ORIGINAL ARTICLE

EFFECT OF *BACILLUS CLAUSII* IN COMBINATION WITH PHOTOTHERAPY IN THE TREATMENT OF NEONATAL HYPERBILIRUBINEMIA: A QUASI-EXPERIMENTAL STUDY IN A TERTIARY CARE HOSPITAL

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Background: Neonatal hyperbilirubinemia is common in the first week of life, and probiotics like *Bacillus clausii* are gaining popularity in bilirubin clearance by modulating gut flora, and shown in previous studies to be safe and well-tolerated in newborns. The aim of this study was to compare the distribution of serum bilirubin levels between neonates receiving phototherapy alone and those receiving phototherapy plus *Bacillus clausii*. **Methods:** This quasi-experimental study using alternate allocation was conducted over six months from 1st Jan to 30th Jun 2025 in the Paediatric Department of PAF Hospital Mushaf, Sargodha. Eighty neonates were assigned to receive either phototherapy with *Bacillus clausii* (Group A) or phototherapy alone (Group B). Serum bilirubin levels were measured twice daily and finally evaluated on day four of treatment and compared between the groups. **Results:** Baseline characteristics were comparable between both treatment groups. Post-therapy serum bilirubin levels were significantly lower in the combined therapy group 'A' compared to the monotherapy group 'B' [median 4.0 (IQR 3.25–6.0) versus. 6.0 (IQR 4.0–9.0); $p<0.001$]. The effect size indicated a moderate treatment effect ($r=0.40$), favouring the combined therapy approach. **Conclusion:** Combining *Bacillus clausii* with phototherapy appears to be a safe and effective approach for managing neonatal hyperbilirubinemia. This combination not only accelerates bilirubin clearance but may also help reduce the duration of phototherapy.

Keywords: *Bacillus clausii*, Bilirubin, Hyperbilirubinemia, Neonatal Jaundice, Phototherapy, Probiotics

Pak J Physiol 2026;22(1):33–6, **DOI:** https://doi.org/10.69656/pjp.v22i1.1909

INTRODUCTION

Neonatal hyperbilirubinemia refers to the clinical state of elevated total serum bilirubin (TSB), which results in deposition of bilirubin into an infant's skin.[1,2] It is one of the leading causes of admission to newborns' nurseries worldwide.[3] It is characterized as yellowish discoloration of the skin, sclera, and mucous membranes.[4] Approximately 60% of term and 80% of preterm neonates develop jaundice during the first 7 days of life,[5] while nearly 10% of exclusively breastfed infants may develop breast milk jaundice.[4] Neonatal hyperbilirubinemia can be physiological when it occurs due to increased red blood cell turnover, immature hepatic conjugation mechanisms, and enhanced enterohepatic circulation, or pathological which may arise from haemolysis, infection, metabolic disorders, or birth trauma.[6] A number of cases are self-limiting, however in some cases, serum bilirubin may cross the blood-brain barrier, deposit in the basal ganglia, and cause permanent neurological damage, resulting in kernicterus.[7,8]

Phototherapy remains the cornerstone of treatment for neonatal hyperbilirubinemia and effectively lowers serum bilirubin levels, thereby reducing the need for exchange transfusion.[9] However, phototherapy is associated with several limitations, including dehydration, temperature instability, mother-infant separation, and increased healthcare costs.[10] Probiotics, particularly *Bacillus clausii*, have emerged as a potential therapeutic option due to their ability to modulate intestinal flora and reduce enterohepatic circulation of bilirubin via β-glucuronidase suppression.[11] Limited regional evidence exists regarding their effectiveness in the management of neonatal jaundice, warranting further investigation.

The rationale of this study was to fill the existing knowledge gap by investigating whether the combined use of *Bacillus clausii* and phototherapy offers clinically meaningful benefits in the management of neonatal jaundice. Specifically, the study aimed to determine whether this adjunctive therapy enhances bilirubin reduction compared with phototherapy alone, thereby potentially reducing the risk of bilirubin-induced neurological dysfunction, including kernicterus, while limiting the duration of exposure to phototherapy and its associated complications.

METHODOLOGY

This quasi experimental study was conducted in Paediatric Department, Pakistan Air Force Hospital Mushaf, Sargodha, after the approval of Ethical

Committee, from 1st Jan to 30th Jun 2025. Sample size was calculated using WHO Sample Size Calculator.[12] With population standard deviation= 38.5, confidence level=95%, and power of test=99%, total sample size calculated was 80 having 40 in each group.

Inclusion criteria was neonatal age ≤3 days, term neonates (gestational age ≥37 weeks), birth weight ≥2.5 Kg and the total serum bilirubin ≥15 mg/dL [hyperbilirubinemia defined as >250 μmol/L (≈14.6 mg/dL) per NICE guidelines].[13] The exclusion criteria included direct hyperbilirubinemia, infants of diabetic mothers, neonatal sepsis. Rh-incompatibility between mothers and neonates was also excluded; therefore, only Rh-compatible pairs (predominantly Rh^+) were included in the study.

Informed consent was obtained from the primary caregivers of all babies before recruiting for study. Non-probability consecutive sampling technique was used, regardless of gender or race. Group allocation was done to ensure equal numbers of participants in each group; odd-numbered neonates were assigned to the combined treatment group and even-numbered neonates to the monotherapy group. Declaration of Helsinki was followed throughout the study.

Birth history and clinical examination was carried out when patient was screened for enrolment. Group A received *Bacillus clausii* 2,000 million spores per 5 ml once daily plus phototherapy up to 15 hours per day. Group B received only phototherapy. Neonates in both groups were on-demand feeding. Serum bilirubin level was assessed twice daily. Sampling was done when phototherapy lights were off to prevent photo-oxidation of the sample. Safety measures were implemented using a standardized nursing flow sheet to record hydration status, temperature, skin changes, and stool pattern, abdominal distension, vomiting, and feeding patterns in order to detect and manage any possible side-effect at the earliest. Serum total bilirubin levels were assessed at 4th day of treatment to detect the treatment outcomes.

Data were entered and analysed on SPSS-25. Descriptive analysis was done for all variables. The tests of statistical significance were Mann-Whitney U test for continuous variables (Shapiro-Wilk <0.05) and Pearson Chi-square test for categorical variables. Effect size was calculated to compare post-therapy outcomes between both the treatment groups. The $p \leq 0.05$ was considered statistically significant.

RESULTS

Both groups, Combined Therapy (A) and Monotherapy (B), included an equal number of participants (40 each). Continuous variables showed a non-normal distribution (Shapiro-Wilk $p<0.05$). Baseline characteristics of participants were comparable (Table-1 and 2) between both the groups. Table-1 demonstrates the descriptive analysis of continuous variables. Table-2 demonstrates the descriptive analysis of categorical variable.

No remarkable side-effects were observed in either treatment group. A statistically significant and clinically meaningful difference was observed in post-therapy serum bilirubin between the groups, showing the superiority of the combined therapy group (Table-3).

Table-1: Analysis of continuous variables

Study Variable	Group A Median (Inter-Quartile Range)*	Group B Median (Inter-Quartile Range)*	Mann-Whitney U (Z)	*p* (2-Tailed)
Age	3.0 (2.0–3.0)	3.0 (2.0–3.0)	780.00 (-0.240)	0.811
Gestational Age	39.5 (38.25–40.0)	39.0 (38.0–40.0)	613.00 (-1.840)	0.066
Baseline Serum Total Bilirubin	17.0 (17.0–19.0)	18.0 (16.25–19.0)	1593.500 (-0.261)	0.794
*Q1–Q3				

Table-2: Analysis of categorical variables

	Group A (n=40)	Group B (n=40)	Chi-square (df)	*p*
Gender				
Males	23 (57.5%)	28 (70%)	1.352 (1)	0.245
Females	17 (42.5%)	12 (30%)		
Mode of Delivery				
SVD	17 (42.5%)	16 (40%)	0.52 (1)	0.82
LSCS	23 (57.5%)	24 (60%)		
Baby's Blood Group				
A^+	5 (12.5%)	7 (17.5%)	3.821 (3)	0.281
B^+	14 (35%)	10 (25%)		
AB^+	16 (40%)	12 (30%)		
O^+	5 (12.5%)	11 (27.5%)		
Mother's Blood Group				
A^+	6(15%)	7 (17.5%)	2.239 (3)	0.524
B^+	13 (32.5%)	14(35%)		
AB^+	15 (37.5%)	17 (42.5%)		
O^+	6 (15%)	2 (5%)		
Type of feeding				
Breast-feeding	15 (37.5%)	14 (35%)	0.054 (1)	0.816
Formula feeding	25 (62.5%)	26 (65%)		
History of jaundice among siblings				
Jaundice	11 (27.5%)	13 (32.5%)	0.238 (1)	0.62

Table-3: Post-therapy analysis of serum bilirubin level

Study Variable	Group A	Group B	Mann-Whitney U (Z)	*p* (2-Tailed)	Effect Size (*r*)
Median Post-therapy Serum Total Bilirubin	4.0 (3.25–6.0)*	6.0 (4.0–9.0)*	433.000 (-3.578)	<0.001	0.40
*Median (Interquartile Range: Q1–Q3)					

DISCUSSION

The present study demonstrated the efficacy of combining *Bacillus clausii* with phototherapy in treating neonatal jaundice. Probiotics like *Bacillus clausii* are thought to enhance phototherapy outcomes by suppressing intestinal β-glucuronidase, thereby promoting bilirubin clearance,[14] thereby promoting bilirubin clearance, preventing its reabsorption, and modulating the gut microbiota to accelerate bilirubin excretion.[15]

The baseline characteristics of both the treatment groups were comparable, indicating appropriate group allocation and minimizing potential confounding bias.

Waheed *et al*, verified the efficacy of combining oral probiotics with phototherapy, they also noted comparable baseline characteristics between both groups, with a higher rate of vaginal deliveries.[16] The baseline equivalence strengthens the internal validity of the study and supports that the observed treatment outcomes are likely due to the therapeutic interventions rather than underlying demographic or clinical differences.

In our study, combined therapy group showed greater improvement in serum bilirubin, decreasing from 17.0 mg/dL to 4.0 mg/dL, compared with 18.0 mg/dL to 6.0 mg/dL in the phototherapy-alone group. Our results are consistent with Eghbalian *et al*[17], who reported a mean (baseline) serum bilirubin of 15.6±1.7 mg/dL in the combination therapy group and 15.8±1.6 mg/dL in the monotherapy group, which decreased to 7.2±0.9 and 7.8±0.7 mg/dL respectively, supporting combination of phototherapy with oral probiotic therapy. Similar evidence is documented by Waheed *et al*[16], who recorded mean bilirubin at the time of admission as 18.14±2.35 mg/dL in the monotherapy group *vs* 19.86±5.85 mg/dL in the combined therapy group, which markedly improved after 72 hours, i.e., 12.31±2.29 mg/dL *vs* 10.75±2.23 mg/dL, supporting the synergistic benefit of phototherapy with oral probiotics.

Tariq *et al*[18] mentioned that duration of therapy was also decreased in combined therapy group *vs* monotherapy (3.13±0.92 *vs* 3.81±1.12 days; p=0.002). It highlights the importance of faster bilirubin reduction which in turn reduces risk of bilirubin encephalopathy, shortens hospital stay, and lowers phototherapy exposure.

Our study indicated a clinically meaningful effect size of 0.40. Quratul Ain *et al*[19] in their randomized controlled trial found combined treatment effectiveness as 53% *vs* only 35% in phototherapy alone group. Our findings are aligned with meta-analysis of Deshmukh *et al*[20] who recorded a significant reduction in serum bilirubin level at 96th hour [MD: -1.74 (-2.92, -0.57); p=0.004] and 7th day [MD: -1.71 (-2.25, -1.17); p<0.00001; LOE: low] after probiotic treatment.

In contrast, Fatima *et al*[21] meta-analysis found a slightly better outcome with phototherapy alone (MD: 0.22; 95% CI: 0.19–0.26), possibly due to differences in probiotic strains, dosing, or population characteristics.

Overall, the findings of this study support that probiotics combined with phototherapy provide an effective approach to neonatal jaundice. Use of a specific probiotic strain (*Bacillus clausii*), clear IQR reporting, and high internal validity were the strengths of our study.

The limitations of our study included its single-centre design, lack of long-term follow-up for rebound jaundice, or the inability to control for exact breastfeeding frequency (which affects gut motility). While our results offer a clinical pathway to reduce hospital occupancy and neonatal stress, larger-scale multi-centre longitudinal studies are essential to establish standardized dosing protocols and evaluate long-term safety profiles.

CONCLUSION

Combining *Bacillus clausii* with phototherapy offers a safe, effective strategy for managing neonatal hyperbilirubinemia while reducing the risk of phototherapy-related complications.

REFERENCES

1. Ashwini S, Pabbati JL, Kumar BR, Yendamuri RM. A study on role of probiotics as an adjuvant therapy in the management of neonatal hyperbilirubinemia. Eur J Cardiovasc Med 2023;13(3):911–17. doi: https://doi.org/10.5083/ejcm/2023
2. Sarwar I, Ayaz A, Ammir K, Bashir N, Muhammad T, Langah A. Etiological risk factors for neonatal jaundice. J Peoples Uni Med Health Sci 2022;12(1):14–22. doi: https://doi.org/10.46536/jpumhs/2022/12.01.344
3. Belay G, Gerbi A, Gebremariam T, Tilahun T, Chimdi E, Etefa T. Jaundice and its associated factors among neonates admitted to selected referral hospitals in southwest oromia, Ethiopia: Multi-center cross-sectional study. Heliyon 2023;9(5):e16019. doi: https://doi.org/10.1016/j.heliyon.2023.e16019.
4. Ansong-Assoku B, Adnan M, Daley SF, *et al*. Neonatal Jaundice. [Updated 2024 Feb 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532930/
5. Jaundice in newborn babies under 28 days. London: National Institute for Health and Care Excellence (NICE); 2023 Oct 31. (NICE Guideline, No. 98). Available from: https://www.ncbi.nlm.nih.gov/books/NBK553311/
6. van der Geest BAM, de Mol MJS, Barendse ISA, de Graaf JP, Bertens LCM, Poley MJ, *et al*. Assessment, management, and incidence of neonatal jaundice in healthy neonates cared for in primary care: a prospective cohort study. Sci Rep 2022;12(1):14385.
7. Shahid M, Zia A, Mazhar A, Hassan FQM, Shahid SM, Aslam N. Correlation between total serum bilirubin levels and transcutaneous bilirubinometer values in neonatal jaundice. Pak Postgrad Med J 2023;35(1):3–6.
8. Par EJ, Hughes CA, DeRico P. Neonatal Hyperbilirubinemia: Evaluation and Treatment. Am Fam Physician 2023;107:525–34.
9. Dani C, Fusco M, Andreini M, Pepe G, Pratesi S. Implementation of the 2022 AAP guidelines for neonatal hyperbilirubinemia could reduce the need for phototherapy in Italy. Ital J Pediatr 2025;51(1):162. doi: https://doi.org/10.1186/s13052-025-02002-x
10. Lai NM, Kamar AA, Lee SW, Ng KT, Fiander M, Cracknell J, *et al*. High-versus low-dose phototherapy for neonatal jaundice. Cochrane Database Syst Rev 2026;3(3):CD003308. doi: https://doi.org/10.1002/14651858.CD003308.pub3
11. Huang HM, Lin BH, Kang YN, Lai NM, Chen KH. Efficacy and safety of probiotic supplementation for neonatal jaundice: a systematic review and meta-analysis. Probiotics Antimicrob Prot 2025. doi: https://doi.org/10.1007/s12602-025-10795-w
12. WHO Sample Size Calculator. Available from: https://cdn.who.int/media/docs/default-source/ncds/ncd-surveillance/steps/sample-size-calculator.xls
13. National Institute for Health and Care Excellence (NICE). Jaundice in newborn babies under 28 days [CG98]. London:

NICE; 2010. [updated 2023 Oct; cited 2026 Mar 8]. Available from: www.nice.org.uk
14. Javadi F, Farhat AS, Saeidi R, Mohammadzadeh A, Bakhtiari E, Kordkatouli M. Effectiveness of probiotics in treating jaundice in low birth weight infants at Imam Reza Hospital. Zahedan J Res Med Sci 2025;27(4):e165142. doi: https://doi.org/10.5812/zjrms-165142
15. Santosa I, Shoji H, Itoh S, Shimizu T. Roles of probiotics in reduction of neonatal jaundice in term newborns. Juntendo Iji Zasshi 2022;68(2):140–6. doi: https://doi.org/10.14789/jmj.JMJ21-0044-OA
16. Waheed A, Shirazi H, Mustafa A, Waheed Y. Therapeutic effects of probiotics in the reduction of neonatal hyperbilirubinemia. Pak Armed Forces Med J 2024;74(1):113–6. doi: https://doi.org/10.51253/pafmj.v74i1.3071
17. Eghbalian F, Sabzehei MK, Talesh ST, Raeisi R, Jenabi E. The effect of probiotics on phototherapy for bilirubin reduction in term neonates: a randomized controlled trial. Curr Pediatr Rev 2024;21(1):85–90. doi: https://doi.org/10.2174/0115733963257942231024100105
18. Tariq B, Shahman M, Mateen A, Kamal MT, Ali AN, Shah AH. Role of probiotics in neonates with hyperbilirubinemia receiving phototherapy. Pak J Med Health Sci 2021;15:312–5. doi: https://doi.org/10.53350/pjmhs2115113112
19. Ain Q, Mahmood I, Manshah A. Efficacy of probiotics in reducing the severity and duration of neonatal hyperbilirubinemia. J Bacha Khan Med Coll 2024;5(2):116–21.
20. Deshmukh J, Deshmukh M, Patole S. Probiotics for the management of neonatal hyperbilirubinemia: a systematic review of randomized controlled trials. J Matern Fetal Neonatal Med 2019;32(1):154–63. doi: https://doi.org/10.1080/14767058.2017.1369520
21. Fatima M, Tufail U, Ahmed A, Waseem H, Simran FNU, Zafar M, *et al*. Efficacy of probiotics and synbiotics combined with phototherapy *vs* phototherapy alone in neonatal hyperbilirubinemia: a systematic review and meta-analysis of randomized controlled trials. Eur J Pediatr 2025;184(12):765. doi: https://doi.org/10.1007/s00431-025-06627-2

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Received: 19 Oct 2025 **Reviewed:** 20 Mar 2026 **Accepted:** 31 Mar 2026

Contribution of Authors:
Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

HBS: Drafting the work **AA:** Analysis **AB:** Interpretation of data
SM: Critical review **NB:** Statistical analysis **SN:** Data collection

Conflict of Interest: No conflict of interest is declared **Funding:** No funds have been received from any agency