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Assessing the temporal relationship between the environment of the neonatal intensive care unit and the early infant gut microbiome

The interactions between the gut microbiome and the human host are instrumental in human health and disease. Unlike the adult gut microbiome, the infant gut microbiome is more easily influenced by external factors like the environment. As such, it is critical that the infant gut microbiome develops with the optimal distribution of microbes for healthy immune function. This is especially important for infants in the neonatal intensive care unit (NICU), who are highly susceptible to gut dysbiosis and severe diseases like necrotizing enterocolitis. Stool evaluation gives a comprehensive look into the makeup of an infant's gut microbiome. We investigated the contributions of microbial communities in the NICU to the infant gut microbial communities using 16S rDNA amplicon sequencing and analyzed using the programming language R. This study involved 1,607 environmental and stool samples curated across 25 patients from the NICUs at Children's Hospital New Orleans and Touro Infirmary tracked over time. Environmental samples came from various sites in the NICU (alarm cancel switch, computer enter key, floor, sink drain, light switch, erase board ledge, incubator, and stethoscope). We hypothesized that the NICU environment has a temporal effect on the early infant gut microbiome.

To characterize the microbial community of each sample, the amplicon reads were denoised, and amplicon sequence variants were inferred, taxonomically classified, and decontaminated using mock community and negative control samples. A phylogenetic tree was generated, and the microbial communities were visualized through sample ordination and alpha diversity. We used a Gibb's sampler to determine the proportions of a stool microbial community that came from environmental sites of the NICU which was done for each stool sample with environmental samples taken within two days of the stool sample. This provided a time series of contributions each site made to stool samples across all patients. The time series were normalized with Gaussian process regression and were used to train a self-organizing map (SOM), a neural network trained using unsupervised learning. The SOM was partitioned into 4 clusters using hierarchical clustering. Dynamic Time Warping Barycenter Averaging was used to find the optimal average time series for each cluster. Clustering captures different trends within the time series and compares the time series between different sites and patients.

We found that most NICU microbial communities were dominated by *Lactobacillus* bacteria. However, samples obtained from stethoscopes were dominated by *Staphylococcus*, and *Pseudomonas* was most abundant in sink drain samples. *Escherichia-Shigella* was most abundant in stool samples, followed by *Staphylococcus*. Alpha diversity of stool samples was lower than those of environmental samples. There was a positive contribution of NICU environmental samples in stool samples with alarm switch, floor, sink drain, and light switch having the lowest average contribution among the sites. Cluster 1 had the highest average contribution, followed by Cluster 4. The time series of incubators, ledges, and stethoscopes were mostly clustered in Clusters 1 and 4 while those of enter keys were in Clusters 2 and 3. Patients had different distributions across the clusters.

These results show that the NICU environment does affect the infant gut microbiome and reveal how different sites of the NICU follow typical temporal trends with the infant gut microbiome. Hence, the NICU can provide an important source of microbes that colonize the early infant gut.