Occupational Microbial Exposures Of Animal Care Workers Measured By 16s Rdna Sequencing

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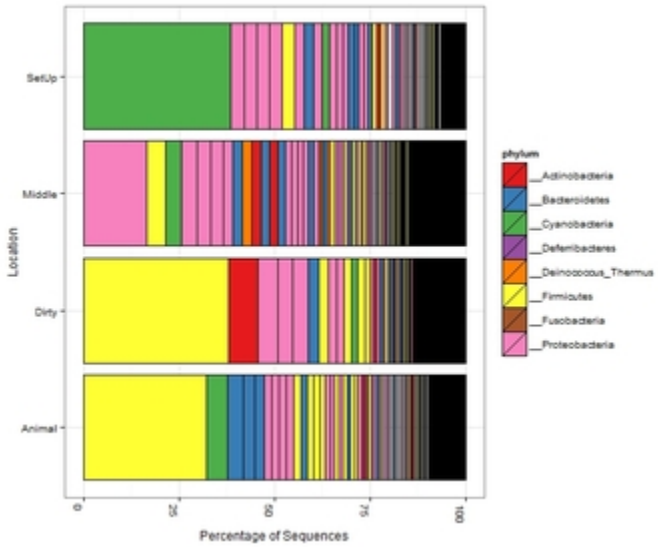
RATIONALE: Animal-related microbial exposures measured by aerosolized endotoxin levels is associated with respiratory symptoms independent of allergic sensitization in animal care workers. These measures of microbial load do not describe the diversity of microbial exposure. Here we compare measures of microbial load (endotoxin) with microbial diversity (16S rDNA sequencing) in animal care facilities.

METHODS: Air sampling was performed in four animal care facilities working primarily with mice. In each facility, samples were collected in the animal room and in three cage wash areas (dirty, middle, and setup). Samples for endotoxin and microbial DNA were obtained using two parallel closed face endotoxin-free cassettes sampled at a rate of 2.5 L/minute for 8 hours. Endotoxin was eluted from filters using pyrogen-free water with 0.05% TWEEN-20 and assayed by the Limulus Amebocyte Lysate assay (Bio-Whittaker, Walkersville, MD). Genomic DNA was extracted using the MOBIO Powersoil Kit (MO BIO Laboratories, Carlsbad, CA, USA), and the 16S rDNA V4 region was amplified by PCR and sequenced on the Illumina MiSeq platform. 16S rRNA gene sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm. OTUs were mapped to the SILVA Database and abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. The *vegan* and *phyloseq* R packages were used for downstream data analysis.

RESULTS: There was no significant difference in airborne endotoxin levels between location (median [interquartile range] endotoxin levels 0 [0-0.15], 1.05 [0.76-1.17], 0.74 [0.41-1.09], 0.45 [0.24-1.03] EU/m^3 for animal room, dirty, middle, and setup locations respectively, p = 0.28, Kruskal-Wallis rank sum test). There were significant differences in alpha diversity between location with the dirty cage wash area having higher alpha diversity in two of four calculated measures (p = 0.04 for observed OTUs, p = 0.03 for Chao1, p = 0.50 for Shannon, and p = 0.25 for Inverse Simpson). There was poor correlation between endotoxin and alpha diversity measures (Spearman ρ from 0.38 to 0.60). When looking at the abundance of different bacterial phyla by location (**Figure 1**), there were significant differences in the relative abundance of Firmicutes (adjusted p = 0.046).

CONCLUSIONS: 16S rDNA sequencing identified differences in microbial exposure whereas measurement of endotoxin levels did not between locations within animal care facilities. Future research should investigate whether measures of microbial diversity rather than microbial load affect the development of occupational respiratory disease.



This abstract is funded by: NIH-NIEHS K23ES023700, Harvard-NIOSH Education and Research Center (T42 OH008416-09) Pilot Grant