Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Correlation Analysis Between Amniotic Fluid Microbiota and Complex Congenital Heart Disease

Yuefeng Cao
Beijing Anzhen Hospital
Guofeng Xing
Beijing Anzhen Hospital
Qiang Wang
190278645@qq.com

Beijing Anzhen Hospital

Research Article

Keywords: Congenital heart defects, Amniotic Fluid, Gut Microbiota, Fetus

Posted Date: March 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8741630/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Correlation Analysis Between Amniotic Fluid Microbiota and Complex Congenital Heart Disease

YuefengCao, GuofengXing, QiangWang

Abstract

Background and aims

Numerous studies have found that dysbiosis of the microbiota is associated with cardiovascular diseases in humans and shows certain links to congenital heart disease (CHD) in children. However, direct evidence regarding whether maternal gut microbiota is related to fetal heart development remains lacking. This study aims to collect amniotic fluid samples during cesarean sections from pregnant women, compare the differences in microbiota between fetuses with complex CHD and normal fetuses, and explore the role of maternal gut microbiota as an environmental pathogenic factor in complex CHD.

Methods and results

Amniotic fluid samples were collected from patients undergoing cesarean sections at a single center between November 2024 and October 2025. The study included 6 cases in the fetal complex congenital heart disease (CCHD) group and 9 cases in the fetal normal (NOR) group. Microbial 16S rRNA sequencing was performed on these samples. A total of 1,908,431 paired-end reads were obtained, which were assembled into 1,897,612 tags, corresponding to 4,057 ASVs/OTUs. No significant differences in microbial diversity were observed between the two groups. However, in the complex congenital heart disease (CCHD) group, the abundance of certain microbial taxa associated with diabetes, inflammatory bowel disease, and periodontal disease was significantly elevated. ($p < 0.01$).

Conclusion

No significant differences were observed in the microbial diversity of amniotic fluid between fetuses with complex congenital heart disease (CCHD) and normal fetuses. However, the abundance of specific microbial taxa was significantly elevated in the CCHD group. Further research is needed to elucidate the impact of these microbial changes on fetal cardiac development.

Keywords

Congenital heart defects, Amniotic Fluid, Gut Microbiota, Fetus

Introduction

The human body harbors a vast number of microorganisms, which establish a stable symbiotic relationship with the host and serve as an indispensable component in maintaining physiological homeostasis. The gut microbiota constitutes a complex ecosystem within the host's intestines, composed of diverse microorganisms. It encompasses approximately 100 trillion bacteria, classified into over 2,000 species[1]. These microorganisms coexist symbiotically and are essential for human health, while many diseases are associated with microbiota dysbiosis. As a critical "environmental factor" influencing fetal development, the maternal microbiota has emerged as a forefront area in perinatal research. Numerous studies have identified correlations between dysbiosis and cardiovascular diseases in humans, as well as certain links to congenital heart disease (CHD) in children.

The incidence of congenital heart disease is approximately 0.8–1%[2]. In China, CHD ranks as the leading category of birth defects in children, with complex CHD severely affecting the quality of life, exhibiting high surgical mortality, and poor prognosis. While current understanding of risk factors for CHD has expanded from traditional genetic factors and established teratogens (e.g., viruses, drugs) to include maternal metabolism, research on broader "intrauterine environmental" factors, particularly those involving the symbiotic microbiota, remains limited. It is well-established that gestational diabetes mellitus (GDM) is a risk factor for CHD, increasing the risk by 1.5–4 times. Extensive studies[3-5] have confirmed characteristic dysbiosis in the gut microbiota of diabetic pregnant women, such as reductions in beneficial bacteria (e.g., short-chain fatty acid producers) and increases in opportunistic pathogens. The microbiota directly contributes to the development of GDM through its metabolites (e.g., short-chain fatty acids, secondary bile acids), modulation of inflammation, and insulin resistance. Therefore, further investigation is needed to determine whether maternal gut microbiota is associated with fetal CHD. Specifically, it is essential to explore whether microbial communities exist in the amniotic fluid—the fetal growth environment—and whether they correlate with fetal cardiac development. Such research will help elucidate microbial environmental pathogenic factors contributing to CHD, potentially reducing its incidence.

Existing studies[6-7] have confirmed differences in gut microbiota between mothers of children with CHD and those without, as well as between children with CHD and healthy children. However, direct evidence remains lacking regarding whether maternal gut microbiota influences fetal cardiac development. Since maternal microbiota rapidly colonizes newborns via the birth canal and maternal skin during delivery, studies on neonatal stool samples are confounded and cannot directly reflect fetal microbiota changes. Amniotic fluid, as the fetal growth environment, can directly reflect the fetal gut microbiota, making it crucial to investigate the maternal-amniotic fluid-fetal gut microbiota relationship.

This study aims to collect amniotic fluid samples during cesarean sections from pregnant women, compare microbiota differences in amniotic fluid between fetuses with complex CHD and normal fetuses, explore maternal gut microbiota-related

environmental pathogenic factors for complex CHD, and provide clinical insights for the prevention of complex CHD.

Materials and Methods

Pregnant women hospitalized in the Department of Obstetrics of our institution between November 2024 and October 2025 were selected for this study. This study was approved by the Medical Ethics Committee of Anzhen hospital (ID: 2024025). All samples and information were obtained from the project biobank. Based on obstetric evaluation and maternal preference, patients scheduled for cesarean delivery were recruited, and their consent was obtained for the collection of amniotic fluid samples during the procedure for 16s rRNA microbial sequencing. Participants were divided into two groups according to preoperative fetal cardiac ultrasound findings: a fetal complex congenital heart disease group (CCHD) and a fetal normal group (NOR). The CHD group was diagnosed prenatally through systematic ultrasound examination by fetal cardiology specialists and cardiac surgeons. The NOR group consisted of fetuses with no prenatally detected congenital anomalies. A total of 20 amniotic fluid samples were planned for inclusion, with 10 cases in each group. Inclusion criteria: Pregnant women scheduled for cesarean delivery. Exclusion criteria: Prenatal diagnosis of fetal malformations other than cardiac defects; prenatal diagnosis of severe chromosomal abnormalities; patients undergoing vaginal delivery.

Bioinformatics analysis

Samples were placed in EP tubes after collection and stored at −80°C for subsequent analysis. DNA was extracted using a DNA isolation kit. Amplicon Generation 16S rRNA/18SrRNA/ITS genes of distinct regions (16SV4/16SV3/16SV3-V4/16SV4- V5, 18SV4/18SV9, ITS1/ITS2, ArcV4) were amplified used specific primer (e.g. 16SV4: 515F- 806R, 18SV4: 528F-706R, 18SV9: 1380F- 1510R, et. al) with the barcode. All PCR reactions were carried out with 15 μL of Phusion High - Fidelity PCR Master Mix; 0.2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98℃for 1 min, followed by 30 cycles of denaturation at 98℃ for 10 s, annealing at 50℃for 30 s, and elongation at 72℃for 30 s and 72℃for 5 min.

The PCR products were purified using magnetic bead purification. Samples were mixed inequi density ratios based on the concentration of PCR products. After thorough mixing, the PCR products were detected and target bands were recovered. For the PCR products generated by amplification, PCR product detection will be performed

Sequencing libraries were generated and indexes were added. The library was checked with Qubit and real-time PCR for quantification and bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on NovaSeq6000 or DNBSEQ-G99 platforms, according to effective library concentration and data amount required.

Statistical analyses

Statistical analysis was performed using SPSS 24.0 software. Categorical variables were expressed as whole numbers and percentages, with between-group

comparisons conducted using Fisher's exact test. Normally distributed continuous variables were presented as mean ± standard error of the mean (SEM) or mean (95% confidence interval), and comparisons between groups were made using t-tests. Non-normally distributed continuous variables were expressed as median and interquartile range (IQR), and between-group comparisons were performed using the Wilcoxon rank-sum test. P-value < 0.05 was considered statistically significant.

Results

A total of 25 samples were collected for the study. After excluding contaminated specimens, 15 cases were ultimately included in the analysis. The average maternal age was 33.3 years (range: 27.8–37.3 years), and the average body mass index (BMI) was 21.7 (range: 20.0–26.6). The cohort comprised 6 cases of fetal complex congenital heart disease (CCHD) and 9 cases of normal fetuses. Regarding conception methods, 3 of the 15 pregnancies resulted from assisted reproductive technology, while the remaining were conceived naturally. In terms of maternal history of adverse habits, one case in the CCHD group had a previous history of smoking.

Based on prenatal fetal cardiac ultrasound, the diagnoses within the CCHD group included Tetralogy of Fallot (2 cases), pulmonary atresia (2 cases), complete transposition of the great arteries (1 case), and severe pulmonary valve stenosis (1 case). In the CCHD group, maternal complications included: 2 cases with preeclampsia and proteinuria, 1 case with obesity and cervical insufficiency, and 1 case with premature rupture of membranes and anemia, who subsequently delivered preterm at 31 weeks and 6 days of gestation. In the normal (NOR) group, maternal conditions consisted of: 1 case with gestational hypertension, 1 case with gestational diabetes mellitus, and 1 case with subaortic stenosis. Detailed baseline characteristics of the pregnant women are presented in Table 1.

A total of 1,908,431 paired-end reads were obtained from the sequencing of 15 samples, yielding 1,897,612 tags after assembly, which corresponded to 4,057 ASVs/OTUs. The number of reads per sample ranged from 99,628 to 113,386. To ensure consistent sequencing depth for comparative analysis across samples, 98,849 reads were randomly selected from each sample for downstream analysis.

Differences in gut microbiota at the family level between the CCHD and NOR groups were analyzed using a Venn diagram (Figure 1A). The results showed that 230 bacterial families (52.63%) were shared between the two groups. Additionally, the CCHD and NOR groups harbored 108 (24.71%) and 99 (22.65%) unique bacterial families, respectively. No statistically significant differences were observed between the two groups.

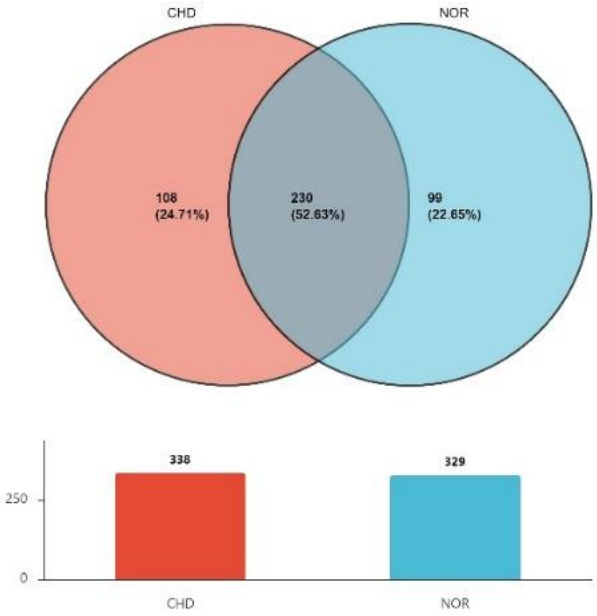


Figure 1. Analysis of Changes in Bacterial Taxa at the Family Level and Indicator Genera in the CCHD and NOR Groups

At the family level, no statistically significant differences were observed in the composition or relative abundance of gut microbiota between the two groups (Figure 2). In the CCHD group, the predominant bacterial families were, in descending order: Aeromonadaceae (47.1%), Pseudomonadaceae (19.0%), Lysobacteraceae (8.5%), and Bacillaceae (4.5%). In the NOR group, the main families were: Aeromonadaceae (52.9%), Pseudomonadaceae (20.0%), Lysobacteraceae (9.0%), and Moraxellaceae (3.1%).

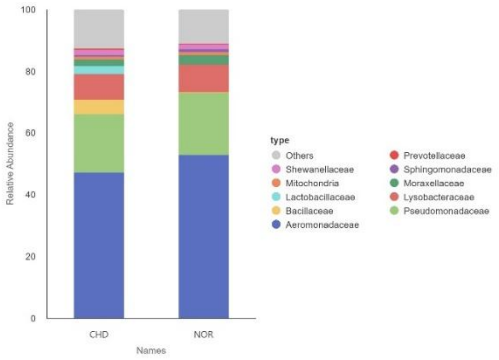
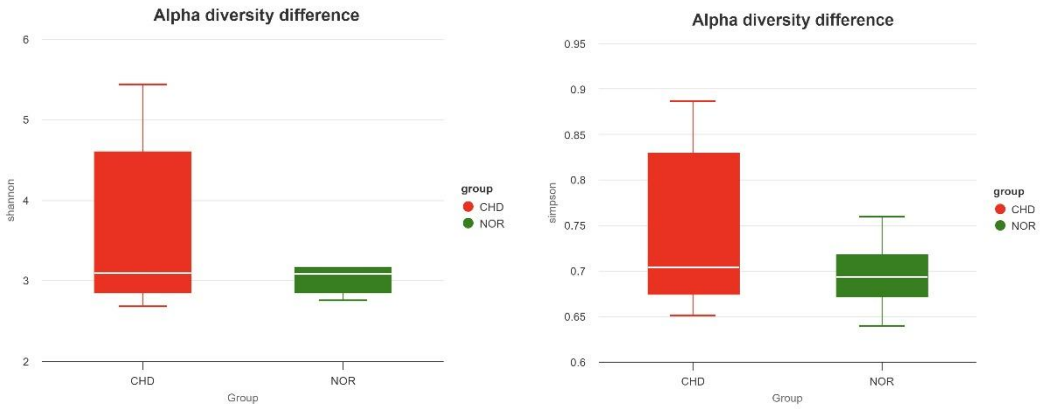


Figure 2. Comparison of the Composition and Relative Abundance of Gut Microbiota at the Family Level Between the Two Groups

Analysis of Alpha Diversity (α-diversity) Index Differences

The α-diversity of gut microbiota in the two sample groups was assessed using the Shannon index and Simpson index. The results indicated no significant clinical differences in the α-diversity levels of gut microbiota between the two groups. The specific findings are presented in Figure 3.



A. shannon B. simpson

Figure 3. Analysis Results of Intergroup Differences in Alpha Diversity Indices Notes: A. shannon, B. simpson.

The Shannon Index is a diversity metric that integrates both species richness and evenness within a community. A higher Shannon index value indicates greater community diversity.

The Simpson Index measures the probability that two individuals randomly selected from a community belong to the same species. A higher Simpson index value (or its complement, 1 − Simpson index, often referred to as the Simpson diversity index) indicates greater dominance of certain species and lower community diversity.

Furthermore, the study analyzed the similarities and differences in community structure between the two sample groups. The NMDS plot based on the Bray-Curtis distance matrix revealed no significant differences in community structure between the groups. (Figure 4)

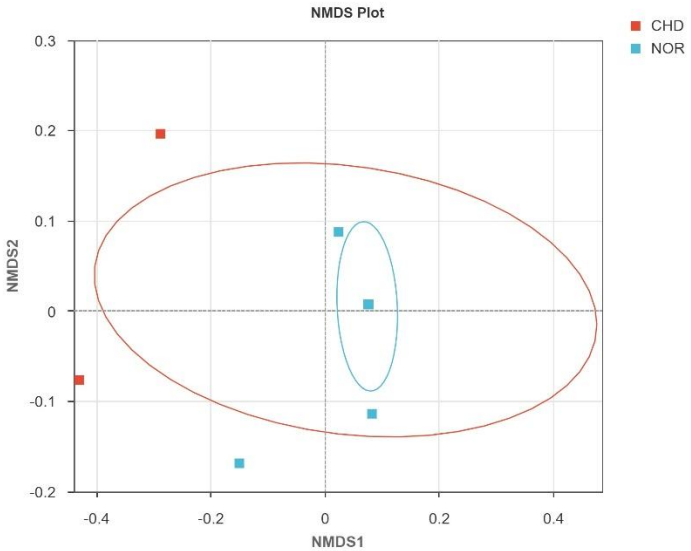
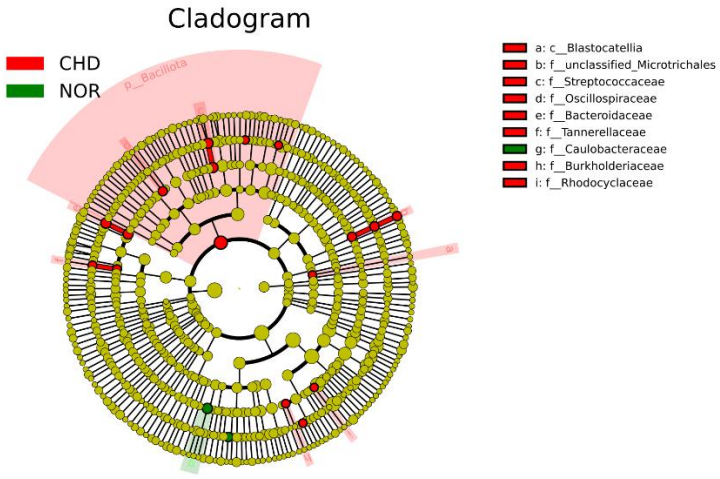


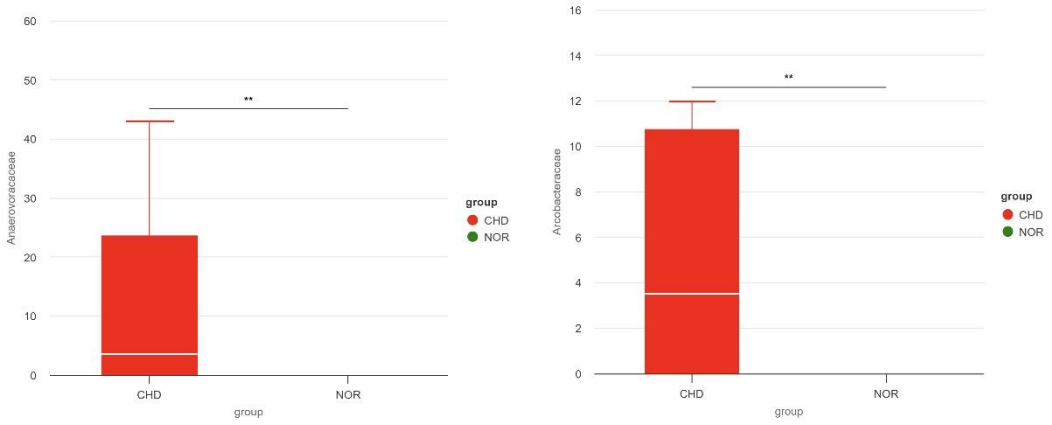
Figure 4, The NMDS plot of beta diversity based on Bray-Curtis distance matrix is shown.

LEfSe (Linear Discriminant Analysis Effect Size) was employed to compare the two groups and identify statistically significant biomarkers at the phylum level. The statistical results of LEfSe are presented in the cladogram in Figure 5. In the fetal complex congenital heart disease group, the abundance of Bacillota (formerly Firmicutes) in amniotic fluid samples was significantly higher compared to that in the normal fetal group, showing a statistically significant difference.



Picture 5, LEfSe Cladogram

Significant differences were observed between the two sample groups at the family and genus levels. Hypothesis testing on species abundance data across groups was conducted using the MetagenomeSeq method to obtain p-values. Statistically significant species with meaningful p-values were screened to identify those exhibiting significant intergroup differences. The analysis identified the following six family-level taxa ($p<0.01$): Anaerovoracaceae, Arcobacteraceae, Idiomarinaceae, Oscillospiraceae, Saprospiraceae, and Tannerellaceae. Additionally, three genus-level taxa ($p<0.01$) were identified: Lachnospiraceae_XPB1014_grou, Selenomonas, and Parabacteroides. As shown in Figure 6 (family level) and Figure 7 (genus level), all of these taxa exhibited significantly higher abundance in the CCHD group ($p < 0.01$).



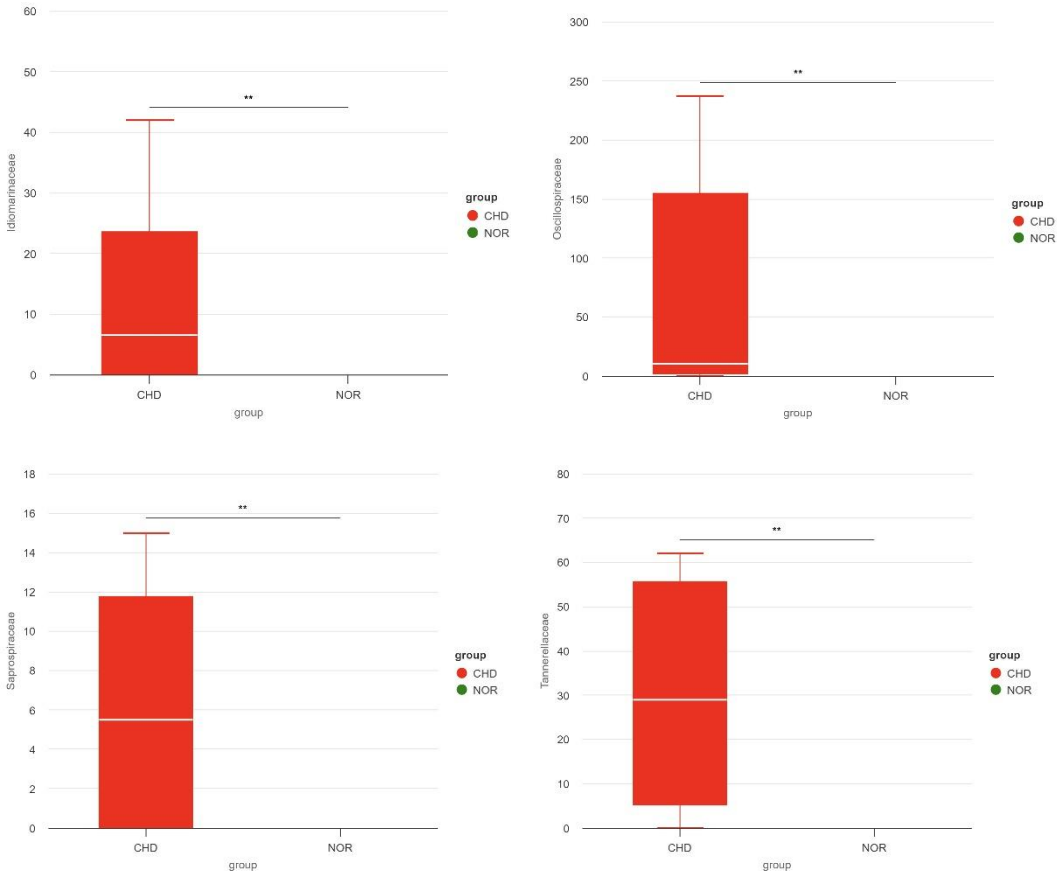


Figure 6. Intergroup Species Difference Analysis at the Family Level by MetagenomeSeq: Anaerovoracaceae (a), Arcobacteraceae(b), Idiomarinaceae(c), Oscillospiraceae(d), Saprospiraceae (e), Tannerellaceae (f)

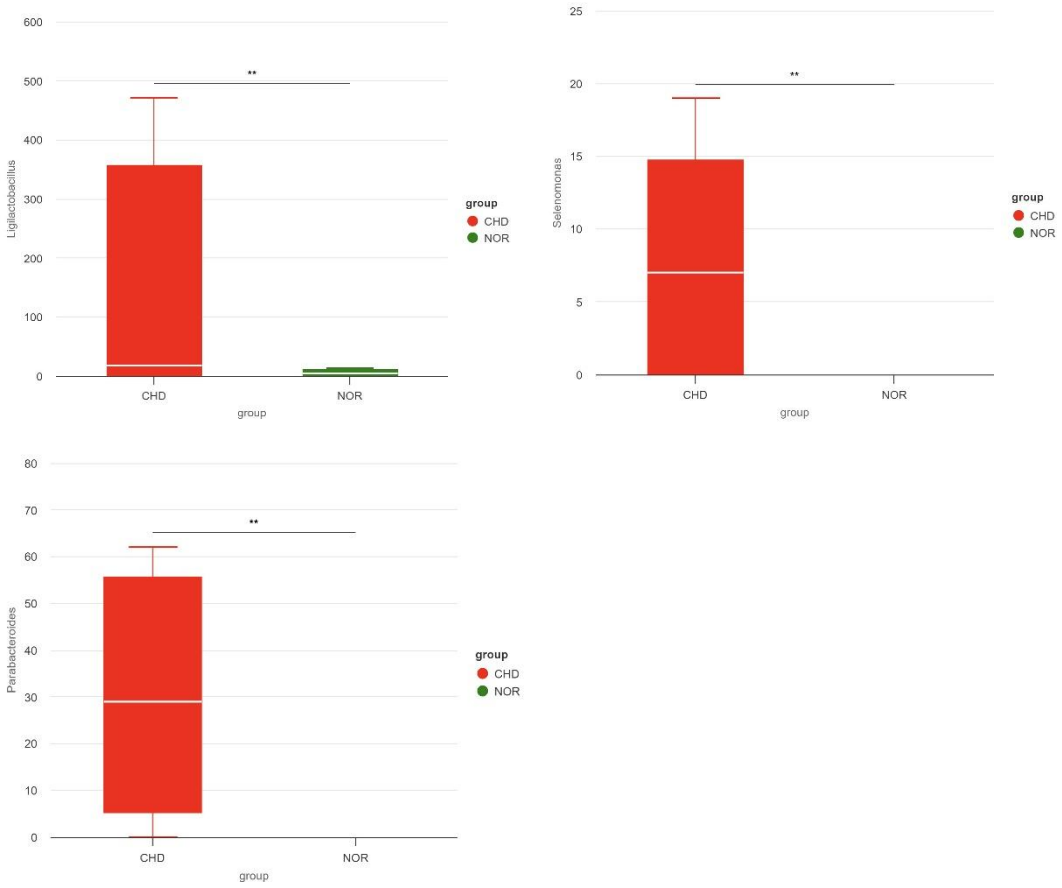


Figure 7. Intergroup Species Difference Analysis at the Genus Level by MetagenomeSeq: Lachnospiraceae_XPB1014_group(a), Selenomonas(b), Parabacteroides(c)

Discussion

This study confirms the presence of microbial communities in amniotic fluid and systematically investigates the potential association between maternal gut microbiota and the risk of fetal complex congenital heart disease (CCHD) through 16S rRNA sequencing. The results indicate that, compared with the control group, the gut microbiota of mothers with CCHD-affected children shows no significant differences in species composition or β-diversity. However, significant differences are observed in certain bacterial genera, providing new insights for research into the mechanisms underlying congenital heart disease.

The existence of microorganisms in amniotic fluid has long been a subject of debate. Traditionally, amniotic fluid was considered sterile. However, recent studies[4,8] have confirmed the presence of microorganisms in amniotic fluid, which share similarities with maternal gut microbiota. The transfer of gut microbiota primarily occurs through extracellular vesicles (EVs) secreted by the gut microbial community. EVs are membrane-enclosed particles secreted by nearly all cell types and can transport various biomolecules across biological barriers, serving as important mediators of host-microbiota communication. As signaling vesicles, EVs play a crucial role in intercellular communication within the host and between

organisms. Anna Kaisanlahti[9] et al. compared fecal and amniotic fluid samples from 28 pregnant women and found a common origin for bacterial EVs in both amniotic fluid and maternal feces. This suggests that bacterial EVs secreted by the maternal gut microbiota enter the intrauterine space during fetal development. Through 16S rRNA gene analysis of amniotic fluid, this study directly confirms the presence of microorganisms in pregnant women's amniotic fluid.

Although the impact of maternal gut microbiota during pregnancy on fetal gut–brain axis development has been widely discussed, its association with fetal heart development remains a relatively new research area. Recent studies[10] have revealed potential links between microbiota and fetal heart development, highlighting the critical role of microbial metabolites such as short-chain fatty acids in cellular differentiation and the development of multiple systems, including the cardiovascular system. This suggests that maternal microbiota may be a key factor shaping offspring cardiovascular health. While many previous studies have analyzed maternal feces, neonatal feces, or meconium to identify factors influencing fetal congenital heart disease, research on amniotic fluid—the direct fetal growth environment—has been limited. Amniotic fluid is a vital environment for fetal growth and development, and its microbial composition and metabolites directly reflect fetal development. One study[4] analyzing amniotic fluid obtained via amniocentesis from mothers carrying fetuses with congenital heart disease found significantly higher α- and β-diversity in the congenital heart disease group, though with a p-value of 0.049. Additionally, amniocentesis itself, as a method for detecting fetal chromosomal abnormalities, introduces potential bias. Another study[7] showed no significant difference in gut microbiota diversity between children with tetralogy of Fallot and healthy children. The gut microbiota plays a key role in regulating human health and homeostasis, and microbiota imbalance is detrimental to fetal development and birth[11–14]. The results of this study indicate no significant difference in microbiota diversity between the CCHD and NOR groups, suggesting that maintaining microbiota diversity homeostasis is beneficial for overall fetal development.

It has been widely demonstrated that the gut microbiota can influence the host in many ways. Specific maternal gut microbiota may affect offspring immune, metabolic, and nervous system development. For example, the presence of Lactobacillus and Bifidobacterium is closely associated with beneficial effects such as promoting immune system development in children and reducing the risk of future allergies and asthma[15,16]. This study found that the abundance of Firmicutes in the CCHD group was significantly higher than in the NOR group. The gut microbiota of healthy individuals is primarily composed of Firmicutes and Bacteroidetes, which produce short-chain fatty acids (e.g., butyrate and propionate) crucial for energy supply and immune regulation. Further analysis revealed significant differences in bacterial taxa at the family and genus levels between the CCHD and NOR groups. Notably, these family- and genus-level bacteria are closely associated with conditions such as inflammatory bowel disease, periodontal disease, and diabetes[18–20]. The pro-inflammatory factors and short-chain fatty acids they produce may influence cardiomyocyte differentiation during fetal cardiac fusion. Certain signaling molecules

produced by the microbiota, such as trimethylamine N-oxide (TMAO), butyrate, and nitric oxide (NO), may interfere with processes related to cardiac fibrosis.[4,17] These significantly enriched microbial taxa show certain associations with maternal health and fetal cardiac development.

By analyzing amniotic fluid, a crucial medium for fetal growth and development, this study provides evidence supporting the correlation between fetal complex congenital heart disease and microorganisms, warranting further investigation. However, this study has certain limitations. Due to the difficulty in collecting amniotic fluid samples, the sample size included in this research is limited. In subsequent studies, we will increase the sample size to further validate our findings.

Conclusion

In summary, by combining 16S rRNA sequencing analysis, we have identified the presence of microorganisms in maternal amniotic fluid. While no significant differences were observed in the overall biodiversity of amniotic fluid microorganisms between the complex congenital heart disease (CCHD) group and the control group, significant variations were found in specific microbial compositions. This study provides evidence supporting the correlation between fetal complex congenital heart disease and maternal microorganisms.

Availability of Data and Materials

All data generated or analysed during this study are included in this published article.

Funding

There was no Funding

Ethics approval and consent to participate

This study was approved by the Medical Ethics Committee of Anzhen hospital (ID: 2024025). All samples and information were obtained from the project biobank. All subjects provided written informed consent.

References:

1. Qin, J.; Li, R.; Raes, J.; Arumugam, M.; Burgdorf, K.S.; Manichanh, C.; Manichanh, C. A human gut microbial genecatalogue established by metagenomic sequencing. Nature 2010, 464, 59–65. [CrossRef]
2. Zhao QM, et al. Prevalence of congenital heart disease at live birth in China. J Pediatr. 2019;204:53–8.
3. Navpreet, Kaur; Pankaj, Kumar; Mahadev, Dhami, et al: Antibiotic-induced gut dysbiosis: unraveling the gut-heart axis and its impact on cardiovascular health. Mol Biol Rep 2025 Mar 17;52(1):319
4. Wenli, Xu; Lu, Li; Hong, Kang, et al: Amniotic fluid microbiota and metabolism with non-syndromic congenital heart defects: a multi-omics analysis. BMC Pregnancy Childbirth 2025 Feb 8;25(1):130
5. Katarzyna, Zych-Krekora; Oskar, Sylwestrzak; Michał, Krekora, et al: Potential Impact of a Pregnant Woman's Microbiota on the Development of Fetal Heart Defects: A Review of the Literature. Biomedicines 2024 Mar 14;12(3)
6. Tingting, Wang; Lizhang, Chen; Peng, Huang.,et al: Association of maternal gut microbiota and plasma metabolism with congenital heart disease in offspring: a multi-omic analysis. Sci Rep 2021 Mar 5;11(1):5339
7. Xiang, Liu; Shaoyou, Lu; Yijia, Shao. Disorders of gut microbiota in children with Tetralogy of Fallot. Transl Pediatr 2022 Mar;11(3):385-395
8. He Q, et al. The meconium microbiota shares more features with the amni otic fluid microbiota than the

maternal fecal and vaginal microbiota. Gut Microbes. 2020;12:1794266.

9. Anna, Kaisanlahti; Jenni, Turunen; Nadiya, Byts, et al: Maternal microbiota communicates with the fetus through microbiota-derived extracellular vesicles. Microbiome 2023 Nov 13;11(1):249
10. Katarzyna, Zych-Krekora; Oskar, Sylwestrzak; Michał, Krekora. Potential Impact of a Pregnant Woman's Microbiota on the Development of Fetal Heart Defects: A Review of the Literature. Biomedicines 2024 Mar 14;12(3)
11. Sender, R.; Fuchs, S.; Milo, R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016, 14, e1002533.
12. Thursby, E.; Juge, N. Introduction to the human intestinal microflora. Biochem. J. 2017, 474, 1823–1836.
13. Valdes, A.M.; Walter, J.; Segal, E.; Spector, T.D. The role of the gut microbiota in nutrition and health. BMJ 2018, 361, k2179.
14. Belkaid, Y.; Hand, T.W. The role of the microbiota in immunity and inflammation. Cell 2014, 157, 121–141.
15. DiGiulio, D.B.; Callahan, B.J.; McMurdie, P.J.; Costello, E.K.; Lyell, D.J.; Robaczewska, A.; Sun, C.L.; Goltsman, D.S.; Wong, R.J.; Shaw, G.; et al. Temporal and spatial variability of the human microbiota during pregnancy. Proc. Natl. Acad. Sci. USA 2015, 112, 11060–11065.
16. 42. Stokholm, J.; Blaser, M.J.; Thorsen, J.; Rasmussen, M.A.; Waage, J.; Vinding, R.K.; Schoos, A.M.M.; Kunøe, A.; Fink, N.R.; Chawes, B.L.; et al. Maturation of the gut microbiome and risk of asthma in childhood. Nat. Commun. 2018, 9, 141.
17. Kaisanlahti A, et al. Maternal microbiota communicates with the fetus through microbiota-derived extracellular vesicles. Microbiome. 2023;11:249.
18. Franziska A, Graef; Bettina, Berger; Lina S, Bahr; et a. Fasting elicits gut microbiome signature changes that extend to type 1 diabetes patients. Front Endocrinol (Lausanne) 2025;16(0):1623800
19. Mei, Li; Ding, Cheng; Chuan, Peng et al. Therapeutic mechanisms of the medicine and food homology formula Xiao-Ke-Yin on glucolipid metabolic dysfunction revealed by transcriptomics, metabolomics and microbiomics in mice. Chin Med 2023 May 18;18(1):57
20. Igor, Venâncio; Ângelo, Luís; Fernanda, Domingues et al. The Prevalence of Arcobacteraceae in Aquatic Environments: A Systematic Review and Meta-Analysis. Pathogens 2022 Feb 13;11(2)

Table 1. Basic Information of Pregnant Women

Number	Group	Age	Conception method	gestational age (W+T)	number of pregnancies	Comorbidity	Fetal Congenital Heart Disease
1	CCHD	24	natural	38	2	Human Papillomavirus	Tetralogy of Fallot
2	CCHD	38	natural	35+6	2	Preeclampsia	Tetralogy of Fallot
3	CCHD	30	assisted conception	35+5	3	Preeclampsia	Pulmonary Atresia
4	CCHD	24	natural	31+6	1	Premature Rupture of Membranes, Anemia	Complete Transposition of the Great Arteries
5	CCHD	34	natural	38+3	1		Pulmonary Atresia
6	CCHD	39	natural	40	2	Obesity, Cervical Insufficiency	Severe Pulmonary Valve Stenosis
7	NOR	35	natural	38+6	2		-
8	NOR	33	natural	37+6	1		-
9	NOR	33	natural	36+2	1		-
10	NOR	39	natural	40	1	Pituitary Adenoma	-
11	NOR	37	natural	36	2	Severe Subaortic Stenosis	-
12	NOR	36	natural	38	1		-
13	NOR	32	assisted conception	37+2	1	Gestational Diabetes Mellitus	-
14	NOR	28	natural	40+1	1		-
15	NOR	27	assisted conception	36+6	1	Gestational Hypertension	