Deviations in the Gut Microbiota of Neonates Affected by Maternal Group B Streptococcus Infection

Yue-feng Li
Shenzhen Luohu Maternity and Child Health Care Hosptial
Xue-lei Gong
Shenzhen Luohu Maternity and Child Health Care Hosptial
Su-xiang Chen
Shenzhen Luohu Maternity and Child Health Care Hospital
Ke-jian Wang
The Third Affiliated Hospital of Shandong First Medical University(Affiliated Hospital of Shandong Academy of Medical Sciences),Jinan
Yan-hua Jiang (1453751387@qq.com)
Department of Obstetrics and Gynecology, Shenzhen Luohu Maternity and Child Health Hospital, 518019, China

Research article

Keywords: Group B Streptococcus (GBS) infection/colonization, gut microbiota, Microarray-based technique

Posted Date: December 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-117284/v1

Abstract

Background: Group B Streptococcus (GBS) infection is the leading cause of septicemia, meningitis, and pneumonia in neonates. Aberrant gut colonization in early life may predispose children to various diseases in adulthood. However, the associations between gut microbial changes and GBS infection is still unclear.

Methods: We adopted a new microarray-based technique on neonatal meconium samples collected from 13 participants with mother's GBS infection/colonization in vaginas and 73 uninfected controls.

Results: The composition and diversity of meconium microbiota in GBS group were similar to that of healthy controls. However, we identified several specific taxa that were differentially abundant between the two groups (linear discriminant analysis (LDA) effect size (LEfSe): $p<0.05$, LDA>2.0). Particularly, the relative abundance of *Lactobacillus paracasei* was significantly reduced, indicating a role in GBS infection.

Conclusions: Our study presented a series of bacterial species affected by GBS, thus providing novel evidence in support of initial intestinal microbiota dysbiosis in the neonates with mother's GBS infection/colonization.

Background

Group B Streptococcus (GBS) are β-hemolytic and Gram-positive bacteria, which are recognized as a leading cause of neonatal early-onset sepsis (EOS), meningitis, and pneumonia [1, 2]. The mother-to-child vertical transmission is the major GBS infection route in neonatal periods. Previous studies have shown that the prevalence of GBS colonization in vagina during pregnancy is approximately 10–30% [3, 4]. And the neonatal morbidity rate for acquiring GBS through birth canal is 60% [5]. The implementation of intrapartum antibiotic prophylaxis (IAP) in pregnant women with GBS colonization is an preventive treatment for reducing the risk of GBS-induced neonatal EOS [6]. However, IAP might also disrupt the balance between microbial members of the gut microbiota [7–9].

Recent evidences indicated that the disturbance of gut microbiome has been involved in potential prenatal and early life of infant [10, 11]. For example, Cassidy-Bushrow et al. observed that *Clostridiaceae*, *Ruminococcoceae*, and *Enterococcaceae* were significantly enriched in infants of GBS positive (GBS+) mothers compared to infants of GBS negative (GBS-) ones [12]. Rosen et al. reported 18 taxa that were found to be significantly associated with GBS carriage [13]. Although previous studies provided clues about how GBS alters the vaginal microbiome of pregnant women, the relationship between the gut microbiota of infants and maternal GBS infection remains largely undetermined.

In this study, we adopted a new microarray-based technique [14] to characterize the fecal samples of the neonates in GBS + group as compared with control group. The study aimed to investigate the influence of

maternal GBS infection on the gut microbiome of newborns, with the intention of improving perinatal infant care.

Results

The clinical information in the study

A total of 104 neonatal fecal specimens were collected during the study period. Of these, 12 fecal samples were undetectable due to inadequate amount of total DNA after extraction and 6 fecal samples were further excluded due to low content of 16S rDNA after amplification. Finally, 86 fecal samples from neonates were analyzed. The flowchart of the study was shown in Figure 1.

The clinical characteristics of the 86 neonates were shown in Table 1. There were no differenference between two groups except in the term of gestational age and antibiotics exposure after birth.

Table 1
The clinical information between GBS + and control groups

Parameters	**GBS + group (n = 13)**	**Control group (n = 73)**	**OR (95%CI)**	***P* value**
Neonatal features				
Gestational age, W	39.5 ± 0.6	38.8 ± 1.9	-	0.009
Birth weight, g	3192 ± 218	3121 ± 515	-	0.4
Male, (n, %)	8(61.5)	39(53.4)	1.4(0.4–4.7)	0.6
Cesarean section (n,%)	2(15.4)	30(41.1)	0.3(0.05–1.3)	0.1
Antibiotics exposure	10(76.9)	30(41.1)	4.8(1.2–18.8)	0.017
Mother complication				
PROM(n,%)	2(15.4)	17(23.3)	0.6(0.1–2.9)	0.8
Intrapartum fever history	0(0)	7(9.6)	0.8(0.8–0.9)	0.5
GDM (n,%)	1(7.7)	27(37.0)	0.1(0.02–1.15)	0.07
Placental abruption	0(0)	1(1.4)	-	1.0[#]
MSAF(n,%)	1(7.7)	23(31.5)	0.2(0.02–1.44)	0.1
IAP exposure	11(84.6)	44(60.3)	3.6(0.7–17.6)	0.17
[#]Fisher exact test				
PROM; prelabor rupture of the membranes; GDM: gestational diabetes mellitus; MSAF: meconium-stained amniotic fluid; IPA: intrapartum antibiotics prophylaxis.				

Comparison of α- and β-diversity between two groups

To evaluate the differences in composition of gut microbiota, we performed α- and β-diversity analyses. Several α-diversity indexes including Chao, Ace, Shannon and Simpson (Fig. 2) indicated no significant difference in species richness and diversity between GBS + and control groups. As to β-diversity, as β-diversity indicators were applied to estimate the dissimilarity between samples. PCoA plots based on weighted Unifrac distance and Bray Curtis distance showed that the controls clustered more tightly than the infants in GBS + group (Fig. 3), indicating similar bacterial compositions in the controls .

Alteration of taxa in the GBS + and control groups

To identify the specific taxa associated with GBS infection, a comparison of the microbiota between the infants in GBS + and control groups was conducted by the Linear discriminant (LDA) and effect size (LEfSe) approach. A cladogram represented the significant structure of the gut microbiota from phylum level to species level (Fig. 4A), which listed a collection of the differential abundant bacteria between two groups. Particularly, the abundance of *Staphylococcus lugdunensis*, *Lactobacillus helveticus*, *Lactobacillus mudanjiangensis*, *Staphylococcus paracasel* in the infants with GBS + group were reduced as compared to the controls.

Discussion

The imbalance of bacterial communities in infants has a profound impact on host's health, but there is insufficient evidence to suggest the associations between dysbiosis in meconium and bacterial infections such as GBS infection. In this context, identification of the factors affecting the morbidity for gestational GBS-related infection is an important issue that needs to be addressed.

In this study, bacterial composition and diversity showed no significant differences between infants in GBS + and control groups. However, we found a lower abundance of *Staphylococcus* and *Lactobacillus* in the infants with GBS + mothers, which was in line with previous studies. *Staphylococcus lugdunensis* is a coagulase-negative *Staphylococcus* [17], which has been implicated as the main pathogen in various infections, including central nervous system infections, urinary tract infections, and systemic infections [17–20]. A study from Japan reported that the GBS detection was correlated with significantly lower probability of coagulase-negative *Staphylococcus* [21]. Furthermore, Altoparlak et al. reported that the decreased level of *Lactobacillus* species was associated with detection of GBS infection [15]. And Kubota et al. demonstrated that GBS positive women had lower percentages of *Lactobacillus* than GBS negative women [21]. It should be noted that certain *Lactobacillus* such as *Lactobacillus paracasei* had the capabilities to prevent GBS adherence to vaginal epithelial cells [22], and antimicrobial activity of *Lactobacillus* against GBS had been documented in vitro [23]. Moreover, this lower *Lactobacillus* species had been detected in the neonatal EOS recently [24]. Thus, the reduced abundance of above genera might limit the protective role of microbiome so as to increase susceptibility to infection.

Currently, intrapartum antibiotics prophylaxis (IAP) implementation is considered as the most effective measurement for reducing the risk of GBS-induced neonatal EOS [6]. However, previous studies indicated that IAP could impact not only infant meconium microbiota at the time of birth [25] but also intestinal microbiota during the first 3 months of life, thus increasing the prevalence of antibiotic resisitance genes [26]. In the present study, although there were a higher proportion of IAP in GBS + group than control group (84.6% vs 60.3%), there were no difference in the aspect of IAP implementation between two groups (Table 1). It was further indicated that this imbalance of bacterial communities within 24 h of life in the neonate in our study might be closely correlated with mother's GBS colonization in vaginal tract, which was in line with previous studies [12].

There were also certain limitations of our results. First, this study was conducted in a single center with a relatively small sample set. Second, potential influence of nutrition intakes during pregnancy was not taken into consideration. Furthermore, we do not have access to the matched maternal microbiome samples, thus evidence tracing the origin of meconium microbiome is required in further study.

Conclusion

In summary, our findings add to a growing body of knowledge about the association between GBS infection and neonatal meconium.Our results demonstrated the potential features of microbiota in GBS-affected infants, which may lead to new biomarkers and innovative therapeutic approaches for perinatal infant care.

Methods

Study Design and Sample Collection

The Ethics Committee of Shenzhen Luohu Maternity and Child Health Hospital has approved all of the research procedures. Under the procedure approved by the Institutional Review Board (registry number: LL2018006), informed consent was given by the parents of the newborns.

The high-risk neonates admitted to the department of neonatology from May, 2018 to Jul, 2019 were enrolled after receiving informed consent from the parents. Preterm infants with extreme asphyxia (stage III), fetal chromosomal abnormalities, cyanotic congenital cardiac failure, congenital intestinal atresia, gastroschisis, omphalocele, excessive upper gastric intestinal bleeding, or parental permission deficiency/refusal were excluded from the study era.

The fecal samples were collected in 30–50 g within 24 hours after birth by senior nurse and transported immediately to lab on ice and stored at − 80°C for further studies. The GBS culture from mother's vaginal swab were conduected at 36 week gestational age for term labor or prior to delivery for premature labor. The clinical information, treatment and lab data of mothers and neonates were extracted from medical records.

Dna Extraction And Labeling

Bacterial DNA from the stool samples was processed following a previously published protocol [14]. In brief, DNA was collected using the Stool DNA Extraction Kit (Halgen, Ltd., Zhongshan, China) and amplified in a PCR reaction with standardized primers that covered the 16S rRNA gene V1-V9 regions. The PCR products were explicitly labeled without purification for array hybridization.

Microarray Hybridization

Again, previous protocols were followed to perform microarray hybridization [14]. In general, Cy5- and Cy3-labeled sample DNA were combined and loaded into a hybridization tank. After 3.5 h incubation, the slides were manually washed and automatically screened using a dual-channel (Genepix 4000B) scanner to calculation the mean signal strength of Cy5/Cy3 ratio, by which the relative abundance of each bacterial species is given.

Data analysis

Alpha-diversity was measured using default parameters and QIIME tools [15]. Wilcoxon rank-sum test was used to measure the disparities in alpha-diversities between classes. Analyzes of PCoA and NMDS were performed using QIIME modules and visualized with R packages (version 3.5.2). Linear Discriminant Analysis (LDA) Impact Size (LEfSe) tool [16] was adopted to analyze the disparity between classes of bacterial organisms.

Declarations

Acknowledgements

Not applicable

Author’s contributions

Conceived and designed experiments: Yue-feng Li and Yan-hua Jiang. Collected fecal samples: Su-xiang Chen. Collected and analyzed clinical data: Xue-lei Gong and Yue-feng Li. Performed fecal DNA extraction, microarray hybridization: Ke-jian Wang. Analyzed and interpreted results: Yan-hua Jiang, Yue-feng Li and Ke-jian Wang. Drafted the manuscript: Yan-hua Jiang, Ke-jian Wang, Yue-feng Li and Xue-lei Gong. All authors read and approved the final manuscript.

Funding

This work was supported by the Department of Luohu Science and Technology [grant No. 2018006] and Key Discipline of Luohu Neonatal Department [grant No. 2019006].

Ethics approval and consent to participate

All procedures were approved by the Ethics Committee of Shenzhen Luohu Maternity and Child Health Hospital. Under the procedure approved by the Institutional Review Board (registry number: LL2018006), informed consent was given by the parents of the newborns.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. Koenig JM, Keenan WJ: **Group B streptococcus and early-onset sepsis in the era of maternal prophylaxis**. *Pediatric Clinics* 2009, **56**(3):689-708.
2. Vornhagen J, Waldorf KMA, Rajagopal L: **Perinatal group B streptococcal infections: virulence factors, immunity, and prevention strategies**. *Trends in microbiology* 2017, **25**(11):919-931.
3. El Beitune P, Duarte G, Maffei CML: **Colonization by Streptococcus agalactiae during pregnancy: maternal and perinatal prognosis**. *Brazilian Journal of Infectious Diseases* 2005, **9**(4):276-282.
4. El Aila NA, Tency I, Claeys G, Saerens B, Cools P, Verstraelen H, Temmerman M, Verhelst R, Vaneechoutte M: **Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women**. *BMC infectious diseases* 2010, **10**(1):285.
5. Matee MI, Massawe FA, Lyamuya EF: **Maternal and neonatal colonisation of group B streptococcus at Muhimbili National Hospital in Dar es Salaam, Tanzania: prevalence, risk factors and antimicrobial resistance**. *BMC public Health* 2009, **9**(1):437.
6. Hasperhoven G, Al-Nasiry S, Bekker V, Villamor E, Kramer B: **Universal screening versus risk-based protocols for antibiotic prophylaxis during childbirth to prevent early-onset group B streptococcal disease: a systematic review and meta-analysis**. *BJOG: An International Journal of Obstetrics & Gynaecology* 2020, **127**(6):680-691.
7. Zou Z-H, Liu D, Li H-D, Zhu D-P, He Y, Hou T, Yu J-L: **Prenatal and postnatal antibiotic exposure influences the gut microbiota of preterm infants in neonatal intensive care units**. *Annals of clinical microbiology and antimicrobials* 2018, **17**(1):9.
8. Marshall BM, Levy SB: **Food animals and antimicrobials: impacts on human health**. *Clinical microbiology reviews* 2011, **24**(4):718-733.
9. Ouwehand AC, Forssten S, Hibberd AA, Lyra A, Stahl B: **Probiotic approach to prevent antibiotic resistance**. *Annals of Medicine* 2016, **48**(4):246-255.

10. Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, Sears MR, Becker AB, Scott JA, Kozyrskyj AL: **Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months**. *Cmaj* 2013, **185**(5):385-394.
11. Gschwendtner S, Kang H, Thiering E, Kublik S, Fösel B, Schulz H, Krauss-Etschmann S, Heinrich J, Schöler A, Schloter M: **Early life determinants induce sustainable changes in the gut microbiome of six-year-old children**. *Scientific reports* 2019, **9**(1):1-9.
12. Cassidy-Bushrow AE, Sitarik A, Levin AM, Lynch SV, Havstad S, Ownby DR, Johnson CC, Wegienka G: **Maternal group B Streptococcus and the infant gut microbiota**. *Journal of developmental origins of health and disease* 2016, **7**(1):45.
13. Rosen GH, Randis TM, Desai PV, Sapra KJ, Ma B, Gajer P, Humphrys MS, Ravel J, Gelber SE, Ratner AJ: **Group B Streptococcus and the vaginal microbiota**. *The Journal of infectious diseases* 2017, **216**(6):744-751.
14. Liu X, Zou Y, Ruan M, Chang L, Chen X, Wang S, Yang W, Zhang L, Guo Y, Chen Y: **Pediatric acute lymphoblastic leukemia patients exhibit distinctive alterations in the gut microbiota**. *Frontiers in Cellular and Infection Microbiology* 2020, **10**.
15. Altoparlak U, Kadanali A, Kadanali S: **Genital flora in pregnancy and its association with group B streptococcal colonization**. *International Journal of Gynecology & Obstetrics* 2004, **87**(3):245-246.
16. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C: **Metagenomic biomarker discovery and explanation**. *Genome biology* 2011, **12**(6):1-18.
17. Babu E, Oropello J: **Staphylococcus lugdunensis: the coagulase-negative staphylococcus you don't want to ignore**. *Expert review of anti-infective therapy* 2011, **9**(10):901-907.
18. Böcher S, Tønning B, Skov RL, Prag J: **Staphylococcus lugdunensis, a common cause of skin and soft tissue infections in the community**. *Journal of clinical microbiology* 2009, **47**(4):946-950.
19. Haile D, Hughes J, Vetter E, Kohner P, Snyder R, Patel R, Cockerill III F: **Frequency of isolation of Staphylococcus lugdunensis in consecutive urine cultures and relationship to urinary tract infection**. *Journal of clinical microbiology* 2002, **40**(2):654-656.
20. Frank KL, Del Pozo JL, Patel R: **From clinical microbiology to infection pathogenesis: how daring to be different works for Staphylococcus lugdunensis**. *Clinical microbiology reviews* 2008, **21**(1):111-133.
21. Kubota T, Nojima M, Itoh S: **Vaginal bacterial flora of pregnant women colonized with group B streptococcus**. *Journal of infection and chemotherapy* 2002, **8**(4):326-330.
22. Zarate G, Nader-Macias M: **Influence of probiotic vaginal lactobacilli on in vitro adhesion of urogenital pathogens to vaginal epithelial cells**. *Letters in Applied Microbiology* 2006, **43**(2):174-180.
23. De Gregorio PR, Tomás MSJ, Terraf MCL, Nader-Macías MEF: **In vitro and in vivo effects of beneficial vaginal lactobacilli on pathogens responsible for urogenital tract infections**. *Journal of medical microbiology* 2014, **63**(5):685-696.
24. Shane A, Sánchez P, Stoll B: **Sepsis Neonatal**. *Lancet* 2017, **390**(10104):1770-1780.

25. Mazzola G, Murphy K, Ross RP, Di Gioia D, Biavati B, Corvaglia LT, Faldella G, Stanton C: **Early gut microbiota perturbations following intrapartum antibiotic prophylaxis to prevent group B streptococcal disease**. *PLoS One* 2016, **11**(6):e0157527.
26. Nogacka A, Salazar N, Suárez M, Milani C, Arboleya S, Solís G, Fernández N, Alaez L, Hernández-Barranco AM, Clara G: **Impact of intrapartum antimicrobial prophylaxis upon the intestinal microbiota and the prevalence of antibiotic resistance genes in vaginally delivered full-term neonates**. *Microbiome* 2017, **5**(1):93.

Figures

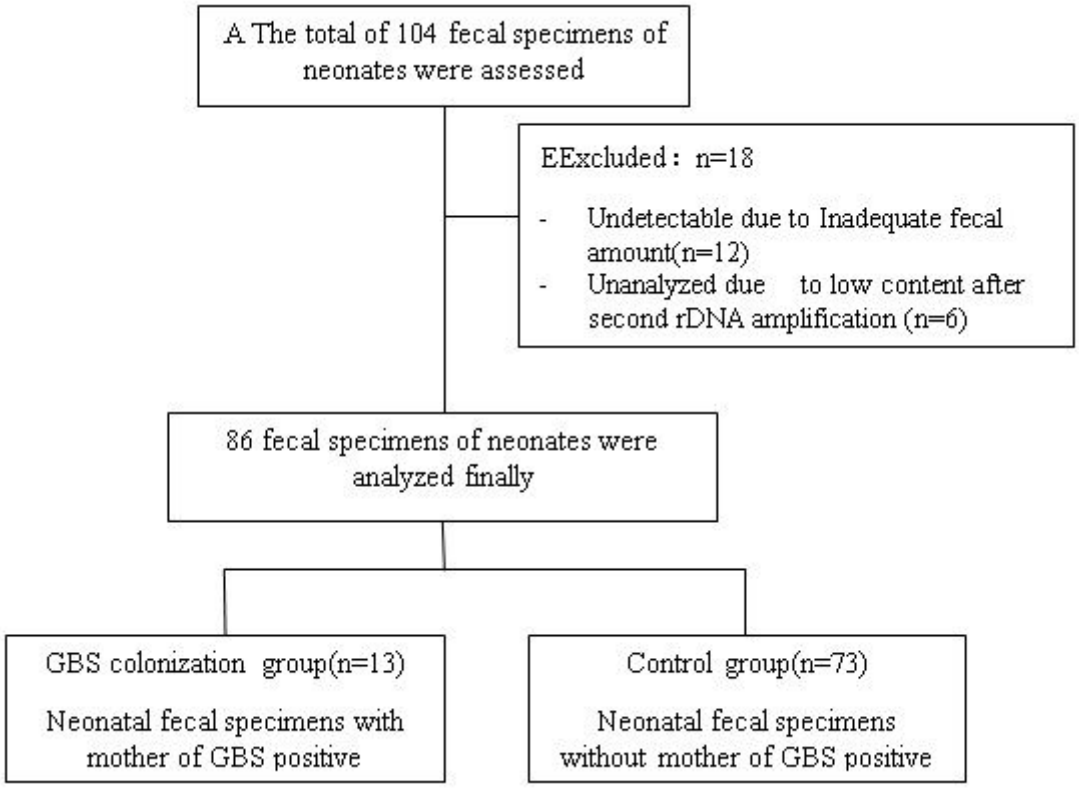

Figure 1

The flowchart of this study.

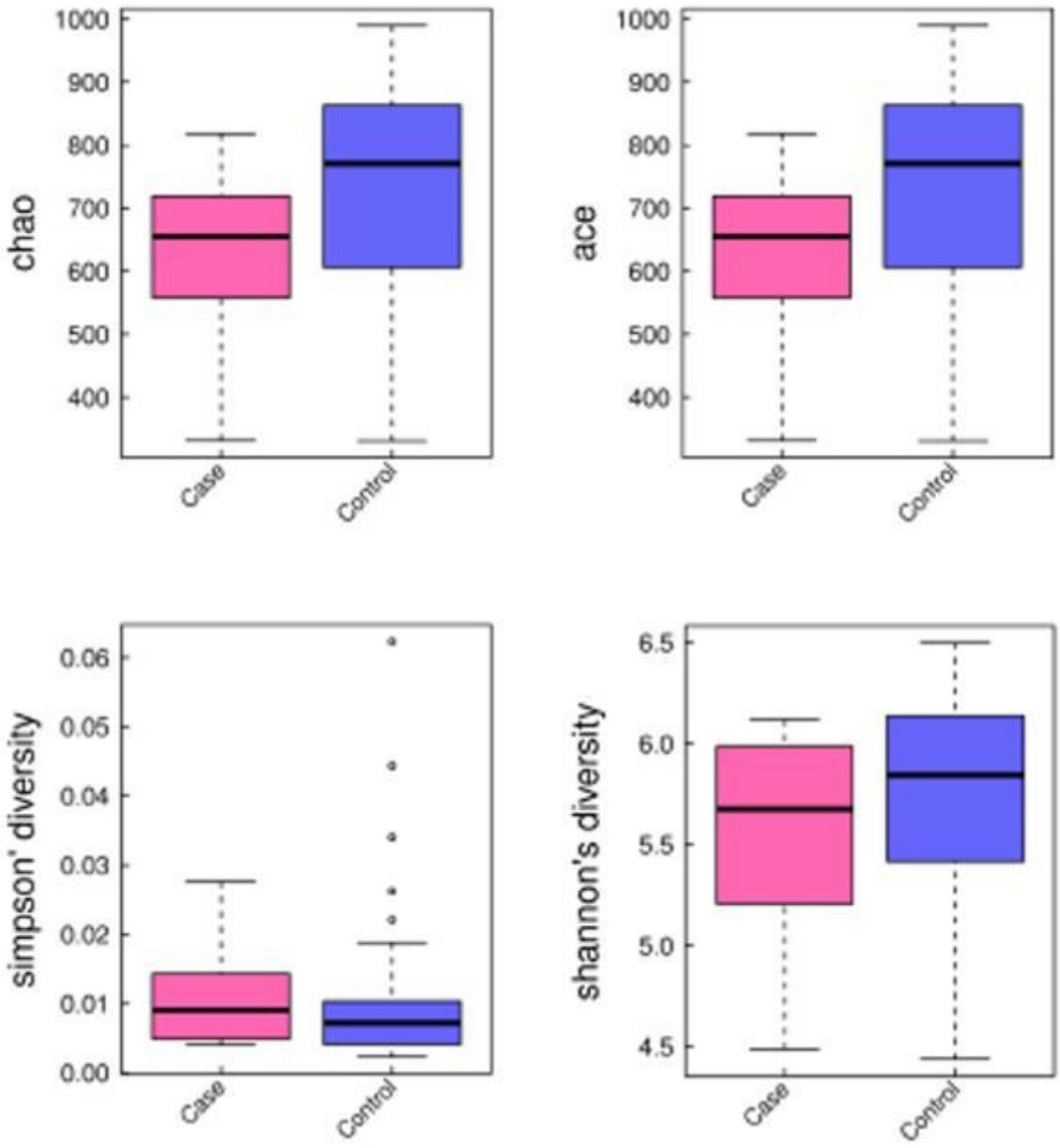

Figure 2

Box plot of Chao, Ace, Shannon and Simpson indexes.

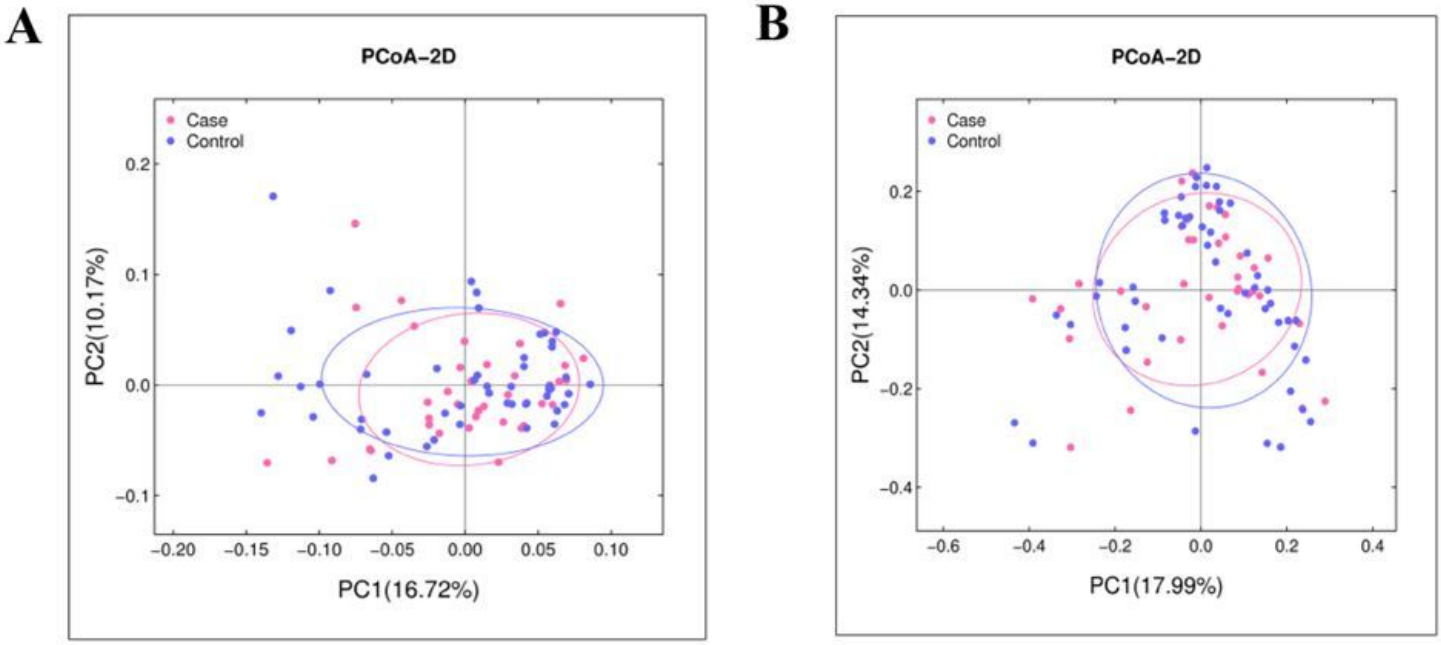

Figure 3

Gut bacterial community analysis of infants in GBS+ and control groups. Principal coordinates analysis (PCoA) plots based on weighted Unifrac distance (A) and Bray Curtis distance (B).

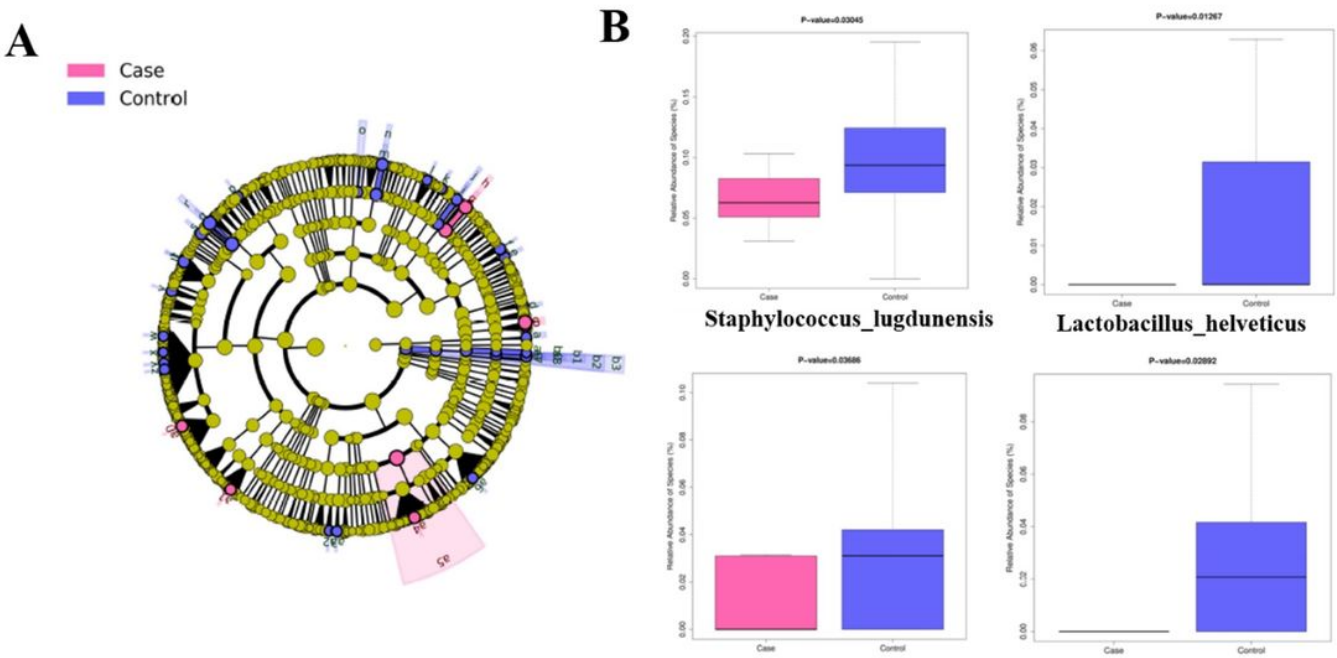

Figure 4

Different profiles of gut microbiota in meconium between infants in GBS+ and control groups. (A) Cladogram of differentially abundant taxa, from the phylum level down to the species level. (B) The

relative abundance of certain taxa associated with GBS infection.