RESEARCH PAPER

Efficacy of *Bacillus clausii* in Pediatric Functional Constipation: A Pilot of a Randomized, Double-Blind, Placebo-Controlled Trial

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Purpose: To evaluate the efficacy of *Bacillus clausii* in the treatment of pediatric constipation.

Methods: A randomized, double-blind, placebo-controlled trial was conducted from January, 2021 to January, 2022 in children aged 1-5 years diagnosed with functional constipation according to Rome IV criteria. They were assigned to receive either *B. clausii* or placebo, once daily for four weeks. The primary out-come was treatment success (defined as ≥3 spontaneous stools per week and stool consistency grade ≥3 on Bristol stool chart). The secondary outcome was a comparison of stool frequency, consistency (defined by Bristol stool grade), and constipation-related symptoms.

Results: This trial enrolled 38 children (*B. clausii, n*=20 and placebo, *n*=18). At 4 weeks, no significant difference was noted in the treatment success between *B. clausii* and placebo groups [45% vs 56%; *P*=0.52). On within-group analyses, the mean (SD) of Bristol stool grade increased in both the *B. clausii* [1.7 (0.5) to 2.8 (1.2); *P*=0.003] and placebo [1.8 (0.5) to 2.8 (1.2); *P*=0.01] groups. Significant increases in the treatment success rate (22% to 56%, *P*=0.01) and mean stool frequency per week [3 (0.9) to 4.2 (1.7), *P*=0.01] were pronounced only in the placebo group. The frequency of painful defecation and large fecal mass were also significantly decreased in both the groups. No serious adverse events were observed.

Conclusion: A 4-week course of *B. clausii* as the sole treatment was not more effective than a placebo for the management of functional constipation in children aged 1-5 years.

Keywords: *Intestinal microbiota*, *Laxatives, Probiotics, Stool frequency*.

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Constipation is documented as a primary complaint in 3-5% of children presenting to pediatricians and 10-20% among pediatric gastroenterologists [1]. A recent study reported 78% children with functional constipation among 316 children with constipation [2]. The use of osmotic laxatives remains the first-line pharmacologic management for children presenting with fecal impaction and maintenance therapy [3]. However, a study demonstrated that more than 50% of the pediatricians felt that the caregivers of constipated children were concerned about tolerance or dependence of the five most commonly used laxatives [4]. Therefore, besides lifestyle and dietary modification, alternative non-pharmacologic management such as probiotics may be advantageous and attractive.

A previous study showed that constipation is associated with gut dysbiosis [5]. Therefore, modifying gut microbiota environment may provide a beneficial effect in patients suffering from functional constipation. Probiotics have therefore been proposed for the management of pediatric functional constipation in the past two decades [6]. Probiotics affect the gut microbiota through various mechanisms including regulating the intraluminal environment, affecting the secretion and absorption of water and electrolytes, enhancing colonic peristalsis and reducing intestinal transit time [7-10]. A recent Cochrane review [11] revealed insufficient evidence to conclude whether probiotics are beneficial in treating constipation. Even the use of probiotics as a sole treatment in this condition remains controversial [3,12].

Invited Commentaries: Pages 429-32.

Most of the *Bacillus* spp. can produce lactic and short-chain fatty acids from carbohydrate fermentation [13], resulting in a lower pH within the colonic lumen and increased peristalsis [14]. *B. clausii*, a non-pathogenic Gram-positive bacteria, has been shown to restore intestinal flora by stimulating immunomodulatory activity [15]. *B. clausii* is also considered safe and has a very low incidence of mild adverse events such as vomiting,

erythematous rash, and stool color change [16]. Therefore, this study aimed to demonstrate the beneficial effects of *B. clausii* on the treatment of functional constipation in young children.

METHODS

This pilot study was a randomized, double-blind, placebo-controlled trial conducted at a quaternary care teaching hospital.

Patients were recruited from both general pediatric clinics and gastroenterology clinics. Inclusion criteria included children aged 1-5 years who were diagnosed with functional constipation according to the Rome IV criteria between January, 2021 and January, 2022. Exclusion criteria were children with suspected or proven organic causes of constipation, drugs that may cause constipation (e.g., antidepressant drugs, opiates), immuno compromised host, neurological impairment, or congenital heart disease. Informed consent was taken for all participants. The study was approved by the institutional review board. The protocol for this study was registered at the Thai Clinical Trials Registry.

During the initial visit, baseline characteristics were recorded, including underlying disease, allergies, and current medication. We collected the characteristics of bowel movement including frequency, consistency defined by the Bristol stool chart on a scale of 1-7 (the scale was noted in the actual stool diary), the frequency of constipation-related symptoms including painful defecation, retentive posturing, large fecal mass (subjectively defined by the caregivers), fecal incontinence (if the child was already toilet-trained), abdominal pain, and rectal bleeding. We used a digital rectal examination for evaluating impacted feces in all children, during the initial visit, before starting the intervention. The children were then randomly assigned into two groups via a computer-generated list, either *B. clausii* or placebo. *B. clausii* (Enterogemina, Sanofi-Aventis), one vial contained *B. clausii* 2 billion spores in 5 mL, odorless, tasteless, colorless, and no refrigeration was needed. The placebo's allocation was concealed by using a vial containing the same volume of sterile water suspension in a plastic container. All caregivers were educated by a single investigator with similar advice on non-pharmacologic management including age- and weight-appropriate fiber and fluid intake, toilet training in developmentally appropriate normal children aged >2-3 years (based on the American Academy of Pediatrics recommendation) with a stool diary on a daily basis. A washout period of two weeks was applied if the child had received any prior treatments for constipation.

Caregivers were instructed to give one vial of the study product to the child once daily for 28 days, and complete a daily diary (provided by the researcher) for stool patterns based on Bristol scale, associated symptoms and adverse events. Parents were also advised to use sodium chloride enema once if the child did not defecate for three or more consecutive days (10 mL for children aged 1-2 years, and 20 mL for children aged 3-5 years). After two weeks, the caregivers sent the daily diary via an online application to the research team, who also telephoned them to ask for further explanations, if needed. Parents returned for a follow-up visit at four weeks, with the diary or via an online application, if the caregivers were unable to visit, especially during the ongoing COVID-19 pandemic. We also defined the compliance and the use of rectal enema. Children who took less than 75% of the total vials in each phase were excluded from the trial.

The primary outcome was treatment success defined as 'at least 3 defecations per week and stool consistency at least grade 3 on the Bristol stool chart' at week 2 and week 4 after the intervention. Secondary outcomes are constipation-related symptoms described above and adverse effects during the study.

Statistical analysis: Statistical analysis was performed by per protocol analysis. Descriptive statistics were performed for baseline characteristics. Continuous variables were described by mean and standard deviation (SD), or in the case of non-normal distributions, by median and interquartile range (IQR). Categorical variables were described by percentage. The significance of difference between independent samples was determined by student *t*-test or Mann-Whitney *U* test for continuous data and by Chi square test or Fisher exact test for categorical data. For paired samples, the significance of difference was determined by paired *t*-test or Wilcoxon sign-rank test for continuous data and by McNemar test for categorical data. Statistical analyses were performed with Stata software version 14.0. For all comparisons, a P value of <0.05 was considered statistically significant.

RESULTS

Caregivers of 50 children agreed to participate and were enrolled in the study. However, 11 patients were excluded during the 2-week washout period due to worsening constipation, and as some children had rectal bleeding. After randomization, 21 children were assigned to receive *B. clausii* and 18 children received a placebo (**Fig.1**). One child in the *B. clausii* group dropped out due to severe abdominal pain and rectal bleeding. Overall, the baseline characteristics of both groups were comparable (**Table I**).

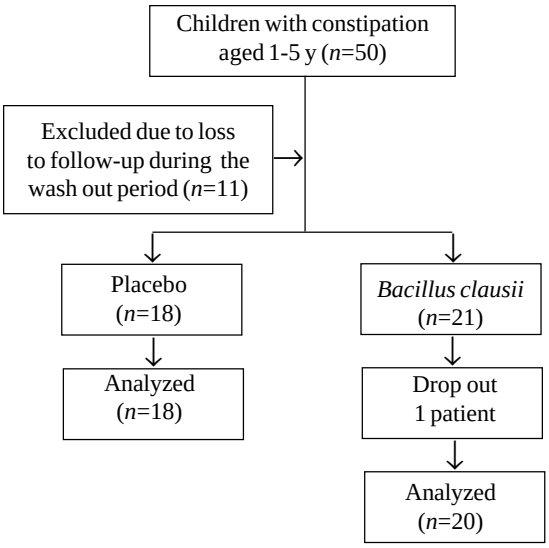


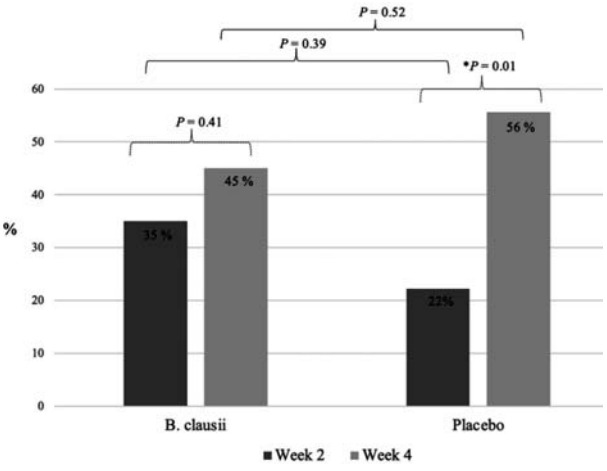
Fig. 1 Flow diagram of the study population.

Two-week follow-up: The treatment success was not significantly different when compared between the *B. clausii* and the placebo groups (35% vs 22%; *P*=0.39) (Fig. 2a). The stool consistency (Fig. 2b), rectal enema used (Fig. 2c), stool frequency (Fig. 3a), painful defecation (Fig. 3b), large fecal mass (Fig. 3c), and retentive posturing (Fig. 3d), as well as fecal incontinence and

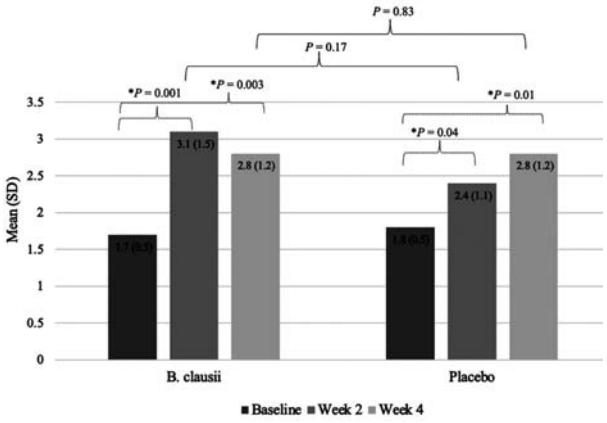
Table I Baseline Characteristics of Children With Functional Constipation Enrolled in the Study

Characteristics	*Bacillus clausii* (*n =20*)	*Placebo* (*n =18*)	*P value*
Male sex	7 (35)	10 (56)	0.21
Age (y)[a]	2.7 (1.0)	2.7 (0.9)	0.91
Body weight (kg)[a]	13.2 (3.9)	13.5 (2.6)	0.76
Duration of constipation (mo)[a]	12.1 (9.5)	12.7 (9.4)	0.69
Previous constipation treatment	15 (75)	15 (83)	0.53
Lactulose	6	12	-
Polyethylene glycol	5	3	-
Unison enema ± laxative	4	-	-
Bristol stool grade[a]	1.7 (0.5)	1.8 (0.5)	0.41
Stool frequency (per wk)[a]	4 (2.1)	3 (0.9)	0.06
Painful defecation (per wk)[b]	3 (2-3)	2 (2-3)	0.55
Large fecal mass (per wk)[b]	2 (2-3.5)	2 (2-3)	0.52
Retentive posture (per wk)[b]	1 (0-1)	1(0-1)	0.43
Fecal incontinence	5 (25)	5 (28)	0.85
Abdominal pain	5 (25)	5 (28)	0.85
Rectal bleeding	6 (30)	5 (28)	0.88
Impacted feces	4 (20)	5 (28)	0.58

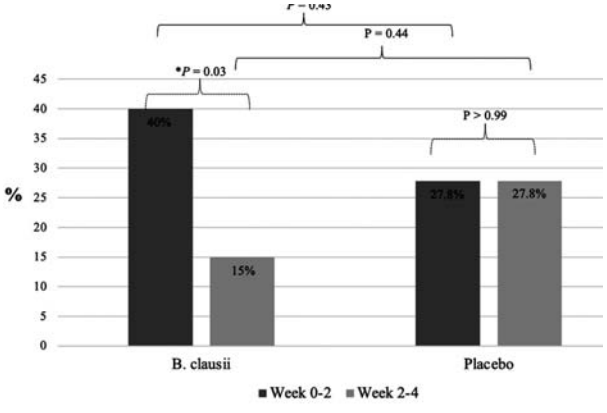
Values in no. (%),[a]mean (SD) or [b]median (IQR).



(*a*)



(*b*)



(*c*)

Fig. 2 Effect of *Bacillus clausii* and placebo on *a*) treatment success (defined by at least 3 defecations/wk and stool consistency at least grade 3 on the Bristol stool chart, *b*) Bristol stool grade, and *c*) rectal enema used during each of the 2-wk study period.

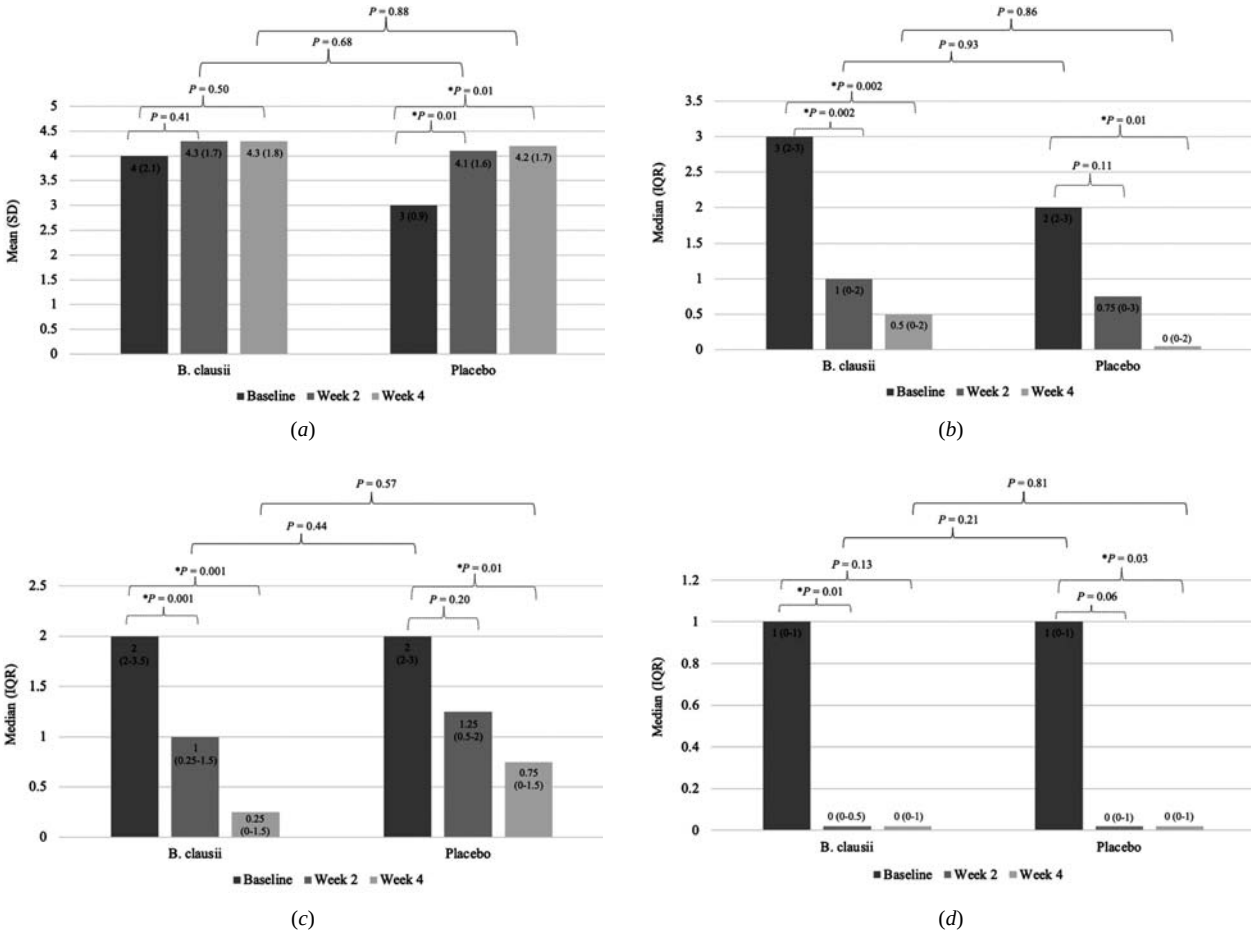


Fig. 3 Effects of *Bacillus clausii* and placebo on *a*) stool frequency, *b*) painful defecation, *c*) large fecal mass, and *d*) retentive posture.

abdominal pain, were not significantly different between groups.

The Bristol stool grade increased in both groups at week 2 as compared to baseline (P=0.001 in the *B. clausii* and P=0.04 in the placebo group) (**Fig. 2b**). Frequencies of painful defecation, large fecal mass, and retentive posture decreased in the *B. clausii* group (**Fig. 3b-d**). However, the stool frequency increased significantly only in the placebo group (**Fig. 3a**).

Four-week follow-up: Treatment success was again not significantly different when compared between the *B. clausii* and placebo groups (45% vs 56%; P=0.52). The treatment success rate of constipated children in the placebo group was even higher than the intervention. The mean stool consistency, rectal enema used, stool frequency, painful defecation, large fecal mass, and retentive posturing as well as other constipation-related symptoms were also not significantly different (all P >0.05).

The Bristol stool grade increased in both groups at week 4 as compared to baseline (P=0.003 in the *B. clausii* and P=0.01 in the placebo group) (**Fig. 2b**). Painful defecation and large fecal mass also decreased in both groups (**Fig 3b, 3c**). While rectal enema used decreased over time only in the *B. clausii* group (**Fig. 2c**), significantly increased treatment success and stool frequency were noted only in the placebo group (**Fig. 2a, 3b**). However, neither group showed any reduction in fecal incontinence nor abdominal pain (data not shown).

Adverse effects: No serious adverse effects were observed. One patient had urticaria and another had abdominal pain in the *B. clausii* group (n=2), and one patient in the placebo group had vomiting.

DISCUSSION

In this prospective randomized study, we found that the efficacy of 4-week probiotic *B. clausii* in the treatment of functional constipation in young children did not differ when compared to those who received a placebo. We also observed no significant differences in the secondary out-

WHAT IS ALREADY KNOWN?

- Besides laxatives and lifestyle modification, alternative non-pharmacologic managements such as probiotics may have a role in managing children with functional constipation.

WHAT THIS STUDY ADDS?

- A 4-week course of *B. clausii* as the sole treatment was not more effective than a placebo in constipated children aged 1-5 years.

comes or adverse effects. With regards to within-group analyses, both groups showed significant improvement in stool consistency, painful defecation, and large fecal mass after 4 weeks. However, the treatment success and stool frequency only improved in the placebo group, while the rescue use of rectal enema decreased only in the *B. clausii* group.

Clinical data support *B.clausii* use for the treatment and prevention of gut barrier impairment [17]. Trials have investigated its use in acute diarrhea [18,19] and prevention of adverse effects from *H. pylori* therapy [20]. To our knowledge, this study is the first trial investigating the efficacy of *B. clausii* as a sole treatment in pediatric functional constipation. Currently, the mainstay management of pediatric functional constipation include lifestyle modification and osmotic laxatives, while the efficacy of most probiotics strain remains in question. A previous systematic review [6] showed no significant difference between the probiotic and placebo groups in children. However, some probiotic strains showed beneficial effects on stool frequency [6].

In pediatric trials [21], four weeks of *Lactobacillus casei rhamnosus* Lcr35 revealed no additional benefit in the treatment success when compared to placebo. However, there was a significant increase in stool frequency. In another study [22], a three-week course of fermented dairy products containing *Bifidobacterium lactis* strain DN-173 010 failed to show an improvement in stool frequency when compared to placebo. The results were consistent with our trial. The lack of an effect of *B. clausii* in this trial may be contributed by an enhanced placebo effect. A high placebo response rate was observed in the earlier trials of pediatric functional constipation, which revealed a 58-70% treatment success rate [21,23]. Moreover, this may likely be a true placebo effect as the participating parents anticipate clinical improvement regardless of the intervention in a non-differential manner.

Recent data has raised concerns regarding probiotic safety. Khatri, et al. [24] reported a case of prolonged *B. clausii* bacteremia in a 17-month-old immunocompetent child, without a definite site of infection or predisposing risk factors. In another case [25], a 5-month-old child with a history of surgically corrected congenital heart disease and malnutrition developed recurrent *B. clausii* bacteremia and consequently succumbed to multidrug-resistant *Klebsiella pneumoniae* sepsis with multiorgan failure [25]. However, no serious adverse events were reported in our trial.

This trial had some limitations that could have caused biases. First, the number of subjects was rather small due to the effect of the COVID-19 pandemic that limited overall outpatient visits to our institution, which may lead to the negative finding of this trial (type 2 error). The follow-up time was also short as it may be worthwhile to wait for a longer period to observe the effect of probiotics, even after the probiotics were ceased. Nevertheless, we tried to avoid the vicious cycle of constipation going for a longer time before initiating an appropriate laxative. Most of the evaluated outcomes were based on a daily stool diary completed by the parents, which might have caused some difficulties, especially when assessing fecal soiling or incontinence and abdominal pain in toddlers. The inability to create a placebo container that was identical to the probiotics container despite the equal volume and similar physical property (clear, odorless, tasteless) of the solution may lead to a minimal bias. We did not have comprehensive data on dietary intake and toilet-related data such as toilet seat. As the gut microbiota analysis was not included in this study, hypothesis regarding the role of gut dysbiosis in children with functional constipation remained speculative. A larger trial of this probiotic strain with diet and toilet-related history, gut microbiota analysis and a longer follow-up period may confirm our finding.

A 4-week course of *B. clausii* as a sole treatment was not more effective than a placebo for the management of functional constipation in a small group of children aged 1-5 years. Larger trials with diet history and a longer follow-up may either confirm or rebut this finding.

Note: The study protocol was retrospectively registered on Feb 19, 2021 and the first patient was enrolled on Jan 5, 2021.
Ethics clearance: Institutional Review Board COA; MURA 2020/253 dated Feb 14, 2020.
Contributors: PL: preparation of the draft manuscript, conception and design, data interpretation of data, revision of the manu-

script; JP: conception and design; PT: conception and design, data interpretation of data, critical review and revision of the manuscript; SG: conception and design; CL: conception and design, revision of the manuscript; ST: conception and design, revision of the manuscript. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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