Research Square

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Effect of Phototherapy on the Structure of the Periocular Skin Microbiota of Jaundiced Newborns Wearing Different Eye Patches

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Research article

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Abstract

Background Phototherapy is a very common method to treat jaundice.This study explored whether phototherapy affected the periocular skin microbiota of newborns and whether the use of different eye patches during phototherapy had different effects on the microbiota.

Methods Twenty neonates with jaundice were randomly divided into experimental (using modified eye patches) and the control (using conventional eye patches) groups. Skin samples around the eyes were collected before phototherapy and 24h and 48h after phototherapy, and 16SDNA high-throughput sequencing was performed to identify bacterial strains in the microbiota in different time periods.

Results No significant difference was found in the basic information, including age, gestational age, body weight, sex, decrease in the bilirubin level, and conjunctivitis, between the two groups ($P > 0.05$). Also, no significant difference was observed in the abundance of different bacterial strains in the control group before and after phototherapy ($P > 0.05$). In the control group, the first three strains in the periocular skin were mainly Staphylococcus, Streptococcus, and Corynebacterium. In the experimental group, the colony abundance in the periocular skin decreased significantly 48 h after phototherapy ($P < 0.05$); the abundance of some bacteria, such as Enterococcus or Enterobacter, around the eyes decreased significantly ($P < 0.05$). The skin around the eyes in the experimental group was also dominated by staphylococci and streptococci before and after phototherapy.

Conclusion Phototherapy can change the flora of the skin around the eyes of newborns.The skin of newborns who wore conventional eye patches exhibited no significant change in the abundance of bacterial strains after phototherapy, but a significant decrease was observed in those who used improved eye patches.

Introduction

Neonatal jaundice is a common disease. Studies have reported that the incidence of jaundice in full-term infants and premature infants is 50% and 80%, respectively [1]. Blue light therapy is a common clinical method for treating neonatal hyperbilirubinemia. However, some adverse reactions of blue light include fundus retinal degeneration. Hence, it is necessary to make infants wear an effective eye patch during phototherapy to prevent damage to the retina [2,3].Studies have shown the short- and long-term side effects of phototherapy. The short-term effects are interference in the mother–infant relationship, imbalance in temperature and water electrolysis, skin damage, paralytic intestinal obstruction in bronze baby syndrome, opening of ductus arteriosus, and circadian rhythm disorders. The long-term effects include allergic diseases such as asthma, rhinitis, conjunctivitis, and so forth [4]. Phototherapy may cause skin lesions, such as macula, papules, and macular papules, including periocular skin, and damage the retina. Therefore, it is necessary to make newborns wear eye patches or eye masks during phototherapy to avoid retinal damage. However, patches may cause periorbital skin irritation, further leading to an eye infection. Yurdakok found that the incidence of conjunctivitis significantly increased during phototherapy. Eye diseases and eye mask friction or phototherapy led to increased allergic reactions[5]. Whether these allergic reactions are related to the changes in the skin microbiota around the eyes during phototherapy is not clear.

Parasitic bacteria are common in all parts of the human body. They are widely found in the skin, oral cavity, nasopharynx, eye conjunctiva, external auditory canal, intestinal tract, vagina, and other corresponding parts of the human body[6]. These bacteria can invade the eye conjunctiva of newborns via any mode of contact. A number of studies have shown that the diversity of skin microorganisms is associated with many diseases [7-9]. The conjunctival bacteria that lead to neonatal conjunctivitis develop after birth, and the pathogenicity of these microorganisms affects the development of conjunctival lesions. Many studies have shown that *Staphylococcus* is the main skin pathogen. Many of these bacteria come from the mother's birth canal, or they are a result of improper care of the newborn skin postnatally[10]. After birth, different bacterial strains colonize in the skin microbiota around the eyes, which may be the direct source of neonatal conjunctivitis. In recent years, high-throughput DNA sequencing has enhanced the understanding of the diversity of microbiota in various parts of the body [11]. The bacterial genome is relatively small, usually with only one circular DNA and plasmid. High-throughput sequencing helps understand all its genetic information. DNA sequencing has also become one of the important means of microbial research, providing relevant information regarding the genetic evolution of bacteria, disease prevention and treatment, and development of vaccines and antibiotics [12-14]. The development of modern technology provides good technical support for the in-depth exploration of skin microbiota around the eyes of newborns. A light-emitting diode (LED) is the common light source (a cold light source) used in phototherapy 15. Whether this kind of phototherapy can affect the changes in the children's skin microbiota, especially around the eyes, and whether the local temperature and humidity around the eyes will be changed with eye patches during phototherapy remain unexplored.

In this study, 16SDNA high-throughput sequencing was used to analyze the effect of conventional phototherapy on the microecology of neonatal periocular skin. At the same time, different eye patches were used to observe the changes in bacterial diversity in the periocular skin, so as done to compare the characteristics of bacterial communities on the periocular skin in the two groups, thus providing a theoretical basis for the rational use of protective eye patches in clinical phototherapy.

Methods

Study design

In this cohort study, patients were selected between October 2018 and March 2019 in the Department of Neonatal Pediatrics, East Hospital of Shanghai Sixth People's Hospital. Full-term newborns with jaundice who needed phototherapy were randomly enrolled. The changes in the periocular skin microbiota were observed after using different eye patches.

Clinical study registration

This study has been registered in the Chinese Clinical Trial Register.The registration number is ChiCRT2000031992.

Study participants

Participants included those needing hospitalization for Jaundice and full-term newborns. The jaundice and phototherapy diagnostic criteria mentioned in the jaundice diagnostic guidelines were used [15]. The inclusion criteria were as follows: (1) gestational age 37–40 weeks; (2) mother and baby transferred to the same room within 7 days of birth; (3) whole-body skin intact, no rash, no redness, no swelling, and no secretions; and (4) phototherapy ≥two times and phototherapy duration ≥12 h.

The exclusion criteria were as follows: patients with birth weight less than 2500 g, infection requiring antibiotics, genetic and metabolic diseases, congenital malformations, and severe complications. The study flow diagram is shown in Figure 1.

Randomization

All patients were divided into two groups according to the principle of complete randomization and by the nonblind method. The digital random method was used to set up the control and experimental groups. The traditional eye patch was used in the control group, and the improved eye patch was used in the experimental group.The lab assistant is responsible for recording and distributing all random numbers.

Interventions

Phototherapy: Both groups needed continuous phototherapy. Samples were collected immediately after phototherapy and 24 h and 48 h after phototherapy. Choice of phototherapy box and phototherapy lamp tube: For LED cold light source treatment, the wavelength of the therapeutic apparatus was set to 475 nm. The effective irradiation area was 40×20 cm^2, and the maximum radiation intensity was 42 $\mu W/cm^2$. The noise was below 60 dB. During the treatment, the lamp holder was placed 40 cm away from the skin of the child. All instruments and equipment were of a unified standard and the same brand. Operation before phototherapy: Both groups received routine care before phototherapy, including preheating the phototherapy box, setting an appropriate box temperature, taking samples before entering the box, applying powder and oil on the skin, cutting nails, and protecting the perineum with phototherapy diapers. The other parts were exposed, and the changes in vital signs were monitored and recorded. The staff cleaned their hands and wore patches during the operation. Phototherapy operation: Control group: Before phototherapy, the medical eye mask was directly covered and fixed with a 3M tape, and the same eye mask was used for phototherapy; the mask was disinfected with alcohol before each use. Experimental group: An 8P skimmed gauze 6 × 8 cm^2 in size, was used as a disposable medical dressing before phototherapy. During phototherapy, the eye patch of the appropriate type was selected according to the size of the head and eye distance. The eye patch was folded and wrapped in a small piece of gauze. The size of the gauze was the same as that of the mask. It was flattened and fixed with a 3M tape. The patch was then covered on both sides with the gauze. A specific eye patch is shown in Figure 2.

Data collection

1. Basic Information

The basic information of hospitalization in the two groups included the age, gestational age, body weight, mode of production, sex, Apgar (5 min), laboratory bilirubin levels before and 24 h and 48 h after phototherapy, and the occurrence of conjunctivitis.

2. Sample collection and microorganism examination and analysis

2.1 Sample collection and retention

Collection: The samples were collected three times around the eyes of each patient: before the first phototherapy, 24 h after the first phototherapy, and 48 h after the second phototherapy. Retention: A sterilized cotton swab was dipped immediately into a small amount of normal saline and rubbed on the skin around the eyes once in a circle from the inside to the outside for more than 15 s [16]. The cotton swab was put into the specimen, kept in the test tube, and frozen in the refrigerator at –80°C immediately.

2.2 High-throughput sequencing

The 16SrRNA high-throughput sequencing technique was used to analyze the changes in the periocular skin microbiota in the two groups before phototherapy, after the first phototherapy, and after the second phototherapy, so as to understand the effect of a phototherapy eye mask on the periocular skin microbiota. The concentration and purity of total bacterial DNA were analyzed using a periocular skin bacterial DNA kit, and the integrity of DNA was detected by agarose gel electrophoresis. The 16SrDNA hypervariable region was amplified using primers.PCR amplification was completed, and the amplification effect of PCR products was detected by agarose gel electrophoresis. The bacterial DNA was sequenced by PCR-DGGE. The sequencing results were compared and analyzed using Blast (http://www.ncbi.nlm.nih.gov/BLAST) on NCBI.

Outcomes

The primary outcome was the difference in the abundance of bacterial strains in the microbiota 24 h and 48 h after phototherapy. The secondary outcome was the decrease in bilirubin levels and conjunctivitis in the two groups before and after phototherapy.

Statistical analysis

Data were analyzed using statistical software SPSS23. The normal data were expressed as mean ± standard deviation. The *t* test, analysis of variance, nonparametric rank-sum test, and *c*2 test were used to analyze the quantitative or qualitative data.

Results

1. Basic details

No significant difference was found in age, gestational age, body weight, mode of production, sex, bilirubin levels, and conjunctivitis between the two groups, as shown in Table 1 ($P > 0.05$).

Table 1. Comparison of the basic situation between the experimental and control groups

	Control group	Experimental group	*P* value
Postpartum days	3.8 ± 1.2	3.9 ± 1.8	＞0.05
Gestational days	38.4 ± 1.5	38.71 ± 0.89	＞0.05
Birth weight	3226.1 ± 493.1	3269 ± 293.6	＞0.05
Mode of birth (vaginal/cesarean section）	8/2	7/3	＞0.05
Male/Female	8/2	7/3	＞0.05
APGAR score(5 min）	10 ± 0	10 ± 0	＞0.05
Bilirubin level before and after phototherapy (mg/dL)	21 ± 3.2/10 ± 1.2	22 ± 2.2/8 ± 2.2	＞0.05
Conjunctivitis	1	0	＞0.05

2. Comparison of the periocular skin microbiota between the experimental group (Ta) and the control group (Ca) before phototherapy

2.1. Abundance of different bacterial strains in the periocular skin microbiota in the two groups before phototherapy

No significant difference was observed in the abundance of different bacterial strains in the periocular skin microbiota between the two groups before phototherapy ($P > 0.05$), as shown in Figure 3.

2.2 Most abundant bacterial strains in the periocular skin microbiota in the two groups before phototherapy

Before phototherapy, the top 20 bacterial strains in the periocular skin microbiota of the 2 groups were staphylococci and streptococci, as shown in Figure 4A and 4B.

3. Changes and comparison of microbiota in the control group after phototherapy

3.1 Abundance of different bacterial strains in the periocular skin microbiota in the control group after phototherapy

The abundance of (Cc) periocular colonies in the control group 24 h after phototherapy (Ca) and 48 h after phototherapy (Cb,) is shown in Figure 5A. It displayed an increasing trend (Fig. 5B) but with no statistically significant difference ($P > 0.05$).

3.2 **Most abundant bacterial strains in the periocular skin microbiota in the control group after phototherapy**

The first 20 genera around the eyes after phototherapy in the control group were also dominated by staphylococci and streptococci, as shown in Figure 6A and 6B.

4. Changes and comparison of microbiota in the experimental group after phototherapy

4.1 **Abundance of different bacterial strains in the periocular skin microbiota in the experimental group after phototherapy**

A significant difference was found in the abundance of (Tc) periocular colonies in the experimental group 24 h after phototherapy (Ta) and 48 h after phototherapy (Tb); the colony count decreased significantly 48 h after phototherapy ($P < 0.05$) (Fig. 7A and 7B).

4.2 **Most abundant bacterial strains in the periocular skin microbiota in the experimental group after phototherapy**

Staphylococci and streptococci were the main bacteria in the first 20 genera around the eyes after phototherapy in the experimental group. The abundance of genera (A) and species (B) decreased 48 h after phototherapy, as shown in Figure 8.

5. Comparison of the microbiota between the experimental and control groups after phototherapy

5.1 Abundance of different bacterial strains in the periocular skin microbiota in the two groups after phototherapy

The abundance of bacterial strains in the periocular skin microbiota in the experimental group followed a downward trend, with a statistically significant difference ($P < 0.05$) (Fig. 9).

5.2 **Most abundant bacterial strains in the periocular skin microbiota in the experimental and control groups after phototherapy**

The first 20 genera in the 2 groups were mainly staphylococci and streptococci, as shown in Figure 10C and 10D.

Discussion

Neonatal jaundice is a common disease in neonatal pediatrics. In clinic, it is not necessary to take measures if jaundice is mild. If the condition is serious, it affects the health of newborns and hence corresponding measures need to be taken. The current treatment methods include phototherapy, drugs, blood exchange, and so on; phototherapy is the most effective treatment at present [17]. The wavelength of light used in phototherapy is most likely to cause damage to the retinal macula. Also, long-term therapy using a strong light can significantly increase the likelihood of conjunctivitis in children [18]. At present, phototherapy protective measures to reduce this side effect include wearing eye patches. A previous study found that phototherapy significantly affected the composition of neonatal intestinal microbiota. The present study explored whether phototherapy might affect the structure of the periocular skin microbiota in newborns who wore protective eye mask during phototherapy and whether different methods of using protective eye patches also affected the periocular skin microbiota. The method of high-throughput sequencing was used to analyze the effect of phototherapy on the periocular skin microbiota of newborns and understand whether the improved eye mask could affect the composition of the ocular skin microbiota.

In this study, the two groups of newborns were kept mainly in the same room with mothers. No statistically significant difference was found between the groups in the abundance of different bacterial strains in the skin around the eyes before phototherapy. The first 20 bacterial strains in the skin of the 2 groups mainly came from the mother and the surrounding environment.Different bacterial strains, mainly staphylococci, streptococci, enterobacteria, and so on, were found in the skin of newborns,which was consistent with the findings of Conlan [19] report. Also, bacteria such as *Lactobacillus* and *Bifidobacterium* may play an important role in maintaining the stability of the neonatal microbiota.

High-throughput sequencing showed that the abundance of different bacterial strains in the periocular skin microbiota of the control group had no significant change before and after phototherapy. In the control group, the number of bacteria increased after phototherapy, but with no statistical significance. This indicated that phototherapy had little effect on the overall skin microbiota around the eyes. However the abundance of different bacterial strains in the microbiota increased progressively with the extension of time. Staphylococci and streptococci were dominant. The abundance of bacterial strains in the periocular skin microbiota increased significantly at the genus level (*Pseudomonas* and *Spirulina*) after phototherapy, while the abundance of defective bacteria changed significantly at the species level. The bacterial strain *Alister* found in the oral cavity, is a Gram-negative coccus, which is difficult to culture and closely related to dental pulp disease and periapical periodontitis [20]. Alternatively, *Pseudomonas* is a Gram-negative bacillus mostly found in marine environments. *Pseudomonas aeruginosa* can significantly inhibit the corrosion of common metals in seawater [21], while MB117 has the ability to produce alkaline cellulose [22]. Spirochetes play a certain role in regulating food allergy. These bacteria are relatively rare [23]. Whether the increase in the abundance of bacteria has an impact on the body needs to be further investigated later.

High-throughput sequencing in the experimental group found that the colony abundance in the periocular skin decreased significantly after phototherapy, following the use of the improved eye mask, indicating that the use of the improved eye mask in phototherapy might inhibit the growth of some bacteria around the eyes. The main bacteria and strains before and after phototherapy were staphylococci, *Pseudomonas*, *Corynebacterium*, *Streptococcus*, and *Haemophilus*. The abundance of bacteria, including *Clostridium*, enterococci, or enterobacteria, decreased significantly because these bacteria were more sensitive to phototherapy. At the same time, the use of improved eye patches can significantly inhibit the growth of these bacteria, reduce the possibility of skin infection, and maintain the stability of the skin microbiota around the eyes. Phototherapy can change the skin colonization around the eyes of newborns, while the use of improved eye patches can reduce the abundance of harmful bacteria, keep the relative number of beneficial bacteria around the eyes stable, and decrease the possibility of skin or eye infection after phototherapy.

A statistically significant difference was observed in the number of colonies between the experimental group and the control group after phototherapy. The abundance of bacteria, such as *Staphylococcus*, *Streptococcus*, and *Corynecoccus*, followed a decreasing trend after phototherapy in the experimental group, while the abundance of *Pseudomonas* and *Haemophilus* increased. The abundance of major bacterial strains in the skin remained unchanged; staphylococci and streptococci were still dominant. In the experimental group, improved eye patches were used at the time of phototherapy to reduce the abundance of these bacterial strains. The change in abundance at the species level was similar to that at the genus level. After phototherapy, the experimental group also showed an increase in the abundance of some bacteria, such as *Proteus*, S*phingomycetes*, and *Flavobacterium*. However, no clinical effect on newborns was found because the rate of increase in abundance was quite low. The number of prominent bacteria in the experimental group was also significantly lower than that in the control group, suggesting that the use of improved eye patches was more beneficial to maintain the stability of the flora around the eyes of newborns treated with phototherapy, thus relatively reducing the possibility of skin and eye infections in newborns.

This study was novel in exploring the changes in the periocular skin microbiota of newborns during phototherapy. It aimed to analyze the effect of two kinds of eye patches on the periocular skin flora of newborns after phototherapy. Phototherapy was found to have no significant change in the number of bacteria in the periocular skin of newborns who wore conventional eye patches. However a significant decrease was observed in the number of bacteria around the eyes of newborns using improved eye patches. The study indicated that more attention should be paid to the selection and application of eye patches in clinical phototherapy. The study also found that the common colonizing bacteria around the eyes of newborns were less affected by the changes in phototherapy. However, some rare bacteria showed statistically significant changes, but these changes had no significant clinical impact on newborns because the number of these rare bacteria was low. This study showed that phototherapy might increase the number of some rare bacteria. Attention should be paid to the hygiene and cleanliness of the eyes during phototherapy. An improved eye mask can be used or a new eye mask can be used every time to keep the skin around the eyes of newborns safe during phototherapy, reduce the effect on the periocular skin microbiota, and decrease the possibility of skin and eye infections among newborns.

This study had some limitations. It was a cohort observational study. The sample size was relatively small, and the time of phototherapy was relatively short. Further, the conclusion could reflect only the changes in the flora around the eyes of newborns under the current conditions. The length of the recovery time for the microbiota in the later stage needs further follow-up research. However, the findings might provide a theoretical basis for clinical studies on phototherapy and eye patches in the future.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of the East Hospital of Shanghai Sixth People's Hospital. Parents or guardians of the infants provided written informed consent before enrollment in the trial.

Consent for publication

All the authors agreed to publish

Availability of data and material

The authors are willing to disclose all the original data

Competing interests

There are not any potential conflict of Interest.There were not any form of honorarium, grant, payment given to Minling Mo and Anping Lv as co-first authors.

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Authors' contributions

Minling Mo, and Anping Lv: performed data curation (lead) and was involved in formal analysis (lead), methodology (lead), writing of the original draft (lead), and editing and review.

Yanan Ma: participated in data curation (supporting), methodology (supporting), writing of the original draft (supporting).

Sainan Fan: was responsible for methodology (supporting), resources (supporting), and writing and review of the manuscript (supporting).

Kun Zhang : was responsible for methodology (supporting), resources (supporting), supervision (supporting), and writing, review, and editing of the manuscript (supporting).

Jingping Zhang: was responsible for funding acquisition (lead), investigation (lead), resources (lead), supervision (lead), writing of the original draft (lead), and writing, review, and editing of the manuscript (lead).

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Declaration of interest:

Author Dr. Hsieh Pei-Shan was employed by the company Glac Biotech Co., Ltd., Taiwan, China; she does not have any interest in the subject. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Bhutani VK, Stark AR, Lazzeroni LC, Poland R, Gourley GR, Kazmierczak S, Meloy L, Burgos AE, Hall JY, Stevenson DK, et al.: **Predischarge screening for severe neonatal hyperbilirubinemia identifies infants who need phototherapy**. *J Pediatr* 2013, **162**:477-482 e471.
2. Silva L, da Silva FS, Turiani M, Juliani CM, Spiri WC: **Development of an eye protector for phototherapy on newborns: a technology**. *Rev Lat Am Enfermagem* 2008, **16**:47-51.
3. Xiong T, Qu Y, Cambier S, Mu D: **The side effects of phototherapy for neonatal jaundice: what do we know? What should we do?***Eur J Pediatr* 2011, **170**:1247-1255.
4. Faulhaber FRS, Procianoy RS, Silveira RC: **Side Effects of Phototherapy on Neonates**. *Am J Perinatol* 2019, **36**:252-257.
5. Yurdakok M: **Phototherapy in the newborn: what's new?***Journal of Pediatric and Neonatal Individualized Medicine* 2015, **4**.
6. Grice EA, Kong HH, Renaud G, Young AC, Program NCS, Bouffard GG, Blakesley RW, Wolfsberg TG, Turner ML, Segre JA: **A diversity profile of the human skin microbiota**. *Genome Res* 2008, **18**:1043-1050.
7. Williams MR, Gallo RL: **The role of the skin microbiome in atopic dermatitis**. *Curr Allergy Asthma Rep* 2015, **15**:65.
8. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, Gallo RL, Leung DY: **Endogenous antimicrobial peptides and skin infections in atopic dermatitis**. *N Engl J Med* 2002, **347**:1151-1160.

9. Owen CM, Chalmers R, O'Sullivan T, Griffiths CE: **WITHDRAWN: Antistreptococcal interventions for guttate and chronic plaque psoriasis**. *Cochrane Database Syst Rev* 2019, **3**:CD001976.
10. Kara M, Kivanc SA, Olcaysu OO, Akova Budak B, Ozmen AT, Kivanc M, Hormet Oz HT: **The newborn conjunctival flora at the post delivery 24 hours**. *J Curr Ophthalmol* 2018, **30**:348-352.
11. Egert M, Simmering R: **The Microbiota of the Human Skin**. *Adv Exp Med Biol* 2016, **902**:61-81.
12. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG: **Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing**. *Nat Methods* 2013, **10**:57-59.
13. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, et al.: **QIIME allows analysis of high-throughput community sequencing data**. *Nat Methods* 2010, **7**:335-336.
14. Jovel J, Patterson J, Wang W, Hotte N, O'Keefe S, Mitchel T, Perry T, Kao D, Mason AL, Madsen KL, et al.: **Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics**. *Front Microbiol* 2016, **7**:459.
15. : In *Jaundice in newborn babies under 28 days*. Edited by; 2010. National Institute for Health and Care Excellence: Clinical Guidelines
16. Gutta S, Shenoy J, Kamath SP, Mithra P, Baliga BS, Sarpangala M, Srinivasan M: **Light Emitting Diode (LED) Phototherapy versus Conventional Phototherapy in Neonatal Hyperbilirubinemia: A Single Blinded Randomized Control Trial from Coastal India**. *Biomed Res Int* 2019, **2019**:6274719.
17. Du L: **[Prevention and intervention strategies for hyperbilirubinemia induced brain injury]**. *Zhonghua Er Ke Za Zhi* 2014, **52**:721-723.
18. Stern RS, Bagheri S, Nichols K, Study PFU: **The persistent risk of genital tumors among men treated with psoralen plus ultraviolet A (PUVA) for psoriasis**. *J Am Acad Dermatol* 2002, **47**:33-39.
19. Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, Schoenfeld D, Nomicos E, Park M, Program NIHISCCS, et al.: **Topographic diversity of fungal and bacterial communities in human skin**. *Nature* 2013, **498**:367-370.
20. Rocas IN, Siqueira JF, Jr.: **Characterization of Dialister species in infected root canals**. *J Endod* 2006, **32**:1057-1061.
21. Jung SW, Kim BH, Katano T, Kong DS, Han MS: **Pseudomonas fluorescens HYK0210-SK09 offers species-specific biological control of winter algal blooms caused by freshwater diatom Stephanodiscus hantzschii**. *J Appl Microbiol* 2008, **105**:186-195.
22. Rezzoagli C, Granato ET, Kummerli R: **In-vivo microscopy reveals the impact of Pseudomonas aeruginosa social interactions on host colonization**. *ISME J* 2019, **13**:2403-2414.
23. Ma JY, Zhang J, Li QH, Shi ZL, Wu HJ, Zhang HQ, Tang LP, Yi R, Su H, Sun X: **Oral administration of a mixture of probiotics protects against food allergy via induction of CD103(+) dendritic cells and modulates the intestinal microbiota**. *Journal of Functional Foods* 2019, **55**:65-75.

Figures

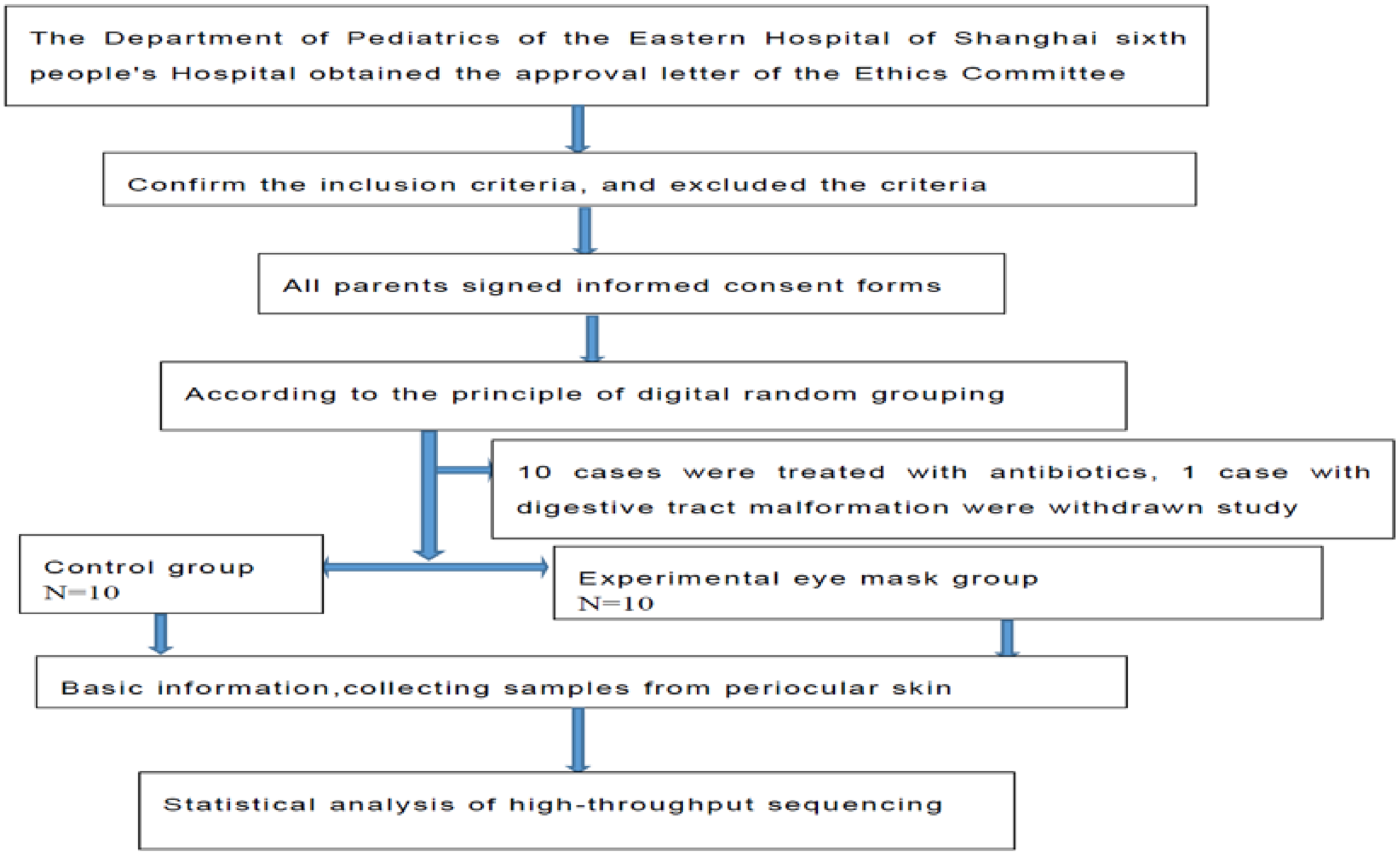

Figure 1

Study flow diagram.

A B

Figure 2

Two kinds of phototherapy eye patches for the (A) control group and (B) the experimental group. (Doll model)

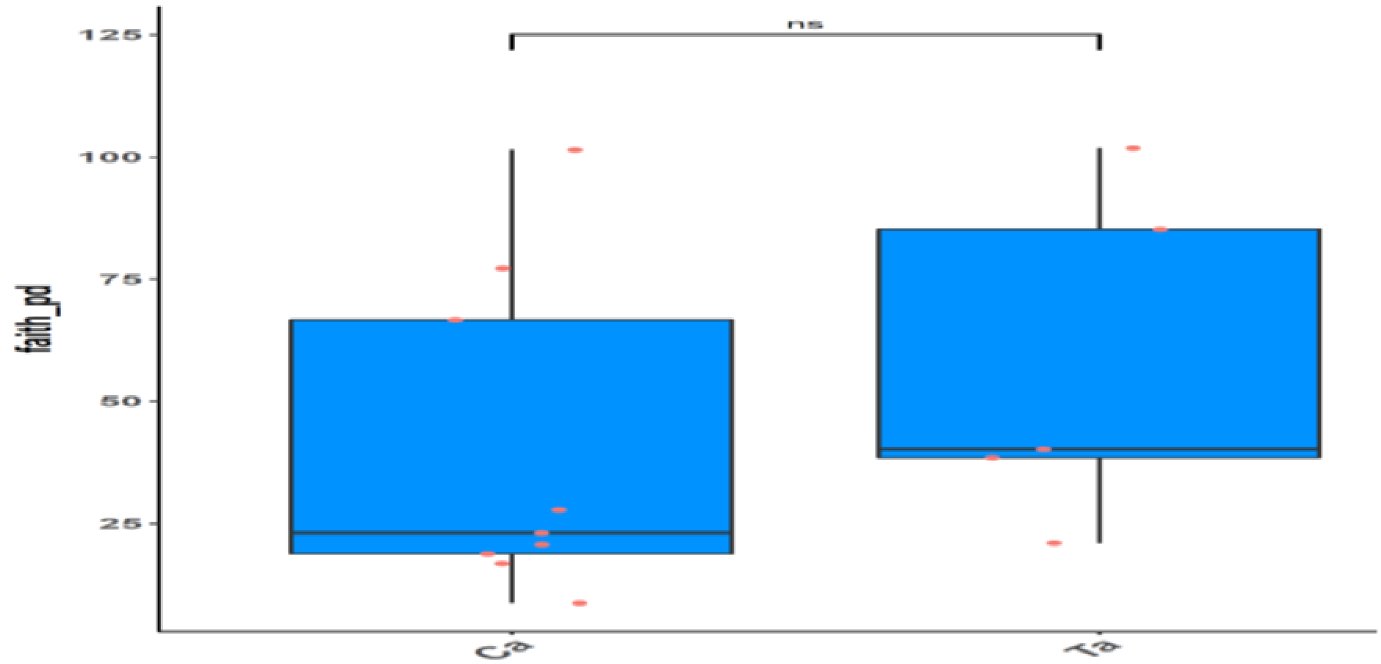

Figure 3

Comparison of periocular skin flora between the experimental and control groups before phototherapy.

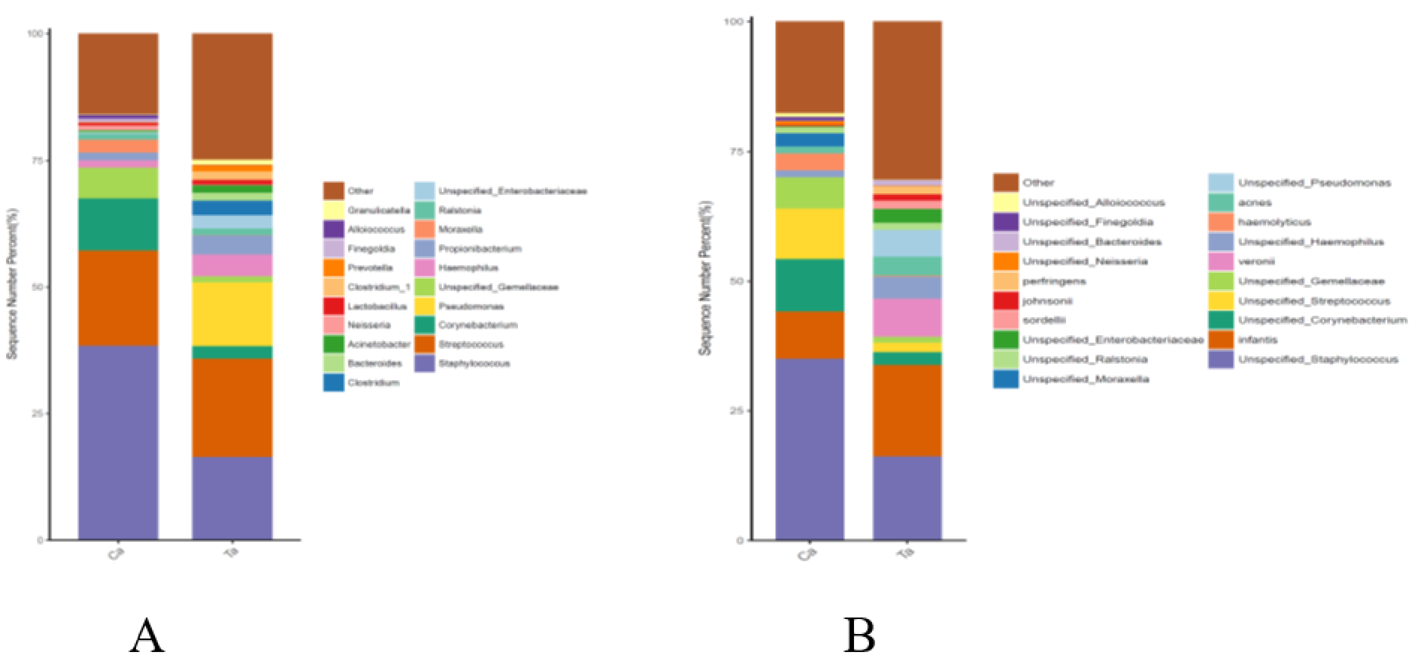

Figure 4

Top 20 genera in the periocular skin of the (A) experimental and (B) control group before phototherapy.

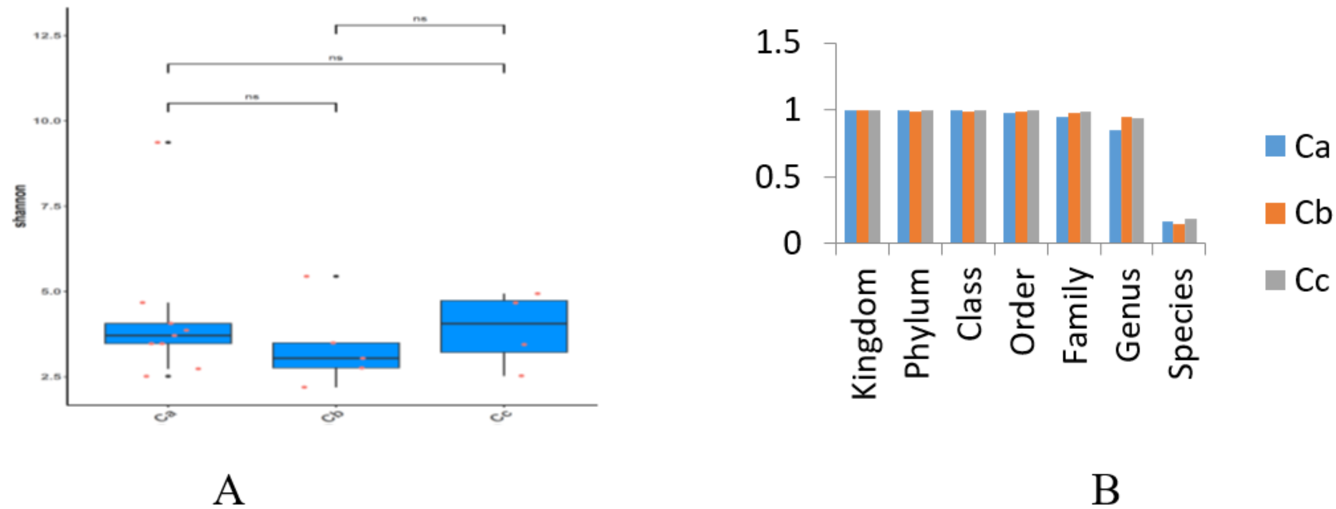

Figure 5

Comparison of the abundance of different bacterial strains in the skin microbiota around the eyes in the control group before and after phototherapy.

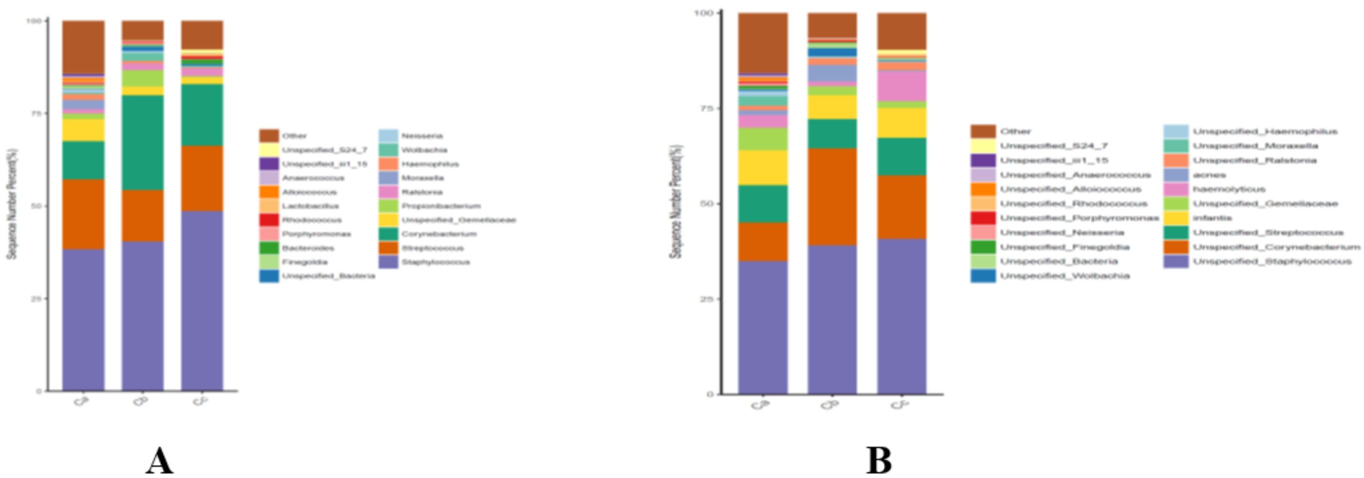

Figure 6

Comparison of the top 20 genera in the periocular skin of the control group (A) before and (B) after phototherapy.

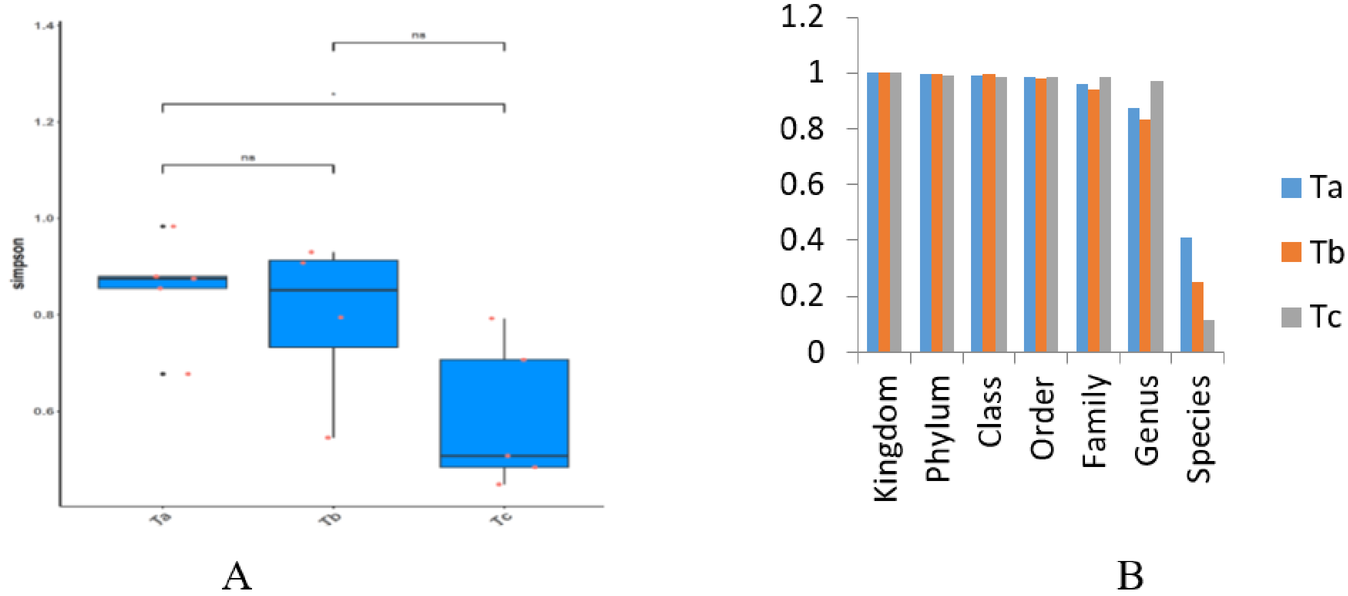

Figure 7

Difference in the abundance of bacterial strains in the periocular skin microbiota of the experimental group before and 48 h after phototherapy.

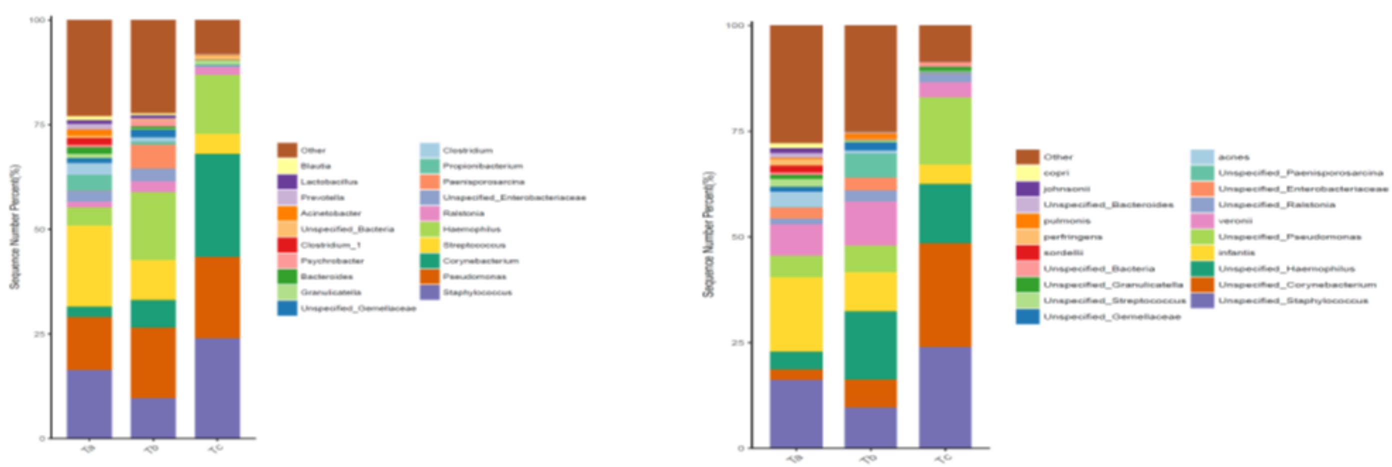

Figure 8

Distribution of the top 20 genera and species in the experimental group after phototherapy.

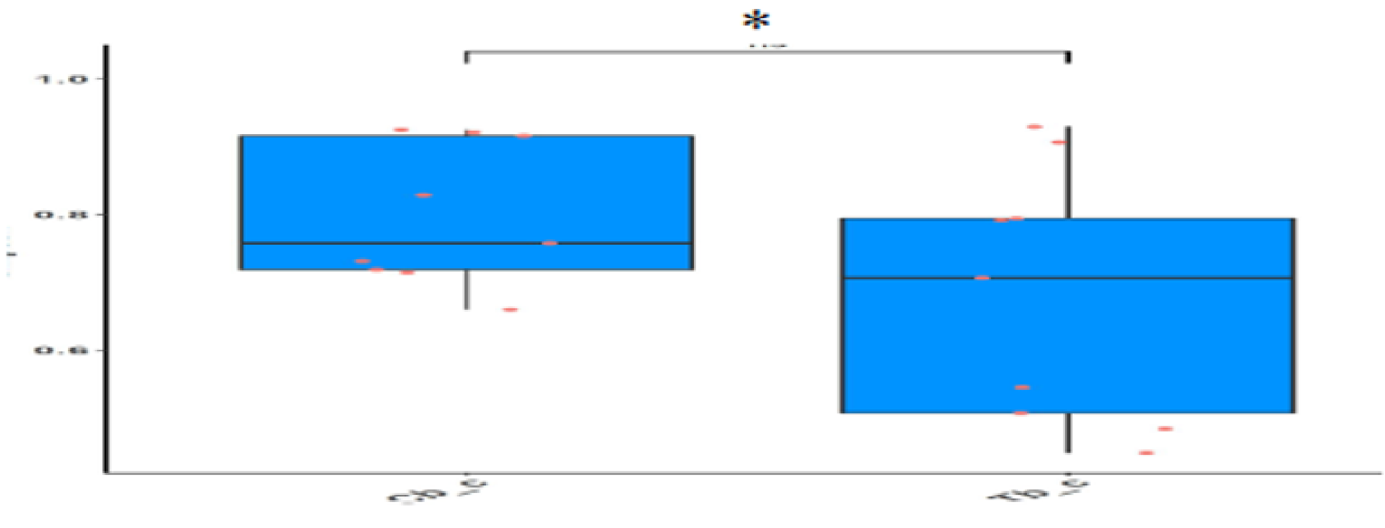

Figure 9

Comparative analysis of the microbiota between the experimental and control groups after phototherapy.

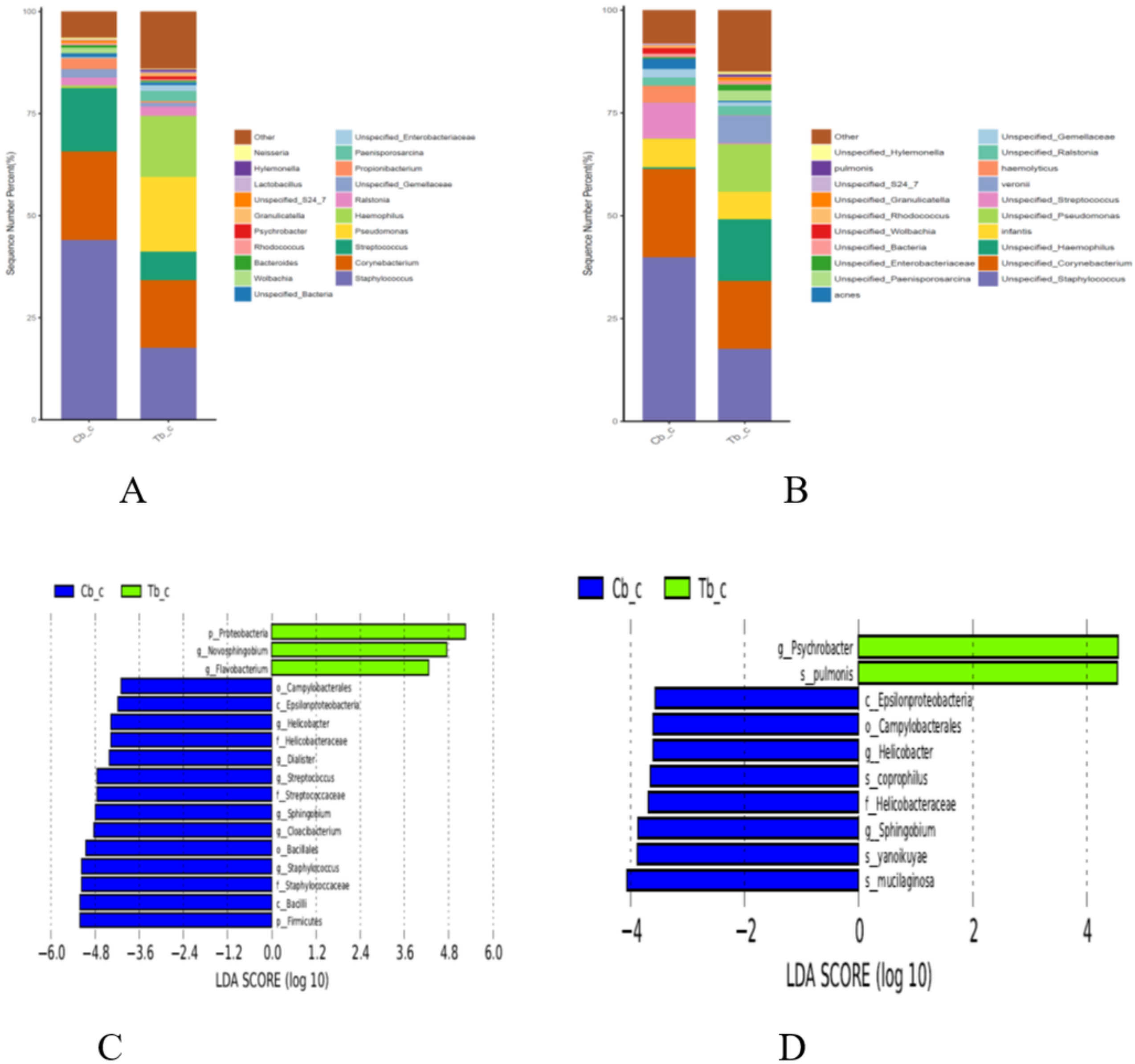

Figure 10

(A and B) Top 20 bacteria in the experimental and control groups after phototherapy (C and D). Different bacterial strains in the two groups after phototherapy.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CONSORT2010Checklist.doc