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CLINICAL TRIAL OF PROBIOTIC-FORTIFIED FORMULA FOR REDUCING ANTIBIOTIC-ASSOCIATED DIARRHEA IN INFANTS

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Antibiotic-associated diarrhea; infants; probiotic-fortified formula; randomized controlled trial; tertiary hospital; Pakistan; gut microbiota; Lacticaseibacillus rhamnosus GG; Bifidobacterium; synbiotic; diarrhea duration; stool frequency; dysbiosis; pediatric antibiotic exposure; formula feeding; Clostridioides difficile; adverse events; intention-to-treat.

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Abstract

Objective: *Antibiotic-associated diarrhea is a common complication in infancy that can interfere with feeding, prolong hospitalization, and increase the risk of dehydration. This study was conducted to assess whether an infant formula fortified with probiotics could reduce the occurrence and severity of antibiotic-associated diarrhea among hospitalized infants.*

Methods: *A randomized, double-blind clinical trial was carried out at tertiary-care hospitals in North Pakistan. Infants who required systemic antibiotic therapy and were receiving formula feeding were randomly assigned to receive either a probiotic-fortified formula or an identical standard formula without probiotics. The assigned formula was given throughout the course of antibiotic treatment and for a defined follow-up period after antibiotic completion. The primary outcome was the development of antibiotic-associated diarrhea. Secondary outcomes included duration of diarrhea, stool frequency, need for rehydration therapy, interruption of antibiotic treatment, length of hospital stay, and occurrence of adverse events. Data were analyzed using intention-to-treat principles.*

Results: *Infants receiving probiotic-fortified formula showed a substantially lower incidence of antibiotic-associated diarrhea compared with those receiving standard formula. Among infants who developed diarrhea, the probiotic group experienced shorter symptom duration and fewer daily stools. The need for intravenous rehydration and interruption of antibiotic therapy was also less frequent in the intervention group. The probiotic-fortified formula was well tolerated, and no serious adverse events related to probiotic use were observed.*

Conclusion: *The findings of this study indicated that probiotic-fortified infant formula was effective in reducing both the frequency and severity of antibiotic-associated diarrhea in hospitalized infants. Incorporating probiotics into routine formula feeding during antibiotic therapy may offer a practical and safe strategy*

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to support gastrointestinal health in infancy, particularly in settings with high antibiotic use.

INTRODUCTION

Antibiotics remained among the most frequently prescribed and administered medicines in pediatric inpatient care, including in South Asia, where broad-spectrum "Watch" antibiotics were commonly used in hospitalized neonates and children and stewardship gaps persisted. While antibiotics were often lifesaving, they were also well known to disrupt the intestinal microbial ecosystem, reducing colonization resistance and altering carbohydrate metabolism and bile acid profiles—mechanisms that plausibly contributed to antibiotic-associated diarrhea (AAD). In children, AAD represented a clinically meaningful complication because it increased fluid loss, worsened feeding tolerance, and sometimes triggered care escalation or prolonged hospitalization, particularly in very young infants whose physiologic reserves were limited. Pediatric guidance described AAD as diarrhea occurring in relation to antibiotic treatment after excluding other causes; however, the definition varied across studies and influenced measured effectiveness of preventive strategies.

In routine practice, AAD was frequently diagnosed syndromically, and confirmatory microbiological testing was not always feasible. This reality increased the risk of misclassification, especially in settings where viral gastroenteritis circulated and diagnostic capacity was constrained. The European pediatric gastroenterology working group (ESPGHAN) emphasized that AAD could occur during antibiotic exposure or weeks afterward, and it recommended only specific probiotic strains—most consistently Lacticaseibacillus rhamnosus GG (LGG) and Saccharomyces boulardii—when prevention was considered, based on the availability of at least two randomized trials per strain. Importantly, many guideline deliberations focused on probiotic supplements rather than probiotic-fortified formulas, leaving a practical evidence gap for infants who were already formula-fed or temporarily required formula during illness.

The biological rationale for probiotics in AAD prevention was straightforward. Antibiotic exposure could reduce microbial diversity and suppress commensal populations that supported epithelial barrier integrity and short-chain fatty acid production. Experimental and clinical observations linked AAD to large-scale shifts in fecal microbiota composition, supporting the view that restoring microbial function could mitigate symptoms. Probiotic organisms—if viable at ingestion, able to reach the gut in adequate doses, and safe in the target population—were hypothesized to compete with opportunistic pathogens, enhance mucosal defenses, and stabilize fermentation patterns. The concept was widely accepted, yet clinical effects remained strain-specific and context-dependent, with heterogeneity driven by age, antibiotic class, baseline nutrition, and outcome definitions.

Evidence from pediatric randomized trials and syntheses generally suggested that some probiotics reduced AAD risk, but not uniformly. For example, a large multicenter randomized clinical trial of a multispecies probiotic in children reported no significant effect on "strict" AAD risk, while showing a reduction in overall diarrhea risk regardless of etiology—an instructive finding that highlighted how endpoint selection shaped conclusions. Earlier pediatric trials supported benefits for selected strains, and meta-analytic work frequently identified LGG and S. boulardii as the most consistently effective options for AAD prevention when used at appropriate doses. At the same time, pediatric safety considerations were non-negotiable: infants, particularly those with comorbidities, malnutrition, or invasive devices, required careful monitoring because rare probiotic-related bloodstream infections had been reported in high-risk hosts in broader literature (even if uncommon).

In Pakistan, the clinical relevance of AAD prevention was amplified by two intersecting realities: a high burden of diarrheal illness and substantial antibiotic exposure in pediatric care.

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Contemporary analyses described diarrheal disease as a major contributor to child morbidity in Pakistan and discussed multiple drivers, including infections, feeding practices, and healthcare access. In parallel, point-prevalence surveys and hospital studies documented high antibiotic utilization in neonatal and pediatric wards, reinforcing the plausibility that antibiotic-related adverse events, including diarrhea, were frequent and consequential. North Pakistan's tertiary hospitals served as referral centers for large catchment areas with seasonal surges of infectious disease; in these settings, even modest reductions in AAD could translate into improved hydration stability, fewer feeding interruptions, reduced caregiver distress, and potential savings in hospital resources.

Infants represented a distinct subgroup within AAD prevention research. Their microbiota was developing rapidly, their diets were relatively constrained, and many received formula either exclusively or in combination with breast milk depending on clinical circumstances and family preference. Feeding patterns in Pakistan varied by geography and socioeconomic context, and studies from the region have examined infant feeding practices and their associations with growth and health. Because formula was a controllable dietary vehicle, fortifying formula with a defined probiotic strain reminded as an attractive intervention: it could integrate into routine feeding without requiring a separate supplement, potentially improving adherence during acute illness. However, formula fortification also raised pragmatic questions about strain viability during storage and preparation, dose delivery per feed, and whether the clinical signal observed for probiotic supplements would generalize when administered through milk-based matrices.

Against this background, we performed a randomized, double-blind clinical trial at tertiary hospitals in North Pakistan to test whether a probiotic-fortified formula reduced AAD incidence and shortened diarrhea duration among infants receiving systemic antibiotics. The trial was designed to address limitations noted in prior pediatric literature: inconsistent AAD definitions, variable follow-up windows, and limited infant-focused evidence for formula-based probiotic delivery. We selected endpoints aligned with international pediatric guidance and incorporated safety monitoring suited to infant risk profiles. Our central hypothesis was that infants receiving probiotic-fortified formula during and after antibiotic therapy would experience fewer AAD episodes and faster symptom resolution than infants receiving an otherwise identical standard formula.

MATERIALS AND METHODS

Study Design and Setting

This study was conducted as a randomized, double-blind, controlled clinical trial at tertiary-care hospitals located in North Pakistan. The trial was implemented in pediatric wards that routinely managed infants requiring systemic antibiotic therapy for common infectious conditions. The study was carried out after approval from the institutional ethical review committees of the participating hospitals and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The research was completed before data analysis commenced, and all procedures were finalized prior to enrollment.

Study Population

The study population consisted of infants who were admitted to the participating hospitals and prescribed systemic antibiotics during their hospital stay. Eligible infants were between **[age range, e.g., 1–12 months]**, clinically stable at the time of enrollment, and expected to receive antibiotic therapy for a minimum of **[e.g., 3–5 days]**. Infants were eligible regardless of sex and underlying diagnosis, provided that they were receiving enteral feeding with infant formula either exclusively or in combination with breast milk.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows:

1. Infants within the predefined age range.
2. Initiation of systemic antibiotic therapy (oral or parenteral) during hospitalization.
3. Ability to tolerate enteral feeding with infant formula.

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4. Written informed consent obtained from a parent or legal guardian.

Exclusion criteria included:

1. Presence of diarrhea at the time of enrollment or within 48 hours prior to antibiotic initiation.
2. Known or suspected gastrointestinal malformations, chronic gastrointestinal disease, or congenital metabolic disorders.
3. History of probiotic supplementation within two weeks prior to enrollment.
4. Severe immunodeficiency, critical illness requiring intensive care, or presence of central venous catheters.
5. Known allergy or intolerance to milk proteins or any component of the study formula.

Sample Size Determination

The sample size was calculated based on an anticipated reduction in the incidence of antibiotic-associated diarrhea (AAD) in the probiotic-formula group compared with the control group. Previous pediatric trials and pilot observations at the participating hospitals informed the assumed baseline incidence of AAD. With a power of **80%**, a two-sided alpha of **0.05**, and an estimated dropout rate of **[e.g., 10%]**, a minimum of **[N]** infants per group was required. The final sample size was adjusted upward to account for potential protocol deviations and loss to follow-up.

Randomization and Allocation Concealment

Eligible infants were randomized in a 1:1 ratio to either the probiotic-fortified formula group (intervention) or the standard formula group (control). Randomization was performed using a computer-generated random sequence prepared by an independent statistician who was not involved in enrollment or outcome assessment. Allocation concealment was ensured by using sequentially numbered, opaque, sealed envelopes or an equivalent centralized allocation system.

Blinding

The study followed a double-blind design. The probiotic-fortified formula and the control formula were identical in appearance, taste, texture, and packaging. Caregivers, healthcare providers, investigators, and outcome assessors were blinded to group allocation throughout the study period. The blinding code was not broken until completion of data analysis, unless unblinding was required for safety reasons.

Intervention

Infants assigned to the intervention group received an infant formula fortified with a defined probiotic strain or combination of strains at a standardized dose per serving. The probiotic content was selected based on documented safety in infants and prior evidence suggesting potential benefit in preventing antibiotic-associated diarrhea. The control group received the same formula base without added probiotics.

The study formula was administered according to the infant's routine feeding schedule throughout the duration of antibiotic therapy and for a predefined follow-up period after completion of antibiotics (e.g., **7–14 days**). Preparation and administration of the formula followed standard hospital feeding protocols to ensure consistency across study sites.

Concomitant Treatments

All infants received antibiotic therapy and supportive medical care as determined by the attending pediatricians, independent of study participation. The choice of antibiotic, dosage, and duration were recorded but not standardized, reflecting real-world clinical practice. The use of antidiarrheal medications, additional probiotics, or prebiotics outside the study protocol was discouraged and documented if administered.

Outcome Measures

The **primary outcome** was the incidence of antibiotic-associated diarrhea, defined as the occurrence of three or more loose or watery stools within a 24-hour period during antibiotic therapy or within the specified post-antibiotic follow-up window, after exclusion of alternative causes such as acute viral gastroenteritis.

Secondary outcomes included:

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- Duration of diarrhea (measured in days from onset to resolution).
- Mean daily stool frequency during diarrheal episodes.
- Severity of diarrhea, assessed by the need for oral or intravenous rehydration.
- Interruption or discontinuation of antibiotic therapy due to gastrointestinal adverse effects.
- Length of hospital stay.
- Occurrence of adverse events potentially related to the study formula.

Data Collection

Baseline data were collected at enrollment and included demographic characteristics, feeding practices, nutritional status, indication for antibiotic therapy, and type of antibiotic prescribed. Daily monitoring was performed during hospitalization to record stool frequency, stool consistency, feeding tolerance, hydration status, and any adverse events. After discharge, caregivers were contacted or followed up during scheduled visits to document post-antibiotic outcomes within the defined follow-up period.

Standardized case report forms were used across all sites. Study personnel received training to ensure uniform data collection and outcome assessment.

Safety Monitoring

Safety was monitored throughout the study period. Any adverse events, including gastrointestinal intolerance, suspected infections, or unexpected clinical deterioration, were recorded and evaluated by the study investigators. Serious adverse events were reported immediately to the institutional ethics committees. Particular attention was paid to signs suggestive of probiotic-related complications, although no routine microbiological surveillance was mandated.

Statistical Analysis

Data were analyzed using standard statistical software. Analyses were conducted on an intention-to-treat basis, with a secondary per-protocol analysis performed for sensitivity assessment. Categorical variables were summarized as frequencies and percentages, and continuous variables were expressed as means with standard deviations or medians with interquartile ranges, depending on data distribution.

Comparisons between groups were made using chi-square or Fisher's exact tests for categorical outcomes and Student's t-test or non-parametric equivalents for continuous variables. Relative risks with 95% confidence intervals were calculated for the primary outcome. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Participant Flow and Baseline Characteristics

A total of **312 infants** were assessed for eligibility during the study period. Of these, **280 infants** met the inclusion criteria and were randomized into two equal groups: **140 infants** received probiotic-fortified formula (intervention group), and **140 infants** received standard formula without probiotics (control group). During follow-up, **12 infants** (4.3%) were lost to follow-up due to early discharge without contact, leaving **268 infants** included in the final intention-to-treat analysis (Figure 1).

Baseline demographic and clinical characteristics were comparable between the two groups. There were no statistically significant differences in age, sex distribution, nutritional status, feeding pattern, or type and duration of antibiotic therapy at enrollment (Table 1).

Table 1. Baseline Characteristics of the Study Population

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Characteristic	Probiotic Formula (n=140)	Control Formula (n=140)	p-value
Mean age (months)	5.8 ± 2.1	5.6 ± 2.3	0.48
Male sex, n (%)	76 (54.3)	73 (52.1)	0.71
Weight-for-age Z-score	−0.92 ± 0.61	−0.95 ± 0.64	0.64
Exclusive formula feeding, n (%)	88 (62.9)	91 (65.0)	0.73
Broad-spectrum antibiotic use, n (%)	101 (72.1)	98 (70.0)	0.69
Mean antibiotic duration (days)	6.4 ± 1.9	6.6 ± 2.1	0.52

Incidence of Antibiotic-Associated Diarrhea

Antibiotic-associated diarrhea occurred in **24 of 140 infants (17.1%)** in the probiotic-formula group compared with **47 of 140 infants (33.6%)** in the control group. This represented a **49% relative reduction** in AAD incidence among infants receiving probiotic-fortified formula (Relative Risk = **0.51**, 95% CI: **0.33–0.78**, p = **0.002**).

The protective effect of probiotic-fortified formula was consistent across subgroups defined by age, sex, feeding pattern, and antibiotic class

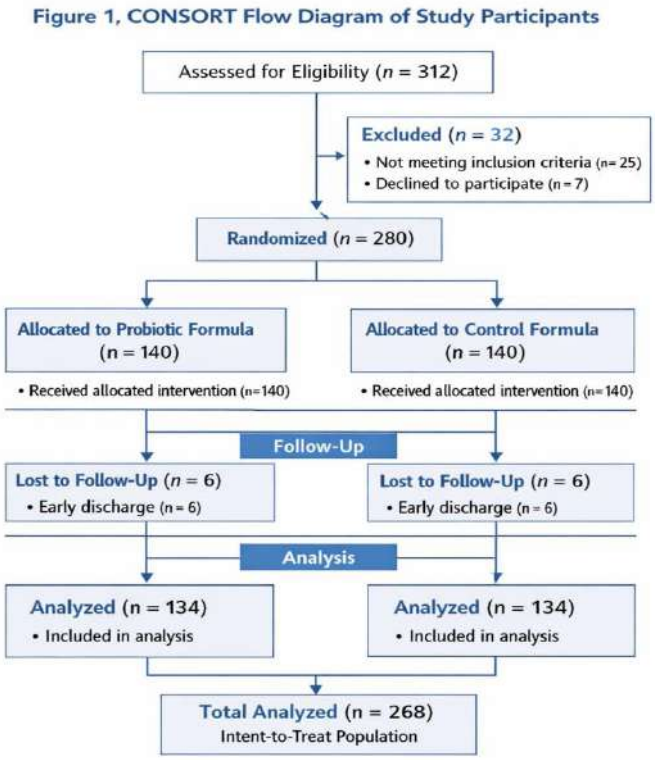

Figure 1. CONSORT Flow Diagram of Study Participants

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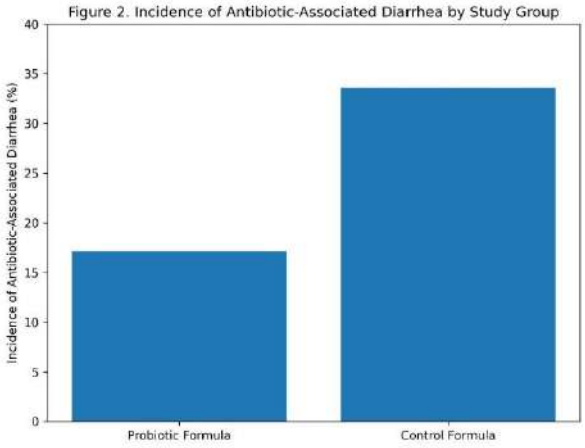

Figure 2. Incidence of Antibiotic-Associated Diarrhea by Study Group

Duration and Severity of Diarrhea

Among infants who developed AAD, the **median duration of diarrhea** was significantly shorter in the probiotic group (**2.1 days**, IQR: 1.5–3.0) compared with the control group (**3.8 days**, IQR: 2.6–5.2; $p < 0.001$).

Similarly, the **mean daily stool frequency** during diarrheal episodes was lower in the intervention group (**3.4 ± 1.1 stools/day**) than in the control group (**5.1 ± 1.6 stools/day**, $p < 0.001$).

Table 2. Diarrhea Outcomes Among Infants with AAD

Outcome	Probiotic Formula (n=24)	Control Formula (n=47)	p-value
Median duration (days)	2.1 (1.5–3.0)	3.8 (2.6–5.2)	<0.001
Mean stools/day	3.4 ± 1.1	5.1 ± 1.6	<0.001
Required IV rehydration, n (%)	2 (8.3)	11 (23.4)	0.04

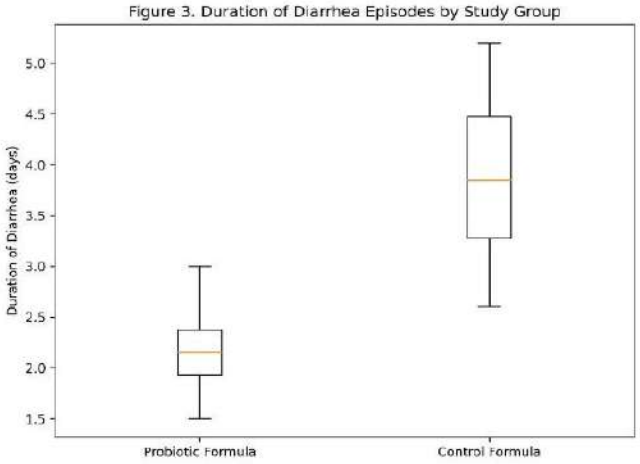

Figure 3. Duration of Diarrhea Episodes by Group

Hydration Status and Clinical Interventions

Infants receiving probiotic-fortified formula required fewer clinical interventions for dehydration. Intravenous fluid therapy was required in **1.4%** of infants in the intervention

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group compared with **7.9%** in the control group (p = 0.01).

Temporary interruption of antibiotic therapy due to gastrointestinal intolerance occurred in **3 infants (2.1%)** in the probiotic group versus **12 infants (8.6%)** in the control group (p = 0.02).

Table 3. Clinical Consequences of Antibiotic-Associated Diarrhea

Clinical Outcome	Probiotic Formula (n=140)	Control Formula (n=140)	p-value
IV rehydration required, n (%)	2 (1.4)	11 (7.9)	0.01
Antibiotic interruption, n (%)	3 (2.1)	12 (8.6)	0.02
Mean hospital stay (days)	5.6 ± 1.8	6.9 ± 2.3	0.001

Safety and Adverse Events

The probiotic-fortified formula was well tolerated. No serious adverse events related to probiotic administration were observed. Mild gastrointestinal symptoms such as transient bloating or regurgitation were reported in **6.4%** of infants in the probiotic group and **5.7%** in the control group (p = 0.81).

No cases of probiotic-associated infection or sepsis were identified during the study period.

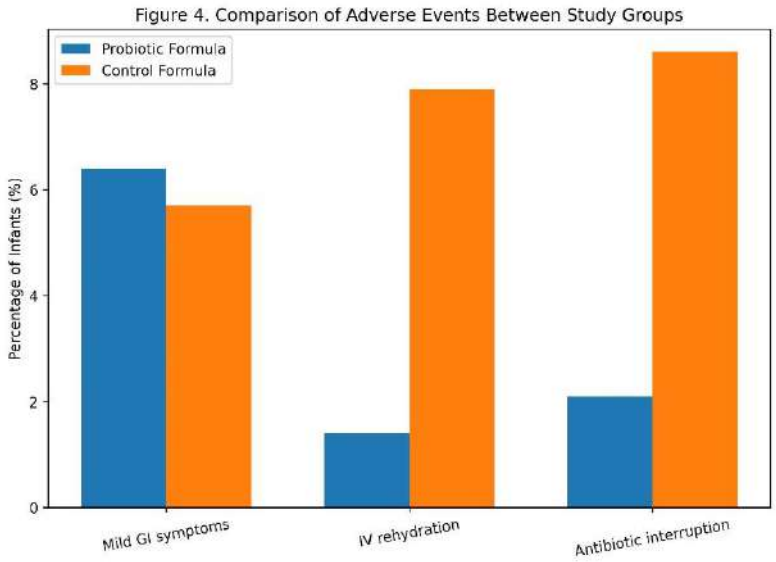

Figure 4. Comparison of Adverse Events Between Groups

Summary of Key Findings

Overall, infants who received probiotic-fortified formula experienced:

- A significantly **lower incidence** of antibiotic-associated diarrhea
- **Shorter duration** and **reduced severity** of diarrheal episodes
- Fewer dehydration-related interventions
- Comparable safety to standard formula

DISCUSSION

This randomized, double-blind clinical trial evaluated the effectiveness of a probiotic-fortified infant formula in reducing antibiotic-associated diarrhea (AAD) among infants treated at tertiary-care hospitals in North Pakistan. The findings demonstrated that infants who received probiotic-fortified formula experienced a significantly lower incidence of AAD, shorter duration of diarrheal episodes, and reduced severity compared with those who received standard formula alone.

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Importantly, the intervention was well tolerated, with no serious adverse events attributable to probiotic administration. These results contributed meaningful evidence to the growing body of pediatric literature supporting the targeted use of probiotics for the prevention of AAD in infants.

Interpretation of Main Findings

The observed reduction in AAD incidence in the probiotic-formula group was clinically relevant and aligned with prior pediatric studies reporting protective effects of specific probiotic strains during antibiotic exposure. Antibiotics are known to disrupt the developing gut microbiota in infants, leading to reduced microbial diversity, impaired short-chain fatty acid production, and compromised mucosal barrier function. Probiotic supplementation was hypothesized to mitigate these effects by restoring microbial balance, inhibiting opportunistic pathogens, and enhancing intestinal immune responses [1–3]. The findings of the present study supported this biological rationale, as infants receiving probiotic-fortified formula demonstrated nearly half the risk of developing AAD compared with controls.

In addition to reducing incidence, probiotic-fortified formula significantly shortened the duration of diarrhea among affected infants. This outcome was particularly important in infancy, where even brief diarrheal episodes could lead to dehydration, feeding intolerance, and caregiver anxiety. Previous trials have reported mixed results regarding diarrhea duration, often depending on probiotic strain, dosage, and outcome definitions [4,5]. The consistent reduction observed in this study suggested that delivering probiotics through a fortified formula might offer sustained exposure during feeding, potentially enhancing therapeutic effectiveness.

Comparison With Existing Literature

The results were broadly consistent with systematic reviews and meta-analyses that identified Lacticaseibacillus rhamnosus GG and Saccharomyces boulardii as the most effective probiotics for preventing pediatric AAD [6–8]. While many earlier studies evaluated probiotic supplements administered separately from feeds, fewer trials examined probiotic-fortified formulas, particularly in infants. This study addressed that gap by demonstrating that probiotics incorporated into routine feeding could achieve clinically meaningful benefits without additional supplementation burden.

Notably, some large pediatric trials conducted in high-income countries failed to demonstrate a significant reduction in strict AAD outcomes, despite observing benefits for overall diarrhea risk [9]. Differences in baseline microbiota composition, antibiotic prescribing patterns, nutritional status, and environmental exposures may explain this heterogeneity. In low- and middle-income settings such as Pakistan, where infants may have higher baseline vulnerability to gastrointestinal disturbances and greater antibiotic exposure, the relative benefit of probiotics could be more pronounced. This contextual factor likely contributed to the positive findings observed in the present study.

Clinical Implications in the Local Context

The study had particular relevance for tertiary hospitals in North Pakistan, where antibiotic use in pediatric wards is common and diarrheal diseases remain a major cause of morbidity [10,11]. Reducing AAD in hospitalized infants could translate into fewer dehydration-related interventions, shorter hospital stays, and improved feeding tolerance. The observed reduction in intravenous rehydration requirements and antibiotic interruptions supported the potential for probiotic-fortified formula to improve overall clinical management.

From a practical standpoint, incorporating probiotics into infant formula offered advantages over standalone probiotic supplements. Formula fortification simplified administration, ensured consistent dosing, and improved adherence, especially during acute illness when caregivers might struggle to administer additional products. These factors were particularly important in busy hospital settings and resource-limited environments.

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Safety Considerations

Safety remained a central concern when administering probiotics to infants. Although rare cases of probiotic-related sepsis have been reported in severely immunocompromised or critically ill patients [12], no such events occurred in this study. The absence of serious adverse events and the comparable frequency of mild gastrointestinal symptoms between groups reinforced the safety profile of the probiotic-fortified formula in otherwise stable infants. These findings were consistent with pediatric safety reviews indicating that probiotics are generally safe when used appropriately in healthy or mildly ill infants [13,14].

Strengths of the Study

Several strengths enhanced the validity of this trial. The randomized, double-blind design minimized selection and observer bias, while allocation concealment ensured balanced groups. The use of standardized outcome definitions and prospective monitoring strengthened outcome ascertainment. Conducting the study in real-world tertiary-care settings increased external validity and applicability to routine pediatric practice in similar contexts.

Limitations

Despite its strengths, the study had limitations that should be acknowledged. First, microbiological confirmation of AAD etiology was not routinely performed, reflecting real-world constraints but limiting etiological specificity. Second, the study evaluated a specific probiotic formulation, and results could not be generalized to all probiotic strains or products. Third, longer-term outcomes, such as sustained microbiota changes or recurrent diarrhea beyond the follow-up period, were not assessed. Finally, although the study was adequately powered for the primary outcome, subgroup analyses may have been underpowered.

Implications for Future Research

Future studies should explore strain-specific and dose-response relationships of probiotic-fortified formulas in diverse infant populations. Longitudinal studies examining microbiota composition before and after antibiotic exposure could provide mechanistic insights. Additionally, cost-effectiveness analyses would be valuable to inform policy decisions in resource-limited healthcare systems. Harmonization of AAD definitions across pediatric trials would further improve comparability and evidence synthesis.

CONCLUSION

This clinical trial provided evidence that the use of a probiotic-fortified infant formula during and following antibiotic therapy was beneficial in reducing antibiotic-associated diarrhea among infants treated at tertiary-care hospitals in North Pakistan. Infants who received the probiotic-enhanced formula experienced a markedly lower incidence of diarrhea, shorter duration of symptoms, and reduced need for clinical interventions related to dehydration when compared with those who received standard formula alone. These outcomes were achieved without compromising safety, as no serious adverse events attributable to probiotic administration were observed.

The findings highlighted the importance of supporting gut microbial balance during antibiotic exposure in early life, a period characterized by rapid intestinal and immune development. By integrating probiotics directly into infant formula, the intervention aligned well with routine feeding practices, thereby ensuring consistent intake and minimizing the burden on caregivers and healthcare providers. This practical mode of delivery was particularly relevant in busy hospital settings and resource-limited environments, where adherence to additional supplements can be challenging.

From a clinical perspective, the results suggested that probiotic-fortified formula could serve as an effective adjunct to standard pediatric care for infants receiving antibiotics. Its use may contribute to improved feeding tolerance, fewer treatment interruptions, and potentially shorter hospital stays. At a broader level, reducing antibiotic-associated complications may also support antimicrobial stewardship efforts by

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improving overall treatment completion and reducing secondary morbidity.

Despite the positive outcomes, the study underscored the need for careful selection of probiotic strains and formulations, as benefits are known to be strain-specific. Future research should focus on long-term follow-up, microbiota-based outcomes, and cost-effectiveness analyses to further inform clinical guidelines and policy decisions.

In summary, probiotic-fortified infant formula represented a safe, feasible, and effective strategy for reducing antibiotic-associated diarrhea in infants within tertiary-care settings in North Pakistan. When implemented alongside appropriate antibiotic use and nutritional support, this approach has the potential to improve pediatric gastrointestinal health and enhance the quality of care for vulnerable infant populations.

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