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Chiranjit Maity & Anil Kumar Gupta

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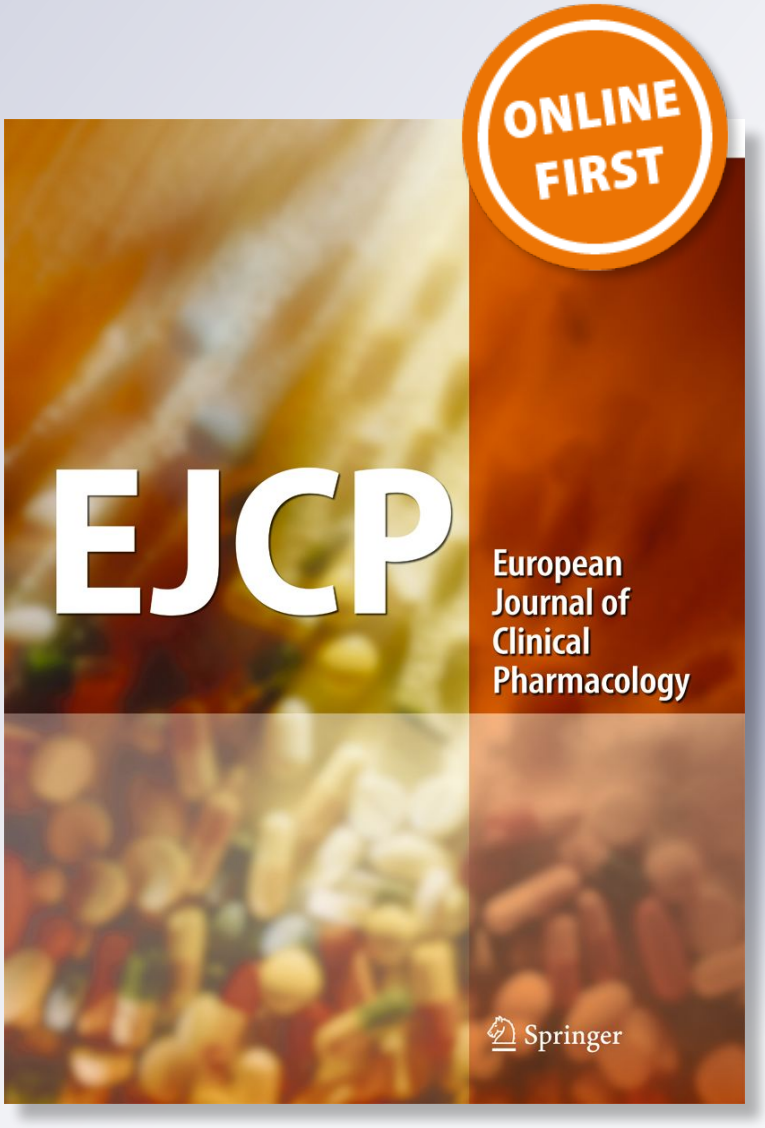

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CLINICAL TRIAL

A prospective, interventional, randomized, double-blind, placebo-controlled clinical study to evaluate the efficacy and safety of *Bacillus coagulans* LBSC in the treatment of acute diarrhea with abdominal discomfort

Chiranjit Maity[1] · Anil Kumar Gupta[1]

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Abstract

Purpose Increasing resistance towards antibiotics has augmented the use of probiotics for the treatment of diarrhea and associated symptoms. Probiotics are active microorganisms which exert some health benefits when consumed in the right amount. This randomized, double-blind, placebo-controlled clinical trial was conducted on 60 "intention to treat" subjects to evaluate the safety and efficacy of probiotic preparation Lactic Acid Bacillus (LAB containing active ingredient *Bacillus coagulans* strain LBSC) for the treatment of acute diarrhea with abdominal discomfort.

Methods The Test-A arm ($n = 30$) was on *B. coagulans* LBSC [2 billion/g] and Placebo-B arm ($n = 30$) was on the carrier. The primary outcomes were the time to last unformed stool (TTLUS), number of unformed stools, change in severity of abdominal pain, time to complete resolution of abdominal discomfort, complete remission of diarrhea, and quality of life (QoL). The secondary outcomes were physical examination and vitals, hematological analysis, and assessment of reported adverse events (AEs) or serious adverse events (SAEs).

Results Trial data showed that the LAB was well-tolerated by participants at the dose provided. The LAB was effective in recovering from acute diarrhea with abdominal pain and discomforts and exhibited improved cluster of QoL. No AEs or SAEs were reported during the trial.

Conclusions It is evident that the test drug, i.e., LAB (*B. coagulans* strain LBSC) is safe and effective for improving the pathophysiological conditions related to acute diarrhea and abdominal discomfort evaluated through stage-II clinical trial.

Keywords *Bacillus coagulans* · Diarrhea · Abdominal pain · Bristol stool scale · Quality of life

Introduction

Use of broad spectrum antibiotics in treatment of diarrhea and related diseases is a common medical practice; however, it is associated with the risk of conferring antibiotic resistance to otherwise sensitive microorganisms [1]. This acquired resistance has increasingly made the antibiotic treatment of microbial pathogens difficult. Additionally, untargeted elimination of gastrointestinal microbiome or narrowing in microbial diversity is a common side effect of antibiotic treatment, which has a magnitude of adverse impacts on human physiology. Alongside antibiotic treatment, oral rehydration salt solution (ORS) and zinc supplementation are commonly recommended adjunct therapy for diarrheal diseases [2]. However, none can restore the depressed gastrointestinal microbiome and microecology; thus, microbiota-associated health benefits.

Probiotic therapy offers a promising route for treating various gastrointestinal ailments like diarrhea, indigestion, nutrient malabsorption, small intestinal bacterial overgrowth (SIBO), irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), pouchitis, ulcerative colitis, and Crohn's

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✉ Chiranjit Maity
chiranjit@advancedenzymes.com

[1] Probiotics Laboratory, Advanced Enzyme Technologies Ltd., 5th Floor, A-Wing, Sun Magnetica, LIC Service Road, Louiswadi, Thane (W), Maharashtra 400 604, India

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disease, without a risk of spreading antibiotic resistance in microorganisms [3]. Probiotics used for diarrheal treatment mainly belong to the genera of *Bacillus*, *Saccharomyces*, *Streptococcus*, *Lactobacillus*, and *Bifidobacterium*.

The systematic interventions of lactic acid-producing bacterial probiotics are well-documented in different community-based meta-analysis and ensued significant reduction of various diarrheal incidences and associated symptoms. Acute diarrhea, antibiotic-associated diarrhea (AAD), rotaviral diarrhea, and *Clostridium difficile*-induced (CDI) diarrhea are significantly improved by monoculture or mixed culture of probiotic formulations [4]. The lactic acid-producing bacterial probiotics, like *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, *Bifidobacterium longum*, *Bifidobacterium lactis*, *Streptococcus thermophillus*, and *Bacillus coagulans* are mainly included in various commercial formulations. In a systematic review conducted by Johnston et al. [5], *Bacillus coagulans* was shown to be the most promising probiotic among all other clinically studied probiotics for preventing antibiotic-associated diarrhea.

Strains of *B. coagulans* are sporogenous, notably stable, and widely studied probiotic for human consumption. The protective coating around the spore allows it to be stable at various unfavorable conditions like high temperature, desiccation, and osmolarity. This highly resilient probiotic can survive and proliferate in acidic microenvironment and the bile condition of gastrointestinal systems. The probiotic activity of *B. coagulans* is highly strain-dependent and may vary in its final formulation, dosages, and medical conditions in order to exhibit the intended health benefits [6].

The ameliorative activity of *B. coagulans* is profound among children for treating antibiotic-associated diarrhea [7]. The significant improvement in abdominal pain and bloating conditions is obtained in irritable bowel syndrome, treated by *B. coagulans* [8, 9]. However, most of these clinical studies have focused on the therapeutic application of *B. coagulans* for diarrheal symptoms under the influence of either antibiotic treatment, association with irritable bowel syndrome or *Clostridium difficile* or other infections. Very limited clinical findings are available about the therapeutic impact of *B. coagulans* on diarrhea, induced due to diet, lifestyle, or patho-ecological causes through randomized placebo controlled blind clinical trials, which led us to conduct the current study.

The present study was undertaken to evaluate the efficacy and safety of a probiotic *Bacillus coagulans* strain LBSC [DSM17654] in the treatment of a common gastrointestinal illness, i.e., acute diarrhea with various abdominal discomfort. *Bacillus coagulans* strain LBSC is the active ingredient used in this study, which is also available under the commercial name Lactic Acid Bacillus (LAB). The diarrheal frequency, time to last unformed stool, complete remission, abdominal discomfort, and quality of life in the intervened group and control group were compared as outcome variables.

Methods

Formulation

Lactic Acid Bacillus (LAB) was the investigational product which contained the active ingredient, *B. coagulans* strain LBSC mixed with excipient. The strength of the active ingredient was two billion spores per gram per sachet, was supplied by Advanced Enzyme Technologies Ltd., Thane, India. The placebo drug was only excipient, maltodextrin (1.0 g). Both the investigational and placebo products had stringently passed through the global specifications (Supplement Method of Analysis); physical appearance, packing, and labeling were same.

Ethics and informed consent

The trial registered with the Clinical Trial Registry India (CTRI/2018/01/011635), was conducted following the written ethical approval by Rajlaxmi Hospital, Bangalore, India (RH/IEC/AP-002/2018). The approved study protocol was in accordance with the Declaration of Helsinki [10], ICH-harmonized tripartite guideline for good clinical practice [11], and Indian Council of Medical Research (ICMR) Guidelines for Biomedical Research on Human subjects [12]. The approved protocol was followed with no further changes or amendments during the trial. The written and oral information about the study were provided to all participating subjects in a language understandable by subjects. Every subject had given the written informed consent to the investigator after understanding the objective of this trial, including possible risks and benefits.

Study design and selection of study subjects

The prospective interventional trial was randomized, double-blinded, parallel group, placebo controlled, and had a total of five visits to the clinical site by the study subjects. Subject selection was based on the defined inclusion and exclusion criteria.

Inclusion criteria Subjects were included based on the following criteria: (1) Male and females (18–65 years) with symptoms of acute diarrhea manifesting within 48 h prior to entering the trial; (2) subjects experienced at least three incidences of unformed stool and last stool passed of unformed consistency (type 7 by Bristol Stool form scale [13]) within 24 h prior to entering the trial; (3) must have complaints of abdominal discomfort within the last hour; and (4) written informed consent by study participants.

Exclusion criteria Subjects with severe form diarrhea or diarrhea as symptoms of more severe disease complexities, purulent or bloody stools, presence of erythrocytes or leukocytes in

stools, require antibiotic treatment, and presence of ulcers, were excluded from the study (Supplement Details of Exclusion Criteria). In addition, subjects having adverse effects or serious adverse effects, pregnancies, disease emergencies, concomitant therapy, and study protocol violation are considered for withdrawal of subjects from the trial.

Sample size calculations, randomization, and treatment allocation and procedures

A total of 60 subjects consisting 30 randomized subjects in each of the test and the placebo group were enrolled for the current study. The test group—A ($n = 30$) was on the Lactic Acid Bacillus (*B. coagulans* LBSC) with two billion colony-forming unit activity (Supplement Method of Analysis) powder (carrier maltodextrin) thrice a day, whereas, control (placebo) group—B ($n = 30$) was only on maltodextrin with similar dosing schedule. Total study duration did not exceed 7 days. The subject randomization (followed by SAS random number generation method), treatment allocation, and procedures are represented in Fig. 1. All supportive treatments were, if required, administered to the subjects as deemed necessary by the investigator, but no antibiotics were recommended along with the study drug. No changes or amendments were made to the approved protocol after the trial commenced and no interim analysis was done during the study period.

Efficacy and safety variables

The efficacy outcomes were determined by measuring the *t*-ime *t*o *l*ast *u*nformed *s*tool (TTLUS), number of unformed stools produced from first IP administration to the end of treatment (EOT), change in severity of abdominal pain, time to complete resolution of abdominal discomfort, complete remission of diarrhea, rate of reoccurrence, and quality of life (QoL) assessment. TTLUS is defined as the number of hours from first IP acceptance time to last instance of unformed stool, after which, participant reported on formed stool [Bristol stool scale] or no stool at all. If no unformed stool was observed at any time during the trial, TTLUS was recorded as 0. The QoL was assessed based on yes or no responses for constipation, diarrhea, blood, mucus in stool, and stain. Results were processed to a binary numerical response and analyzed through principal component analysis (PCA).

Safety outcomes were measured by evaluating physical examination and vitals, hematological analysis and assessing reported adverse events (AEs) or serious adverse events (SAEs). Hematological and biochemical analyses were performed following the standard medical protocol. The adverse event (AE) is defined as any medically untoward event detected in clinical study subject after use of the study agents, whether or not caused by the use of the agents. Whereas, serious adverse event (SAE) is defined as any untoward medical incidence which is life-threatening and

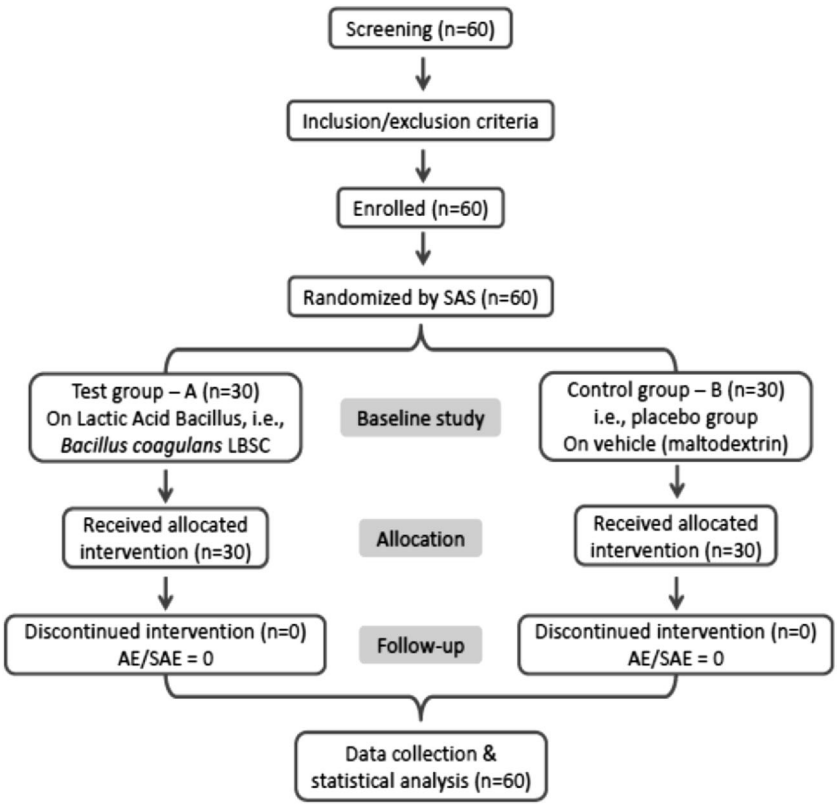

Fig. 1 The schematic diagram of current clinical study. The study enrolled "intension to treat" (ITT) 60 subjects through the inclusion and exclusion criteria screening followed by the SAS random number generation method. No subject reported on discontinuation and adverse events (AEs) or serious adverse events (SAEs). The study compliance was checked at every follow-up visit alongside study evaluation and assessment by a team of physician-investigator and officers

results into death or hospitalization, disability or incapacity, and congenital anomaly.

Statistical analysis

The data were analyzed with 5% significance level and maintaining a minimum power of 80% for study using SAS® software, version 9.1. Differences within the groups were assessed using *t* test or Wilcoxon signed-rank test. Separate analyses were performed for safety and efficacy outcomes. The entire statistical analysis was performed as per the statistical analysis plan (SAP). Principal component analysis (PCA) was performed using ClustVis, a web tool for visualizing clustering of multivariate data.

Results

First patient enrollment and last patient treatment were completed in January 2018 and March 2018, respectively. Total sixty (60) "intention to treat" (ITT) patients participated; and with no drop out and/or withdrawal, the same conferred as "per protocol" (PP) population in this study. Of two treatment arms, i.e., Test-A and Placebo-B, each arm was allocated with 30 subjects. Twenty (20) females and forty (40) males took part in the trial. Further, both the arms had ten (10) females and twenty (20) males. No significant difference was observed in the demographic parameters of both treatment groups (Table 1). Principal investigator and clinical trial team assessed study regulations at each visit along with all the safety and efficacy assays as per the schedule of events (Table 2). The clinical trial was concluded after the final checkup (at Visit 04) of the last enrolled patient and completion of target sample size according to the study procedures.

Safety, adverse events and serious adverse events of *B. coagulans* strain LBSC

Vital examinations were carried out for all the subjects at each visit by the principal investigator. The vital examination included the following parameters; pulse, respiratory rate, systolic blood pressure, diastolic blood pressure, and temperature (Supplement Table 1). The vital sign measures of participants from Test-A and Placebo-B arm remained within the normal range. No statistical significant difference was found among different visits in Test-A ($F = 0.00098$; $p = 0.99$) and Placebo-B ($F = 0.00127$; $p = 0.99$).

Various biochemical and hematological parameters were studied at screening and at the end of the treatment in both Test-A and Placebo-B groups. Biochemical parameters included serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, creatinine, blood urea nitrogen, sodium, and random blood sugar whereas, hematological parameters include red blood cells and total leucocyte count, eosinophils, basophils, neutrophils, lymphocytes, monocytes, hematocrit, erythrocyte sedimentation rate, and platelet counts (Supplement Table 2). The study revealed no statistically significant differences at $p < 0.05$ in hematological and biochemical parameters between the results at screening and at EOT in Test-A ($F = 0.00337$; $p = 0.95$) and in Placebo-B groups ($F = 0.00071$; $p = 0.98$). The parametric values for all the hematological and biochemical studies were also within the reference values as determined.

Efficacy of Lactic Acid Bacillus (*B. coagulans* strain LBSC)

Total number of unformed stools

Total number of unformed stools was reduced by 100% after 120 h of treatment in the Test-A group compared to 47% in the Placebo-B group from first introduction of the investigational

Table 1 The demographic details of subjects of two treatment arms participated in the current clinical trial and their descriptive statistics. Values expressed as mean ± SD

Parameters	Treatment groups	
	Test-A	Placebo-B
Subjects number (*n*)	30	30
Gender [*n* (%)]		
Male	20 (66.67%)	20 (66.67%)
Female	10 (33.33%)	10 (33.33%)
Age (years) [min/max]	32.2 ± 10.4 [18/60]	33.4 ± 13.0 [18/63]
Height (cm) [min/max]	151.9 ± 8.8 [130/167.6]	152.6 ± 13.6 [120/176.7]
Weight (kg) [min/max]	62.3 ± 10.6 [46/81]	59.7 ± 11.3 [38/82]
Body mass index (kg m^{-2}) [min/max]	26.9 ± 4.9 [19.6/37.12]	25.9 ± 5.4 [17.6/37.19]
Diet		
Mixed	29 (96.67%)	28 (93.33%)
Vegetarian	1 (3.33%)	2 (6.67%)

Table 2 The schedule of the clinical trial conducted for acute diarrhea patients

Visits	Visit 01 (day 0)[a]	Visit 02 (day 1)[b]	Visit 03 (day 3)[c]	Visit 04 (day 5)[d]	Visit 05 (day 10 ± 3)[e]	Visit 06[f]
Obtain informed consent	√					
Demography	√					
Inclusion/exclusion criteria	√					
Vital signs	√	√	√	√		
Physical examination	√	√	√	√		
Medical/surgical history	√					
Pregnancy test	√					
Investigator symptoms assessment	√	√	√	√		
Laboratory tests	√			√		
IP dispensing		√	√			
Issue dairy card		√	√			
Concomitant medications		√	√	√		
QOL questionnaire		√	√	√		
Compliance check		√	√	√		
AE/SAE assessment		√	√	√	√	√

[a] Visit 01 was the screening day

[b] Visit 02 was the baseline day, i.e., 0 h of treatment

[c] Visit 03 was the second day of treatment, i.e., 48 h of treatment

[d] Visit 04 was the fifth day of treatment, i.e., 120 h of treatment

[e] Visit 05 was the telephonic visit, and

[f] Visit 06 was the unscheduled visit

product to EOT ($p < 0.0001$) (Fig. 2). The sum of unformed stools, from the first IP administration to EOT, was recorded as 306 and 488 in Test-A and Placebo-B groups, respectively (95% CI, 180.62–183.32). Number of unformed stool in Test-A was remarkably decreased in 48 h, complete arrest of both the diarrheal frequency and conditions was observed after 120 h (Fig. 2). In the Placebo B group, total number of unformed stools decreased slightly indicating no statistically significant improvement in diarrheal conditions, except slight improvement in frequency per subject.

Time to last unformed stool

Study prevailed that 40% [95% CI, 0.40 (0.25–0.58)] and 60% [95% CI, 0.60 (0.42–0.75)] of subjects in Test-A arm reported on the time to last unformed stool (TTLUS) as 48 and 120 h, respectively (Fig. 3). Comparatively, no remedial response was obtained after 48 h in the Placebo-B group and only 23.3% [95% CI, 0.23 (0.12–0.41)] reported on their last unformed stool after 120 h which indicated that 77.7% patients are still persisting with diarrhea. Comparatively, significant improvement in

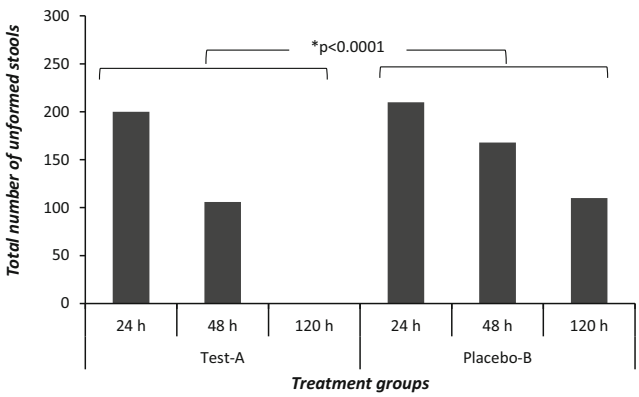

Fig. 2 Total number of unformed stools produced in Test-A and Placebo-B groups from the first IP administration to the end of treatment

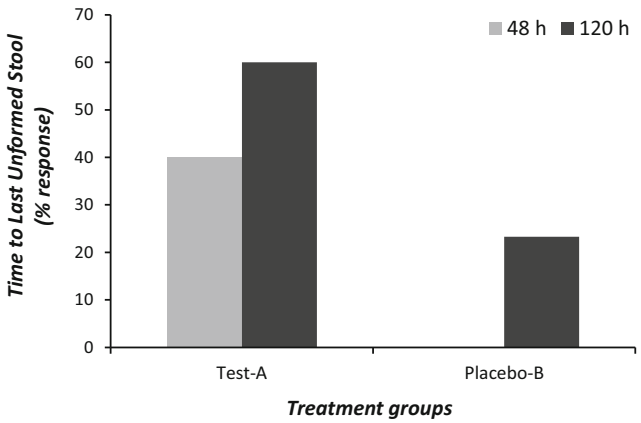

Fig. 3 Response (%) on time to last unformed stool (TTLUS) in Test-A and Placebo-B group after the IP administration to the end of treatment

TTLUS in Test-A to Placebo-B was noted clearly on visit 4 [ARR, −0.37 (95% CI, −0.56–0.12); RR, 2.57 (95% CI, 1.26–5.24); OR, 4.98 (95% CI, 1.61–15.07)].

Change in severity of abdominal pain

Abdominal pain during diarrhea was classified into three different severity categories, viz., mild, moderate, and severe. The activity tolerance scale under the universal pain assessment scale was the main assessor for pain rankings as investigated through physical examinations done by the investigator. The ratio of severe, moderate, and mild abdominal pain was 26:02:02 at screening in the Test-A arm out of 30 patients, which was improved with a ratio of 05:16:09 within 24 h of LAB treatment. In the Placebo-B arm, the ratio of severe, moderate, and mild abdominal pain was 24:04:02 at screening, which remained unchanged after 24 h of treatment with a ratio of 23:03:04, respectively (Fig. 4). According to pain scale, severe abdominal pain in Test-A was reduced by 70% (86.7% at screening and 16.7% at visit 02) after LAB treatment [ARR, 0.70 (95% CI, 0.46–0.82); RR, 0.19 (95% CI, 0.09–0.43); OR, 0.03 (95% CI, 0.01–0.13), whereas, only 3.3% reduced (80% at screening and 76.7% at visit 02) in Placebo-B [ARR, 0.03 (95% CI, −0.17–0.24); RR, 0.96 (0.73–1.25); OR, 0.82 (95%CI, 0.24–2.81)] after 24 h of treatment duration. Results indicated that the LAB has significantly reduced pain severity during diarrhea treatment in Test-A arm in comparison to Placebo-B [ARR, 0.60 (95% CI, 0.36–0.75); RR, 0.22 (95% CI, 0.09–0.49); OR, 0.06, (0.02–0.22)].

Time to complete resolution of abdominal discomfort

Time to complete resolution from abdominal discomfort was assessed using physical examinations done by the investigator. When patients reported for "No Diarrhea" as well as for "No Abdominal pain", the visit hours were captured to measure the time for complete resolution from abdominal discomfort. In Test-A group, 9 [95% CI, 0.30 (0.17–0.48)] and 19 [95% CI, 0.63 (0.45–0.78)] patients were completely relieved of abdominal discomfort after 48 and 120 h, respectively, whereas, only 3 [95% CI, 0.10 (0.03–0.26)] and 4 [95% CI, 0.13 (0.05–0.29)] patients were relieved respectively after 48 and 120 h in Placebo-B (Fig. 5). Results clearly reflected that Test-A is more efficacious in comparison to Placebo-B after 48 h [ARR, −0.20 (95% CI, −0.39–0.01); RR, 3.00 (95% CI, 0.89–10.01); OR, 3.86, (0.93–16.05)] and in 120 h [ARR, −0.50 (95% CI, −0.67–0.26); RR, 4.75 (95% CI, 1.83–12.31); OR, 11.23, (3.09–40.71)] of treatment duration.

Complete remission of diarrhea [by Bristol Stool Scale]

Complete remission of diarrheal symptoms within 120 h was assessed using "Bristol Stool Test" and "Investigator's assessment questionnaire". At screening, subjects from Test-A and Placebo-B arm had Bristol type-7 stool which is watery without solid pieces. Subjects from Test-A (30/30) showed improvement in diarrheal condition after 120 h of treatment, whereas in Placebo-B arm, 22 subjects reported for diarrhea, seven patients reported for "tend to diarrhea", and only one patient relieved from diarrhea (Fig. 6). Trial data showed diarrheal condition was completely remitted for 100% subjects

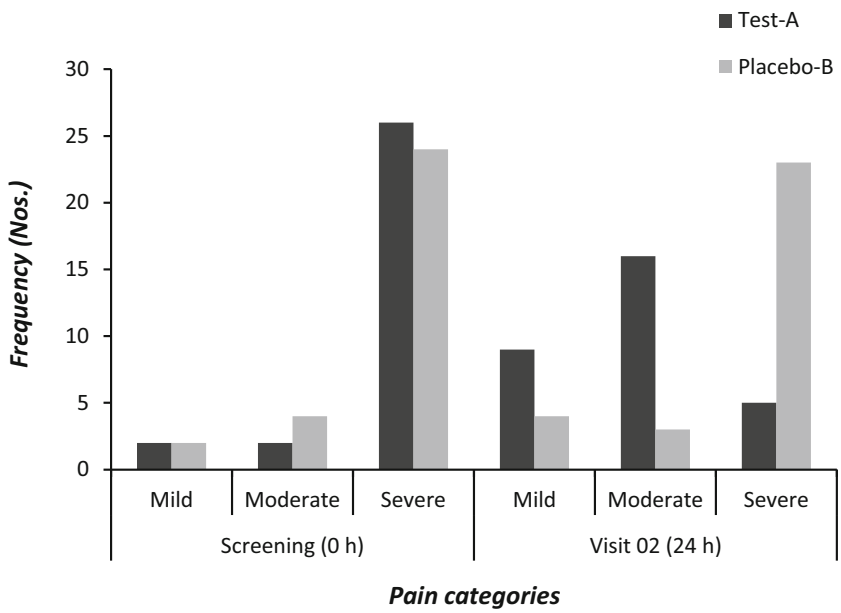

Fig. 4 Change in severity of abdominal pain within 24 h of treatment for Test-A and Placebo-B. The universal pain scale considered three pain categories, viz., mild, moderate, and severe as examined by the investigator

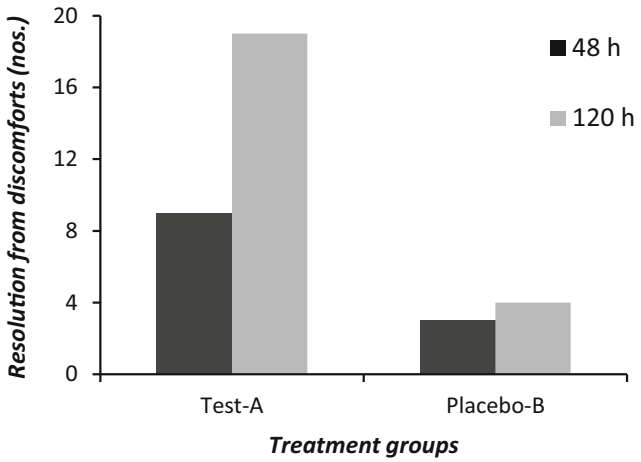

Fig. 5 Complete resolution of abdominal discomforts associated with the acute diarrhea within 48 h and 120 h of treatment in Test-A and Placebo-B arm

in Test-A (95% CI, 0.89–1.00), compared to only 3.33% in Placebo-B (95% CI, 0.01–0.17) ($p < 0.0001$).

Assessment of quality of life (QoL)

Physician's observations indicated improved recovery with absence of constipation and no change in mucus of stool in Test-A. However, no improvement in constipation, diarrhea, and blood and mucus condition was observed in Placebo-B arm with worsened stain condition during diarrhea (Fig. 7).

Discussion

In the current intervention study, safety and efficacy of Lactic Acid Bacillus (LAB) [containing active probiotic agent, *Bacillus coagulans* strain LBSC (formulated with the inert carrier, maltodextrin)] is evaluated for the treatment of acute diarrhea in human. *Bacillus coagulans* strain LBSC [DSM17654; GenBank, KX355750.1] is an extensively studied probiotic bacteria, has been evaluated for safety and efficacy following WHO/FAO (2001) guidelines [14] and whole genome sequence analysis [GenBank CP022701.1]. The "Gram-positive spore-forming bacteria" *Bacillus coagulans* also appears in the Qualified Presumption of Safety (QPS) list for intentional addition into the food and feed chain by European Food Safety Authority (EFSA, 2018) [15].

Subjects in the current clinical trial were randomly distributed into two groups, i.e., Test-A and Placebo-B with female to male ratio of 1:2. The vital parameters (Supplement Table 1), hematological and biochemical (Supplement Table 2) parameters were within the normal range and no significant differences were observed between baseline and final visit of Test-A and Placebo-B arms. This clinically important observation determines the safety of *B. coagulans* strain LBSC. Simultaneously, no adverse events (AEs) or serious adverse events (SAEs) noted during the trial, hence, the investigational product is safe to use. Safety of another *B. coagulans* MTCC 5856 was previously demonstrated by Majeed et al. [16] studied through a human trial ($n = 40$) by administering 2 billion cfu/day for 30 days. Similar to the observations in

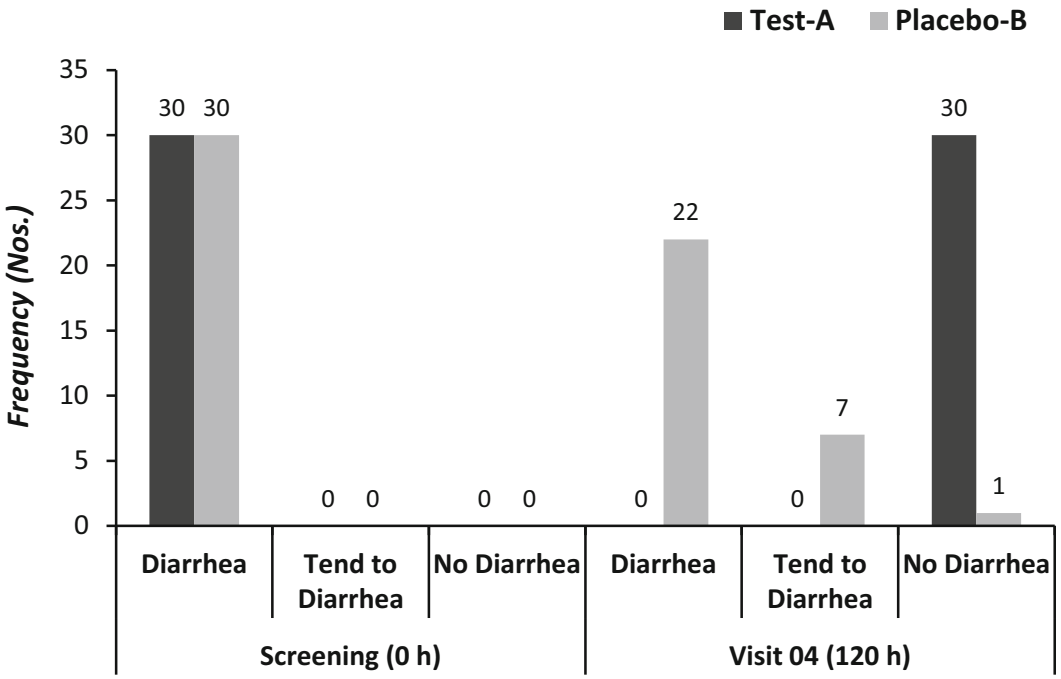

Fig. 6 Graphical representation of stool frequency as per Bristol Stool form scale in Test-A and Placebo-B groups after visit 04

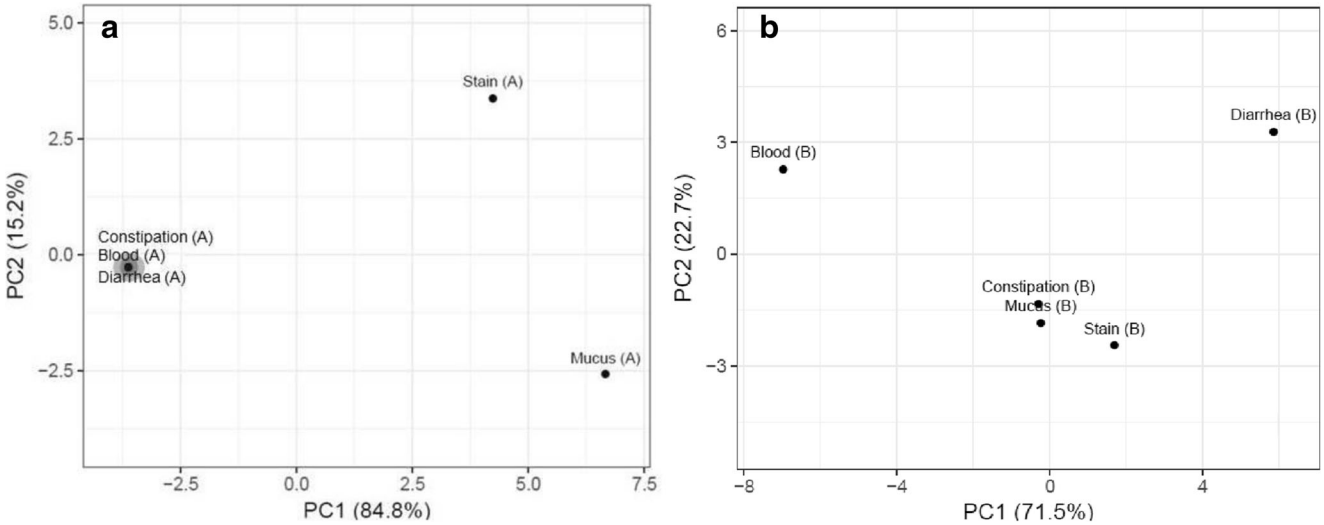

Fig. 7 PCA analysis of responses for different variables of quality of life (QoL) from Test-A (**A**) and Placebo-B (**B**) groups after visit 04. Variables considered are—presence of constipation, mucus in stool, stool is stained, presence of blood in stool, and presence of diarrhea

the present study, Majeed et a [16] reported anthropometric and vital sign measures of participants, both from Placebo and Test, within normal range and found no statistical significant difference. Additionally, therapeutic dosage of *B. coagulans* is ranged between 0.1 and 20 billion cfu/day for human application in various clinical trial studies [17]. Subsequently, 2 billion cfu for thrice of *B. coagulans* strain LBSC were seen to be well-tolerated in the current study for 7 days duration.

Therapeutic effects of *B. coagulans* are evident from significant decrease of total number of unformed stools along with reduction of TTLUS in Test-A compared to Placebo-B, resulting in improvement of diarrheal conditions. Similar findings on improvement of diarrheal condition were reported earlier for other strains of *B. coagulans* [18–21]. A number of different probiotic strains have been reported for improving various gastrointestinal illness, especially, diarrhea and other associated symptoms, though at varying degrees. Stefano et al. [22] demonstrated the antidiarrheal effects of *Lactobacillus* GG through a clinical trial on children of 1 month to 3 years of age ($n = 147$). Different meta-analysis studies also evaluated the impact of probiotics on diarrhea in different population groups and reported significant reduction in duration of diarrhea and other related symptoms [23–25]. In a separate meta-analysis study, Sazawal et al. [26] demonstrated that the antidiarrheal effects of probiotics are dependent on probiotic strain and age of subjects. In the present study, *B. coagulans* strain LBSC was found to significantly reduce diarrheal duration to 48 h, whereas other studies reported a treatment duration of 5–7 days when treated with a daily 4-billion-cfu dosage [20]. This difference could be due to higher dosage used in the present study compared to the other reports [20]. The strong therapeutic potential of current investigating strain *B. coagulans* LBSC, hence, can be used to counter as well as for alternative or preventive treatment of acute diarrhea.

Often, diarrhea is associated with bloating, nausea, cramping, pain, digestive upset, constipation, distension, flatulence, and excessive movement of the intestine. These collective abdominal discomforts, called as functional gastrointestinal disorders (FGIDs), are the result of altered microbiome (or dysbiosis) that has been identified for misbalancing the gut-brain axis (GBA) via central, autonomic, and enteric nervous systems [27]. Various lactic-acid-producing bacteria have shown promising health effects by remodeling gut microbiome and improving GBA and functional gastrointestinal disorders [28, 29]. *Lactobacillus reuteri* DSM17938, *L. acidophilus* AD031, *L. paracasei* subsp. *paracasei* IBS041, *L. rhamnosus* GG, *Bifidobacterium bifidum* BGN4, *Bifidobacterium animalis* subsp. *lactis* AD011, *Bifidobacterium breve* Bb99, *Propionibacterium freudenreichii* subsp. *shermanii* JS, *Bacillus coagulans* GBI-30, 6086, *S. thermophillus*, and *Saccharomyces boulardii* are some clinically tested probiotic [30–32]. *B. coagulans* being available in spore forms offers additional advantages over other probiotics as they are stable over wide range of process conditions, temperatures, gastric acidity, and intestinal biliary microenvironment [33, 34]. The endospores of *Bacillus* probiotics can germinate in host intestine and may alleviate FGIDs and abdominal pains upon establishment and immune stimulation [35]. Probiotics may decrease visceral hypersensitivity through expression of mu-opioid and cannabinoid receptors in intestinal epithelial cells [36] or activate antinociceptic activity through inhibition of transient receptor potential vanilloid 1 (TRPV1) channels [37] for reliving abdominal pain symptoms. Modulating integrity of tight junction proteins (claudin-1, occludin, JAM-A, and ZO-1) may facilitate epithelial barrier against pathogen infection and arresting translocation of toxins as evidenced from animal model study treated with single strain of probiotics like *Bacillus*

subtilis, *B. coagulans*, or a mixed formulation containing *L. casei*, *Lactobacillus plantarum*, *L. acidophilus, Lactobacillus delbrueckii*, *B. longum*, *Bifidobacterium infantis, B. breve*, and *Streptococcus salivarius* [38].

Absence of diarrhea and abdominal pain was considered as relief from the abdominal discomfort. Test-A arm showed complete resolution of abdominal discomfort by visit 04 in comparison to Placebo-B arm, where 76.7% of subjects were not relieved from abdominal discomforts. Several reports demonstrated the relief from abdominal discomforts and improvement in QoL among children with IBS, when treated with a mixture of *B. infantis* M-63, *B. breve* M-16V, and *B. longum* BB536 [39]. Among spore-forming microbes, *B. subtilis*, *B. coagulans*, and *Bacillus clausii* are the most studied probiotics against diarrhea, where varied degree of resolution is noted from associated abdominal discomforts [40–42]. This study reports a considerable higher degree of efficacy of *B. coagulans* strain LBSC in relieving abdominal discomforts associated with diarrhea.

Alongside, Bristol type-7 stool was improved to Bristol type-1 to type-4 in Test-A arm, which indicates that watery liquid stool was recovered to smooth soft sausage to separate solid lumps by rightly balancing the stool water content. However, Bristol type-7 stool in Placebo-B arm was slightly shifted to Bristol type-7 to type-4 which is soft blobs of stools to watery stools indicating no significant improvement of stool texture (Table 3). Improvements in Bristol stool scale have been reported by several other researchers in their studies on irritable bowel syndrome (IBS), small intestinal bacterial overgrowth (SIBO), *Clostridium difficile* infection (CDI), antibiotic associated diarrhea (AAD), and radiation [4, 43–50]. Improvement in stool consistency was previously reported by Sudha and Bhonagiri [20], using *B. coagulans* Unique IS-2; however, these results were stool scoring system rather than the Bristol stool scale. In another study, Minamida et al. [51] examined the efficacy of *B. coagulans* lilac-01 on bowel movement and fecal properties of healthy human subjects ($n = 297$). Subjects showed an improved fecal size, odor, and sensation of evacuation when received the strain lilac-01 (0.1 billion cfu/day) for 2 weeks. A downshift in Bristol stool score was demonstrated in a 36-subject trial treated with *B. coagulans* SBC-37-01, MTCC 5856 (2 billion cfu/day) indicating the stool consistency improvement in diarrhea-predominant irritable bowel syndrome [52].

Improvement in life conditions was checked through quality of life (QoL) responses from subjects on presence of constipation, blood, diarrhea, mucus, and stain in stool during treatment. Responses were converted into binary responses and analyzed through principal component analysis (PCA). The first principal component (PC1) represented about 84.8% of total variance in Test-A arm and it was strongly correlated with all five variables. The first principal component decreases with increasing constipation, diarrhea, and blood scores and increases with mucus and stain scores, whereas the first principal component in Placebo-B (71.5%) strongly correlated with increasing scores of diarrhea, blood, and stain during the diarrhea (Fig. 7). This suggested that five different variables vary individually according to individual score contribution. The improvement in quality of life by probiotics in various health-related conditions like IBS, ulcerative colitis, and colorectal cancer has previously been reported with positive impact by several research groups [53–56]. This study for the first time evaluated the impact of sporogenous probiotic on quality of life (QoL) conditions in acute

Table 3 Frequency of stool appearance as per Bristol Stool scale in Test-A and Placebo-B group after visit 04

Treatment arms	Visit (event)	Bristol stool type	Symptoms	Frequency (nos.)
Test-A	Visit 01 (baseline)	Type 7	Diarrhea	30
	Visit04 (follow-ups)	Type 1	No diarrhea	1
		Type 2	No diarrhea	17
		Type 3	No diarrhea	9
		Type 4	No diarrhea	3
		Type 5	No diarrhea	0
		Type 6	No diarrhea	0
		Type 7	No diarrhea	0
Placebo-B	Visit 01 (baseline)	Type 7	Diarrhea	30
	Visit04 (follow-ups)	Type 1	No diarrhea	0
		Type 2	No diarrhea	0
		Type 3	No diarrhea	0
		Type 4	No diarrhea	1
		Type 5	Tending to diarrhea	7
		Type 6	Diarrhea	15
		Type 7	Diarrhea	7

diarrhea, which is relatively shorter in duration than other diseases studied. The study clearly revealed an improvement on QoL by probiotic *B. coagulans* strain LBSC (Test-A arm) compared to Placebo-B arm.

In conclusion, the Lactic Acid Bacillus [LAB, *Bacillus coagulans* strain LBSC] was well-tolerated by participants (two billion active spores per gram). It is evident from the presented data that the test drug, i.e., LAB (*B. coagulans* LBSC) is safe and effective for improving the acute diarrhea and abdominal discomfort as evaluated through stage-II human clinical trial.

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Author contribution statement CM designed the study protocol, and monitored and prepared the manuscript. AKG reviewed the protocol and manuscript, and approved with technical inputs.

Compliance with ethical standards

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

Ethical approval This study is conducted in compliance with the applicable ethical standards Harmonized Tripartite Guidelines for Good Clinical Practice (GCP), the relevant sections of Good Laboratory Practice (GLP), local laws and regulations, and the provisions of the Declaration of Helsinki. The EC Approval [Reference No.: RH/IEC/AP-002/2018 and Dated: 16/01/2018] and registration of the Clinical Trials Registry—India (CTRI) [Reference No.: CTRI/2018/01/011635 and Dated: 31/01/2018] were obtained prior to the clinical trial study.

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