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Comparison between Lactobacillus Reuteri Probiotic in Addition to Standard Care versus Standard Care Alone in the Treatment of Infantile Colic

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ABSTRACT

Excessive crying is one of the most common problems in the first three months of life, accounting for nearly 20% of pediatric consultations. **Objectives:** To evaluate the effectiveness of *Lactobacillus reuteri* in infants with infantile colic compared with standard care alone. **Methods:** A prospective quasi-experimental study was used to enroll 172 infants (<13 weeks of age) with clinically diagnosed infantile colic who were enrolled. Group A received probiotic *L. reuteri* in addition to standard care, while Group B received standard care alone. Outcomes included mean daily crying time, crying episodes/week, and daily sleep duration, measured at baseline, days 7, 14, 21, and 28. Between-group comparisons were performed using the Mann–Whitney U test, and within-group changes were analyzed using the Friedman test. **Results:** Over 28 days, Group A showed greater improvement than Group B in reducing crying and increasing sleep. Daily crying decreased by −3.53 ± 1.05 hours in Group A vs. −1.79 ± 1.19 hours in Group B ($p<0.001$), weekly crying episodes by −3.60 ± 0.69 vs. −3.36 ± 0.97 ($p=0.041$), and daily sleep increased by 5.38 ± 2.08 hours vs. 2.80 ± 3.52 hours ($p<0.001$). **Conclusions:** *L. reuteri* supplementation in addition to standard care significantly reduced crying time and episodes, and improved sleep-in infants with colic compared to standard care alone.

INTRODUCTION

Excessive crying is the most common problem in the initial 3 months of infantile age. Nearly 20% of pediatric consultations by parents are for this reason. Although it is a self-limited problem, parents and caregivers face huge stress and anxiety due to this complaint [1]. Excessive crying can be attributed to various factors, including pain, hunger, discomfort, or the infant's need for emotional reassurance. Infantile colic is a common cause of excessive crying in infants, impacting 10-40% of this population. It is typically observed as early as 2 weeks of life and gradually diminishes by 6 months of age in infants. Both boys and girls are impacted in the same manner [2]. A healthy, well-nourished newborn under five months old suffering from fits of uncontrolled weeping is said to be suffering from infantile colic. Cry out loud for at least three hours every day, three times a week, for at least three weeks straight [3]. Infantile colic is a crying spell with no obvious etiology. Due to stress, parents use analgesics as

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well as sedative drugs to calm the infant, and sometimes this excessive and non-stop crying leads to shaken baby syndrome [4]. Very few (<5%) babies have an underlying organic cause of colic, e.g., lactose intolerance, constipation, or gastroesophageal reflux disease (GERD) [5]. Another school of thought postulates that colic is associated with increased peristaltic activity of the gut, supported by the fact that symptoms are relieved by the use of anticholinergic medications [6]. As large numbers of coliform bacteria are found in colicky babies, studies were conducted to try to modify the gut flora, and these studies have shown a positive impact in treating the symptoms of colic. Few measures can provide some degree of comfort, e.g., improve sleep hygiene and organize parents' and baby's routine [7], and use of fennel, chamomile, and peppermint [8].

Although extensive evidence is being gained in the world on the benefits of *L. reuteri* in the treatment of infantile colic, little local data is available in Pakistan. Practically all trials are initiated by Western populations, who have various feeding habits, access to healthcare, or gut microbiota profiles. This is the gap that restricts the development of evidence-based guidelines on the local level. Also, very limited research compared probiotic adjunct therapy to standard care only using longitudinal, multi-timepoint outcome measures. These gaps are addressed through this study as it provides strong local comparative data. This study aimed to evaluate the effectiveness of *Lactobacillus reuteri* in infants with infantile colic compared with standard care alone.

METHODS

This prospective quasi-experimental study aims to compare the efficacy of standard treatment with *Lactobacillus reuteri* for infantile colic conducted at Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU), Children's Hospital, Pakistan Institute of Medical Sciences (PIMS), Islamabad, from January 2020 to June 2020. The study was approved by the ethical committee of the institute vide letter number F.1-1/2015/ERB/SZABMU/406. The sample size was calculated using the formula for comparing two means, with α = 0.05, power = 80%, expected mean difference (Δ) = 58 minutes/day, and standard deviation (σ) = 135 minutes, based on Savino *et al.* This yielded 72 infants per group; allowing 20% for attrition, the final target was 86 per group (total n=172) [9]. Eligible participants were infants under 13 weeks old, breastfed or formula-fed, diagnosed with infantile colic, and meeting study criteria. Exclusions included infants under 2500 grams, those with failure to thrive, or with other medical conditions. Also excluded were infants and mothers who used **Lactobacillus reuteri** before the trial, infants starting solid foods, and those who received antibiotics in the last two weeks. Informed consent was provided by the parents. All the infants on probiotic *Lactobacillus reuteri*, along with the standard care, were enrolled in Group A (probiotic group), and all taking standard care alone in the form of conservative management in Group B (control group). Parents documented daily crying episodes and sleep duration in a provided diary. Follow-up occurred on days 7, 14, 21, and 28, with final assessments at day 28 when medication and diaries were returned. To address attrition, an additional 20% of participants were recruited. Effectiveness Definition: Treatment effectiveness was defined as a statistically significant improvement ($p \leq 0.05$) in at least two of the three primary outcomes (1) reduction in mean daily crying time, (2) reduction in mean weekly crying episodes, and (3) increase in mean daily sleep duration at day 28 compared with baseline, with greater improvement in the probiotic group than in the control group. Data analysis was performed using SPSS version 26.0. The Shapiro-Wilk test was used to test the normality of continuous variables. Since the majority of the variables were not normally distributed, the results were represented as median (interquartile range) of the baseline and follow-up measurements, as well as means with standard deviation of the change scores. The Mann-Whitney U test was used in between-group comparison, and the Friedman test was used in within-group differences over time. The Chi-square test was used to analyze categorical variables and was presented in the form of frequencies and percentages. The significance value of $p<0.05$ was taken as significant.

RESULTS

A little over half were aged 31–40 years (83 women, 53.5%), while 72 women (46.5%) were 20–30 years, with a mean age of 31.03 ± 5.89 years. Regarding BMI, 84 women (54.2%) had a normal BMI, 51 (32.9%) were overweight, and 20 (12.9%) were obese, giving a mean BMI of 25.31 ± 3.38 kg/m^2. Slightly more participants were multiparous (82, 52.9%) than nulliparous/primiparous (73, 47.1%). Education levels varied: 51 (32.9%) had secondary, 50 (32.2%) primary, 39 (25.2%) tertiary, and 15 (9.7%) no formal schooling. Socioeconomic status was predominantly low (80, 51.6%), followed by middle (52, 33.5%) and high (23, 14.9%). Residence was evenly distributed between urban (80, 51.6%) and rural (75, 48.4%). Only 1 woman (0.6%) reported smoking, while 154 (99.4%) were non-smokers. The average number of antenatal care (ANC) visits was 4.55 ± 2.27, and the mean gestational age at delivery was 33.88 ± 1.43 weeks, confirming the preterm status. All participants delivered preterm, confirming that the study population exclusively comprised women with preterm labor (Table 1).

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Table 1: The Baseline Measurements Data

Variables	Group A (Median [IQR])	Group B (Median [IQR])	p-value
Age (Weeks)	9.00 (6.00–10.00)	9.00 (7.00–10.00)	0.3311*
Crying Time (Hours) at Baseline	4.00 (4.00–5.00)	5.00 (4.00–5.00)	0.4298*
Crying Episodes/ Week at Baseline	5.00 (4.00–6.00)	5.00 (4.00–6.00)	0.0702*
Sleep (Hours/24h) at Baseline	11.00 (10.00–12.75)	11.00 (10.00–12.00)	0.4786*
Gender			
Male	47 (54.7%)	40 (46.5%)	0.2857**
Female	39 (45.3%)	46 (53.5%)	
Feeding			
Breast	47 (54.7%)	43 (50.0%)	0.7104**
Formula	24 (27.9%)	29 (33.7%)	
Mixed	15 (17.4%)	14 (16.3%)	

*Mann–Whitney U test. ** Chi-square test

The study compares the treatment outcomes between infants receiving *Lactobacillus reuteri* in addition to standard care (Group A) and those receiving standard care alone (Group B). At baseline, median crying time was similar between groups [4.00 (IQR: 4.00–5.00) vs. 5.00 (IQR: 4.00–5.00), p=0.430]. However, by day 7, Group A demonstrated a significant reduction in crying time compared to Group B [3.00 (IQR: 2.00–3.00) vs. 4.00 (IQR: 3.00–5.00), p<0.001], with this difference widening at day 14 [2.00 (IQR: 1.00–2.00) vs. 3.00 (IQR: 2.00–4.00), p<0.001], day 21 [1.00 (IQR: 1.00–2.00) vs. 3.00 (IQR: 3.00–4.00), p<0.001], and day 28 [1.00 (IQR: 1.00–1.00) vs. 3.00 (IQR: 2.00–4.00), p<0.001]. Within-group Friedman tests confirmed significant reductions over time in both groups (p<0.001). For crying episodes per week, baseline medians were comparable [5.00 (IQR: 4.00–6.00) in both groups, p=0.070], but Group A achieved significantly lower values from day 7 onward, with differences persisting through day 28 (all p≤0.002). Both groups showed significant reductions over time (p<0.001). Regarding sleep duration, no significant difference was observed at baseline [11.00 (IQR: 10.00–12.75) vs. 11.00 (IQR: 10.00–12.00), p=0.479]. From day 7 onwards, Group A demonstrated markedly greater increases in sleep hours compared to Group B, with median sleep at day 28 reaching 17.00 (IQR: 16.00–17.00) in Group A versus 13.50 (IQR: 12.00–16.00) in Group B (p<0.001). Within-group changes over time were significant for both groups (p<0.001). Mean change analysis from baseline to day 28 showed that Group A had a greater reduction in crying time (-3.53 ± 1.05 hours) compared to Group B (-1.79 ± 1.19 hours, p<0.001) and a greater increase in daily sleep (5.38 ± 2.08 hours vs. 2.80 ± 3.52 hours, p<0.001). The change in crying episodes per week was similar between groups (-3.60 ± 0.69 vs. -3.36 ± 0.97, p=0.567) (Table 2).

Table 2: Comparison between Lactobacillus reuteri Probiotic in Addition to Standard Care Versus Standard Care Alone in the Treatment of Infantile Colic

Outcomes	Time Point	Group A (Median [IQR])	Group B (Median [IQR])	Between Group	Within Group A	Within Group B
Crying Time (Hours)	Baseline	4.00 (4.00–5.00)	5.00 (4.00–5.00)	0.430	<0.001	<0.001
	Day 7	3.00 (2.00–3.00)	4.00 (3.00–5.00)	<0.001	<0.001	<0.001
	Day 14	2.00 (1.00–2.00)	3.00 (2.00–4.00)	<0.001	<0.001	<0.001
	Day 21	1.00 (1.00–2.00)	3.00 (3.00–4.00)	<0.001	<0.001	<0.001
	Day 28	1.00 (1.00–1.00)	3.00 (2.00–4.00)	<0.001	<0.001	<0.001
Crying Episodes/Week	Baseline	5.00 (4.00–6.00)	5.00 (4.00–6.00)	0.070	<0.001	<0.001
	Day 7	3.00 (3.00–4.00)	4.00 (3.25–5.00)	0.002	<0.001	<0.001
	Day 14	2.00 (2.00–3.00)	3.00 (2.00–3.00)	<0.001	<0.001	<0.001
	Day 21	1.00 (1.00–2.00)	2.00 (1.00–2.00)	<0.001	<0.001	<0.001
	Day 28	1.00 (1.00–1.00)	2.00 (1.00–2.00)	<0.001	<0.001	<0.001
Sleep (Hours/24h)	Baseline	11.00 (10.00–12.75)	11.00 (10.00–12.00)	0.479	<0.001	<0.001
	Day 7	12.00 (11.00–13.75)	11.00 (10.00–13.00)	<0.001	<0.001	<0.001
	Day 14	13.00 (12.00–14.00)	12.00 (10.00–12.00)	<0.001	<0.001	<0.001
	Day 21	14.00 (12.00–16.00)	12.00 (11.00–13.00)	<0.001	<0.001	<0.001
	Day 28	17.00 (16.00–17.00)	13.50 (12.00–16.00)	<0.001	<0.001	<0.001
Crying Time (Hours) Mean Change	Baseline to Day 28	-3.53 ± 1.05	-1.79 ± 1.19	<0.001	–	–
Crying Episodes/Week Mean Change	Baseline to Day 28	-3.60 ± 0.69	-3.36 ± 0.97	0.567	–	–
Sleep (Hours/24h) Mean Change	Baseline to Day 28	5.38 ± 2.08	2.80 ± 3.52	<0.001	–	–

Note: Values are presented as median (IQR). Between-group comparisons were performed using the Mann–Whitney U test. Within-group changes over time were analyzed using the Friedman test. A p-value < 0.05 was considered statistically significant.

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DISCUSSION

This study assessed the efficacy of *Lactobacillus reuteri* DSM 17938 in the management of infantile colic. At baseline, both treatment groups demonstrated comparable crying duration, crying episodes, and sleep patterns. By days 7, 14, 21, and 28, *L. reuteri*, in addition to standard care, significantly reduced crying time and crying episodes, while improving sleep duration, compared to standard care alone ($p<0.05$). At day 28, the mean crying-time reduction was -3.53 ± 1.05 hours in Group A versus -1.79 ± 1.19 hours in Group B ($p=0.000$), the mean crying-episode reduction was -3.60 ± 0.69 versus -3.36 ± 0.97 ($p=0.567$), and the mean increase in sleep duration was 5.38 ± 2.08 hours versus 2.80 ± 3.52 hours ($p=0.000$). These results indicate statistically significant changes as well as clinically significant improvements in symptom burden. According to a randomized controlled trial carried out by Jalal *et al.* in the Benazir Bhutto Hospital of Rawalpindi, probiotics that consisted of *L. reuteri* proved to significantly decrease infantile colic cases compared to simethicone [10]. It is also suggested by the Latin American Experts Group that *L. reuteri* DSM 17938 can be used in infantile colic [11]. During 28 days, Savino *et al.* randomly assigned 90 infants with colicky behavior who were breast-fed to either simethicone or *L. reuteri*. During day seven, the mean duration of crying in the probiotic and simethicone groups was 159 and 177 minutes, respectively. These values were 51 minutes and 145 minutes on day 28, and 95 percent of the probiotic group and 7 percent of the simethicone group improved [12]. Statistically significant differences are observed in our findings already on day 7 and throughout day 28, which is consistent with this temporal improvement. In comparison, the study conducted in the Department of Pediatrics of the University indicated that the enhanced parental knowledge of normal infant crying behavior can help to decrease the number of crying incidents as well, which prioritizes the role of non-pharmacologic and educational approaches, as well as therapeutic interventions [13]. Some other hypotheses suggest that alterations of faecal microbiota could be possible causes of infantile colic [8]. In that respect, lower faecal calprotectin concentrations have been noted in responders to *L. reuteri* treatment [14], which is in line with an anti-inflammatory effect, which is in line with our results of improvements in gastrointestinal symptoms and sleep duration. *L. reuteri* DSM 17938 has been the most widely researched strain in the management of infantile colic, and several randomized controlled trials have been performed in different settings. Szajewska *et al.* reported much better responder rates that were determined as a 50 percent decrease in daily crying time in the probiotic group at all time points ($p<0.05$) [15]. More responder rates were also reported in an Italian trial on days 7, 14, and 21 with microbiological evidence of higher faecal lactobacilli and lower *E. coli* [16]. Muller *et al.* showed that the probiotic group had less crying time and a shorter median crying period in contrast to the placebo group [2]. A study carried out in China also documented significant decreases in crying time by probiotics. Such uniform advantages in various settings prove the external validity of our findings [17]. These findings are further put into perspective through systematic reviews and meta-analyses. Schreck *et al.* found a pooled mean decrease in the duration of crying of about 56 minutes/day with a two-week favorable *L. reuteri* effect, with results meandering during the first week and reaching its peak in the third week- a nearly similar result to that in our trial [17]. Similar results were also found by Xu *et al.* with two and three-week benefits at six randomized trials [18]. Although no advantage was reported in a Canadian cohort at one month [19], Sung *et al.* reported that a subsequent meta-analysis of the same group reported consistent reductions in crying/fussing time in four randomized trials [20]. Variability could be attributed to dissimilarity in feeding condition, baseline symptom intensity, strain dosing, and methodology of analysis. The non-parametric analyses of our cohort established statistically significant between-group effects as well as significant within-group time effects, with both crying-time reduction and sleep gain by day 28 having clinically relevant magnitudes. The multifactorial effects of *L. reuteri* DSM 17938, such as improvement of gut microbial homeostasis through an increase in Lactobacilli colonization, inhibition of pathogenic *E. coli*, and minimization of intestinal inflammation, could explain the following benefits that were observed [16]. A reduction in the faecal calprotectin levels in the responders, as mentioned above [14], is an additional indicator of an anti-inflammatory effect. Also, the betterment of sleep patterns recorded here and in earlier studies [18] implies the possible regulation of the gut-brain axis. Clinically, the changes are meaningful, since they not only discuss infant distress, but also parental stress, difficulties feeding, and possible cessation of breastfeeding. Our study, especially the decrease in crying time by greater than 3.5 hours and increase in sleep by greater than 5 hours by day 28, is indicative of the ability of *L. reuteri* to be used as an adjunct in infantile colic, particularly in the first line.

This research has a number of limitations. The quasi-experimental non-randomized design does not make it possible to infer causation and can create selection bias. The single-center design limits generalizability. Parental diary reporting is associated with the possibility of recall bias. Follow up period of 28 days does not determine long-term outcomes and recurrence. It should be followed by

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future multicenter randomised controlled trials involving a longer follow-up, blinding, and objective biomarkers (e.g., fecal calprotectin, microbiome analysis). The priority should be put on the subgroup analysis based on feeding (breastfed vs. formula-fed). There is a need for health economic analyses to determine cost-effectiveness in low-resource environments.

CONCLUSIONS

Mean daily crying time and crying episodes per week were significantly reduced, and mean daily sleep duration was significantly improved at days 7, 14, 21, and 28 in infants receiving probiotic *L. reuteri* in addition to standard care compared with those receiving standard care alone. The probiotic therapy was well tolerated and demonstrated cost-effectiveness. Future studies with larger sample sizes, extended follow-up, and assessment of additional variables such as fecal calprotectin levels and gut microflora changes are recommended to further validate and elucidate these findings.

Authors' Contribution

Conceptualization: MA
Methodology: SM, SH, SN, RW
Formal analysis: HN, YK, RW, MA
Writing and Drafting: SM, SH, SN, MA
Review and Editing: SM, HN, YK, SH, SN, RW, MA

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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