frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 15 May 2023
DOI 10.3389/fmicb.2023.1148466

OPEN ACCESS

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RECEIVED 20 January 2023
ACCEPTED 17 April 2023
PUBLISHED 15 May 2023

CITATION
Cha T, Kim HH, Keum J, Kwak M-J, Park JY, Hoh JK, Kim C-R, Jeon B-H and Park H-K (2023) Gut microbiome profiling of neonates using Nanopore MinION and Illumina MiSeq sequencing.
Front. Microbiol. 14:1148466.
doi: 10.3389/fmicb.2023.1148466

Gut microbiome profiling of neonates using Nanopore MinION and Illumina MiSeq sequencing

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This study aimed to evaluate the difference in gut microbiomes between preterm and term infants using third-generation long-read sequencing (Oxford Nanopore Technologies, ONT) compared with an established gold standard, Illumina (second-generation short-read sequencing). A total of 69 fecal samples from 51 term (T) and preterm (P) infants were collected at 7 and 28days of life. Gut colonization profiling was performed by 16S rRNA gene sequencing using ONT. We used Illumina to validate and compare the patterns in 13 neonates. Using bioinformatic analysis, we identified features that differed between P and T. Both T1 and P1 microbiomes were dominated by *Firmicutes* (*Staphylococcus* and *Enterococcus*), whereas sequentially showed dominant transitions to *Lactobacillus* ($p<0.001$) and *Streptococcus* in T2 ($p=0.001$), and pathogenic bacteria (*Klebsiella*) in P2 ($p=0.001$). The abundance of beneficial bacteria (*Bifidobacterium* and *Lactobacillus*) increased in T2 ($p=0.026$ and $p<0.001$, respectively). These assignments were correlated with the abundance at the species-level. Bacterial α-diversity increased in T ($p=0.005$) but not in P ($p=0.156$), and P2 showed distinct β-diversity clustering than T2 ($p=0.001$). The ONT reliably identified pathogenic bacteria at the genus level, and taxonomic profiles were comparable to those identified by Illumina at the genus level. This study shows that ONT and Illumina are highly correlated. P and T had different microbiome profiles, and the α- and β-diversity varied. ONT sequencing has potential for pathogen detection in neonates in clinical settings.

KEYWORDS

gut microbiome, neonates, Illumina MiSeq®, Nanopore MinION, preterm infants

Introduction

Perturbations in the infant gut microbiome during early life affect growth, development, and long-term health (Sherman, 2010; Carl et al., 2014; Tarr and Warner, 2016). Preterm infants have a physiologically and anatomically immature gastrointestinal tract that is known to be more permeable than that of term infants, and their microbiota colonization is challenged by environmental factors, such as antibiotic use, hospital stay, and enteral feeding (Dominguez-Bello et al., 2010; Fallani et al., 2010; DiGiulio, 2012; Healy et al., 2022). Although previous studies have