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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

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Abstract

Background: Probiotics had been used to decreased bilirubin level in neonatal jaundice (NJ) without being further studied mechanism and stratification. The intestinal pathogen *Escherichia coli* produced β-glucuronidase would increase enterohepatic circulation and elevate serum bilirubin levels (SBLs) which might worsen the disease process of NJ.

Study objective: We hypothesized that some probiotics could decrease bilirubin level through inhibiting the growth of *E. coli*. It's assumed that adjuvant probiotic intervention might accelerate the phototherapy for NJ and alleviate the severity of the NJ. Besides, it's further study the efficacy of the probiotic intervention in NJ among the full-term and preterm newborns.

Materials and methods: Firstly, the *Bifidobacterium animalis* subsp. *lactis* CP-9 was screened for its anti-*E. coli* activity. Then, it was orally administered to newborns with NJ in combination with conventional phototherapy (wavelength 425–457 nm) to determine its efficacy. 83 neonatal patients whose serum bilirubinemia was at a concentration of ≥ 15 mg/dL were participated the double-blind randomized trial and conducted in the neonatal ward of China Medical University Children's Hospital (CMUCH, Taichung, Taiwan). The test was conducted in 2 groups: experimental group: phototherapy + *B. animalis* subsp. *lactis* CP-9 (n = 43; 5 × 10^9 CFU/capsule) and control group: phototherapy + placebo (n = 40). The SBL and total phototherapy duration were measured.

Results: The experimental group showed improved serum bilirubin decline rate (–0.16 ± 0.02 mg/dL/h; P = .009, 95% CI -0.12 to -0.2), particularly in the first 24 hour of in-hospital care, and reduced total phototherapy duration (44.82 ± 3.23 h; P = .011, 95% CI: 51.3–38.2) compared with the control group. Especially, probiotics had a significant therapeutic effect (serum bilirubin decline rate: –0.18 ± 0.02 mg/dL/h, 95% CI -0.12 to -0.23, P = .014; phototherapy duration: 43.17 ± 22.72 h, 95% CI 51.9–34.3, P = .019) in the low-risk subgroup (full-term newborns).

Conclusions: In conclusion, *B. animalis* subsp. *lactis* CP-9 synergistically improves treatment outcomes of NJ during in-hospital phototherapy including reduced total phototherapy duration and improved serum bilirubin decline rate, particularly in full-term newborns.

Abbreviations: *B. animalis* CP-9 = *Bifidobacterium animalis* subsp. *lactis* CP-9, *E. coli* = *Escherichia coli*, MRS agar plates = de Man, Rogosa, and Sharpe agar plates, NJ = neonatal jaundice, SBLs = serum bilirubin levels.

Keywords: adjuvant probiotics, conventional phototherapy, hyperbilirubinemia, low-risk subgroup (full-term newborns), neonatal jaundice

W-YL contributed equally to this work.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files]

This clinical trial complied with the Declaration of Helsinki, and it was reviewed and approved by the Research Ethics Committee of the Affiliated Hospital of China Medical University (CMUH107-REC1-136). All guardians provided signed informed consent before their baby starting the trial (ClinicalTrials.gov Identifier: NCT03876678).

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1. Introduction

Neonatal jaundice (NJ) is common in neonates, which is usually caused by the inability of newborns' immature livers to metabolize excess serum bilirubin and excrete processed bilirubin into bile.[1] Several hypotheses are proposed for the mechanism underlying breast milk jaundice, including the inhibition of hepatic glucuronyl transferase, excess beta-glucuronidase activity deconjugating the conjugated bilirubin, and damaging the intestinal wall resulting in an increase in enterohepatic circulation of bilirubin.[2] In 2% of term neonates, bilirubin levels can reach 20 mg/dL, which requires therapeutic intervention; if left untreated, it can lead to bilirubin-induced neurologic dysfunction and chronic neurological sequela.[3] Besides, the incidence of NJ in Taiwan has a steady increasing trend during the entire study period, 2000 to 2010, both before and after the guideline modification. According to the report of Taiwan Birth Registry, the number of live births with <37 weeks of gestation had slightly increased from 8.92% in 2004 to 9.13% in 2010.[4]

The goal of NJ treatment is to prevent bilirubin encephalopathy. Phototherapy is the safest and most effective treatment for NJ.[5] However, newborns with excessively high bilirubin levels may require exchange transfusion.[6] Some novel treatments for jaundice have been discussed, such as activated charcoal, agar, cholestyramine metalloporphyrins, phenobarbital, clofibrate, and intravenous immunoglobulin, but their clinical benefits are not proven.[7,8]

Probiotics are generally considered a safe food additive[9] and are used for reducing necrotizing enterocolitis risk in preterm infants.[10] Researchers have evaluated its effectiveness in NJ treatment.[11,12] Moreover, researchers have discovered that decreased levels of intestinal bacterial in newborn babies may result in increased serum unconjugated bilirubin levels during the first week of life.[13,14] Funda et al suggested that *Bifidobacterium* species may protect against breast milk jaundice, thus protecting against hyperbilirubinemia, through the alteration of intestinal motility and intestinal microbial flora.[15] Additionally, most pathogenic *Escherichia coli* secrete beta-glucuronidase, which may increase enterohepatic circulation and cause the NJ.[16]

Adjuvant Probiotics strategy in NJ treatment is a new area of interest for researchers and clinicians.[17] However, limited evidence is available regarding the effect of probiotics on the bilirubin level in neonates. Some studies have evaluated the effect of probiotics on serum bilirubin levels (SBLs) and have reported that probiotics reduce the phototherapy duration.[18] Additionally, whether probiotics benefit preterm newborns with jaundice remains unclear. On the basis of available evidence, first, we hypothesized that probiotic strains that inhibit *E. coli* might aid in neonatal bilirubin metabolism. Second, we assumed that the selected probiotic strains, which exert excellent anti-*E. coli* activity, would synergistically improve phototherapeutic efficiency in NJ. This study was further evaluated the efficacy of supplemented probiotics in combination with phototherapy in the jaundice treatment of the full-term (38–40 weeks; low risk group) and preterm (35–37 weeks; high risk group) infants. This study may improve the progress of personal medicine in the treatment of neonatal hyperbilirubinemia among full-term newborns or pre-term newborns.

2. Materials and methods

2.1. Probiotic strain

Five probiotic strains were selected, namely *Lactobacillus rhamnosus* L-134, *Lactobacillus salivarius* L-1, *Lactobacillus johnsonii* L-3, *Bifidobacterium longum* L-337, and *Bifidobacterium animalis* subsp. *lactis* CP-9, which were obtained from Glac biotech Co. Ltd. (Tainan, Taiwan). *B. animalis* subsp. *lactis* CP-9 was isolated from human breast milk and deposited at China Center for Type Culture Collection (M2014588, Wuhan, China) and Bioresource Collection and Research Center (BCRC910645, Hsinchu, Taiwan).

2.2. In vitro screening of probiotics through E. coli inhibition test

Probiotic strains were subcultured to their third generation at 37°C for 20 hour. Selected probiotics were seeded (10^9/mL) over de Man, Rogosa, and Sharpe (MRS) agar plates (110,660, Merck, Darmstadt, Germany) with a 2-cm-wide growth zone and were cultured under semianaerobic conditions at 37°C for 48 h. Then, the probiotic strains were isolated from the probiotic MRS plate, and 14 mL of the culture was used for the co-cultivation of pathogenic bacteria. After the pathogenic bacteria culture solidified, the second generation of pathogenic *E. coli* was obtained by evenly spreading the cultured bacteria (BCRC11634, Hsinchu, Taiwan) on it with a cotton swab. Then, the co-cultured plate was further incubated for 48 hour at 37°C. The width of the inhibition zones was measured for quantifying antipathogenic activity.

2.3. Determination of β-glucuronidase activity

The API ZYM kit (API system, BioM´erieux, France) was used to determine the β-glucuronidase activity of the 5 probiotic strains, namely *L. rhamnosus* L-134, *L. salivarius* L-1, *L. johnsonii* L-3, *B. longum* L-337, and *B. animalis* CP-9. All experimental procedures were conducted according to the manufacturer's instructions.[12]

2.4. Participants

This double-blind randomized trial on NJ treatment with oral probiotics was conducted from January 1, 2019, to December 31, 2020. This trial was performed in the neonatal ward of China Medical University Children's Hospital (CMUCH, Taichung, Taiwan). The study was initiated and approved by Institutional Ethical Committee (CMUH107-REC1-136). Yi-Hao Weng et al defined the l hyperbilirubinemia at a total bilirubin ≥15 mg/dL.[19] A total of 98 patients were initially recruited, 2 of whom withdrew from the study.

Thus, 96 patients were enrolled. The sample size calculation was analyzed according to previous study.[20,21] It's presumed that the mean value of phototherapy duration after intervention was decreased 30% with a standard deviation of 0.88 day for both groups. For a power of 80% and a type 1 error of 0.05, the number of subjects needed for each arm of the study is 39. Assuming a dropout rate of 20%, approximately 49 subjects was required for each arm in the study. 47 patients were randomly assigned to the probiotic group and

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49 patients were randomly assigned to the placebo group via computer-generated random numbering with double blinding allocation, out of which 4 patients withdrew due to loss to follow-up in probiotic group and 9 patients withdraw from placebo group. Finally, a total of 83 patients were included for analysis, among which 43 patients in the probiotic group and 40 patients in the placebo group (Fig. 1). Moreover, the study followed the principle of per-protocol analysis (PP).

2.5. Inclusion criteria and low/high risk definition

The neonatal newborns in China Medical University Children's Hospital (CMUCH, Taichung, Taiwan) diagnosed with NJ were recruited in this study. Inclusion criteria were birth in CMUCH, gestational age > 35 weeks, and SBL > 15 mg/dL on the fourth day after birth. Exclusion criteria were congenital hypothyroidism, chromosomal anomaly, maternal ABO incompatibility, gastrointestinal disease, Glucose-6-phosphate Dehydrogenase (G6PD) deficiency, hemangioma, cephalohematoma or subgaleal hemorrhages, severe perinatal asphyxia (stage III), cyanotic congenital heart disease, omphalocele, early-onset sepsis, and liver failure. All newborns whose parents provided written informed consent and who met the inclusion and exclusion criteria were enrolled in the randomized double-blinded study. The participants were further categorized based on their risk status into high- (gestational age of 35–37 weeks) and low-risk (gestational age of 38–40 weeks without other complications; full-term newborns with jaundice) NJ subgroups.[22]

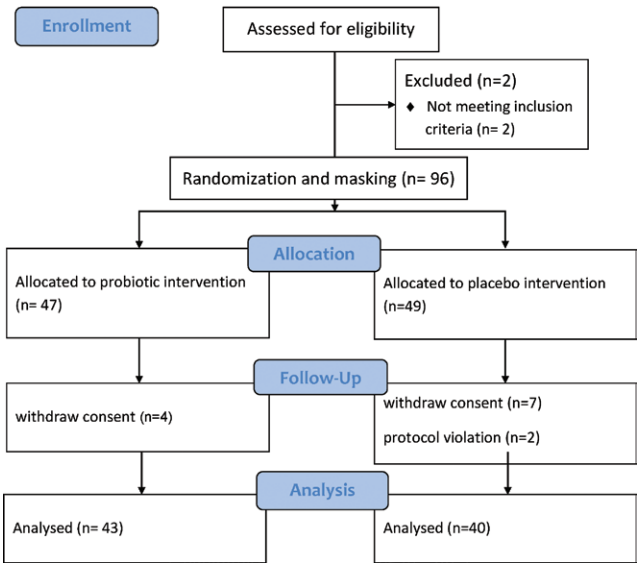

Figure 1. Consort of flow diagram.

2.6. Intervention

All recruited newborns with jaundice were randomly assigned to 2 groups via computer-generated random numbering with double blinding allocation (Fig. 2): experimental and control groups. The experimental group received phototherapy (wavelength 425–457 nm; BiliBed Medela Phototherapy Lamp, Switzerland) and adjuvant probiotic intervention (*B. animalis* CP-9). The control group received phototherapy with placebo (maltodextrin). Probiotic strain *B. animalis* CP-9 (5×10^9 CFU/capsule; 2 capsules per day) and placebo (2 capsules per day) were blindly distributed to participating infants. The probiotic and placebo capsules were prescribed twice a day and added to breast milk or formula milk. The blinding procedure was descripted as follows: the probiotics provider (Glac biotech Co., Ltd., Taiwan) prepared the probiotic capsules and placebo capsules into the same format plastic zipper bag. The plastic zipper bag filled with the probiotic capsules were labeled symbol # on the top of the bag. The plastic zipper bag filled with the placebo capsules were labeled symbol % on the top of the bag. The probiotic provider offered the labeled plastic zipper bags to the clinical administrative staff without telling the grouping. Then, the clinical administrative staff blindly and randomly distribute the plastic zipper bag filled with the placebo or probiotic capsules to the participants.

All babies were monitored for bilirubin levels, adverse reactions, such as vomiting, diarrhea, or bloating. Conventional phototherapy was performed according to the guideline of the American Academy of Pediatrics monogram for neonatal hyperbilirubinemia treatment[22] and Taiwan society of neonatology (http://www.tsn-neonatology.com/health/). The intensity of phototherapy was ≧ 30 μW/cm²/nm. During the phototherapy, the distance between LED light tube and patients who covered with eye masks and diapers was around 30 cm. The clinician stopped phototherapy while the bilirubin levels decreased 3 mg/dL by comparing to the baseline. The adjuvant probiotic intervention was provided until patients

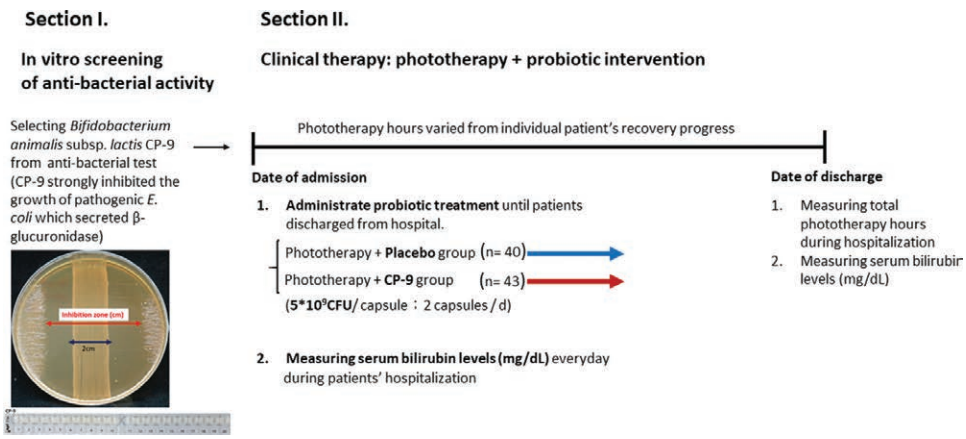

Figure 2. Experimental design. The double headed arrow in blue is schematic probiotic seeding area. The double headed arrow in red is schematic inhibition zone for pathogenic bacteria.

Table 1

Basic information of the participants (before treatment).

	CP-9	Placebo	*P* value
N	43	40	-
Gender (M/F)	20/23	25/15	.187
Birth weight (g)	3055.70 ± 50.12	3114.38 ± 54.99	.43
Gestational age (wks)	38.0 ± 0.20	37.88 ± 0.18	.64
Baseline serum bilirubin (mg/dL)	16.78 ± 0.41	16.13 ± 0.41	.267
Endpoint serum bilirubin (mg/dL)	11.27 ± 0.21	11.04 ± 0.19	.417
Duration of hospital stay (hours)	62.20 ± 3.93	65.42 ± 3.87	.561
Delivery mode (NSD/C/S)	38/5	35/5	.999
Number of hospital discharges (24-48 h, N)	22	14	.184
Number of hospital discharges (48-72 h, N)	15	21	.125
Number of hospital discharges (>72 h, N)	6	5	.999

P values of <.05 were considered significant. The statistic result revealed no significant difference between the probiotic treatment and placebo groups.

were stopped receiving phototherapy. Besides, previous study has demonstrated the safety use of the *B. animalis* CP-9[23] and there were no clinical symptom or sign recorded in this clinical trial.

2.7. SBL measurements

Using the heel stick method (BD Quikhee, Becton Dickinson, NJ,), 300 μL of whole blood was obtained from infants. The SBL was quantified using a capillary tube-directed optics color method with a bilirubin meter (APEL BR-5200 spectrophotometry, Saitama, Japan) at the CMUH laboratory. The protocol for measuring serum bilirubin was according to a previous study.[24] The experiment was performed by experienced technicians, and therefore, SBL was measured at an accuracy of ± 0.5 mg/dL.

Serum bilirubin decline efficiency = ΔSerum bilirubin/ phototherapy duration

ΔSerum bilirubin = SBL on the date of admission – SBL on the date of discharge

2.8. Statistical analyses

The research data were based on IBM SPSS for analytical statistics. The Kolmogorov-Smirnov test was used to analyze the normality of the dataset. The sample size calculation was analyzed according to previous study.[20] Normally distributed data were analyzed using Student's *t* test 2-tailed assay. Repeated measures were performed to obtain changes in bilirubin levels before and after intervention. The independent *t* test was applied for the comparison of bilirubin levels and phototherapy duration between the experimental and control groups. *P* values <.05 were considered significant.

3. Results

3.1. B. animalis CP-9 exhibited strong E. coli inhibition ability in the in vitro antibacterial test

This study included 2 major sections: screening the potential of the probiotic strain with an in vitro antibacterial test and clinical trial (Fig. 2). *B. animalis* CP-9, *B. longum* L-337, *L. salivarius* L-1, *L. johnsonii* L-3, and *L rhamnosus* L-134 presented inhibition zones of 5.3, 4.3, 3.4, 2.8, and 3.1 cm, respectively. Thus, among the 5 probiotic strains, *B. animalis* CP-9 showed the strongest antibacterial activity (see Fig. S1, http://links.lww.com/MD/H577, which demonstrates the antibacterial activity of CP-9). Furthermore, API ZYM results revealed that none of the 5 strains produced β-glucuronidase (see Fig. S2, http://links.lww.com/MD/H578, which demonstrates the API ZYM results; see Table S1, http://links.lww.com/MD/H579, which demonstrates the API ZYM reading table). Therefore, CP-9 was selected as the candidate probiotic strain for NJ treatment.

3.2. Basic characteristics of the participants

In this study, 98 newborns with jaundice were enrolled. However, 15 participants were excluded, and finally, 83 newborns with jaundice participated in this clinical trial. The participants were randomly assigned to the experimental group (probiotic *B. animalis* in combination with phototherapy; N = 43) and control group (phototherapy plus placebo; N = 40) (Fig. 1). The demographic and clinical characteristics of the participants are presented in Table 1. No significant difference was observed in serum bilirubin, hospital stay period, sex, delivery mode, birth weight, gestational age, and number of hospital discharges between the 2 groups.

3.3. Probiotic B. animalis facilitated the reduction in serum bilirubin and phototherapy duration among all participants

Figure 3 presents the clinical effect of probiotic *B. animalis* combined with phototherapy on bilirubin metabolism in comparison with the placebo. The rate of serum bilirubin decline of the experimental group was significantly higher (–0.16 ± 0.02 mg/dL/h; 95% CI -0.12 to -0.2; *P* = .009) than that of the control group (–0.10 ± 0.01 mg/dL/h; 95% CI -0.08 to -0.12; Fig. 3a). The duration of phototherapy was significantly reduced in the experimental group (44.82 ± 3.23 h; 95% CI 51.3–38.2; *P* = .011) compared with the control group (57.86 ± 3.83 h; 95% CI 65.6–50.1; Fig. 3b). Moreover, the serum bilirubin decline rate in the experimental group (–0.13 ± 0.01 mg/dL/h; 95% CI -0.09 to -0.17; *P* = .03) significantly increased compared with the control group (–0.08 ± 0.01 mg/dL/h; 95% CI -0.05 to -0.11) during the first 24 h but revealed no difference during 24 to 48 and 48 to 72 hour between these 2 groups (Fig. 3c).

3.4. Probiotic B. animalis facilitated serum bilirubin decline in the low-risk group (Full-term newborns with jaundice)

We further analyzed the influence of probiotic *B. animalis* in the high- and low-risk subgroups. The result revealed that the initial bilirubin levels before treatment and hospital stay hours did not statistically differ between the 2 risk groups (Table 2). After therapeutic intervention, the serum bilirubin decline rate was higher in the experimental group (–0.18 ± 0.02 mg/dL/h; 95% CI -0.12 to -0.23) than in the control group (–0.10 ± 0.01 mg/dL/h; 95% CI -0.07 to -0.12;*P* = .014) among low-risk participants (Fig. 4a), but high-risk participants showed no significant change in serum bilirubin (high-risk experimental group:

–0.13 ± 0.02 mg/dL/h, 95% CI -0.17 to -0.07; high-risk control group: –0.10 ± 0.02 mg/dL/h, 95% CI -0.06 to -0.14; *P* = .415; Fig. 4b).

3.5. Probiotic B. animalis reduced phototherapy duration in the low-risk group

Probiotic *B. animalis* reduced phototherapy duration (43.17 ± 22.72 h; 95% CI 51.9–34.3; *P* = .019) compared with placebo (58.54 ± 23.07 h; 95% CI 68.2–48.8) in low-risk NJ participants (Fig. 4c), but high-risk NJ participants showed no significant serum bilirubin change on treatment with probiotic *B. animalis* (high-risk experimental group: 47.91 ± 18.38 h, 95% CI 58.08–37.7; high-risk control group: 56.84 ± 26.55 h, 95% CI 70.9–42.6; *P* = .288; Fig. 4d).

4. Discussion

In our study, probiotic *B. animalis* CP-9 in combination with phototherapy significantly decreased the serum bilirubin decline rate and phototherapy duration in the experimental group compared with the control group. Additionally, the probiotic strain effectively benefited the serum bilirubin decline rate in combination with phototherapy in the first 24 h. *E. coli* has been reported to facilitate the production of urobilin in vitro and β-glucuronidase.[16,25] Thus, we screened probiotic candidates for NJ treatment based on their anti-*E. coli* activity. Section 1 of our study (in vitro screening part; Fig. 2) reveals that the probiotic *B. animalis* CP-9 efficiently inhibited the growth of *E. coli*. A semiquantitative miniaturized system, API ZYM strip, was used to determine 19 enzymatic activities.[26] The API ZYM test revealed that *B. animalis* CP-9 secretes minimal amounts of β-glucuronidase (Fig. S2). Therefore, probiotic *B. animalis* CP-9 was used to study the clinical benefit of probiotics in NJ. Besides, the safety used of the probiotic *B. animalis* CP-9 has been reported in previous study.[23]

According to strict medical order, the clinical staff regularly supply probiotic or placebo capsules to all participated newborn. Therefore, the percentage of treatment compliance rate should be 100%. During study period, all recruited newborns were monitored for bilirubin levels, adverse reactions, such as vomiting, diarrhea, or bloating. There were no clinical symptoms or signs observed in this clinical trial. Thus, the rate of adverse effects was 0%. Moreover, the dropout rate (13.54%; 13/96) fell into the pre-estimated dropout range (20%), which didn't affect the precision and reliability of this per-protocol analysis (PP) study. Further analysis indicated that probiotic

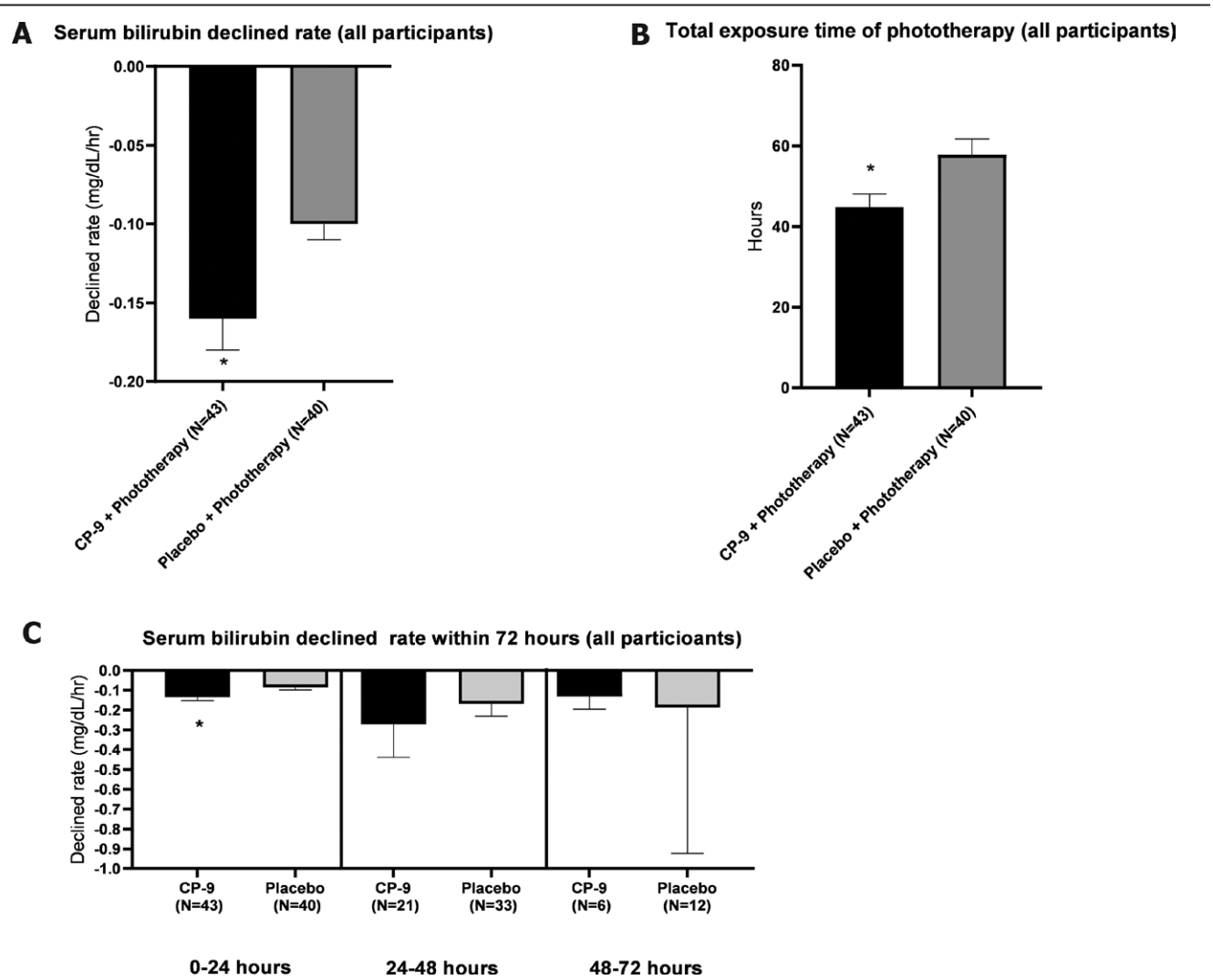

Figure 3. Probiotic supplementation decreased the (a) serum bilirubin declined rate, (b) phototherapy duration, and (c) serum bilirubin decline rate at 24, 48, and 72 h among the participants. The independent *t* test was applied for comparison of bilirubin levels and phototherapy duration between the experimental and control groups. *P* values <.05 (*) were considered significant. Serum bilirubin declined rate = ΔSerum bilirubin/ phototherapy time; ΔSerum bilirubin = Serum bilirubin level date of admission - Serum bilirubin level date of discharge.

Table 2

Basic information of the high- and low-risk participants (before treatment).

	High risk$		Low risk$	
Probiotic intervention	***B.* CP-9**	**Placebo**	***B.* CP-9**	**Placebo**
N	15	16	28	24
Baseline serum bilirubin (mg/dL)	16.93 ± 0.71	16.24 ± 0.63	16.70 ± 0.51	16.07 ± 0.59
Duration of hospital stay (h)	61.73 ± 5.05	68.34 ± 8.65	62.45 ± 5.46	65.10 ± 3.80

$High-risk group: gestational age of 35 to 37 weeks; Low-risk group: gestational age of 38 to 40 weeks without other complications.

The independent *t* test was applied for comparison of N numbers, bilirubin levels, and phototherapy duration between high- and low-risk groups. *P* values of <.05 were considered significant. The statistic result revealed no significant difference between the high- and low-risk groups.

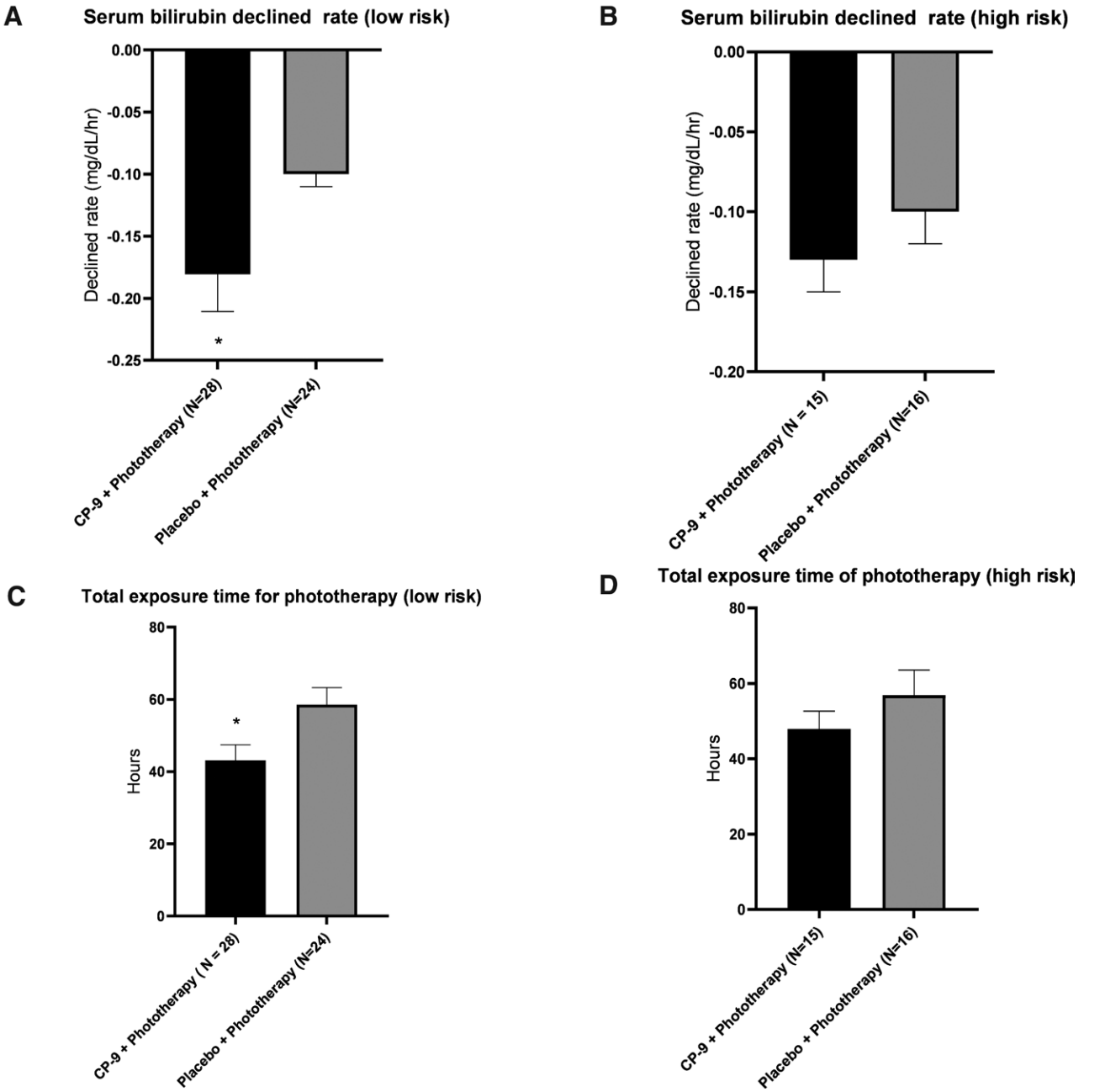

Figure 4. Probiotic supplementation suppressed serum bilirubin decline rate in (a) low-risk participants (full-term newborns with gestational age of 38–40 weeks without other complications) and (b) high-risk participants (preterm newborns with gestational age of 35–37 weeks). Probiotic supplementation decreased phototherapy duration in (c) low-risk participants and (d) high-risk participants. The independent *t* test was applied for comparison of bilirubin levels between the experimental and control groups. *P* values <.05 (*) were considered significant. Serum bilirubin declined rate = ΔSerum bilirubin/ phototherapy time; ΔSerum bilirubin = Serum bilirubin level date of admission - Serum bilirubin level date of discharge.

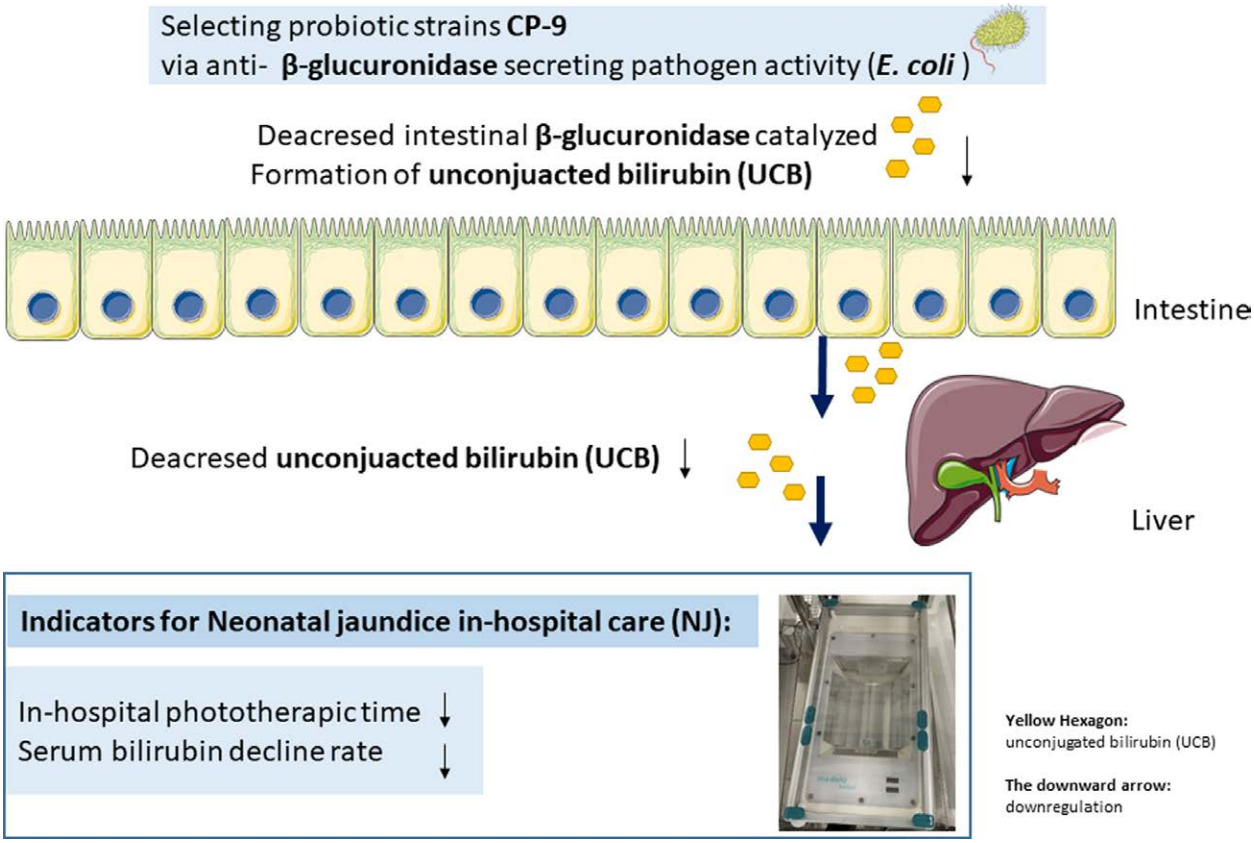

Figure 5. The hypothesis of probiotic CP-9 improved the in-hospital care for neonatal jaundice. Thew yellow hexagon represents unconjugated bilirubin (UCB); The downward arrow represents downregulation.

supplementation was not advantageous in high-risk newborns with NJ. However, it worked in synergy with phototherapy in low-risk newborns with NJ. The reason for probiotic *B. animalis* CP-9 not benefiting high-risk newborns with NJ is unclear. A large sample size of high-risk newborns should be recruited and analyzed in future. Additionally, researchers have reported that preterm infants have weaker physiological and metabolic functions than full-term newborns.[27] Henderick et al hypothesized that preterm infants with an immature gastrointestinal tract would have a poor gut microbiota immune system, which may require high nutritional supplementation to achieve optimal feeding for the growth and development of preterm infants.[28]

No clinical evidence indicates that probiotics can directly metabolize bilirubin. Researchers suggest that the major mechanism by which probiotics decrease SBL is through the reduction of intestinal urobilinogen mixtures, such as urobilinogen, mesobilirubin, half-stercobilinogen, stercobilinogen, dihydrobilirubin, and dihydromesobilirubin. Besides, it's reported that the lower activity of mucosal β-glucuronidase and higher amount of gut flora lead to more effective bilirubin metabolism.[29] In a rat jaundice model, a *Bifidobacterium* strain metabolized bilirubin directly, which correlated with the activity of oral β-glucuronidase.[30] Probiotics may accelerate bilirubin metabolism through the suppression of bile acid reabsorption into enterohepatic circulation.[31] The levels of microbiota changes, urobilinogens and β-glucuronidase in feces should be measured between the 2 groups in the future.

Probiotics with a long history of safe use in foods or those obtained from healthy gastrointestinal tracts are unlikely to harm the human body, such as *Lactobacillus* species (*acidophilus*, *casei*, *fermentum*, *gasseri*, *johnsonii*, *paracasei*, *plantarum*, *rhamnosus*, and *salivarius*) and *Bifidobacterium* species (*adolescentis*, *animalis*, *bifidum*, *breve*, and *longum*).[32] Studies have reported various functions of *B. animalis* CP-9, such as inhibition of extended-spectrum β-lactamase–producing *E. coli*[33]; inhibition of pathogenic group B *Streptococcus*, which is highly correlated with newborn survival rate[34,35]; suppression of pathogenic *Staphylococcus aureus*, which may cause infant atopic dermatitis[36,37]; protection against oral pathogens to improve oral immunity in subhealthy adults[38,39]; and reduction of inflammation and glycemic index in a type 2 diabetes animal model.[40] In the present study, food-grade probiotic strain *B. animalis* CP-9 in synergy with phototherapy was effective in NJ treatment.

The results of the present study indicated that *B. animalis* CP-9 reduces phototherapy duration. Studies have revealed that phototherapy involves a risk of hypocalcemia.[41–43] Thus, the risk of hypocalcemia induced by long courses of phototherapy maybe decreased by shorting the duration of phototherapic time through supplementation with the supplementation of *B. animalis* CP-9, particularly in the first 24 h of the therapeutic period. However, several limitations of study should be discussed. For example, the experiment should be tested more different dosages of probiotic supplementation; it should record and report the feeding and digestive conditions of the participated newborns; it's suggested the administrative staff to track the long-term neuro-pathogenic condition of the participated NJ newborns in the future study; The alteration of intestinal microbiota and metabolomic profile by probiotic intervention should be tested in future. Moreover, food-grade probiotic strains (other probiotic strains in combination with *B. animalis* CP-9) may be evaluated in preterm newborns with jaundice.

In conclusion, this study demonstrated that *B. animalis* subsp. *lactis* CP-9 facilitates serum bilirubin decline, particularly in the first 24 h of hospital care, and reduces phototherapy duration in NJ. Especially, the *B. animalis* subsp. *lactis* CP-9 in synergy with

phototherapeutic treatment among full-term newborns with jaundice (Fig. 5).

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