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Research Article

Evaluation of Bacillus clausii Probiotic Formulations in the Prevention of Antibiotic-Associated Diarrhea in Infants and Children: A Randomized, Double-Blind, Placebo-Controlled Study

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Background & Objective: Antibiotic-associated diarrhea (AAD), is the most common side effect of antibiotics, affecting up to 30% of children receiving antibiotic treatment. We assessed the efficacy and safety of Sanzyme Biologics' probiotic formulations of Bacillus clausii (B. clausii) in preventing AAD in children receiving antibiotics. **Methods:** This was a randomized, double-blind, placebo-controlled three-arm study conducted in children aged 6 months to 12 years old, with mild to moderate bacterial infections requiring β-lactam antibiotic treatment. Participants (N=90) were randomized 1:1:1 (30 per group) to receive either a multi-strain B. clausii probiotic [SNZCLB 1, SNZCLB 2, SNZCLB 3, SNZCLB 4 (B. clausii SNZ 1971)], a single-strain B. clausii SNZ 1971 probiotic, or a placebo, each at 2 billion CFU twice daily for up to 14 days along with antibiotic therapy. **Results:** The incidence of AAD was lower in the probiotic groups compared to the placebo group (2 [6.67%] in each probiotic arm, 6 [20.0%]), yielding a relative risk of 0.33 for both probiotic groups compared to placebo (P=0.20). Among participants who developed AAD (n=10), probiotic-treated children experienced significantly fewer diarrheal episodes in the first 24 hours (multi-strain vs. placebo: P=0.0111; single-strain vs. placebo: P=0.0419) and faster symptom resolution (multi-strain vs. placebo: P=0.0169). Gastrointestinal symptoms, including abdominal pain (P=0.6065) and nausea/vomiting (P=0.7836), were numerically lower in probiotic groups. Additionally, the mean total number of AAD episodes until resolution was significantly lower in the B. clausii groups as compared to placebo (multistrain B. clausii probiotic group vs placebo: P=0.0169; single-strain probiotic B. clausii group vs placebo: P=0.0639). **Conclusion:** Supplementation with either multistrain or single-strain B. clausii, along with β-lactam antibiotics, showed a favorable safety profile and reduced incidence of AAD and related gastrointestinal symptoms as compared to placebo. Sanzyme Biologics' B. clausii probiotic formulations have shown comparable protective effects, supporting their use as adjunctive therapy from the first day of antibiotic treatment to prevent AAD.

Keywords: Antibiotic-Associated Diarrhea, Bacillus Clausii, Infants and Children, Probiotics.

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INTRODUCTION

Diarrhea remains a major cause of morbidity and mortality in children worldwide, even though it is preventable. It leads to around 443832 deaths each year among children under 5 years old. Diarrhea is the most common adverse effect of antibiotic treatments. Antibiotic-associated diarrhea (AAD) is defined as diarrhea that starts from a few hours after beginning antibiotic therapy up to eight weeks after stopping it3,4. It affects up to 30% of children, either early during antibiotic therapy or up to two months after the end of the treatment. Antibiotics can have direct toxic effects on the intestines, such as altering digestion due to an imbalance of gut bacteria or allowing pathogenic microorganisms to overgrow. The bacterial diversity of the intestinal lumen is also diminished after the administration of antibiotics, and these alterations further disrupt the microbiota's

function and composition4,6,7.

The composition of microbiota is also altered in certain disease states, including enteric infections, Helicobacter Pylori and Clostridium Difficile infection, and AAD, resulting in dysbiosis. Antibiotics are one of the main causes of dysbiosis, and their effects on the gut microbiome can last longer than expected. As AAD often results from an imbalance in the normal intestinal flora, studies have looked at using probiotics to restore the normal balance in the gut ecosystem8.

Probiotics are live microorganisms that, when administered in adequate amounts, provide health benefits to the host, as defined by the World Health Organization (WHO)9. The mechanisms of action of different probiotics include providing a physical barrier from pathogens, promoting goblet cell mucus secretion, maintaining the integrity of intestinal epithelial tight junctions, producing antimicrobial factors, and stimulating the immune system. There is growing interest in how probiotics interact with gut microbiota and affect human health10-14. Given the increasing use of antibiotics and the potential gastrointestinal side effects associated with their consumption, there is an unmet need to explore alternative strategies to prevent the AAD15. Over the years, prospective clinical trials concluded that probiotics, especially Bacillus clausii (B. clausii) strains, were found to be effective and safe in the treatment and prevention of AAD in children and also for antibiotic-associated side-effects16-19. In the present study, we evaluated the safety and efficacy of different B. clausii strains (Multi-strain B. Clausii SNZCLB 1, SNZCLB 2, SNZCLB 3, SNZCLB 4 (B. clausii SNZ 1971), and single-strain B. clausii SNZ 1971) in the prevention of AAD, as compared to placebo. The aim was to determine if co-administration of these probiotic strains with antibiotics can serve as a safe and effective way to prevent the AAD in infants and children.

METHODS AND MATERIALS

Study design and participants

This was a randomized, double blind, placebo-controlled three-arm study conducted to evaluate the efficacy and safety of different B. clausii probiotics strains in preventing AAD in infants and children aged 6 months to 12 years. This study was performed in compliance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice, including the archiving of essential documents. The study was registered at the Clinical Trials Registry India CTRI/2024/01/061328 [registered on: 10/01/2024] and was registered prospectively before initiating screening of the subjects (before enrolment).

The primary objective was to assess the effectiveness and safety of B. clausii strains in preventing AAD. Secondary objectives included evaluating the reduction in the incidence of diarrhea events, severity of GI symptoms (nausea, vomiting, abdominal pain), duration of diarrhea days, and overall hospitalization days among participants with AAD. Eligible participants were clinically stable infants and children aged 6 months to 12 years, with confirmed or suspected mild to moderate bacterial infections (respiratory tract, genitourinary, skin or soft tissues) requiring treatment with β-lactam antibiotics for 5 to 14 days. Written informed consent was obtained from a parent or legal guardian before enrollment, with assent from capable children where appropriate.

Exclusion criteria include: clinically unstable infants and children; critical illness; chronic diseases of the endocrine, cardiovascular, renal, or respiratory systems (or any other clinically significant condition that might jeopardize a patient's condition or study outcomes per investigator judgement); history or current immunodeficiency (congenital or acquired, including immunosuppressant therapy); participation in another clinical trial within the past 3 months; hypersensitivity to B. clausii or excipients in the study medication or to other probiotics; and long-term use of oral or intravenous corticosteroids within the 6 months of enrolment.

Study treatments and the supplementation procedure

Participants were randomized in a 1:1:1 ratio into three groups to receive one of the following interventions:

Group A (Multi-strain): Probiotic B. clausii SNZCLB 1, SNZCLB 2, SNZCLB 3, SNZCLB 4 (B. clausii SNZ 1971) containing 2 billion CFU/5mL + β-lactam antibiotic; twice daily for up to14 days.

Group B (Single-strain): Probiotic B. clausii SNZ 1971 containing 2 billion CFU/5mL + β-lactam antibiotic; twice daily for up to 14 days.

Group C (Placebo): Placebo liquid + β-lactam antibiotic; twice daily for up to 14 days.

The probiotic or placebo supplementation was started within 24 hours of antibiotic initiation and continued until the last day of antibiotic therapy (maximum 14 days). All participants received standard care β-lactam antibiotics for their infection. Interventions were dispensed at the clinical site, with study staff recording administration details, including date, dose, and timing. Compliance was monitored through returned packaging and direct observation where possible, with regular reconciliation.

Participants attended the clinical facility for baseline randomization (Visit 1). Follow-up visits occurred at 2 weeks post-antibiotic treatment completion (Visit 2), and then at 2 weeks (Visit 3), 4 weeks (Visit 4), or 6 weeks (Visit 5) after discontinuation of antibiotic treatment, for a total observation period of up to 8 weeks (Figure 1). At each visit, clinical assessments included stool frequency/consistency, GI symptom evaluation, vital signs, and adverse event reporting. AAD was defined as ≥3 loose or watery stools per day during or up to 8 weeks after antibiotic therapy, excluding other causes.

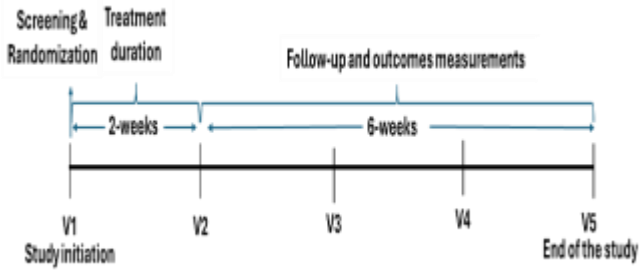


Figure 1: Study Design

Outcomes

The primary outcome was the incidence of the AAD. Secondary outcomes included the number of diarrhea episodes per day, incidence of GI symptoms (nausea, vomiting, and abdominal pain), duration of diarrhea days, and hospitalization days related to AAD. Safety was assessed by monitoring adverse events (AEs), serious AEs, and vital signs throughout the study and 6-week follow-up.

Statistical analysis

The full analysis set (FAS) included all randomized participants who received at least one dose of study intervention (placebo or probiotic over the 12-week duration. Continuous variables were summarized using means, standard deviations, and ranges, while categorical data were described using frequencies and percentages. Between-group comparisons used one-way analysis of variance (ANOVA) with Tukey's honest significant difference post-hoc test for continuous outcomes and Chi-square tests for categorical outcomes.

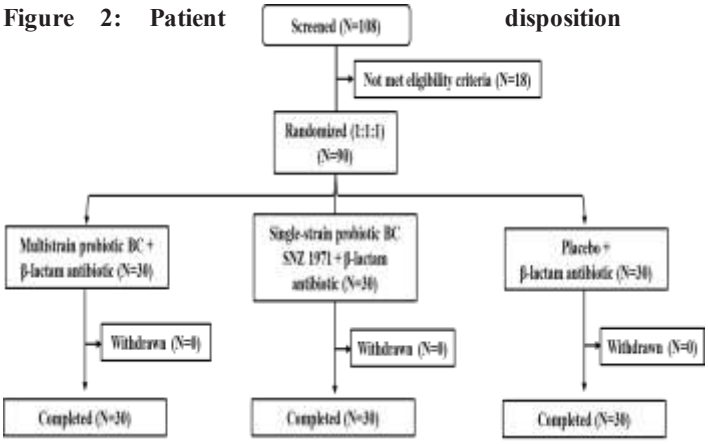
Relative risk, 95% confidence intervals were calculated for AAD incidence. Subgroup analyses (e.g., among those developing AAD) used t-tests or ANOVA as appropriate. Statistical significance was set at $p < 0.05$ (two-sided). Sample size calculated to detect a 20% reduction in AAD incidence (from 30% in placebo to 10% in probiotic groups) with 80% power at $\alpha = 0.05$, requiring at least 30 completed subjects per group. Accounting for a 20% dropout rate, 108 participants were screened for enrollment, and 90 were subsequently randomized in a 1:1:1 ratio (30 per group). Analyses were conducted using R open-source software (version 4.3.2).

RESULTS

Participant disposition

A total of 108 children aged 6 months to 12 years requiring β-lactam antibiotic therapy were screened to be enrolled in the study. Out of these, 90 participants met the eligibility criteria and were randomized with 30 in each of the three treatment arms and comprised the full analysis set (FAS). All 90 participants completed the study as per the protocol, with no withdrawals or losses to follow-up recorded (Figure 2). No protocol deviations were reported during the study conduct.

Figure 2: Patient disposition



BC, *Bacillus clausii*; N, number of patients.

Baseline characteristics

Baseline demographic and anthropometric characteristics, including age, body weight, and height, were assessed before initiation of study treatment. At baseline, the mean age of participants in the Multistrain B. clausii group was 58.93 ± 35.85 months, compared to 60.83 ± 34.53 months in the B. clausii SNZ 1971 group and 60.33 ± 38.59 months in the Placebo group. The age ranges were 9-128 months, 9-120 months, and 9-130 months, respectively. The mean body weight at baseline was 20.13 ± 8.49 kg in the Multistrain Bacillus clausii group, 20.63 ± 7.53 kg in the B. clausii SNZ 1971 group, and 20.04 ± 7.56 kg in the Placebo group. Corresponding weight ranges were 9.2-41 kg, 9-35 kg, and 9-34.5 kg, respectively. Mean height was recorded as 108.47 ± 21.59 cm in the multistrain B. clausii group, 111.10 ± 20.00 cm in the B. clausii SNZ 1971 group, and 109.33 ± 21.14 cm in the Placebo group. The height ranges were 72–145 cm in both active treatment groups and 73–142 cm in the Placebo group.

The clinical indications requiring antibiotic therapy were similarly distributed across treatment groups. Upper respiratory tract infections (URTI) and lower respiratory tract infections (LRTI) were the most common indications, accounting for 26.67% and 23.33–26.67% of cases, respectively, across all groups.

Ear, nose, and throat (ENT) infections represented 20.0–23.3% of cases, followed by skin and soft tissue infections (16.67–20.0%) and genitourinary infections (6.67–10.0%). β-lactam antibiotic regimen usage was comparable across groups, with amoxicillin-clavulanic acid being the most frequently prescribed (43.3–46.7%), followed by cefixime (30.0–40.0%), cefuroxime (6.67–16.67%), and cefpodoxime (3.33–13.33%).

The duration of antibiotic therapy ranged from 3 to 10 days, with the majority of participants (43.3–60.0%) receiving a 5-day course. Overall, baseline demographic and anthropometric characteristics were comparable across the three treatment groups (Table 1), indicating that randomization was adequate and no clinically meaningful differences were observed before study treatment initiation. Clinical Indications for antibiotic therapy initiation in children are presented in Table 2.

Table1: Baseline demographic and clinical characteristics.

Parameters	Group A: Multistrain *Bacillus clausii* (n=30)	Group B: *Bacillus Clausii* SNZ 1971 (n=30)	Group C: Placebo (n=30)
Age (months), mean±SD	58.93±35.85	60.83±34.53	60.33±38.59
Age distribution:			
≤ 4 years	14	12	15
5-8 years	8	11	9
9-12 years	8	7	6
Gender: Male/female, n	19/11	20/10	20/10
Weight (kg) Mean (SD)	20.13 (8.49)	20.63 (7.53)	20.04 (7.56)
Weight (kg) Range	9.2-41	9-35	9-34.5
Indication of antibiotic use: n (%)			
Upper respiratory tract infections	8 (26.67)	8 (26.67)	8 (26.67)
Ear, nose, and throat infections	6 (20.0)	7 (23.33)	6 (20.0)
Lower respiratory tract infections	8 (26.67)	8 (26.67)	7 (23.33)
Genitourinary Infection	2 (6.67)	2 (6.67)	3 (10.0)
Skin and soft tissue infection	6 (20.0)	5 (16.67)	6 (20.0)
Type of β-lactam antibiotic used, n (%)			
Amoxicillin + Clavulanic acid	14 (46.67)	13 (43.33)	13 (43.33)
Ampicillin + Cloxacillin	1 (3.33)	0 (0.0)	2 (6.67)
Cefixime	9 (30.0)	12 (40.0)	9 (30.0)
Cefpodoxime	1 (3.33)	3 (10.0)	4 (13.33)
Cefuroxime	5 (16.67)	2 (6.67)	2 (6.67)
Duration of antibiotic use (days) n (%)			
Up to 3 days	3 (10.0)	1 (3.33)	0 (0.0)
Up to 5 days	13 (43.33)	16 (53.33)	18 (60.0)
Up to 7 days	9 (30.0)	9 (30.0)	9 (30.0)
Up to 10 days	5 (16.67)	4 (13.33)	3 (10.0)

SD, standard deviation.

Table 2. Clinical Indication/condition for antibiotic therapy initiation in children aged 6 months to 12 years.

Clinical condition	Group A: Multistrain *Bacillus Clausii* (n=30)	Group B: *Bacillus Clausii* SNZ 1971 (n=30)	Group C: Placebo (n=30)	Total (n=90)
Upper respiratory tract infections, n (%)	8 (26.67%)	8 (26.67%)	8 (26.67%)	24 (26.67%)
Common cold; n (%)	5 (16.67%)	4 (13.33%)	5 (16.67%)	14 (15.56%)
Whooping Cough; n (%)	1 (3.33%)	-	1 (3.33%)	2 (2.22%)
Pharyngitis (Sore throat); n (%)	1 (3.33%)	3 (10%)	2 (6.67%)	6 (6.67%)
Rhinitis; n (%)	1 (3.33%)	1 (3.33%)	-	2 (2.22%)
Ear nose and throat infections, n (%)	6 (20%)	7 (23.33%)	6 (20%)	19 (21.11%)
Sinusitis; n (%)	2 (6.67%)	4 (13.33%)	3 (10%)	9 (10%)
Otitis; n (%)	2 (6.67%)	1 (3.33%)	2 (6.67%)	5 (5.56%)
Tonsillitis; n (%)	1 (3.33%)	2 (6.67%)	1 (3.33%)	4 (4.44%)
Laryngitis; n (%)	1 (3.33%)	-	-	1 (1.11%)
Lower respiratory tract Infection, n (%)	8 (26.67%)	8 (26.67%)	7 (23.33%)	23 (25.56%)
Bronchiolitis; n (%)	3 (10%)	5 (16.67%)	4 (13.33%)	12 (13.33%)

Bronchitis; n (%)	2 (6.67%)	1 (3.33%)	-	3 (3.33%)
Pneumonia; n (%)	3 (10%)	2 (6.67%)	3 (10%)	8 (8.89%)
Genitourinary infections n (%)	2 (6.67%)	2 (6.67%)	3 (10%)	7 (7.78%)
UTI; n (%)	2 (6.67%)	1 (3.33%)	2 (6.67%)	5 (5.56%)
Cystitis; n (%)	-	1 (3.33%)	1 (3.33%)	2 (2.22%)
Skin and soft tissue Infections, n (%)	6 (20%)	5 (16.67%)	6 (20%)	17 (18.89%)
Impetigo; n (%)	3 (10%)	3 (10%)	2 (6.67%)	8 (8.89%)
Abscess; n (%)	2 (6.67%)	1 (3.33%)	2 (6.67%)	5 (5.56%)
Furunculitis; n (%)	1 (3.33%)	1 (3.33%)	2 (6.67%)	4 (4.44%)

UTI, urinary tract infection

Efficacy

Primary efficacy outcome: Incidence of AAD

The incidence of AAD in children was numerically lower in the probiotic groups as compared to placebo, with a total of 10 diarrhea events in this study. The multistrain B. clausii and single strain B. clausii SNZ 1971 had 2 AAD cases each with a relative risk (95% confidence interval [CI]) of 0.33 (0.14-0.51) as compared to the placebo group with 6 AAD cases (χ^2 test, P=0.2038). Similarly, the incidence of abdominal pain was also numerically lower in probiotic groups as compared to placebo, with a relative risk (95% CI) ranging from 0.33 (0.14-0.51) to 0.7 (0.53-0.86), having a P value of 0.6065. The incidence of nausea and/or vomiting was lower in the probiotic groups as compared to placebo, with a relative risk of 0.50 (0.32-0.67), having a P value of 0.7836 (Table 3).

Table 3. Incidence of AAD and other gastrointestinal-related symptoms.

Parameter	Multistrain BC n (%)	BC SNZ 1971 n (%)	Placebo n (%)	RR (A vs C)	RR (B vs C)	P value
Diarrhea	2 (6.67)	2 (6.67)	6 (20)	0.33	0.33	0.2038
Abdominal pain	1 (3.33)	2 (6.67)	3 (10)	0.33	0.70	0.6065
Nausea/Vomiting	1 (3.33)	1 (3.33)	2 (6.67)	0.50	0.50	0.7836

Relative risk <1 shows probiotic groups has decreased risk of diarrhea/NV/Abdominal pain compared to placebo. AAD, antibiotic-associated diarrhea; BC, *Bacillus Clausii;* vs, versus.

Secondary efficacy outcomes: Subgroup analysis of participants with AAD

Among the participants who developed AAD (n=10), baseline characteristics were comparable across groups. The mean age ranged from 21 to 25.33 across the groups, with a mean duration of antibiotic therapy of 8.5 to 10.0 days. In all cases, AAD onset occurred within 2 weeks of antibiotic initiation (Table 4).

Table 4. Mean age and antibiotic therapy of infants and children presenting with AAD.

Parameter	Multistrain BC (Group A, n=2; mean ± SD)	BC SNZ 1971 (Group B, n=2; mean ± SD)	Placebo (Group C, n=6; mean ± SD)	P value
Mean age (months)	22.0 ± 5.65	21.0 ± 4.24	25.33 ± 11.27	0.8348
Duration of antibiotic use (days)	10.0 ± 0.0	10.0 ± 0.0	8.5 ± 1.64	0.3080
Occurrence of AAD after antibiotic initiation	Within 2 weeks	Within 2 weeks	Within 2 weeks	NA

*P<0.05 was considered statistically significant. AAD, antibiotic-associated diarrhea; BC, *Bacillus clausii*; n, number; NA, not applicable; SD, standard deviation.

Antibiotic	Multistrain BC (n=2)	BC SNZ 1971 (n=2)	Placebo (n=6)
Amoxicillin + Clavulanic acid	1 (50%)	2 (100%)	3 (50%)
Ampicillin + Cloxacillin	–	–	–
Cefixime	1 (50%)	–	2 (33.33%)
Cefpodoxime	–	–	1 (16.67%)
Cefuroxime	–	–	–

A post hoc analysis using one-way ANOVA followed by Tukey's honest significant difference (HSD) test revealed significant differences in AAD severity between probiotic and placebo groups (Table 4). The mean number of AAD episodes in the first 24 hours was lower in the B. clausii groups as compared to placebo (multistrain B. clausii probiotic group vs placebo: P=0.0111; single-strain probiotic B. clausii group vs placebo: P=0.0419) and in the total number of episodes until resolution was significantly lower in the B. clausii groups as compared to placebo (multistrain B. clausii probiotic group vs placebo: P=0.0169; single-strain probiotic B. clausii group vs placebo: P=0.0639) (Table 5)

Table 5. Sub-group analysis among the patients who developed AAD.

Parameter	Multistrain BC (Group A, n=2; mean ± SD)	Single-strain BC SNZ 1971 (Group B, n=2; mean ± SD)	Placebo (Group C, n=6; mean ± SD)	Mean difference (95% CI) vs Placebo	P value
Episodes of diarrhea in first 24 h	3.0 ± 0.0	3.5 ± 0.71	5.0 ± 0.63	A–C: −2.0 (−4.14, −1.85); B–C: −1.5 (−2.78, −0.21)	0.0075**
Duration of diarrhea (days)	3.5 ± 0.71	3.5 ± 0.71	4.33 ± 0.52	−0.83 (−1.94, 0.28)	0.1516
Total episodes until resolution	11 ± 1.41	12.5 ± 0.71	16.67 ± 2.07	A–C: −5.67 (−9.61, −1.72); B–C: −4.17 (−7.98, −0.35)	0.0121*
Duration of abdominal pain (days)	3.0	3.0	3.67 ± 0.58	−0.67 (−1.72, 0.38)	0.3535
Duration of nausea/vomiting (days)	1.0	1.0	2.0	−1.0	NA

*P<0.05 was considered statistically significant. AAD, antibiotic-associated diarrhea; BC, *Bacillus clausii;* CI, confidence interval; NA, not applicable; SD, standard deviation; vs, versus.

Safety

Overall, no treatment-related adverse events were reported during the study and post-study safety assessment. No serious adverse events or deaths were reported during the study. The probiotic strains, both multistrain and single-strain B. clausii treatments, were well-tolerated, with no safety concerns identified throughout the duration of the study.

DISCUSSION

The present study demonstrated that supplementation with Sanzyme Biologics' probiotic formulations, either multi-strain B. clausii (SNZCLB 1, SNZCLB 2, SNZCLB 3, SNZCLB 4) or single-strain B. clausii (SNZ 1971) reduced AAD incidence in pediatric patients (aged 6 months to 12 years) receiving β-lactam antibiotics, compared to placebo (6.7% vs. 20%). Similarly, gastrointestinal symptoms such as abdominal pain and nausea and/or vomiting were less frequent in the probiotic groups, though the differences were nonsignificant. Additionally, analysis of children who developed AAD showed statistically significant benefits: probiotic-treated children had fewer diarrheal episodes within the first 24 hours and faster symptom resolution, demonstrating the clinical utility of B. clausii in reducing AAD severity.

The findings of this study further support the role of Sanzyme Biologics' probiotic strains of Bacillus clausii in reducing the risk and clinical impact of Antibiotic-Associated Diarrhea (AAD). In addition, these strains exhibit exceptional stability under adverse environmental conditions, including gastric acidity, bile salts, and temperature fluctuations. This innate resilience enhances its survival through the gastrointestinal tract and ensures that a sufficient number of viable cells reach the intestine, facilitates persistent colonization and activity during treatment, thereby contributing to the prevention of dysbiosis-induced AAD. Furthermore, the rapid germination of spores in the small intestine allows B. clausii to produce enzymes and immunomodulatory molecules that suppress pathogenic bacteria and promote mucosal barrier integrity. These mechanisms may collectively explain the faster symptom resolution noted in this clinical study with Sanzyme Biologics' probiotic strains of Bacillus clausii.

The observed reduction in the incidence and severity of AAD in subjects receiving B. clausii aligns with previous clinical evidence demonstrating improvements in stool frequency, symptom duration, and overall gastrointestinal well-being. Our findings are building upon broader evidence for B. clausii probiotics in pediatric populations. In a multicenter, randomized, open-label trial among 323 Filipino infants and children (aged 6 months to 12 years) receiving β-lactam antibiotics, Enterogermina (B. clausii O/C, N/R, SIN, T strains; Sanofi) reduced the risk of AAD by 57% (RR 0.43; 95% CI: 0.11-1.62) compared to control (incidence: 1.9% vs. 4.3%; P=0.22)20,21. In comparison, the Sanzyme Biologics' formulations in our placebo-controlled trial demonstrated a 67% risk reduction (RR 0.33; 95% CI: 0.14-0.51), with tighter confidence intervals showing more precise effect estimates. While direct comparison is limited by differences in study design, our double-blind, placebo-controlled approach versus an open-label design, both studies support the protective efficacy of B. clausii strains in AAD prevention20,21.

Beyond prevention, a recent comprehensive meta-analysis by Michel and Pellegrino (2025) evaluated Enterogermina in 11 RCTs involving 2057 children with acute gastroenteritis, demonstrating significant reductions in duration of diarrhea, number of stools, and hospital stay duration22. While that meta-analysis focused on treatment of established gastroenteritis, our study similarly demonstrated that B. clausii prophylaxis significantly reduced AAD episode frequency and severity parameters in affected children, with fewer episodes in the first 24 hours (multistrain: P=0.0111; single-strain: P=0.0419) and faster resolution (multistrain: P=0.0169; single-strain: P=0.0639)22.

Furthermore, our study findings align with existing evidence supporting probiotic use in AAD prophylaxis. A Cochrane review including 6352 children across 33 studies showed that probiotics moderately prevent AAD, with a number needed to treat of 9 (95% CI: 7-13)23. Our relative risk of 0.33 is consistent with this protective effect, though our smaller sample size likely limited statistical power to detect significance in the primary outcome. The modest effect size observed may reflect factors such as different types of infections, variations in antibiotic regimens, and potential use of oral rehydration solutions during diarrheal episodes.

Species-specific data further support the efficacy of B. clausii. A 2025 pooled analysis of three RCTs (n = 435; probiotic group: n = 218; control group: n = 217) demonstrated a significant reduction in AAD incidence in children receiving probiotic supplementation alongside antibiotics (1.8% vs. 6.5%; P= 0.017)24. The 2018 meta-analysis by Ianiro and colleagues, which included six randomized controlled trials with 1298 children, showed that B. clausii significantly reduced diarrhea duration by -9.12 hours (95% CI: -16.49 to -1.75; P=0.015) and hospital stay by 0.85 days (95% CI: -1.56 to -0.15; P=0.017)17. Our results extend these findings by showing reduced episode frequency and faster resolution in probiotic-treated children, supporting B. clausii's ability to reduce AAD severity.

The safety assessment in our study showed no treatment-related adverse events, serious adverse events, or deaths reported during the study or follow-up periods. These observations are consistent with the

2018 meta-analysis of six trials (n=1298), which reported no serious adverse events related to B. clausii across the included studies, supporting its use as an add-on treatment in children receiving antibiotics17.

Additionally, our findings support the 2020 recommendations from an Asian expert panel of pediatricians, pediatric gastroenterologists, and infectious disease specialists, which recommended B. clausii as add-on therapy with oral rehydration solution for pediatric acute diarrhea and as a preventive strategy against AAD25. The panel emphasized considering factors such as antibiotic type, treatment duration, age, other medical conditions, and prior AAD episodes when prescribing probiotics in pediatric patients25.

In conclusion, building on the precedent research data17,21,22,24,26, our study provides additional evidence that supplementation of B. clausii probiotics, either multistrain or single-strain formulations, represents a safe and potentially effective preventive strategy for AAD in children, with a notable decrease in the incidence of AAD with a relative risk of 0.33 as compared to the placebo. Additionally, subgroup analysis of subjects presented with AAD revealed that the mean number of AAD episodes in the first 24 hours was significantly lower in the B. clausii groups as compared to placebo, with a P value of 0.0075 (statistically significant P value achieved by Tukey's HSD). The mean total number of AAD until resolution was significantly lower in the B. clausii groups as compared to placebo, with a P value of 0.0121 (statistically significant P value achieved by Tukey's HSD). These consistent protective trends, significant benefits in the subgroup analysis, and excellent safety profile support B. clausii's role as an add-on therapy in reducing the incidence, frequency, and duration of AAD episodes among pediatric populations. These study findings, combined with existing meta-analytic evidence and expert recommendations, further validate Sanzyme Biologics' probiotic formulations of B. clausii's clinical benefits in the prevention of AAD.

DISCLOSURES

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Author contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Conflicts of interest

Shashidhar Thakur and Anvesh G. have no conflicts of interest to declare. Raunak J Soman, Dhruv Soman and Kishan PV are employees of Sanzyme Biologics P. Ltd, Hyderabad, India.

Data availability statement

The data presented in this study are available within the article.

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