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Effects of phototherapy combined with *Lactobacillus salivarius* AP-32 or *Bifidobacterium animalis* subsp. *lactis* CP-9 on improving neonatal jaundice and gut microbiome health: a randomized double-blind clinical study

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Abstract

Neonatal jaundice is a common condition observed in newborns shortly after birth, making it one of the most frequent health concerns during the first two weeks of life. This study, conducted between May 2019 and July 2023, enrolled 300 full-term infants with bilirubin levels exceeding 15 mg/dL on the fourth day after birth. The infants were recruited and randomly assigned in equal numbers to one of three groups for further investigation. In addition to the control group, the other two groups of infants received probiotic supplementation administered twice daily, with each capsule delivering 5×10^9 CFU of either *Lactobacillus salivarius* AP-32 or *Bifidobacterium animalis* subsp. *lactis* CP-9. Both probiotic groups significantly reduced the overall duration of phototherapy and accelerated the rate of bilirubin reduction compared to the control group. The AP-32 group experienced a significant reduction in hospitalization duration, staying seven hours less than the placebo group ($P=0.024$). Analysis of gut microbiota revealed that the probiotic groups significantly enhanced microbial diversity in the intestines of neonates. The AP-32 group showed a significant increase in the abundance of *L. salivarius*, while the CP-9 group demonstrated a notable enhancement in the abundance of *B. animalis*. These findings suggest

Trial registration: The trial was registered in the US Library of Medicine (clinicaltrials.gov) with the number NCT03876678 on March 12, 2019.

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that integrating phototherapy with probiotic supplementation may enhance jaundice clearance increasing the abundance of beneficial gut bacteria, thereby facilitating the recovery of neonates.

Keywords Probiotic adjuvant, Safety, Neonatal jaundice, Bilirubin

Introduction

Neonatal jaundice can be a benign physiological process, or it can also be the first sign of serious illness with bilirubin-associated toxicity manifested in the nervous system. The practice guidelines released by the American Academy of Pediatrics (AAP) have been well accepted to monitor and identify newborns who might have severe unconjugated hyperbilirubinaemia, and even acute bilirubin encephalopathy or kernicterus [1]. Severe neonatal hyperbilirubinaemia still contributes significantly to neonatal morbidity and mortality, especially in low/middle-income countries [2]. The incidence of neonatal jaundice is around 60–70% in Western countries [3], and even higher among newborns of Asian ethnicity [4]. Phototherapy remains the preferred treatment for neonatal jaundice, utilizing blue light within the 450–470 nm wavelength range to catalyze the isomerization of bilirubin into water-soluble derivatives, thereby facilitating its excretion [5]. The efficacy of this therapy depends on factors such as light intensity, the type of light source and the extent of the infant's skin exposure [5, 6]. While phototherapy effectively lowers bilirubin levels and reduces the risk of neurotoxicity, its documented side effects include thermoregulatory imbalance, diarrhea, sleep disturbances, and the development of bronze-like skin discoloration [5–7].

It has been demonstrated that probiotics could be applied to regulating the gastrointestinal disease, such as diarrhea, neonatal necrotizing enterocolitis, *Helicobacter pylori* eradication [8]. Recently, probiotics and their products are well studied for their health benefit effects on immunomodulatory, type 2 diabetes, hypertension, neonatal jaundice and so on [9]. The efficacy of probiotics depends upon their ability to pass through the stomach and duodenum, and colonize in the intestinal lumen, to reduce the overgrowth of bacteria in the small bowel, restore gastrointestinal barrier function and modulate the immune system [10]. In recent years, probiotics have been studied to treat neonatal jaundice. Bifidobacteria have been shown to increase frequency of defecation, reduce enterohepatic circulation, regulate of intestinal pH, and inhibit of β-glucuronidase activity, thereby promoting the excretion of bilirubin [11–13]. *Lactobacillus* can upregulate the expression of tight junction proteins in intestinal epithelial cells, thereby mitigating bilirubin leakage and inhibiting its reabsorption [13, 14]. The immediate administration of probiotics, such as *L. rhamnosus* GG, after birth positively influences bilirubin metabolism and may reduce the risk of hyperbilirubinemia [15]. Chen YJ et al. have shown that probiotics inhibit the activity of intestinal β-glucuronidase [16], preventing the conversion of conjugated bilirubin to unconjugated bilirubin, thereby reducing the level of unconjugated bilirubin in the blood. Gut microbiota is important in this context as it may play a major role in bilirubin efflux. The supplementation of Bifidobacteria and Lactobacilli in neonates significantly enhances intestinal metabolic function, promotes immune system maturation, and facilitates the development of the intestinal mucosa along with its associated mucus layer [17]. These probiotics significantly influence the development of the neonatal gut microbiome, particularly in relation to the management of jaundice, where they may offer potential protective benefits.

The primary objective of this study was to evaluate the efficacy of two probiotics in reducing the duration of phototherapy in neonatal jaundice. Combining phototherapy with probiotics may enhance gut microbiome health, promoting the degradation and excretion of bilirubin.

Materials and methods

Probiotic protocol

The two probiotic strains used were *Lactobacillus salivarius* AP-32 (BCRC 910437, CCTCC M2011127), originally isolated from the gastrointestinal tract of healthy individuals, and *Bifidobacterium animalis* subsp. *lactis* CP-9 (BCRC 911195, CCTCC M2014588), sourced from the breast milk of healthy mothers. Each probiotic capsule, containing 5×10^9 CFU, was provided by Glac Biotech Co., Ltd. (Tainan, Taiwan) and was visually indistinguishable from the placebo capsules, which contained only maltodextrin. The capsules were administered to the infants twice daily by a consistent team of skilled nurses, who uniformly mixed the probiotic or placebo powder into either breast milk or formula. This regimen continued until the infants were discharged from the hospital.

Study population

The study population for this trial included full-term infants (≥ 37 weeks gestation) who had bilirubin levels exceeding 15 mg/dL on the fourth postnatal day. The jaundice index measurement is based on the methodology described by Tsai et al. (2022) [18]. Infants with hypothyroidism, Down syndrome, ABO hemolytic disease, gastrointestinal disorders, Glucose-6-phosphate dehydrogenase (G6PD) deficiency (favism), vascular tumors, cephalohematoma or bleeding, perinatal asphyxia with

severe hypoxic-ischemia encephalopathy, fetal chromosomal abnormalities, cyanotic congenital heart disease, umbilical hernia, early-onset sepsis, or liver failure were excluded from participation in this study.

Study design

The experimental design is illustrated in Fig. 1A. This study adhered to the ethical principles outlined in the Helsinki Declaration. It received approval from the Institutional Ethical Committee of China Medical University Children's Hospital (CMUCH, Taichung, Taiwan), with the approval number CMUH107-REC1-136. Additionally, the study was registered with ClinicalTrials.gov under the registration number NCT03876678. In estimating the required sample size for this clinical trial, the study by Demirel et al. (2013) was referenced [19]. We assumed that the anticipated effect size between the experimental and placebo groups would be 0.3 units,

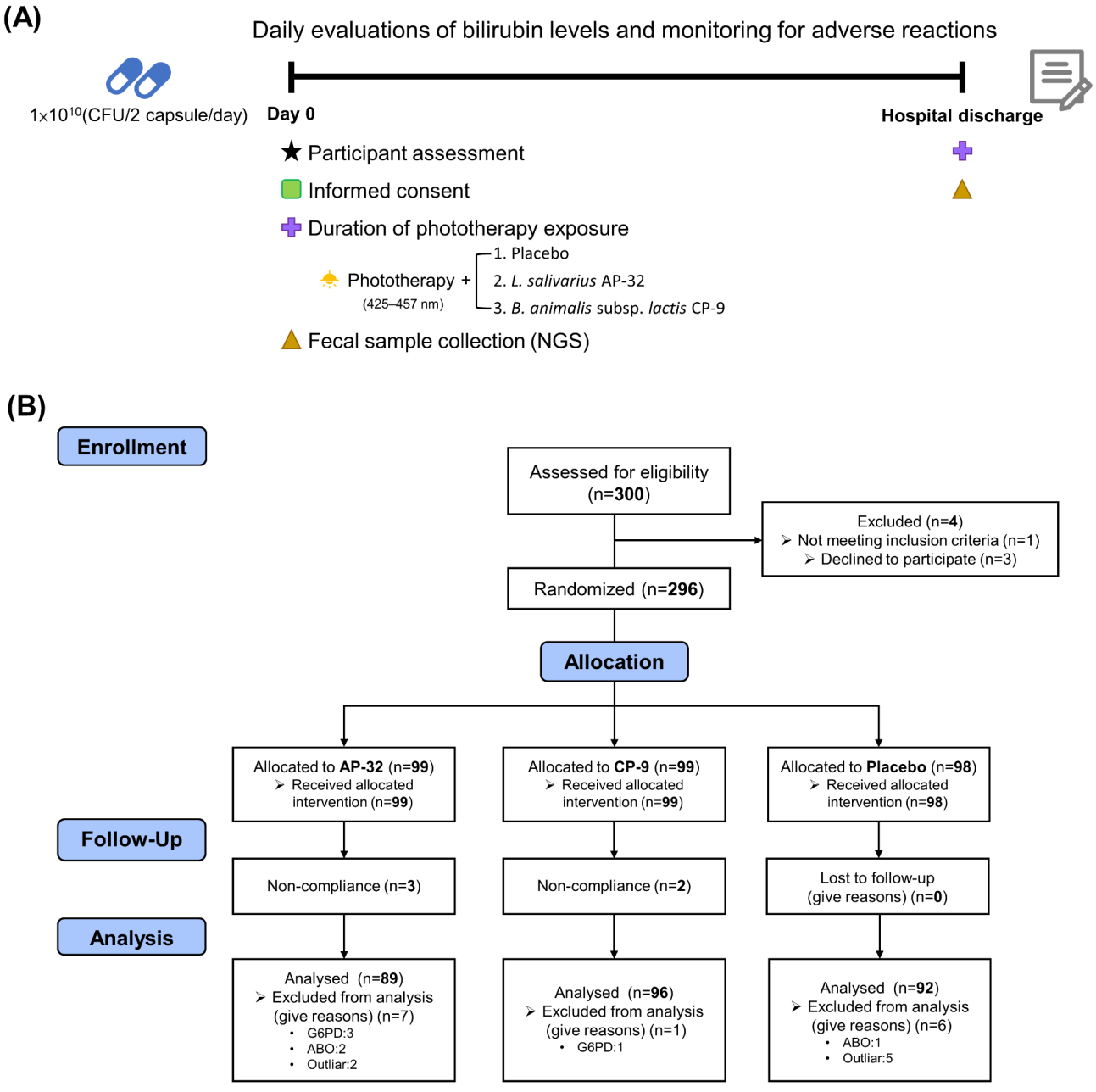


Fig. 1 Participant recruitment and trial flowchart. (A) The trial enrolled full-term neonates (≥ 37 weeks) who had serum bilirubin levels exceeding 15 mg/dL on postnatal day 4. These infants were given two probiotic capsules daily and were closely monitored for changes in bilirubin levels and any adverse reactions until their discharge. (B) A total of 300 participants were randomized into three groups: the AP-32 group ($n = 99$), the CP-9 group ($n = 99$), and the placebo group ($n = 98$). Of these, 277 participants completed the trial

with a standard deviation of 0.63 units. Given a α (type I) error of 0.05 and a statistical power (1-β) of 0.90, the sample size calculation indicated that 85 participants per group were required. Considering the study duration and an expected dropout rate of 15%, the sample size was adjusted to 100 participants per group, resulting in a total of 300 participants for the trial. Participants were recruited based on predefined eligibility criteria. After obtaining consent from their legal guardians, participants were randomly assigned to one of three groups using a randomization table. The allocation to treatment groups was concealed from parents, nurses, and physicians to ensure blinding. Each group received phototherapy (wavelength 425–457 nm; BiliBed Medela Phototherapy Lamp, Switzerland) along with one of the following interventions: AP-32, CP-9, or a placebo. Phototherapy for managing hyperbilirubinemia was administered according to the guidelines established by the AAP [1] and the Taiwanese Society of Neonatology (http://www.tsn-neonatology.com/health/). The phototherapy intensity was maintained ≥ 30 μW/cm²/nm. During the treatment, LED light sources were positioned approximately 30 cm from the patient, who was protected by eye shields and a diaper. Adverse events in the infants were documented throughout the study. Discharge criteria were met when the phototherapy indicator dropped by two units without any subsequent rebound, and participants became eligible for discharge following the physician's clinical evaluation. The primary outcome measures included the duration of phototherapy and the rate of jaundice reduction. Secondary outcome measures encompassed the incidence of adverse reactions and an analysis of changes in the gut microbiota.

Statistical analysis

The primary outcome of this study involved analyzing physiological data through a per-protocol analysis approach. Data were presented as means ± standard error of the mean (SEM). Categorical variables were examined with Pearson's chi-squared test, while continuous variables were assessed using one-way analysis of variance (ANOVA), followed by post-hoc comparisons with the Least Significant Difference (LSD) test. The criterion for statistical significance was set at $P < 0.05$. All statistical analyses were conducted with SPSS version 19.0 (IBM, Armonk, NY, USA).

Next generation sequencing (NGS)

Before and after the probiotic intervention, fecal samples were collected from participants, and total DNA was extracted according to the protocol provided by the QIAamp®DNA Mini Kit (QIAGEN Canada, Mississauga, ON, Canada) manufacturer. To prepare the DNA libraries for each sample, the V3-V4 region of the 16S rRNA gene was amplified using specific primers 314F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') and 805R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'). The PCR reaction mixture consisted of 2.5 μL of DNA template, 5 μL of each primer, and 12.5 μL of KAPA HiFi HotStart ReadyMix (Roche Sequencing Solutions, Pleasanton, CA, USA [KK2601]), yielding a final volume of 25 μL. The thermal cycling conditions were as follows: 95°C for 5 min; 30 cycles of 95°C for 30s, 60°C for 30s, and 72°C for 30s; 72°C for 5 min. After constructing the DNA libraries, sequencing was performed by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) using the Illumina MiSeq platform (Illumina, San Diego, USA) with paired-end sequencing (2 × 300 bp).

Bioinformatics analysis and statistics

The raw sequence data were initially processed using the 16S Metagenomics application on the Basespace platform (Illumina, San Diego, CA, USA). Following this, the sequence data were classified into operational taxonomic units (OTUs) based on the Greengenes classification database (version May 2013) provided by Illumina. Alpha and beta diversity of participants' gut microbiota were assessed using the Shannon index and the Jaccard index, respectively. Alpha diversity calculations were performed with the "vegan" package in R (https://cran.r-project.org/), while beta diversity was estimated via MicrobiomeAnalyst (https://www.microbiomeanalyst.ca/) and analyzed via Principal Coordinates Analysis (PCoA) to explore variations in gut microbiota composition richness across different samples [20, 21]. To evaluate statistical differences in beta diversity, we used Permutational Multivariate Analysis of Variance (PERMANOVA) within QIIME2. A significance threshold of $P < 0.05$ was applied to determine statistical significance.

Results

Participant recruitment and basic characteristics

This study was conducted at China Medical University Children's Hospital. The flow chart is illustrated in Fig. 1B. During the recruitment phase, 300 participants were enrolled. During the screening phase, four participants withdrew. In the trial phase, five participants did not adhere to the study protocol. A total of 291 participants completed the trial. Among them, four participants were found to have G6PD deficiency, three had ABO hemolysis, and seven were identified as outliers in the bilirubin decline rate based on the interquartile range (IQR) criterion (i.e., values falling outside the range of Q1 – 1.5×IQR or Q3 + 1.5×IQR [22]). Consequently, 277 participants were included in the final statistical analysis. The AP-32 probiotic group consisted of 89 participants,

Table 1 Baseline characteristics and primary outcomes

	AP-32 (*N* = 89)	CP-9 (*N* = 96)	Placebo (*N* = 92)	*P*-value
Gender (M/F)	46/43	41/55	51/41	0.198
BBW (g)	3069.96 ± 36.10	3087.29 ± 33.94	3097.54 ± 35.71	0.858
Delivery method (NSD/C/S)	65/24	76/20	77/15	0.214
Gestational age (weeks)	37.93 ± 0.12	37.99 ± 0.12	38.08 ± 0.12	0.702
Baseline serum bilirubin (mg/dL)	15.84 ± 0.25	16.08 ± 0.23	15.67 ± 0.23	0.466
Endpoint serum bilirubin (mg/dL)	11.00 ± 0.15	11.21 ± 0.14	10.99 ± 0.14	0.464
Duration of hospital stay (hours)	57.06 ± 2.05*	62.34 ± 2.12	64.30 ± 2.51	0.066
Δ Jaundice index (mg/dL)	−4.84 ± 0.27	−4.88 ± 0.25	−4.68 ± 0.27	0.859

Abbreviations: BBW, Birth body weight; NSD, Normal spontaneous delivery; C/S, Cesarean section

*Significant difference between placebo, $P < 0.05$

Δ Jaundice index = Endpoint serum bilirubin − Baseline serum bilirubin

Table 2 The number and percentage of discharged participants

	AP-32 (*N* = 89)	CP-9 (*N* = 96)	Placebo (*N* = 92)	*P*-value
24 h post intervention (N/%)	1/1.1	0/0	1/1.1	0.586
48 h post intervention (N/%)	50/56.2	42/43.8	37/40.2	0.078
72 h post intervention (N/%)	30/33.7	44/45.8	43/46.7	0.140
> 72 h post intervention (N/%)	8/9	10/10.4	11/12.0	0.808

the CP-9 probiotic group had 96 participants, and the placebo group included 92 participants. No adverse reactions were recorded during the study period. The study findings indicate that the clinical characteristics of the study groups were comparable, with no significant differences observed in baseline data, including gender, birth weight, delivery method, gestational age, and baseline serum bilirubin levels (Table 1). Although no significant differences were observed in the changes in jaundice index or the number of discharges per 24 h among the three groups, the length of hospital stay was notably reduced in the AP-32 group ($P = 0.024$).

Probiotics showed no effect on the hospital discharge rates of participants

The timing of discharge following the study intervention, as illustrated in Table 2, also revealed no significant differences within the first 24 h post-intervention across the groups. Specifically, the number of discharges within 24 h was minimal and comparable among the AP-32, CP-9, and placebo groups. The number of discharges between 24 and 48 h showed a trend toward earlier discharge in the AP-32 group compared to the CP-9 and placebo groups, the *P*-value of 0.078. Discharge timings between 48 and 72 h and beyond 72 h did not differ significantly among the groups.

Probiotics effectively reduced the duration of phototherapy and improved jaundice

Figure 2 highlights the duration of phototherapy across the three groups. Figure 2A shows that the total duration of phototherapy was significantly shorter in the AP-32 and CP-9 groups compared to the placebo group, with statistically significant differences (*P*-values of 0.003 and 0.015, respectively). This reduction in phototherapy duration suggests a potential therapeutic benefit of the probiotics. Additionally, the rate of decline in the jaundice index, as shown in Fig. 2B, was faster in both the AP-32 and CP-9 groups compared to the placebo group. The decline rates were significantly greater in the probiotic groups, indicating that probiotic supplementation may enhance the reduction of jaundice severity in affected infants ($P < 0.05$). Figure 3 demonstrates the cumulative amount of phototherapy, quantified as the total hours of phototherapy multiplied by the number of light banks. A significant reduction in light exposure observed in both the AP-32 and CP-9 groups. The AP-32 group, in particular, exhibited a more pronounced reduction, with *P*-values less than 0.01 on the 1st and 2nd days. This indicates that probiotics, especially AP-32, may effectively reduce the need for prolonged phototherapy in jaundiced infants, potentially mitigating the risks associated with extended light exposure.

Probiotics enhanced the diversity and abundance of beneficial bacteria in the gastrointestinal tract

The evaluation of microbiome distribution, as illustrated in Fig. 4, reveals that after three days of probiotic treatment, the bacterial diversity became more enriched in both study groups. The alpha diversity analysis results, presented in Fig. 4A, show a statistically significant increase in the Shannon index within the AP-32 group ($P = 0.044$). Moreover, the CP-9 group displayed an upward trend in alpha diversity. This suggests that supplementation with the AP-32 and CP-9 probiotics enhances the diversity of the gut microbiota in jaundiced

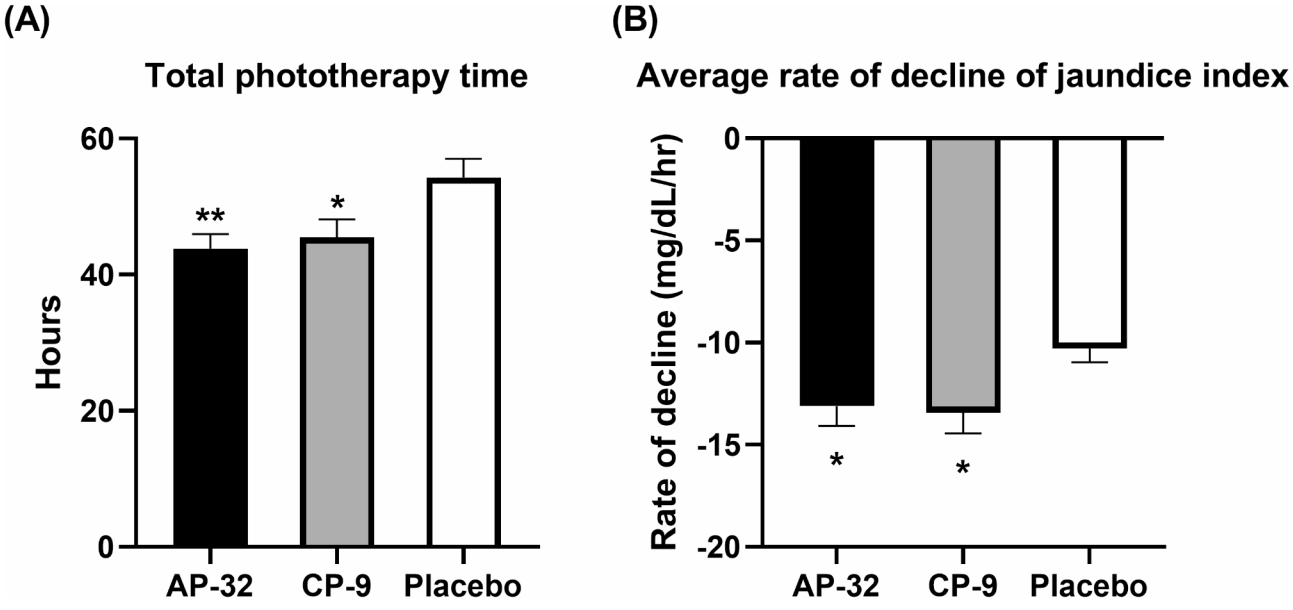


Fig. 2 During the trial period, both (**A**) the total duration of phototherapy and (**B**) the bilirubin declined rate were evaluated. Jaundice index declined rate = (jaundice level at discharge − jaundice level at admission) / duration of phototherapy. Significance levels were indicated as follows: $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to placebo

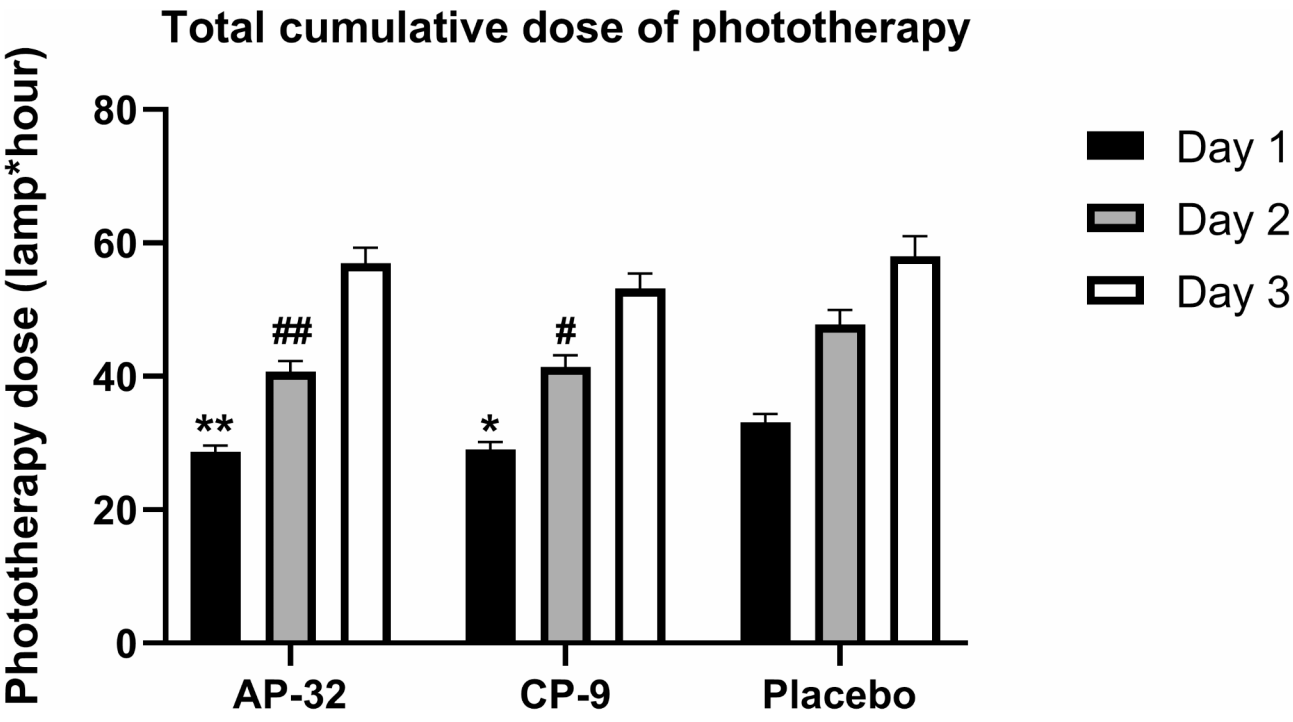
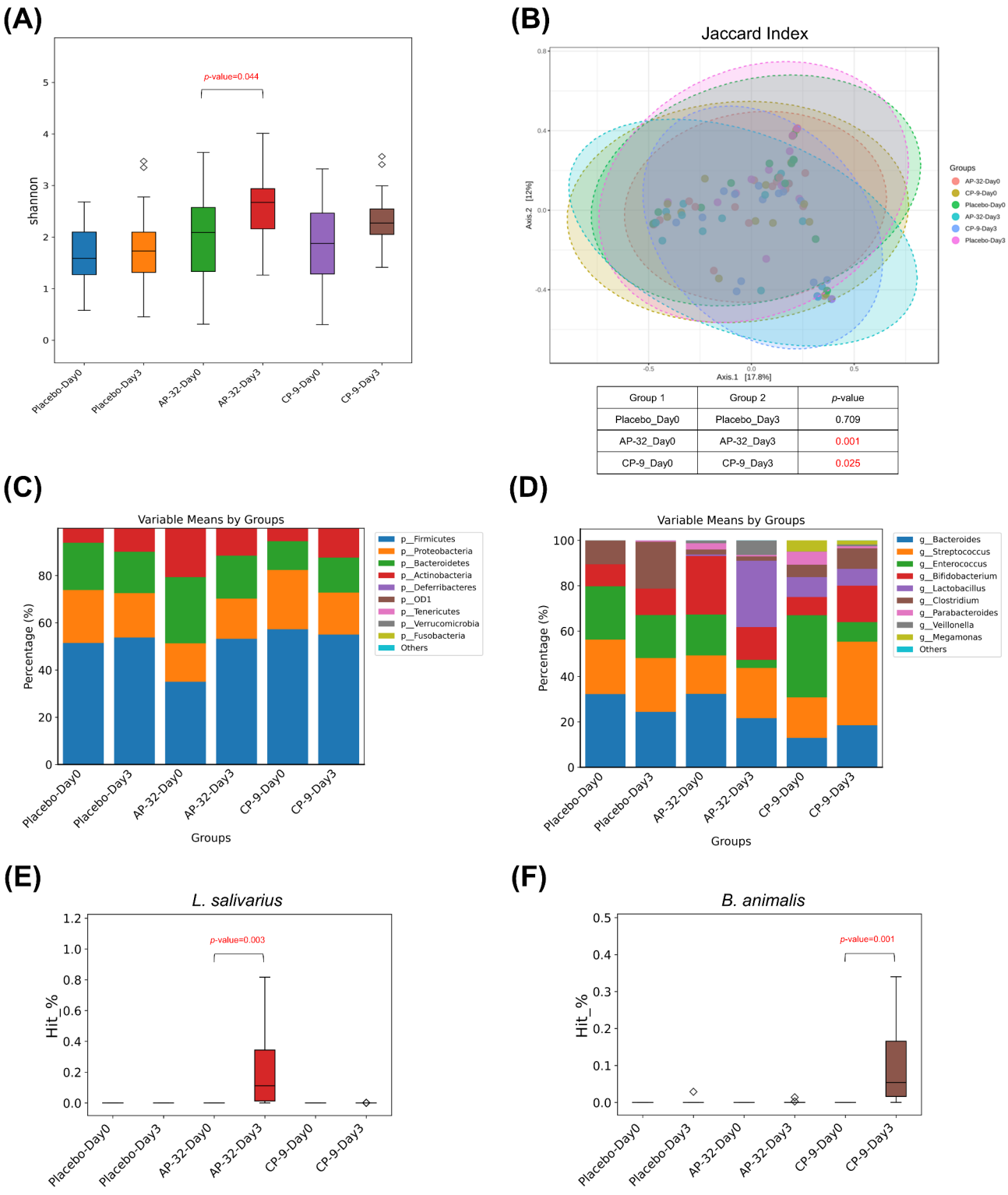


Fig. 3 During the first three days of hospitalization, the total cumulative dose of phototherapy administered were assessed. Significance levels were indicated as follows: $^{*}P < 0.05$, and $^{**}P < 0.01$ compared to placebo $_{\text{Day 1}}$; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared to placebo $_{\text{Day 2}}$. Phototherapy dose (lamp*hour) = number of lamps × duration of phototherapy

infants, potentially contributing to better gut health and more effective management of jaundice. The beta diversity analysis, as shown in Fig. 4B, further corroborates these findings. Significant differences in microbial communities were observed in the Jaccard analysis, both the AP-32 ($P = 0.001$) and CP-9 ($P = 0.025$) groups demonstrated a significant change over the three days which was not seen in the placebo group.

Figure 4C shows the relative abundance of bacterial phyla as a percentage in different groups at three time



Group 1	Group 2	*p*-value
Placebo_Day0	Placebo_Day3	0.709
AP-32_Day0	AP-32_Day3	0.001
CP-9_Day0	CP-9_Day3	0.025

Fig. 4 Changes in gut microbiota composition before and after probiotic intervention. Several indices were evaluated, including (**A**) alpha diversity, (**B**) beta diversity, the top ten bacterial (**C**) phyla and (**D**) genera, as well as the relative abundance of (**E**) *L. salivarius* and (**F**) *B. animalis*

points. The top three bacterial phyla identified were Firmicutes, Proteobacteria, and Bacteroidetes, with predominant genera including *Bacteroides*, *Streptococcus*, *Enterococcus*, *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Parabacteroides*, *Veillonella*, and *Megamonas* (Fig. 4D). This increase in diversity is a positive indicator of a healthier gut environment, which could be linked to the observed clinical benefits. At the genus level (Fig. 4D), *Bacteroides* increased only in the CP-9 probiotic group, while it decreased in the AP-32 and placebo groups. *Streptococcus* increased after supplementation in all groups, and *Enterococcus* showed a slight decrease in the placebo group. A more pronounced reduction in *Enterococcus* was observed in the AP-32 and CP-9 groups. The AP-32 group showed a significant increase in the relative abundance of *Lactobacillus*, especially *L. salivarius* ($P = 0.003$) (Fig. 4D and E). Conversely, the CP-9 group demonstrated an increase in *Bifidobacterium*, specifically *B. animalis* ($P = 0.001$) (Fig. 4D and F). These changes in microbiota reflect the specific effects of different probiotic strains on the gut microbiota, with potential implications for their therapeutic use in managing neonatal jaundice.

Discussion

The study findings provide a comprehensive overview of the effects of probiotic supplementation on bilirubin levels, phototherapy requirements, and gut microbiota diversity in jaundiced infants. This study demonstrates that probiotics can accelerate the reduction of bilirubin levels in jaundiced infants and reduce the duration of phototherapy. A safety study has demonstrated that both AP-32 and CP-9 do not have any adverse effects on the growth and development of infants [23]. Previous literature has indicated that excessive phototherapy may lead to skin hypersensitivity, including effects on the immune and inflammatory systems, as well as potential genotoxic and other side effects [24]. The imbalance of gut microbiota—known as dysbiosis—has also been considered as a pathogenic factor for neonatal jaundice [13]. The bilirubin formed by the destruction of erythrocytes first enters the liver and then combines with UDP-glucuronic acid (UDPGA) to form water-soluble conjugated bilirubin. Subsequently, bilirubin is secreted into the biliary tract and enters the intestine for excretion. Likewise, pathogenic *Escherichia coli* in the intestine produced bacterial enzymes, β-glucuronidase, would also slow down the efficiency of metabolism of bilirubin [13, 25]. Oral administration of probiotics was found to increase alpha diversity, which may explain the accelerated reduction in bilirubin levels through the inhibition of *E. coli* expression [18]. Alpha and beta diversity analysis revealed significant community differences and increased microbial variety in the AP-32 and CP-9 groups after probiotic supplementation. These results highlight the distinct shifts in the gut microbiota composition following probiotic intervention with both strains.

In the early stages of life, anaerobic bacteria typically colonize the intestinal tract of neonates within the first 3 to 7 days after birth [26, 27]. During this developmental phase, the primary anaerobic genera that emerge include *Bifidobacterium*, *Clostridium*, and *Bacteroides* [13, 28]. Beneficial bacteria for jaundice are those capable of metabolizing bilirubin into urobilinoids, with most strains belonging to the genera *Bacteroides* and *Clostridium* [27]. In the control group, changes in the abundance of these two genera were observed to after the experiment. The abundance of *Streptococcus* does not exhibit significant differences between healthy individuals and those with jaundice [29, 30]. Administering of probiotics, including *L. acidophilus*, *S. thermophilus*, and *B. longum*, in conjunction with phototherapy, led to a more rapid reduction of jaundice compared to phototherapy alone [31]. This finding suggests that *Streptococcus* may interact with other gut microbiota, potentially influencing the overall composition and functionality of the microbial community.

Urinary tract infections (UTIs) are recognized as potential contributors to hyperbilirubinemia in newborns [32]. Recent research has identified a significant correlation between congenital anomalies of the kidney and urinary tract (CAKUT) and an elevated risk of both neonatal jaundice and UTIs [33]. Microbial analyses of urine frequently identify *Enterococcus faecalis* and other *Enterococcus* species among the top five pathogens [32, 34, 35]. Moreover, the intestinal microbiome of jaundiced neonates is often characterized by a decreased abundance of Bifidobacteriaceae and an increased presence of Enterococcaceae [25]. *Bifidobacterium* is the most common genus in the infant gut microbiota, and its ability to inhibit β-glucuronidase activity promotes the enhanced dissociation of conjugated bilirubin within the gastrointestinal tract [25]. *Lactobacillus*, a common microbial inhabitants in the gastrointestinal tract of healthy infants, is significantly less abundant in those with jaundice, potentially due to its involvement in bilirubin metabolism [36]. However, a review indicates that most probiotics exhibit minimal β-glucuronidase activity and lack the capacity to convert bilirubin, suggesting a limited or indirect role in bilirubin metabolism [27]. A recent study revealed that alterations in the gut microbiota were observed 24 and 48 h after the initiation of phototherapy [37]. Notably, there was a significant reduction in the abundance of *Bifidobacterium* and *Lactobacillus*, particularly *B. animalis*, *B. breve*, and *L. fermentum*. Additionally, the abundance of *L. salivarius* also decreased. Our research findings indicated that supplementation with *B. animalis* CP-9 or *L. salivarius* AP-32 significantly

increased the abundance of these probiotics in the intestines of jaundiced infants. Appropriate probiotic supplementation may enhance the efficacy of phototherapy in these infants while simultaneously supporting the growth of beneficial gut microorganisms and promoting a more balanced, functional microbiota.

The studies mentioned provide compelling evidence supporting the role of probiotics in managing jaundice and enhancing gut health. Previous studies have suggested that probiotics such as *Bifidobacterium* and *Saccharomyces boulardii* may play a role in preventing hyperbilirubinemia by modulating intestinal motility, improve intestinal barrier function, and regulating gut microbiota [38–40]. The efficacy of probiotics lies in their ability to reduce pathogenic bacterial overgrowth by producing antibacterial substances in the small intestine, restoring gastrointestinal barrier function, and regulating the immune system [10]. These mechanisms underline the potential of probiotics as a therapeutic tool in managing conditions related to jaundice and gut health.

This study has several limitations that should be acknowledged. First, we did not collect and analyze data regarding weight loss among the infants, which could be an important factor influencing the outcomes of jaundice treatment. Second, the study did not differentiate between infants who were fed with formula milk and those who were breastfed. Different feeding methods and types of milk can have varying effects on bilirubin levels and the progression of jaundice. The lack of detailed feeding information limits our ability to fully understand and interpret the influence of diet on the efficacy of probiotic supplementation in jaundiced infants.

Future research should aim to include comprehensive data on feeding practices and weight changes to better assess their impact on jaundice outcomes. Exploring the effects of various probiotic strains in conjunction with different types of milk feeding could provide more nuanced insights into optimizing treatment strategies for neonatal jaundice. Investigating the interactions between specific microbial communities and feeding methods may help in developing targeted interventions that enhance therapeutic efficacy and promote better health outcomes for affected infants.

Conclusion

In conclusion, the integration of *L. salivarius* AP-32 and *B. animalis* CP-9 probiotics with phototherapy presents a promising approach to reducing the treatment duration for neonatal jaundice. This combined therapy not only accelerates bilirubin reduction but also appears to do so without significant side effects. The potential to shorten the duration of jaundice through this synergistic treatment could have lasting benefits on the growth and development of affected infants, making it a valuable addition to current therapeutic strategies.

Abbreviations

AAP	American Academy of Pediatrics
ANOVA	One-way analysis of variance
CAKUT	Kidney and urinary tract
G6PD	Glucose-6-phosphate dehydrogenase
LED	Light-emitting diode
LSD	Least Significant Difference
PCoA	Principal Coordinates Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
SEM	Standard error of the mean
UDPGA	UDP-glucuronic acid
UTIs	Urinary tract infections

Author contributions

Conceptualization, H.H. Ho, Y.W. Kuo; Methodology, H.S. Wang, Y.H. Chin; Validation, Y.W. Kuo, J.H. Lin; Project administration, M.L. Tsai, S.P. Shen, Y.T. Chen, H.Y. Chiu, H.Y. Lin, H.W. Cheng, H.C. Lin, Y.W. Kuo, J.H. Lin; Formal analysis, H.S. Wang, Y.Y. Huang; Investigation, H.S. Wang, Y.Y. Huang; Writing - Original Draft, M.L. Tsai, C.M. Li; Writing - Review & Editing, Y.H. Chin; Visualization, H.S. Wang, C.M. Li; Supervision, H.H. Ho, H.C. Lin. All authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

Glac Biotech Co., Ltd. provided financial support in the form of salaries for Y.W. Kuo, J.H. Lin, H.S. Wang, Y.Y. Huang, C.M. Li, Y.H. Chin and H.H. Ho, but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The remaining authors declare no conflict of interest.

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References

1. Burke B, Robbins J, Hobbs C. American academy of pediatrics subcommittee on hyperbilirubinemia management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics. 2004;114:297–316.
2. Kaplan M, Bromiker R, Hammerman C. Severe neonatal hyperbilirubinemia and Kernicterus: are these still problems in the third Millennium?? *Neonatology* 2011, 100:354–62.
3. Maisels MJ, McDonagh AF. Phototherapy for neonatal jaundice. N Engl J Med. 2008;358:920–8.
4. Chou H-C, Chien C-T, Tsao P-N, Hsieh W-S, Chen C-Y, Chang M-H. Prediction of severe neonatal hyperbilirubinemia using cord blood hydrogen peroxide: a prospective study. PLoS ONE. 2014;9:e86797.

5. Shoris I, Gover A, Toropine A, Iofe A, Zoabi-Safadi R, Tsuprun S, Riskin A. Light on Phototherapy—Complications and strategies for shortening its duration, A review of the literature. Children. 2023;10:1699.
6. Kemper AR, Newman TB, Slaughter JL, Maisels MJ, Watchko JF, Downs SM, Grout RW, Bundy DG, Stark AR, Bogen DL. Clinical practice guideline revision: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. Pediatrics 2022, 150.
7. Wu R, Jiang Y, Yan J, Shen N, Liu S, Yin H, Zhu S, Qiao J. Beneficial changes in gut microbiota after phototherapy for neonatal hyperbilirubinemia. Biomedical Rep. 2024;20:1–7.
8. Behnsen J, Deriu E, Sassone-Corsi M, Raffatellu M. Probiotics: properties, examples, and specific applications. Cold Spring Harbor Perspect Med. 2013;3:a010074.
9. Kasińska MA, Drzewoski J. Effectiveness of probiotics in type 2 diabetes: a meta-analysis. Pol Arch Med Wewn. 2015;125:803–13.
10. Quigley EM. Gut microbiota and the role of probiotics in therapy. Curr Opin Pharmacol. 2011;11:593–603.
11. An HM, Park SY, Lee DK, Kim JR, Cha MK, Lee SW, Lim HT, Kim KJ, Ha NJ. Antiobesity and lipid-lowering effects of bifidobacterium spp. In high fat diet-induced obese rats. Lipids Health Dis. 2011;10:1–8.
12. Deshmukh J, Deshmukh M, Patole S. Probiotics for the management of neonatal hyperbilirubinemia: a systematic review of randomized controlled trials. J Maternal-Fetal Neonatal Med. 2019;32:154–63.
13. Chen K, Yuan T. The role of microbiota in neonatal hyperbilirubinemia. Am J Translational Res. 2020;12:7459.
14. Zhou Y, Qin H, Zhang M, Shen T, Chen H, Ma Y, Chu Z, Zhang P, Liu Z. Lactobacillus plantarum inhibits intestinal epithelial barrier dysfunction induced by unconjugated bilirubin. Br J Nutr. 2010;104:390–401.
15. Mutlu M, Irmak E, Aslan Y, Kader Ş. Effects of Lactobacillus rhamnosus GG as a probiotic on neonatal hyperbilirubinemia. Turk J Pediatr 2018, 60.
16. Yiji C, Zuo-yi S-xW, Shi-Xiao W. Characteristics of enterohepatic circulation in neonates and mechanism of using Microbiological Preparation to treat neonatal jaundice. J Pediatr Pharm {Internet} 2003, 9.
17. Matera M: Bifidobacteria, Lactobacilli… when, how and why to use them. Global Pediatrics. 2024;8: 100139. 2024.
18. Tsai M-L, Lin W-Y, Chen Y-T, Lin H-Y, Ho H-H, Kuo Y-W, Lin J-H, Huang Y-Y, Wang H-S, Chiu H-Y. Adjuvant probiotic bifidobacterium animalis subsp. Lactis CP-9 improve phototherapeutic treatment outcomes in neonatal jaundice among full-term newborns: A randomized double-blind clinical study. Medicine. 2022;101:e31030.
19. Demirel G, Celik IH, Erdeve O, Dilmen U. Impact of probiotics on the course of indirect hyperbilirubinemia and phototherapy duration in very low birth weight infants. J maternal-fetal Neonatal Med. 2013;26:215–8.
20. Chong J, Liu P, Zhou G, Xia J. Using Microbiomeanalyst for comprehensive statistical, functional, and meta-analysis of Microbiome data. Nat Protoc. 2020;15:799–821.
21. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of Microbiome data. Nucleic Acids Res. 2017;45:W180–8.
22. Larson MG. Descriptive statistics and graphical displays. Circulation. 2006;114:76–81.
23. Chen J-F, Ou-Yang M-C, Hsia K-C, Use NP, Group SR, Li C-M, Yeh Y-T, Ho H-H. A Three-Arm, randomized, Double-Blind, Placebo-Controlled study to evaluate the safety of Lactobacillus salivarius AP-32 and bifidobacterium animalis CP-9 used individually in healthy infants. Nutrients. 2023;15:3426.
24. Faulhaber FR, Procianoy RS, Silveira RC. Side effects of phototherapy on neonates. Am J Perinatol. 2019;36:252–7.
25. Akagawa S, Akagawa Y, Yamanouchi S, Teramoto Y, Yasuda M, Fujishiro S, Kino J, Hirabayashi M, Mine K, Kimata T. Association of neonatal jaundice with gut dysbiosis characterized by decreased bifidobacteriales. Metabolites. 2021;11:887.
26. Orrhage K, Nord C. Factors controlling the bacterial colonization of the intestine in breastfed infants. Acta Paediatr. 1999;88:47–57.
27. Su H, Yang S, Chen S, Chen X, Guo M, Zhu L, Xu W, Liu H. What happens in the gut during the formation of neonatal Jaundice—Underhand manipulation of gut microbiota?? Int J Mol Sci. 2024;25:8582.
28. Moore R, Townsend S. Temporal development of the infant gut Microbiome. Open Biol. 2019;9:190128.
29. Li Y, Shen N, Li J, Hu R, Mo X, Xu L. Changes in intestinal flora and metabolites in neonates with breast milk jaundice. Front Pead. 2020;8:177.
30. You JJ, Qiu J, Li GN, Peng XM, Ma Y, Zhou CC, Fang SW, Huang RW, Xiao ZH. The relationship between gut microbiota and neonatal pathologic jaundice: A pilot case-control study. Front Microbiol. 2023;14:1122172.
31. Abbas P, Mayangsari CP, Gunawan R. The effect of Lactobacillus acidophilus, Streptococcus thermophilus, and bifidobacterium longum probiotics on bilirubin levels of neonates with hyperbilirubinemia. Sains Medika J Med Health 2023, 14.
32. Chen L-J, Chen P-J, Yang S-F, Chen J-Y. Causative organisms and antimicrobial susceptibility in jaundiced infants with significant bacteriuria. J Chin Med Association. 2022;85:514–8.
33. Chou H-H, Huang L-C, Shen S-P, Tsai M-L, Chang Y-C, Lin H-C. Neonatal jaundice is associated with increased risks of congenital anomalies of the kidney and urinary tract and concomitant urinary tract infection. Sci Rep. 2024;14:9520.
34. Sagheb S, Mosayebi Z, Nikseresht Z. Unexpected neonatal jaundice as an early diagnostic sign of urinary tract infection. J Iran Med Council. 2021;4:7–11.
35. Chen I, Hsu L-S, Yao C-S, Chang J-T, Wang H-P, Fang N-W. Risk factors for urinary tract infection in infants with unexplained hyperbilirubinemia: a single center case-control study. Front Pead. 2024;12:1332052.
36. Zhang X, Zeng S, Cheng G, He L, Chen M, Wang M, Zhou W, Qiu H, Wang Z. Clinical manifestations of neonatal hyperbilirubinemia are related to alterations in the gut microbiota. Children. 2022;9:764.
37. Fan S, Zhang K, Zhang J, Zhang L, Liu L, Lv A, Ma Y, Fang X, Zheng F, Wu Z. Analysis of the effect of phototherapy on intestinal probiotics and metabolism in newborns with jaundice. Front Pead. 2022;10:878473.
38. Tuzun F, Kumral A, Duman N, Ozkan H. Breast milk jaundice: effect of bacteria present in breast milk and infant feces. J Pediatr Gastroenterol Nutr. 2013;56:328–32.
39. Geyik MF, Aldemir M, Hosoglu S, Ayaz C, Satilmis S, Buyukbayram H, Kokoglu OF. The effects of Saccharomyces boulardii on bacterial translocation in rats with obstructive jaundice. Annals Royal Coll Surg Engl. 2006;88:176–80.
40. Jones C, Badger SA, Regan M, Clements BW, Diamond T, Parks RW, Taylor MA. Modulation of gut barrier function in patients with obstructive jaundice using probiotic LP299v. Eur J Gastroenterol Hepatol. 2013;25:1424–30.

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