Research article

Microbiota of pregnancy, placenta and newborns in the third trimester: A randomized controlled study

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A R T I C L E   I N F O

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A B S T R A C T

Microbiota in pregnant time is vital to healthy of pregnant women and their offspring. However, few study evaluate the composition of the microbiota of health pregnancy, placenta and their newborns at different stages and the origin of the placental microbiota. Samples were obtained from a total of 31 pregnant individuals and their offspring, analyzing by 16S rRNA amplicon sequencing of the V4 region to evaluate the composition and variation of them. We found that the microbiota of pregnant individuals changes in the third trimester. The placental microbiota has its own specific dominant microbiota. The placental microbiota is correlated with the pregnancy microbiota in the gut and vagina at 32–34 weeks but not at full term. The gut microbiota in newborns changes over the first 14 days.

1. Introduction

In recent years, new knowledge about digestive tract health has emerged. Intestinal microorganisms play an important role in daily life [1]. The gut microbiota is important to human health, including pregnancies and their infants. These microbiota have an effect on immunity and metabolisms among pregnant individuals and their offspring [2]. Vuong firstly conduct an animal experiment to elucidate the molecular mechanism underlying the effect of the gut microbiota in female rats on the growth of offspring's nervous system [3].

Previous research has revealed that the composition of the gut microbiota is constantly changing [4,5] and can be influenced by age, sex, genetics, dietary habits, and medicine [5]. During pregnancy, the gut microbiota is constantly changing too [6,7].

Actinobacteria and *Proteobacteria* increase in the early stage of pregnancy, but alpha diversity decreases [7]. In the late stage of pregnancy, as pregnancy weight increases, the beta diversity of the gut microbiota also increases [7]. The gut microbiota will return to normal one month after delivery compared to nonpregnant time [6].

The fetal and newborn periods are important for the development of the immune system and the mucosal barrier [2]. In previous studies, meconium was found to be sterile [8,9]; however, a recent study found germ-carrying meconium [8,9]. It seems that the gut microbiota grew in the fetal gut [8,9]. Therefore, aim of this study was systematically explored the variation in the gut and vaginal microbiota during the 3rd trimesters of pregnancy. We also aimed to determine whether this variation influence the placenta microbiota.

2. Material and methods

2.1. Study design and participants

Samples were obtained from pregnant individuals, without antibiotics and probiotics usage, receiving antenatal care in the 1st Affiliated Hospital of Jinan University. Informed consent was obtained from pregnant women (at least 32 gestational weeks) who met the inclusion criteria. Pregnant women were followed up from gestational 32 weeks to delivery. Newborns were followed up until 14 days after delivery (Table 1). Pregnant inclusive criteria were: 1. Chinese woman who is pregnant with a single fetus; 2. First pregnancy and term delivery. Newborns' inclusive criteria were: 1. Normal weight (>2500 g, <4000 g); 2. Term Infant (>37 weeks, <42 weeks). 3. Natural birth. exclusive criteria were: 1. Gastrointestinal disease or family history; 2. Vaginitis before pregnancy; 3. Antibiotic usage during pregnancy; 4. Hypertension, Diabetes Mellitus, Hyperthyroidism, Hypothyroidism, Autoimmune Disease, or Other Endocrine and Metabolic Disease; 5. Gestational Hypertensive Disease, Gestational Diabetes Mellitus or Other Gestational Disease; 6. Transfusion History, Organ Transplantation History or Immunotherapy History. Newborn's exclusive criteria were: 1. Abnormal weight (>4000 g, <2500 g); 2. With Congenital Diseases; 3. Intrapartum Fetal Complication.

2.2. Feces collection, vaginal secretion collection, placenta collection, meconium collection

Sampling operations were executed by trained professionals under strict aseptic conditions and a uniform protocol.

2.2.1. Feces collection
Feces from pregnancy were collected twice between weeks 32–34 and before labor. Feces collected at weeks 32–34 were collected internally by sterile and dedicated collecting box, thereby avoiding any contamination with foreign material. Feces were stored in a domestic refrigerator (-20 °C) and transferred to the laboratory freezer in frozen pipe at $-80°$ centigrade within 24 h. Feces collected before labor were obtained in the hospital and transferred to the laboratory freezer within 30 min of collection.

2.2.2. Vaginal secretion collection
Vaginal secretions were collected twice between weeks 32 and 34 and before labor. The pregnant woman was asked to not have engage in sexual behavior, clean the vulva, clean the vagina, or use vaginal medicine within 48 h before the sample collection. Vaginal secretion before labor was collected before membrane rupture occurred. The samples were collected at posterior fornix of virgina by sterile cotton swab, stored in frozen pipe and transferred to the laboratory freezer within 30 min of collection, thereby avoiding any contamination by foreign material.

2.2.3. Placenta collection
Placenta were collected ranging from the umbilical cord to 3 cm by stripping the amniotic membrane after birth within 30 min. Blood was rinsed with sterile saline liquid. Four to six pieces of placenta were sampled from the fetal surface. Each piece had a volume of approximately 1 cm^3. The placenta sample were immediately placed in a dedicated specimen box and transferred to the laboratory freezer within 30 min of collection.

2.2.4. Meconium collection
Meconium was collected three times on the 1st day, 3rd day, and 14th day. On the 1st day and 3rd day, meconium was collected

Table 1
Grouping name illustration.

Group	Sample from
PMF	Feces from pregnancy at 32–34 weeks
PML	Vaginal secretions from pregnancy at 32–34 weeks
PLF	Feces from pregnancy at full term
PLL	Vaginal secretions from pregnancy at full term
PP	Placenta from pregnancy
B1F	Meconium of newborn
B2F	Feces from newborn at 3rd day after birth
B3F	Feces from newborn at 14th day after birth

internally by sterile and dedicated collecting box, thereby avoiding any contamination with foreign material in the hospital, and transferred to the laboratory freezer within 30 min of collection. On the 14th day, meconium was collected, stored in a domestic refrigerator, and then transferred to a laboratory freezer infrozen pipe within 24 h of collection.

3. Experimental Procedure

3.1. DNA extraction and Sample quality control

For soil, feces, and intestinal content samples, DNA was extracted by using a Magnetic Soil and Stool DNA Kit (TianGen, China, Catalog #: DP712). For other types of samples, DNA was extracted by using the CTAB extraction method. Please refer to the QC Report for methods of sample quality control.

3.2. Amplicon generation

16S rRNA/18SrRNA/ITS genes of distinct regions (16SV4/16SV3/16SV3– V4/16SV4– V5, 18SV4/18SV9, ITS1/ITS2, ArcV4) were amplified using a specific primer (e.g., 16SV4: 515F- 806R, 18SV4: 528F-706R, 18SV9: 1380F- 1510R) with the barcode. All PCRs were conducted out with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM forward and reverse primers, and approximately 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s and 72 °C for 5 min.

3.3. PCR product quantification and Qualification

The same volume of 1X loading buffer (containing SYB green) was mixed with PCR products, and electrophoresis was performed on a 2 % agarose gel for detection. PCR products were mixed in ratios with equal densities. Then, the mixture of PCR products was purified with a Universal DNA Purification Kit (TianGen, China, Catalog #: DP214).

3.4. Library Preparation and sequencing

Sequencing libraries were generated using the NEB Next® Ultra™ II FS DNA PCR-free Library Prep Kit (New England Biolabs, USA, Catalog#: E7430L) in accordance with the manufacturer's recommendations, and indexes were added. The library was checked with Qubit and real-time PCR for quantification and a bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on Illumina platforms according to the needed effective library concentration and data amount.

3.5. Data analysis

Single-end reads assembly and quality control.

3.6. Data split

Single-end reads was assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence.

3.7. Data Filtration

Quality filtering on the raw reads were performed under specific filtering conditions to obtain the high-quality clean reads according to the Cutadapt (Martin M. , 2011) (V1.9.1, http://cutadapt.readthedocs.io/en/stable/) quality controlled process.

3.8. Chimera removal

The reads were compared with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html) using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) to detect chimera sequences, and then the chimera sequences were removed. Then the Effective Tags finally obtained.

4. OTU(Operational taxonomic Units) cluster and species annotation

4.1. OTU Production

Sequences analysis were performed by Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/). Sequences with ≥97 % similarity were assigned to the same OTUs. Representative sequence for each OTU was screened for furtherannotation.

4.2. Species annotation

For each representative sequence, the Silva Database (https://www.arb-silva.de/)was used based on RDP classifier (Version 2.2, http://sourceforge.net/projects/rdp-classifier/) algorithmto annotate taxonomic information.

4.3. Phylogenetic relationship construction

In order to study phylogenetic relationship of different OTUs, and the difference of the dominant species in different samples (groups), multiple sequence alignment wereconducted using the MUSCLE software (Version 3.8.31, http://www.drive5.com/muscle/).

4.4. Data normalization

OTUs abundance information were normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity were all performed basing on this output normalized data.

4.5. Alpha diversity

Alpha diversity is applied in analyzing complexity of species diversity for a sample, calculated with QIIME (Version1.7.0) and displayed with R software (Version 2.15.3).

4.6. Statistical analysis

Difference analysis of each group. All measurement data are presented as the median ± interquartile range. Assessments of OTU differences in the pregnancy and newborn gut microbiota, pregnancy vaginal microbiota, and placental microbiota were analyzed using non-parametric tests (Mann-Whitney *U* test), and $p < 0.05$ was considered statistically significant. Other outcomes were analyzed using multiple linear regression. All data were analyzed using SPSS 19.0 statistical software (SPSS, Inc., Chicago, IL, USA).

5. Results

The anthropometrics for maternal and infant, as well as other clinical characteristics were recorded as below (Table 2).

5.1. The constitution of microbiota of pregnancies and their offspring

5.1.1. Gut microbiota of pregnancy in different trimesters

Firmicutes (52.1 %), *Bacteroidetes* (26.6 %), and *Actinobacteria* (11.2 %) were the three most common components in the gut microbiota at weeks 32–34 (PMF) at the phylum level. Similarly, *Firmicutes* (59.8 %), *Bacteroidetes* (21.1 %) and *Actinobacteria* (10.4 %) were the three most common components in the gut microbiota of full-term women (PLF) (Fig. 1a).

Bacteroides (16.6 %), *Faecalibacterium* (10.3 %), *Bifidobacterium* (8.8 %), *Prevotella_9* (6.2 %), and *Escherichia-Shigella* (5.2 %) were the five most common components in the gut microbiota of the PMF group at the genus level. In the PLF group, the five most common genera were *Bacteroides* (14.0 %), *Faecalibacterium* (11.8 %), *Bifidobacterium* (10.2 %), *Blautia* (8.9 %) and *Dialister* (4.5 %) (Fig. 1b).

5.1.2. Vaginal microbiota of pregnancy in different trimesters

Firmicutes (92.4 %), *Actinobacteria* (7.0 %), and *Bacteroidetes* (0.25 %) were the three most common components in the vaginal microbiota at weeks 32–34 (PML) at the phylum level. *Firmicutes* (83.4 %), *Actinobacteria* (10.4 %), and *Bacteroidetes* (2.6 %) were also the three most common components in the vaginal microbiota of full-term women (PLL) at the phylum level (Fig. 1c).

Lactobacillus (91.7 %), *Gardnerella* (6.5 %), *Atopobium* (0.3 %), *Streptococcus* (0.07 %), and *Prevotella* (0.06 %) were the five most common components in the vaginal microbiota in the PML group at the genus level. In the PLL group, the five most common components were *Lactobacillus* (81.1 %), *Gardnerella* (9.2 %), *Prevotella* (2.1 %), *Sneathia* (1.9 %), and *Atopobium* (0.8 %) (Fig. 1d).

Table 2
Clinical characteristics fornaternal and infant.

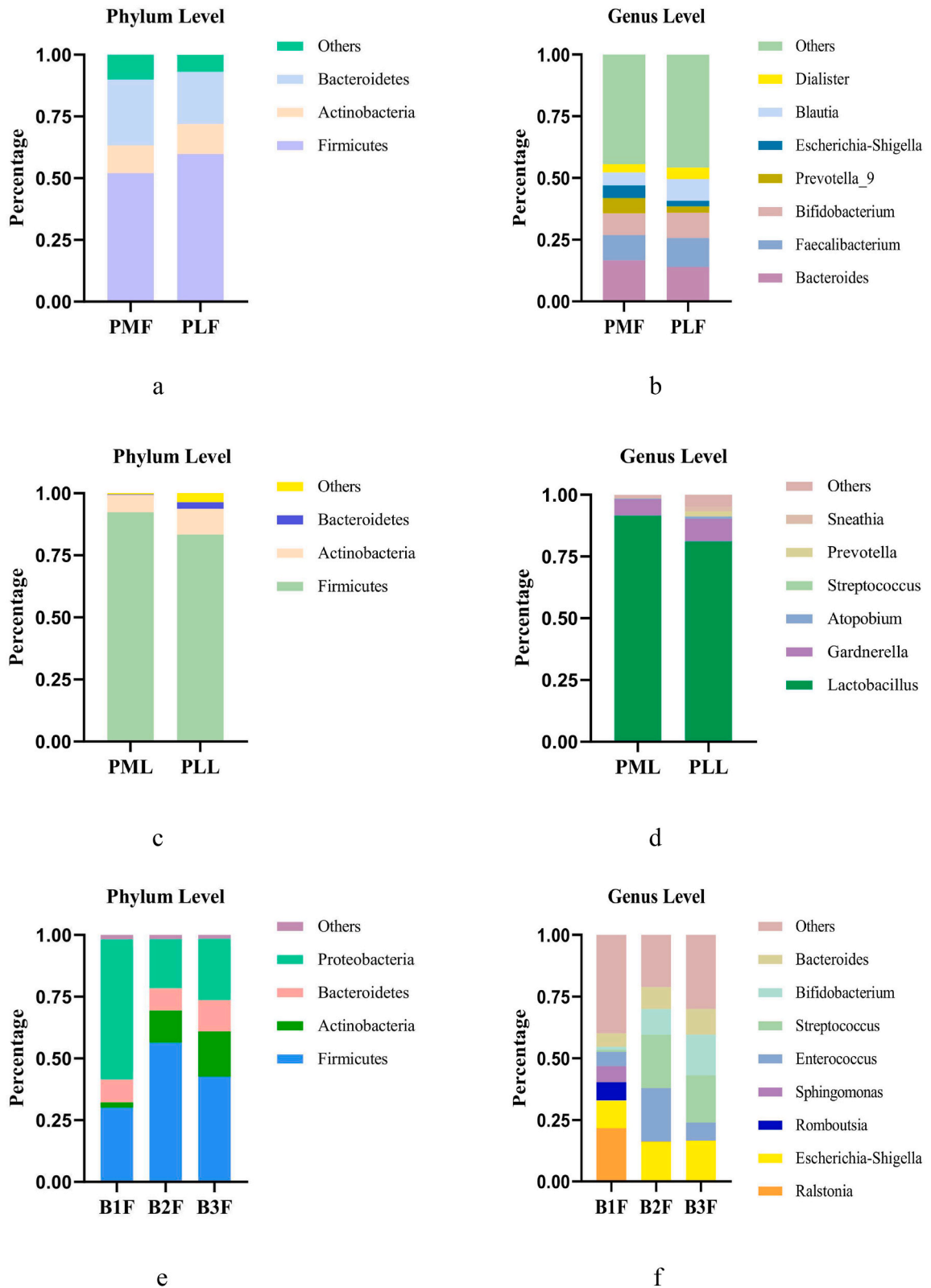|	Average	Range
Maternal age	27	23–35
Maternal height	165.0 cm	145.0–178.0 cm
Maternal weight before labor	65.7 kg	51–80.4 kg
Maternal BMI	25.56 kg/m2	19.36–29.35 kg/m2
Neonatal weight	3.31 kg	2.70–3.89 kg
Neonatal body length	49.7 cm	47.0–53.0 cm

Fig. 1. a–1f: Percentage histogram of gut microbiota, vaginal microbiota of pregnancy in different time and newborn's gut microbiota.

5.1.3. Fetal and newborn gut microbiota

Actinobacteria (56.7 %), *Firmicutes* (30.0 %), and *Bacteroidetes* (9.3 %) were the three most common components in meconium (B1F) at the phylum level. In the gut microbiota of newborns on the 3rd day (B2F), the three most common components were *Firmicutes* (56.4
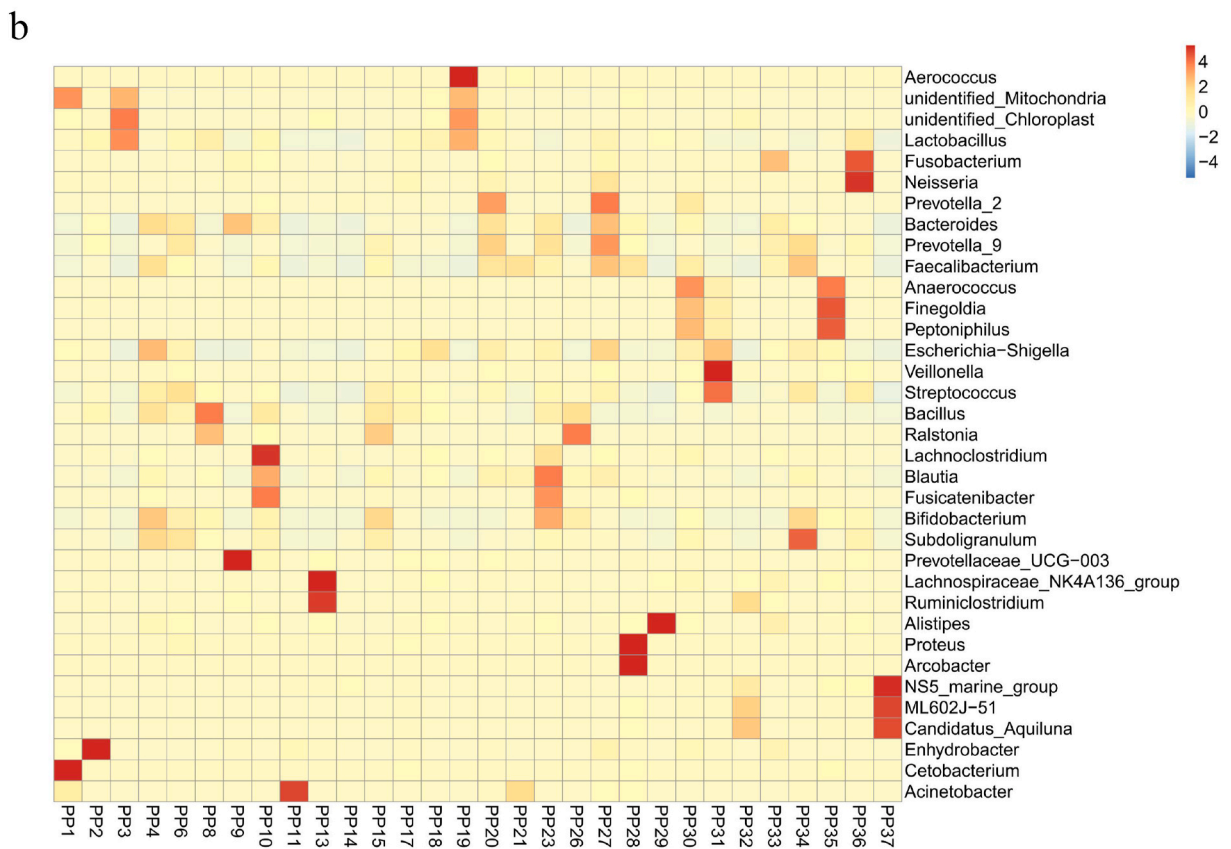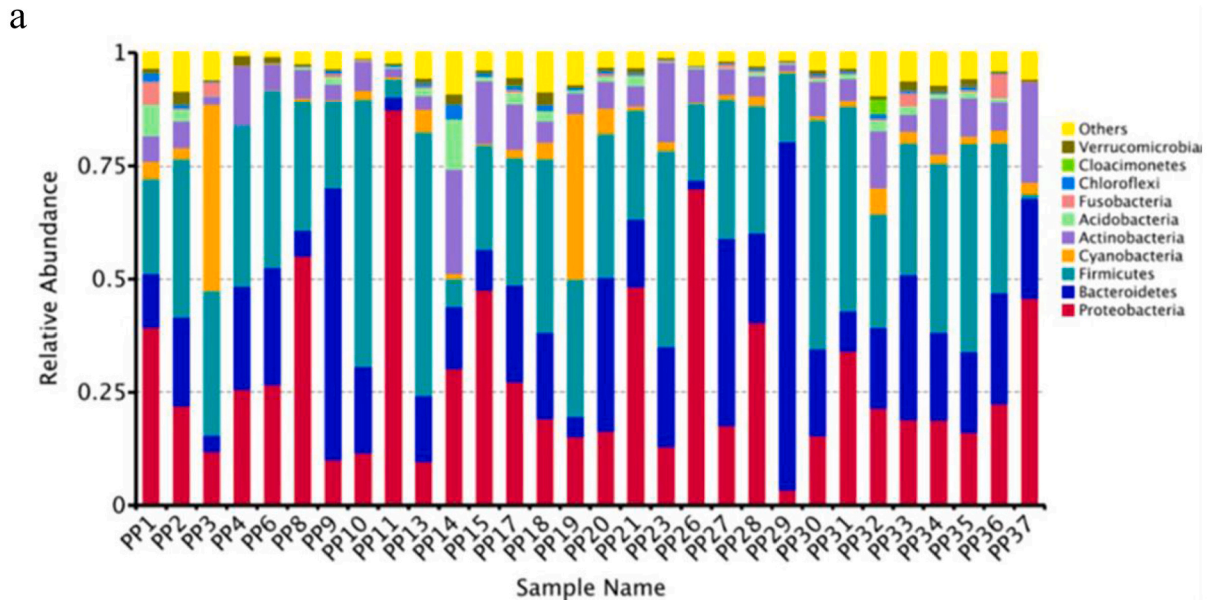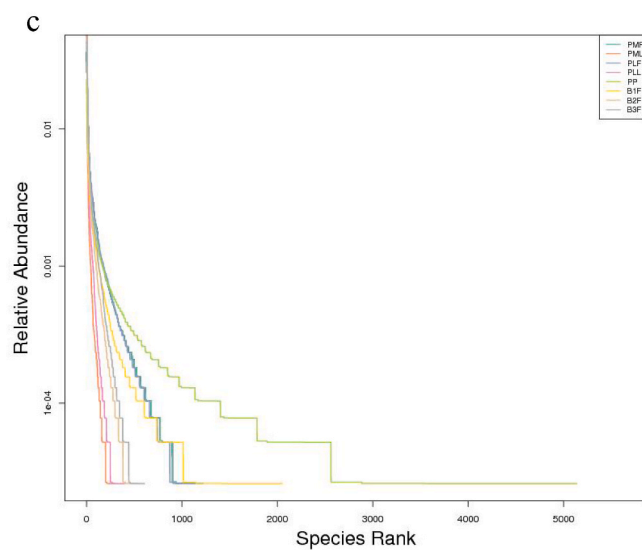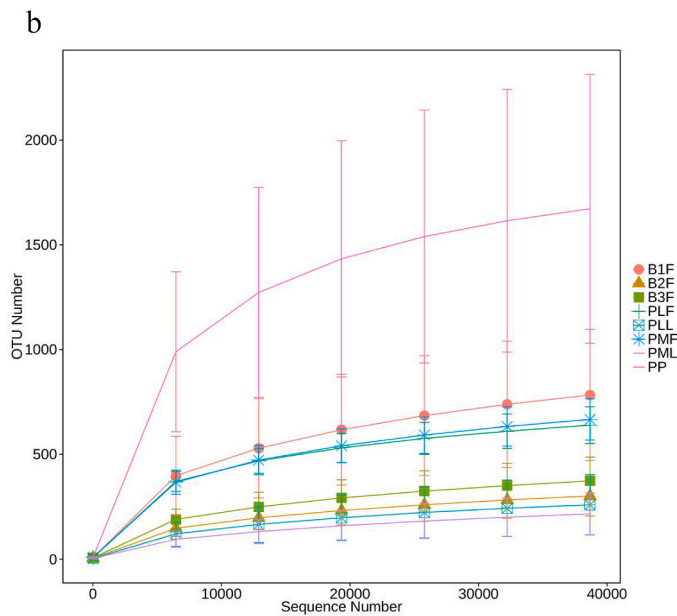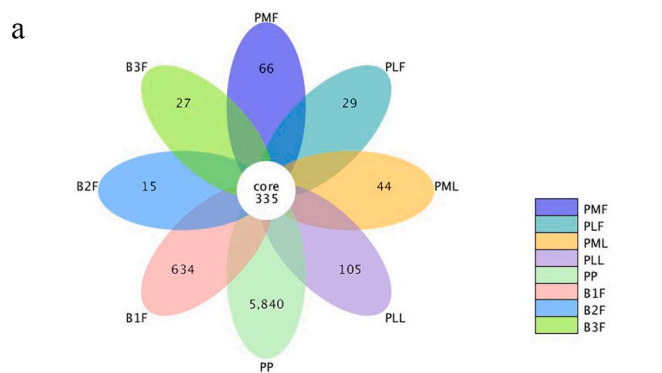
Fig. 2. aAbundance of bacterial phylum in 30 placental samples
Fig. 2bHeatmap showing the bacterial genus abundance (Z score) of the total population of 30 placental samples.

a

b

c

(caption on next page)

Fig. 3. a Each petal in the diagram represents a group sample; different colors represent different groups. The core number represents the number of species in all samples, and the number on the petal represents the number of species unique to the group. Fig. 3bRarefaction Curve. Each curve in the diagram represents a group sample; different colors represent different groups. Flat curve means more sequence number will get less OTU number. Fig. 3c Rank Abundance Curve. Each curve in the diagram represents a group sample; different colors represent different groups. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

%), *Proteobacteria* (19.9 %) and *Actinobacteria* (13.0 %). On the 14th day (B3F), the 3 most common phylum were *Firmicutes* (42.6 %), *Proteobacteria* (19.9 %) and *Actinobacteria* (18.4 %) (Fig. 1e).

Ralstonia (21.7 %), *Escherichia-Shigella* (11.3 %), *Romboutsia* (7.4 %), *Sphingomonas* (6.5 %), and *Enterococcus* (3.8 %) were the five most common components in meconium (B1F) at the genus level. On the 3rd day (B2F), the five most common components were *Enterococcus* (21.6 %), *Streptococcus* (21.5 %), *Escherichia-Shigella* (16.0 %), *Bifidobacterium* (10.6 %) and *Bacteroides* (8.8 %). On the 14th day (B3F), the five most common components were *Streptococcus* (19.1 %), *Escherichia-Shigella* (16.5 %), *Bifidobacterium* (16.4 %), *Bacaeroides* (10.5 %) and *Enterococcus* (7.4 %) (Fig. 1f).

5.1.4. Placenta microbiota

Firmicutes (30.6 %), *Actinobacteria* (28.1 %), and *Bacteroidetes* (20.9 %) were the top three components in the placenta at the phylum level (Fig. 2a). However, the proportions widely varied across samples. *Bacteroides* (6.5 %), *Acinetobacter* (5.5 %), *Ralstonia* (4.7 %), *Lactobacillus* (3.5 %), and *Bifidobacterium* (2.9 %) were the five most common components in the placenta at the genus level (Fig. 2b).

5.2. Diversity of each microbiota

There were 66 specific species in the gut microbiota at 32–34 weeks (PMF). There were only 29 specific species in full-term women (PLF). Regarding the vaginal microbiota, there were 44 specific species at 32–34 weeks (PML) and 105 specific species in full-term women (PLL). In meconium, the number of specific species was 105. This amount decreased to 15 and 27 on the 3rd day and 14th day, respectively (Fig. 3a).

Rank Abundance Curve represent richness and evenness of all samples. In vertical axis, more flat means more evenness. In X axis, more x-axis span means more richness. Rarefaction Curve and Rank Abundance curve were used to analyse the α-diversity of all groups. From these two figures we could see that, the α-diversity of maternal gut microbiota in full-term decreased than those in gestational 32–34 weeks. The α-diversity of maternal virginal microbiota in full-term increased than those in gestational 32–34 weeks. The α-diversity of newborn' gut microbiota in 3rd and 14th day decreased than those in meconium. The α-diversity of placenta microbiota were more than those of other samples. (Fig. 3b and c)

5.3. Difference comparison of pregnancy microbiota in different stages

Among the 10 most common phylum, *Acidobacteria* and *Chloroflexi* were significantly less abundant in the gut of full-term women than those at 32–34 weeks at the phylum level **(Table 3a).**

Actinobacteria and *Fusobacteria* were significantly more abundant in the prenatal vagina among full-term women than among those at 32–34 weeks at the phylum level. **(Table 3b).**

Holdemanella was more abundant in the gut of full-term women than among those at 32–34 weeks at the genus level, but this difference has no statistical significance. **(Table 4a).**

Gardenerella, Atopobium, Prevotella, Sneathia, Prevotella_6, Dialister, Staphylococcus and *Ureaplasma* were significantly more abundant in the prenatal vagina of full-term women than those at 32–34 weeks at the genus level. *Prevotella_9* were significantly less abundant in the vagina of full-term women than those at 32–34 weeks. (Table 4b).

5.4. Newborn's gut microbiota comparison

Firmicutes were significantly more abundant in the feces at day 3 than at day 1 than at the phylum level. *Proteobacteria, Bacteroidetes, Cyanobacteria, Verrucomicrobia and Acidobacteria* were significantly less abundant in the feces at day 3 than at day 1 (Table 5a). The microbiota in the feces was not significantly different between the days 3 and 14 (Table 5c).

At the genus level, the fecal microbiota on the 3rd day had many differences from that at day 1. *Streptococcus* and *Enterococcus* were

Table 3a

Comparison of fecal OTUs between PMF and PLF at the phylum level (n = 16).

Different species of bacteria OTUs	PMF		PLF		p
	Median	IQR	Median	IQR	
Acidobacteria	.0001163	.0003490	.0000000	.0000453	.000*
Chloroflexi	.0000130	.0000776	.0000000	.0000000	.022*

*$p < 0.05$.

Table 3b
Comparison of fecal OTUs between PML and PLL at the phylum level (n = 15).

Different species of bacteria OTUs	PML		PLL		p
	Median	IQR	Median	IQR	
Actinobacteria	.0017323	.0031544	.0035940	.0417055	.038*
Fusobacteria	.0000905	.0002004	.0002586	.0002586	.000*

*p < 0.05.

Table 4a
Comparison of fecal OTUs between PMF and PLF at the genus level (n = 16).

Different species of bacteria OTUs	PMF		PLF		p
	Median	IQR	Median	IQR	
Holdemanella	.0001164	.0109564	.0003103	.0283057	.069

*p < 0.05.

Table 4b
Comparison of fecal OTUs between PML and PLL at the genus level (n = 15).

Different species of bacteria OTUs	PML		PLL		p
	Median	IQR	Median	IQR	
Gardnerella	.0005171	.0012863	.0013445	.0046282	.013 *
Atopobium	.0000000	.0000711	.0001293	.0003361	.001*
Prevotella	.0000905	.0002521	.0003878	.0048609	.007*
Sneathia	.0000000	.0000000	.0001810	.0002844	.000*
Prevotella_6	.0000000	.0000000	.0000259	.0003361	.004*
Dialister	.0000776	.0001745	.0002327	.0018874	.041*
Prevotella_9	.0000517	.0001874	.0000000	.0000517	.008*
Staphylococcus	.0000776	.0002068	.0003361	.0012411	.001*
Ureaplasma	.0000259	.0002327	.0001551	.0016806	.005*

*p < 0.05.

Table 5a
Comparison of fecal OTUs between B1F and B2F at the phylum level (n = 31).

Different species of bacteria OTUs	B1F		B2F		p
	Median	IQR	Median	IQR	
Proteobacteria	.7,704,002	.7,043,645	.0897456	.2,252,560	.000*
Firmicutes	.0935205	.5,835,660	.7,584,549	.8,496,742	.016*
Bacteroidetes	.0402058	.0822732	.0028441	.0102648	.000*
Cyanobacteria	.00131865	.0046540	.0000000	.0000517	.000*
Verrucomicrobia	.00087910	.0012152	.0000000	.0000517	.000*
Acidobacteria	.00064640	.0018875	.0000259	.0000052	.000*

*p < 0.05.

significantly more abundant in the feces on day 3 than on day 1. *Ralstonia, Romboutsia, Sphingomonas, Bacteroides, Acinetobacter* and many microbiota in the genus were significantly less abundant in the feces on day 3 than at day 1 (Table 5b). *Bifidobacterium, Clostridium_sensu_stricto_1, Veillonella, Lactobacillus* and *Parabacteroides* were significantly more abundant in the feces on day 14 than at day 3. *Megamonas* was significantly less abundant on day 14 than on day 3 (Table 5d).

5.5. Correlation analysis of placental microbiota

We used stepwise multiple regression analysis to identify the origin of placental microbiota; the dependent variable was the placental microbiota OTUs, and the independent variables were gut microbiota and vaginal microbiota at 32–34 weeks and at full term. We found that *Proteobacteria* correlated with the vaginal microbiota at 32–34 weeks. The multiple correlation coefficient was 0.381 (*p < 0.05*). *Tenericutes* were positively correlated with the gut microbiota at 32–34 weeks and negatively correlated with the gut microbiota at full term (*p < 0.05*) (Table 6a).

At the genus level, *Clostridium sensu stricto* was positively correlated with gut microbiota at 32–34 weeks (*p < 0.05*). *Fusobacterium* and *Gardnerella* were correlated with the vaginal microbiota at 32–34 weeks (*p < 0.05*). *Sphingomonas* was positively correlated with

Table 5b

Comparison of fecal OTUs between B1F and B2F at the genus level (n = 31).

Different species of bacteria OTUs	B1F		B2F		p
	Median	IQR	Median	IQR	
Ralstonia	.2,472,851	.4,146,241	.0005947	.0021460	.000*
Romboutsia	.0012410	.0007498	.0000000	.0000000	.000*
Sphingomonas	.0556934	.1,152,653	.0001034	.0003620	.000*
Enterococcus	.0028959	.0023529	.0071362	.5,932,361	.012*
Bacteroides	.0212018	.0395336	.0018875	.0079636	.001*
Acinetobacter	.0091530	.0217448	.0001293	.0005947	.000*
Lactobacillus	.0033354	.0053263	.0002844	.0010342	.000*
Clostridium_sensu_stricto_1	.0024304	.0050677	.0009567	.0007240	.009*
Streptococcus	.0045506	.0061278	.0703537	.4,136,415	.000*
Prevotella_9	.0033354	.0043696	.0000517	.0002068	.000*
Faecalibacterium	.0019392	.0045506	.0000517	.0001034	.000*
Parabacteroides	.0014221	.0003723	.0000776	.0003102	.000*
Blautia	.0010601	.0029217	.0000259	.0001034	.000*
unidentified_Chloroplast	.0006981	.0030251	.0000000	.0000259	.000*
Akkermansia	.0003361	.0011635	.0000000	.0000259	.000*
Veillonella	.0003878	.0012669	.0001034	.0001810	.020*

*$p < 0.05$.

Table 5c

Comparison of fecal OTUs between B2F and B3F at the phylum level (n = 30).

Different species of bacteria OTUs	B2F		B3F		p
	Median	IQR	Median	IQR	
Actinobacteria	.0156686	.1,090,857	.0943996	.2,978,591	.062
Proteobacteria	.7,704,002	.7,043,645	.1,943,533	.2,389,531	.063

*$p < 0.05$.

Table 5d

Comparison of fecal OTUs between B2F and B3F at the genus level (n = 30).

Different species of bacteria OTUs	B2F		B3F		p
	Median	IQR	Median	IQR	
Bifidobacterium	.0024822	.0280536	.0576456	.2,776,076	.025*
Megamonas	.0006723	.0011377	.0000260	.0000517	.000*
Clostridium_sensu_stricto_1	.0009567	.0007240	.0023012	.0682788	.001*
Veillonella	.0001034	.0001810	.0013704	.0209238	.000*
Lactobacillus	.0002844	.0010342	.0008274	.0010278	.000*
Parabacteroides	.0000776	.0003102	.0003620	.0012088	.000*

*$p < 0.05$.

Table 6a

Stepwise multiple regression analysis results of the source of placental microbiota at the phylum level.

The source of microbiota	Placental microbiota	Beta	Std Err	R	p Value
PML	*Proteobacteria*	26.617	12.902	0.381	0.048*
PMF	*Tenericutes*	0.323	0.049	1.255	0.000*
PLF	*Tenericutes*	−0.119	0.030	−0.763	0.001*

*$p < 0.05$.

Table 6b

Results of multiple linear regression of placental anaerobic Clostridium.

The source of microbiota	Placental microbiota	Beta	Std Err	R	p Value
PMF	*Clostridium_sensu_stricto_1*	0.095	0.045	0.387	0.046*
PML	*Fusobacterium*	128.670	32.087	0.626	0.000*
	Gardnerella	0.002	0.000	0.634	0.000*
	Sphingomonas	59.587	15.581	0.722	0.001*
PLF	*Sphingomonas*	−18.927	7.877	−0.454	0.024*

*$p < 0.05$.

the vaginal microbiota at 32–34 weeks and negatively correlated with the gut microbiota at full term ($p < 0.05$) (Table 6b)

6. Discussion

In previous work, the gut microbiota in the third trimester of pregnancy is different from that in the second trimester [6]. In our study, the gut microbiota at 32–34 weeks was similar to that at full term at the phylum level, consisting of *Firmicutes, Bacteroides*, and *Actinobacteria*. They are also similar at the genus level, consisting of *Bacteroides, Faecalibacterium*, and *Bifidobacterium*. This result was different from Koren's study. They found that in the third stage of pregnancy, *Proteobacteria* and *Actinobacteria* are the main microbiota [6]. This difference may be related to race, region, diet habits, weight of pregnancy, and metabolism [5,7]. Therefore, it is important to identify the composition of the gut microbiota in different regions. We found that α-diversity of maternal gut microbiota in full-term decreased than those in gestational 32–34 weeks. We also found that in the late stage of pregnancy, *Acidobacteria* and *Chloroflexi* were less abundant at full term than at 32–34 weeks, and *Holdemanella* was more abundant at full term than at 32–34 weeks, but this difference had no statistical significance. However, research on these microbiota is rare. Therefore, the variation and meaning of these microbiota are still unclear. However, previous work showed that gut microbiota had relationship with mellitus, gestational hypertensive disorder and other complications of pregnancy [10,11]. This relationship between microbiota variation and complication of pregnancy need further investigation.

Vaginal microbiota are unique to females. We found that the constitution of the vaginal microbiota is similar to that in the gut. However, the proportions are significantly different. The dominant bacteria in the vagina are *Firmicutes* at the phylum level and *Lactobacillus* at the genus level. The vaginal microbiota of pregnancy varied with gestational weeks. As pregnant females approach full term, they have less diversity, more stability, and more *Lactobacillus* [12]. However, in our study, we first found that the α-diversity of maternal virginal microbiota in full-term increased than those in gestational 32–34 weeks. *Actinobacteria* and *Fusobacteria* were significantly more abundant in the prenatal vagina among full-term women than among those at 32–34 weeks at the phylum level. *Gardenerella, Atopobium, Prevotella, Sneathia, Prevotella_6, Dialister, Staphylococcus* and *Ureaplasma* were significantly more abundant in the prenatal vagina of full-term women than those at 32–34 weeks at the genus level. Only *Prevotella_9* were significantly less abundant in the vagina of full-term women than those at 32–34 weeks. In previous work, some scholars found that *Lactobacilli* were less abundant in puerperium [13]. They also found that *Actinobacteria*, related to bacterial vaginitis, was more abundant in puerperium [13]. This variation could be sustained until one year after labor [14]. We thought that this variation was due to hormone changes, leading to variation in the vaginal environment and inducing labor onset. However, the mechanism of this variation is still unclear.

Collado found that in the placenta, *Proteobacteria* is the main component at the phylum level. *Enterobacter, Escherichia* and *Propionibacterium* are the main components in the placenta at the genus level [15]. In Xu's study, there were *Lactobacteriaceae* and M*icrococcaceae* in the placenta [16]. In our study, we found that the constitution of the placental microbiota is much different from that of the gut and vaginal microbiota. The main component of the placental microbiota is *Proteobacteria* at the phylum level. The main genus-level microbiota in the placenta were *Lactobacillus, Bacteroides, Ralstonia, Acinetobacter* and *Bifidobacterium*.

In 2008, Jimenez found that *Enterococcus* of pregnant mice with gene marks can be found in the meconium of their offspring after cesarean section. These results show that pregnant women and their offspring may have microbiota translocation. This translocation may occur before labor. Although there has been no consensus about the constitution of meconium, most studies have shown that the main components of meconium are *Enterococcus, Escherichia, Lactobacillus* and *Streptococcus* at the genus level [17–19]. In our study, we found that there was a large difference between feces on the 3rd day and meconium. Although the components of a newborn's feces on the 3rd day 14th day are different, they have similar constitutions. The main components of their feces are *Enterococcus, Escherichia* and *Streptococcus*. However, the special microbiota of newborns were less abundant than those in meconium. The α-diversity of newborn' gut microbiota in 3rd and 14th day decreased than those in meconium. The constitution of the newborn's gut microbiota was different than that in pregnancy. There are also many intraindividual differences. We thought these differences were related to their delivery mode, breastfeeding, environment, and other factors [20–22]. We also suggest that the variation in a newborn's gut microbiota takes a long time. Their gut microbiota may be stable in the short term, but further changes need a long time to be investigated.

In our study, we tried to find the correlation between placental microbiota and pregnancy microbiota for the first time. We found that at the phylum level, *Proteobacteria* were correlated with the vaginal microbiota at 32–34 weeks. *Tenericutes* were positively correlated with the gut microbiota at 32–34 weeks and negatively correlated with the gut microbiota at full term. At the genus level, *Clostridium* was positively correlated with gut microbiota at 32–34 weeks. *Fusobacterium* was correlated with the vaginal microbiota at 32–34 weeks. Interestingly, we found that the placental microbiota was correlated with the vaginal and gut microbiota at 32–34 weeks only. Vaginal microbiota at full term seems to have no relationship with placental microbiota. The gut microbiota has a negative correlation with the placental microbiota. We also found that the placental microbiota has high diversity. The 5 most common microbiota in the placenta accounted for only 23.1 % of all placental microbiota. The number of peculiar species in the placental microbiota is 5840. Currently, whether the placenta and fetus have stable microbiota colonization is still controversial. The influence of this colonization is difficult to determine [23]. Therefore, further research is needed to fully elucidate the diversity of placental microbiota.

To our knowledge, our study has several strengths. It is the first time we evaluate the origin of placenta microbiota excluding pathological pregnancies and deliveries. We also used randomized controlled trial to observed the microbiota from pregnancies to their offspring, including gut, vagina and placenta.

Our study have several limitations: small sample size was the main limitation of the study. Secondly, we did not collect oral and mother milk microbiota. Thirdly, further study is needed to investigate the relationship between maternal each microbiota and their

offspring's microbiota.

7. Conclusions

In summary, we analyzed the gut microbiota, vaginal microbiota, and placental microbiota at different gestational weeks and the composition of gut microbiota in different neonatal periods. We found that the gut and vaginal microbiota at term are different from those at 32–34 weeks of gestation, with a decrease in the endemic species of the gut microbiota and an increase in the endemic species of the vaginal microbiota. After birth, the gut microbiota of neonates is significantly different from that of fetuses, and the number of endemic species decreases sharply. The placental microbiota may be derived from the gut and vaginal microbiota of pregnant women at 32–34 weeks, and its composition has great individual specificity and many endemic species. These results demonstrate the importance of maternal microbiota during pregnancy in shaping placental and neonatal metabolomics. Further research is needed to explore the possible mechanisms.

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Ethics approval

Our research was approved by the Ethics Committee of the First Affiliated Hospital of Jinan University (NO.2019-011)

Data Availability

All data generated or analyzed during this study are included in this published article. The sequence data reported in this study was archived in the Sequence Read Archive with the accession number PRJNA1053345.

CRediT authorship contribution statement

Zhe Li: Methodology, Investigation, Conceptualization. **Yiwen Zhang:** Writing – original draft, Formal analysis, Data curation. **Li Wang:** Writing – review & editing, Supervision. **Tye Kian Deng:** Validation. **Wei-Hsiu Chiu:** Software, Resources. **Wai-kit Ming:** Supervision, Investigation. **Chengfang Xu:** Resources, Funding acquisition. **Xiaomin Xiao:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Zhe Li reports financial support was provided by The Department of Science and Technology of Guangzhou. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24698.

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