean FCLP before and after treatment with probiotic (164 ± 70 and $78 \pm 54 \mu\text{g/g}$, respectively; $p < 0.05^*$; MD 86)
Campo [16]	8–44 years	<i>Lactobacillus reuteri</i> (10^8 CFU) DSM 17938 vs. placebo	Primary outcome: intestinal inflammation (FCLP, IL-6, IL-8, TNF), GI discomfort. Secondary outcome: gut microbial balance	Mean FCLP before and after probiotic (33.8 and $20.3 \mu\text{g/ml}$); $p = 0.003$ improved gut microbial balance. Decreased <i>Proteobacteria</i> (30.7 %), increased <i>Firmicutes</i> (38.2 %), increased <i>Bacteroides</i> (16.9 %)
Di Nardo [18]	6–29 years	<i>L. reuteri</i> (10^{10} CFU) vs. placebo	Primary outcome: PEs, hospital admissions, GI and upper respiratory tract infection (URTI). Secondary outcome: FEV1, FCLP, plasma cytokine	Decreased PEs with probiotic ($p < 0.01$); odds ratio, 0.06 (95 % CI, 0–0.4), URTI reduced in probiotic group ($p < 0.05$; odds ratio, 0.14 (95 % CI, 0–0.96)). No significant difference in FEV1, FCLP, and plasma cytokines.
Fallahi [23]	8–12 years	<i>Lactobacillus casei</i> (1×10^9 CFU), <i>L. rhamnosus</i> , <i>L. acidophilus</i> , <i>S. thermophilus</i> , <i>Bifidobacterium breve</i> , <i>Bifidobacterium infantis</i> vs. placebo	Primary outcome: intestinal inflammation FCLP > 50 $\mu\text{g/g}$	Mean FCLP before and after probiotic (101.38 and 56.2 $\mu\text{g/g}$, MD = 45), mean FCLP before and after placebo (70.22 and 182.1 $\mu\text{g/g}$, MD = -112)
Seyed Ali Jafari [40]	2–12 years	<i>L. casei</i> (2×10^9 CFU), <i>L. rhamnosus</i> , <i>Lactobacillus bulgaricus</i> , <i>S. thermophilus</i> , <i>B. breve</i> , <i>B. infantis</i> vs. placebo	Primary outcome: quality of life, rate of pulmonary exacerbation	Quality of life at 3 months improved in PG (86.8 vs. 80.2; $p = 0.01$), mean number of pulmonary exacerbations decreased in probiotic group ($p = 0.01$)
Bruzzese [6]	5–18 years	<i>L. rhamnosus</i> GG (6×10^9 CFU/day) vs. ORS	Primary outcome: PEs, hospital admission. Secondary outcome: FEV1, modification of body weight	Decreased pulmonary exacerbations median 1 vs. 2 (median difference, 1 (95 % CI, 0.1–2; $p = 0.0035$); decreased hospital admission median 0 vs. 1 (median difference, 1 (95 % CI, 1.0–1.5; $p = 0.00$); increase in mean FEV1 (3.6 % \pm 5.2 vs. 0.9 % \pm 5; $p = 0.02$); increase in body weight (1.5 \pm 1.8 vs. 0.7 kg \pm 1.8; $p = 0.02$)

CFU colony forming units, CI confidence interval, FCLP fecal calprotectin, FEV1 forced expiratory volume at 1 s, IL interleukin, MD mean difference, OR odds ratio, ORS oral rehydration solution, RNO rectal nitric oxide, TNF tumor necrosis factor

* $p < 0.05$ significant

Table 2 Characteristics of included non-randomized controlled studies

Study ID and design	Sample size (age)	Intervention	Outcome	Key results
Weiss [70] (prospective open pilot study)	N = 10 (13–29 years)	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus bulgaricus</i> , <i>Bifidobacterium bifidum</i> , <i>Streptococcus thermophilus</i> (6×10^9 CFU)	Primary outcome: pulmonary exacerbations, FEV1, body weight. Secondary outcome: bacterial strain in sputum, cell counts in sputum, sputum IL-8 Primary outcome: bacterial overgrowth, stool appearance, stool frequency, intestinal comfort. Secondary outcome: intestinal function	Decreased pulmonary exacerbation, 1.3 ± 1 to 0.6 ± 0.7 ($p = 0.002$); no significant change in FEV1, BMI, sputum cell count
De Infante Pina [38] (matched pair analysis)	N = 20 (5–17 years)	<i>Lactobacillus rhamnosus GG</i> (2×10^{11} CFU)	Primary outcome: intestinal inflammation (FCLP, RNO)	50 % showed bacterial overgrowth, 81.25 % patients had improved stool appearance, intestinal comfort, 56.25 % had decreased number of stools, improved intestinal function ($p < 0.05$) FCLP, 2.10 ± 42 to 140 ± 43 $\mu\text{g/g}$ ($p < 0.01$); RNO, 21.2 ± 12.6 to 4.3 ± 1.7 μM ($p < 0.05$)
Bruzzese [5] (matched pair analysis)	N = 60 (1–16 years)	<i>L. rhamnosus GG</i> (5×10^9 CFU)	Primary outcome: Intestinal inflammation (FCLP, RNO)	

BMI body mass index, CFU colony forming units, FEV1 forced expiratory volume in 1 s, IL interleukin, TNF tumor necrosis factor, FCLP fecal calprotectin, RNO rectal nitric oxide

Table 3 Characteristics of excluded studies available as abstracts only

Study ID and design	Sample size (age)	Intervention	Outcome	Key results
Garriga ^a [26] (RCT)	8–44 years	<i>Lactobacillus reuteri</i> vs. placebo	Primary outcome: FCLP, IL-1b levels, GI health. Secondary outcome: gut microbial balance.	Decreased FCLP (0.75 to 0.64 mg/L), decreased in IL-1B levels 28 to 40 pg/mL improved gut microbial balance.
Di Benedetto ^a [17] (RCT)	4–11 years	<i>Lactobacillus rhamnosus GG</i> (6×10^6 CFU/day) vs. ORS	Primary outcome: body weight, abdominal pain, infection needing antibiotic. Secondary outcome: steatorrhoea, iron levels	Increase in body weight compared with ORS (8.7 ± 8.1 vs. 2.7 ± 2.5 %; $p < 0.05$), decreased abdominal pain ($p < 0.05$), decreased rate of infection (0.9 ± 0.06 episodes per child on <i>L. rhamnosus GG</i> and 1.7 ± 0.3 episodes on ORS).
Scotto ^a [58] (prospective cohort study)	N = 20 (7–14 years)	<i>L. rhamnosus GG</i> (6×10^9 CFU)	Primary outcome: intestinal inflammation (FCLP, RNO), respiratory inflammation (NO, IL-8), systemic inflammation (TNF- α , IL-8)	Decreased FCLP in 56 %, decreased RNO in 63 %, decreased nasal NO, serum TNF- α in 100 %
Navas-Lopez ^a [49] (prospective cohort study)	N = 42 (5–13 years)	VSL no. 3	Primary outcome: intestinal inflammation (FCLP, intestinal permeability)	Increased FCLP from 221 ± 273 to 256 ± 298 $\mu\text{g/g}$

RCT randomized controlled trial, CFU colony forming units, FCLP fecal calprotectin, IL interleukin, MD mean difference, ORS oral rehydration solution, RNO rectal nitric oxide, TNF tumor necrosis factor

^a Available as abstract only

reported decrease in intestinal inflammation after probiotic supplementation (58.1 to 27.6 %) by using FCLP as the sole predictor of intestinal inflammation.

Jafari [40]: This RCT enrolled 37 study participants (17 in probiotic group, 20 in placebo group) who received either probiotic supplementation or placebo for 1 month. The primary outcomes measured were quality of life and rate of pulmonary exacerbation. At the start point, quality of life was assessed in all study subjects using the Pediatric Quality of Life Inventory 4.0 short-form questionnaire [68]. The mean total score of parent-reported quality of life was significantly higher in the probiotic group (86.8 vs. 80.2; $p = 0.01$) at 3 months with no significant difference at 6 months. The mean number of pulmonary exacerbations significantly decreased in the probiotic group after intervention ($p = 0.01$). The significant improvement in the quality of life at 3 months is questionable since the significance did not persist after 6 months.

Bruzzese [6]: In this cross-over trial, 38 children with CF who were chronically infected with *Pseudomonas* were randomized to receive probiotic supplementation in period 1 followed by ORS in period 2 or vice versa for 6 months in each period. The primary outcomes measured were incidence of pulmonary exacerbation, hospital admission, FEV1, and modification of body weight. The study found decreased rate of pulmonary exacerbations in both periods, decreased rate of hospital admissions in period 1, increased in mean FEV1, and increased in body weight with probiotic supplementation compared with placebo.

Weiss [70]: This prospective open pilot study included 10 study participants who received probiotic supplementation for 6 months. The primary outcomes included number and severity of PEs, FEV1, body weight by body mass index, bacterial strain in sputum, cell counts in sputum, and sputum IL-8 levels. The study reported decrease in the rate of pulmonary exacerbation. There were no significant changes in FEV1 and BMI, no difference in sputum bacterial strain, no change in sputum cell count and sputum IL-8.

De Infante Pina [38]: This matched pair analysis study recruited 20 children who received probiotic supplementation for 4 weeks. The primary outcomes included water, fat, nitrogen, and sugar content of feces as a measure of stool appearance, stool frequency, and bacterial overgrowth. The study reported improved stool appearance, intestinal comfort, and decrease in the number of daily stools with probiotic treatment. Significant improvement in the intestinal function was noted with probiotic supplementation which was shown by reduction in stool fat, sugar, and nitrogen.

Bruzzese [5]: This prospective matched pair analysis enrolled 60 children (30 with CF and 30 healthy controls, subset of 10 CF children compared before and after probiotic supplementation) who received probiotic supplementation for 4 weeks. The primary outcomes measured were intestinal inflammation defined by FCLP levels (0–50 $\mu\text{g/g}$ normal; 50–

100 $\mu\text{g/g}$ as intermediate, and >100 $\mu\text{g/g}$ abnormal) and RNO levels. The study showed a significant decrease in FCLP and RNO levels with probiotic supplementation. The mean FCLP in children with CF ($n = 60$) was four times higher, almost reaching the levels of children with inflammatory bowel disease compared with controls. Probiotic supplementation reduced FCLP and RNO in vast majority of CF children although in none did the FCLP return to normal.

Ongoing studies A RCT of *Lactobacillus* GG (LGG) supplementation (6×10^9 CFU/day \times 12 months) in children with CF has recently been completed and is titled as “Probiotics in Cystic Fibrosis.” The study has recruited 110 participants aged 2 to 18 years [31]. The trial evaluates the rate of PE, incidence of hospital admissions, pulmonary function, and markers of intestinal inflammation in participants.

A double-blinded placebo-controlled randomized cross-over trial of Bio-25 probiotic supplementation titled “The effects of probiotic on sputum bacteria, sputum inflammation and pulmonary infections in patients with cystic fibrosis” was terminated due to severe allergic reaction in one patient [69]. The study targeted to recruit 12 participants aged 5–40 years with CF. The aim of the trial was to evaluate the effect of probiotic supplementation on the rate of pulmonary infections and the rate of pulmonary exacerbations requiring antibiotic therapy. The effects of probiotic supplementation on sputum bacteria, sputum inflammatory markers, and GI inflammation were the secondary outcomes. It was not clear from the published information on the clinicaltrials.gov website if the allergic reaction occurred in the probiotic arm or the placebo.

Recently, a randomized controlled trial of supplementing 1 g of oral probiotic powder daily for 12 months in children with CF has been registered and is titled “Probiotics and the Early Life effects on intestinal bacteria and inflammation in Children with Cystic Fibrosis (PEARL-CF)” [52]. The study aims to recruit 66 children of age ≤ 6 years. The trial evaluates the effect of probiotic supplementation on the intestinal microbial profile and intestinal inflammation.

Details of excluded studies (available as abstracts only)

Garriga [26]: This randomized cross-over trial enrolled 33 children with CF to receive probiotic supplementation or placebo (16 received 6 months of probiotics in period 1 followed by placebo in period 2, 17 received 6 months of placebo in period 1 followed by probiotics in period 2). The primary outcome measured were FCLP, IL-1b levels, GI health, and gut microbial balance. The study found improvement in GI health, decrease in FCLP and IL-1B levels, and significant changes in the gut flora (*Actinobacteria*, *Firmicutes*, and *Bacteroidetes*). Further analysis of the trial was limited by non-availability of detailed results since only the abstract was available.

Di Benedetto [17]: This cross-over trial randomized 24 children with CF to receive probiotics in period 1 followed by ORS in period 2 or vice versa for 6 months in each period. The primary outcomes included modification of body weight, incidence of abdominal pain, number of episodes of infection needing antibiotics, steatorrhoea, and serum iron levels. The study showed increase in body weight, decreased incidence of abdominal pain, and decreased number of infection requiring antibiotics with probiotic supplementation. The serum iron levels and the incidence of steatorrhoea did not show significant difference with probiotic supplementation. Further details of the study could not be obtained since only the abstract was available.

Scotto [58]: This prospective cohort study enrolled 20 children (ten CF children and ten healthy control) to receive probiotic supplementation for 1 month. The primary outcomes included intestinal inflammation using FCLP and RNO, respiratory inflammation using NO and IL8 in nasal brushings, and systemic inflammation by serum TNF- α and IL-8. Both FCLP and RNO decreased after probiotic supplementation. Nasal NO and serum TNF- α decreased in 100 % patients after probiotic administration. Further details of the study could not be obtained since only abstract was available. The analysis of respiratory inflammation using nasal NO is questionable.

Navas-Lopez [49]: This prospective cohort study enrolled 42 children (17 in probiotic group, 25 in mesalamine group) to receive probiotics for 1 month. The primary outcomes included intestinal inflammation by FCLP and intestinal permeability by dual test with lactulose and mannitol. No statistically significant difference was found after treatment in both groups.

Primary outcomes The beneficial effects of probiotic supplementation on the primary outcomes are shown in Tables 1 and 2. The overall pooled estimate suggested that probiotic supplementation decreased the rate of PE in children with CF (MD, 0.25 (95 % CI, 0.15, 0.41); $p < 0.00001$; heterogeneity: $\chi^2 = 1.46$, $I^2 = 32$ %; Fig. 2). The remaining data were not pooled due to the significant heterogeneity in outcome measures.

Secondary outcomes The effect of probiotic supplementation on GI symptoms was reported in one non-RCT. One RCT and one non-RCT reported improvement in gut microbial imbalance, four RCTs and one non-RCT reported on laboratory markers of intestinal inflammation. One RCT reported on plasma/blood cytokine levels and one cross-over trial and one non-RCT reported on the change in body weight after probiotic supplementation (Tables 1 and 2). The overall pooled estimate showed a decrease in the level of FCLP after probiotic supplementation (MD, -16.71 (95 % CI, -27.30, -6.13); $p = 0.002$; heterogeneity: $\chi^2 = 6.92$, $I^2 = 71$ %; Fig. 3).

Risk of bias Four out of six RCTs had some methodological weaknesses. Allocation concealment was unclear in four [6, 7, 23, 40], information about random sequence generation was unclear in two [23, 40], and the risk of attrition bias was high in two trials [6, 16]. The risk of bias summary of the included RCTs is shown in Fig. 4. The mean NOS score of the included non-RCTs was 7 out of 9, indicating that they were of sufficient quality for meta-analysis (Table 4).

Publication bias Creating a funnel plot was not possible given the inability to pool the data due to the heterogeneity of outcomes reported in the included studies. Therefore, the risk of publication bias could not be ruled out.

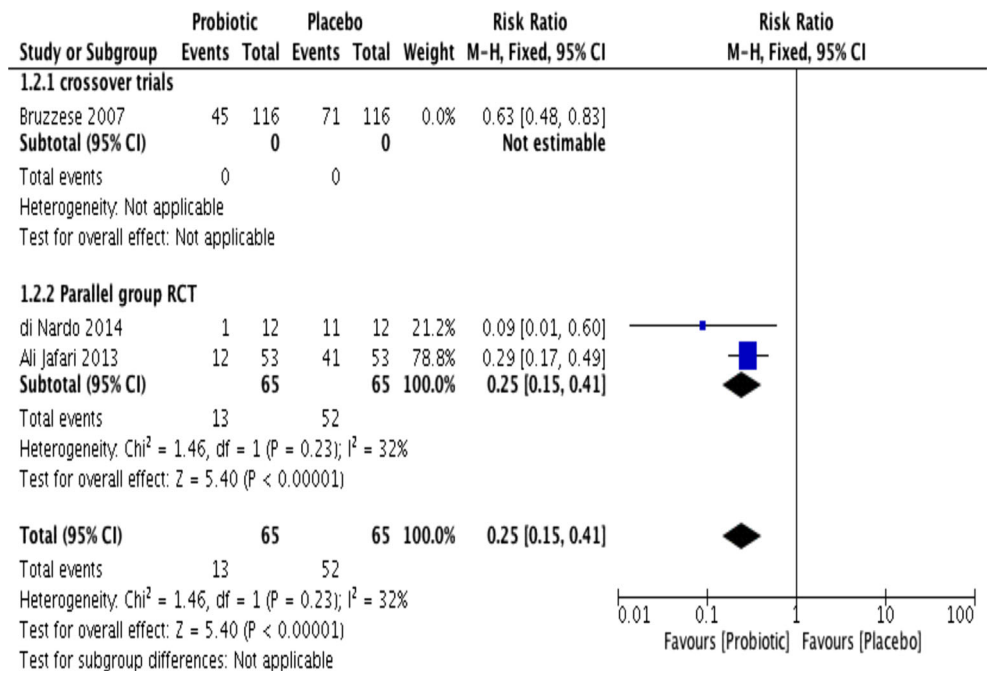
Discussion

Our systematic review showed six RCTs and three non-RCTs ($N = 275$), reporting on the effects of probiotic supplementation in children with CF. Significant improvement in the respiratory parameters (pulmonary exacerbations, infection) and non-respiratory parameters (body weight, abdominal pain,

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Ali Jafari 2013	?	?	+	+	+	+	-
Bruzzese 2007	+	?	+	+	-	+	-
Bruzzese 2014	+	?	+	+	+	+	-
del Campo 2014	+	+	+	+	-	+	?
di Nardo 2014	+	+	+	+	?	+	+
Fallahi 2013	?	?	+	+	+	+	-

Fig. 2 Risk of bias summary. Reviewer judgment about each risk of bias item presented as present (positive sign), unknown (question mark), and absent (negative sign)

Fig. 3 Effect of probiotic supplementation on the rate of pulmonary exacerbation



and GI health) was observed. The overall pooled estimate showed a decrease in the rate of pulmonary exacerbations and reduction in the FCLP levels after probiotic supplementation in children with CF.

Of the three studies which analyzed the effect of probiotic supplementation on the rate of pulmonary exacerbation, the pooled estimate was based on two studies. The third study was a cross-over trial and was not pooled to avoid the influence of carryover of the probiotic effect. The pooled estimate was based on the number of exacerbations rather than the number of participants in the study. This was due to small sample size in the included studies and the rarity of the outcome. Hence, the results should be interpreted with caution. A single study showed the outcome of decreased incidence of respiratory tract infection [18] and the rate of hospitalization [6] significantly after probiotic supplementation and hence the data could not be pooled.

GI health, gut microbial dysbiosis, and body weight showed improvement after probiotic supplementation; however, the data could not be pooled due to difference in the study design and outcome measures. Hence, the validity of

these primary outcome measures could not be ascertained which demands further studies addressing the issues.

The pooled estimate of the FCLP levels was based on decrement in the FCLP levels before and after probiotic supplementation in the treatment group rather than direct comparison between probiotic and placebo groups. There was marked variability in the baseline values of FCLP among the included studies prior to probiotic supplementation. The only study which compared the reduction in FCLP levels between probiotic and placebo groups by Di Nardo et al. showed no significant reduction in the FCLP levels. FCLP is a sensitive marker for intestinal inflammation, but the lack of specificity and baseline variability in the population limits its predictive value in CF [15, 23, 25, 44, 45]. In the absence of a validated marker for gut inflammation, it is important to study the effect of probiotic supplementation on GI signs and symptoms (e.g., diarrhea, constipation, abdominal pain, steatorrhoea) in CF. To our knowledge, this is the first comprehensive systematic review of probiotic supplementation in children with CF—a disease with significant health burden. The strength of our review relates to its comprehensive nature and robust methodology.

Fig. 4 Effect of probiotic supplementation on FCLP levels

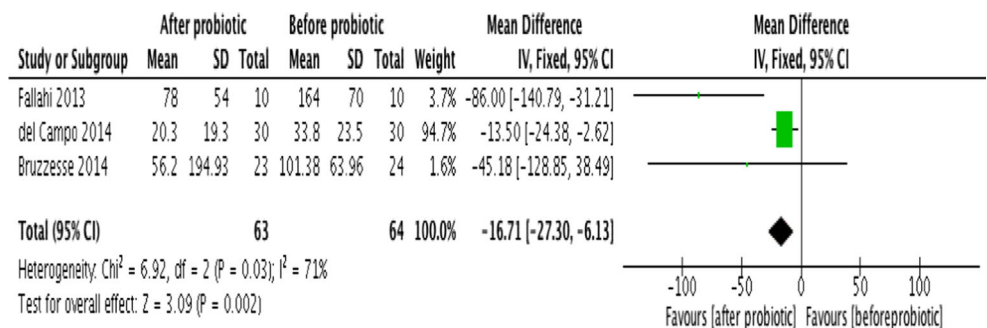


Table 4 Methodological quality of the observational studies

Studies	Selection	Comparability	Outcome/exposure	Total
Weiss 2010	****	*	***	8
Infant pina 2008	***		***	6
Bruzzese 2004	****	*	**	7

Newcastle-Ottawa scoring

Significant benefits were observed irrespective of the variations in patient characteristics, the probiotics, and its supplementation protocol. None of the studies reported adverse effects of probiotics. The most important limitations in our systematic review were the large variability in ages of the patients and that most of the studies were not including only children [16, 18, 70], the lack of information of the pancreatic status and disease severity of the patients, and the rather short study duration in most of the studies (4 weeks) [5, 7, 23, 38, 40].

Considering the potential benefits of probiotics in CF [2–4, 7, 14, 35, 41, 46, 47, 61, 66–68] and the health burden of this disease, further research is important in this area. Few issues need to be discussed with regard to the design and conduct of such studies. The duration of probiotic supplementation is important for optimal benefits. Previous studies have shown increased benefits when probiotics are administered for 6 to 12 months in chronic immune-mediated conditions like eczema and Crohn's disease [29, 32, 43]. Monitoring for complications during long-term probiotic supplementation (e.g., endocarditis, probiotic sepsis) is necessary [21, 24, 63, 64, 73, 74].

Comparing the benefits of single vs. multi-strain probiotics is important. In a systematic review [11], 12/16 included studies showed better benefits following supplementation with a multi-strain compared with single strain probiotics in conditions such as irritable bowel syndrome, diarrhea, atopic disease, respiratory infections, inflammatory bowel disease, and helicobacter pylori infection. Strains with a documented ability to decrease intestinal inflammation and gut dysbiosis (e.g., LGG and *Bifidobacterium breve*) or multispecies products such as VSL no. 3 may be suitable [27, 30]. Given the low incidence of CF in children, recruitment for adequately powered RCTs will be difficult. Cross-over trials have the disadvantage that the residual effect of the probiotics administered in the initial phase could be a confounding factor when interpreting data from the second phase. Deciding an adequate washout period for individual probiotic strains will be difficult. Considering that early intervention may optimize the benefits, future studies of probiotics in CF need to include infants under 12 months age. Further research is needed to assess the optimal dose, strain selection/combination, and duration of probiotics in children with CF.

In summary, current evidence on the safety and efficacy of probiotics in children with CF is limited and of low quality. Well-designed and adequately powered RCTs assessing

clinically important outcomes are required considering the health burden of CF and the potential benefits of probiotics. A comprehensive assessment of gut flora is important to study the pathways of benefits of probiotics in CF. Patient compliance during the study and long-term follow-up need to be ensured.

Author's contribution AA is responsible for literature review, analysis, and writing the first draft. HB is responsible for literature review and analysis. SR is responsible for synthesis and checking the first and the final versions of the manuscript. SP is responsible for concept, supervision, interpretation of results, and checking the first and the final versions of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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