Protocol of sampling of feces in preterm infants

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ABSTRACT

This protocol refer to a study having the aim to study on the variation of gut microbiota in preterm infants in the process of feeding intolerance after birth.

In this study, fecal samples were collected from the preterm infants for several times and performed on the bacterial DNA extraction,PCR amplification in the lab, and 16S rRNA sequencing on the Miseq PE300 of the Illumina Platform.

EXTERNAL LINK

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PROTOCOL STATUS

Working

GUIDELINES

A total of 174 sterile tubes with sterile swabs were available for collecting the fecal samples from the diapers of preterm infants. The bacterial genomic DNA from samples was extracted by E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocols (http://omegabiotek.com/store/wp-content/uploads/2013/04/D5625-Soil-DNA-Kit-120216-Online-2). The bacterial primer set of forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the DNA fragment of the 16S rRNA genes. TransStart Fastpfu DNA Polymerase and thermocycler PCR system (GeneAmp 9700, ABI, USA) were available for PCR. 16S rRNA genes sequencing was performed on an Illumina Miseq 300 Platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China.

MATERIALS TEXT

sterile swabs,sterile tubes,E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) ,NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA),1% agarose,2% agarose,TransStart Fastpfu DNA Polymerase,thermocycler PCR system (GeneAmp 9700, ABI, USA),AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA),QuantiFluor™-ST Real-time PCR System (Promega, USA),Illumina Miseq 300 Platform (Illumina, San Diego, USA).

SAFETY WARNINGS

Parents need to be well informed about this study and informed consents need to be obtained from the parents for the participation of each infant. Patient anonymity should be preserved in this study. The researchers need to ensure that there was no extra force or drug to promote the defecation of infants and the collection caused no pain or hurt to the infants. This study should not cause any interference on the clinical judgement and decision of the physician. All the procedures of this study need to be approved by the Ethics Committee of the hospital.

BEFORE STARTING

In this study, preterm infants diagnosed with feeding intolerance were classified into the feeding intolerance group (FIG) and normal infants were classified into the control group (CG).

The inclusion criteria of this study was as follows:

(1)The gestational age of the infant was less than 37 weeks. (2)The preterm infant classified into the FIG was diagnosed as feeding intolerance and got improved to attain the full enteral feeding by clinical treatments during the hospitalization. (3)The preterm infant classified into the CG was never diagnosed as feeding intolerance during the hospitalization. (4)The preterm infants classified into the FIG and the CG never left the incubators before transferred to another hospital or discharged from the hospital.

The exclusion criteria of this study was as follows:
(1)Infants who suffered from systemic inflammatory reaction, Crohn's disease, IBD (inflammatory bowel disease), congenital gastrointestinal anomalies, necrotizing enterocolitis (NEC), pneumonia or severe diarrhea during the hospitalization. (2) Infants who had severe asphyxia, coagulation disorders or received abdominal surgery operation during the hospitalization. (3)Infants whose mothers received antibiotic treatment in antenatal. (4)Infants whose representations of feeding intolerance reappeared within 7 days after feeding intolerance finished.
Fecal samples were collected by disposable sterile swabs from the diapers of preterm infants, transferred to sterile tubes, and stored at -80℃ for the subsequent DNA extraction.

Fecal sampling

1 Fecal samples were collected by disposable sterile swabs from the diapers of preterm infants, transferred to sterile tubes, and stored at -80℃ for the subsequent DNA extraction.

DNA extraction

2 The bacterial genomic DNA from samples was extracted by E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis.

PCR

3 The bacterial primer set of forward primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the DNA fragment of the 16S rRNA genes. TransStart Fastpfu DNA Polymerase and thermocycler PCR system (GeneAmp 9700, ABI, USA) were available for PCR. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. Amplification conditions were as follows: An initial denaturation step of 95℃ for 3 min, followed by 27 cycles of the denaturation at 95℃ for 30 s, annealing at 55℃ for 30 s, elongating at 72℃ for 45 s with a final extension step at 72℃ for 5 min, halted at 10℃. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST Real-time PCR System (Promega, USA) according to the manufacturer's protocol before sequencing.

16S rRNA sequencing

4 16S rRNA genes sequencing was performed on an Illumina Miseq 300 Platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database.

Data analysis

5 Raw fastq files were quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (1)The reads were truncated at any site receiving an average quality score <20 over a 50bp sliding window. (2) Sequences whose overlap being longer than 10bp were merged according to their overlap with mismatch no more than 2bp. (3)Sequences of each sample were separated according to barcodes (exactly matching) and Primers (allowing 2 nucleotide mismatching), and reads containing ambiguous bases were removed. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE version 7.1 http://drive5.com/uparse/) with a novel "greedy" algorithm that performs chimera filtering and OTU clustering simultaneously. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva (SSU123) 16S rRNA database using confidence threshold of 70%.