December 5, 2022

To whom it may concern:

We are pleased to submit for your consideration our paper entitled, “Post-mortem Nasopharyngeal Microbiome Analysis of Zambian Infants with and without Respiratory Syncytial Virus: A nested case control study”. This paper was previously submitted to medRxiv for pre-print.

This paper describes the analysis completed to compare the nasopharyngeal microbiome of infants who died with respiratory syncytial virus (RSV) and those who died without RSV. Other analyses have focused on similar questions, but our paper uniquely captures a population at the extreme end of RSV symptoms, namely death. This allows us to explore whether changes in the microbiome are consistent across RSV severity and provides a time point after acute infection. Additionally, our sample cohort focuses on infants who died in the community and thus is less likely to be confounded by effects related to antibiotic or nosocomial exposure. Our cohort also comes from a Zambian infant study and focuses on a population that is more burdened with RSV complications, unlike other studies which focus on high- or middle-income countries. We acknowledge that a key limitation to our study is demographic information was often collected from next of kin and may not always be completely accurate. However, our study still adds to the body of knowledge on how the nasopharyngeal microbiome of infants with RSV differs from infants without RSV.

Our key findings include that alpha diversity remains consistent between groups (implying that the number of species present in each group is similar), however beta diversity differs. Specifically, when comparing relative abundance of the two groups, RSV+ infants have higher abundances of *Moraxella* and *Haemophilus* and lower abundances of *Staphylococcus* and *Gemella*. These findings are consistent with previous reports.

These results back previous reports and indicate that previous findings may be universal, rather than dependent on microbiome differences due to environmental factors based on location of the study. We believe that these results strengthen previous findings and add to the body of knowledge in regard to nasopharyngeal microbiome difference between RSV+ infants and RSV- infants.

Yours sincerely,

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