Investigation of RNA concentration in three layered or surgical masks among those with and without asthma

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Introduction

Asthma is the most common chronic illness for children (Zahran). It has been recorded that within one year, a child with asthma will have at least one or more asthma attacks (Zahran). Yet, in recent times, with the COVID-19 pandemic, there has been a decrease in the hospitalization of children due to asthma attacks (Kenyon, Zhang, Krivec). This change is believed to be explained by the use of masks as a protectant against COVID-19 which inturn protects against harmful pathogens or other air pollutants that exacerbate asthma attacks (Zhang, Saraya, Chatkin). Furthermore, it has been found that people with asthma are more likely to hold pathogens within the microbiome of their airways that can exacerbate asthma (Hilty, Kim). Therefore when looking at masks it would be expected to find a difference in the concentration of RNA within each mask layer. However, we only have studied the concentration of RNA on the outside layer of the surgical masks (Chughtai). So, there is a lack of understanding on where in the three layered or surgical masks, holds the most and least concentration of RNA.

Therefore this study aims to take a look at the RNA concentration within the inner, middle, and outside layers of masks. The study will analyze the concentrations of RNA taken from used masks by children at Hartford public school. Also, the masks will be taken from children with and without asthma which will allow for a better understanding of how masks work with those with asthma and those without. For reasons stated above, it is expected that there will be a

difference. Still, if no difference presents itself then this study still provides a crucial understanding of masks especially as COVID-19 continues to impact the world. Also, by achieving a better understanding of where the higher concentration of RNA, which could be any disease, pathogen, harmful bacteria, etc., within maks, could present an opportunity for rethinking the design of masks. Lastly, by including asthma as another variable, the study both furthers the understanding of asthma, and could show how masks are benefiting those with asthma.

Proposed Methods

(This part has already been done by the research team listed below)

Sampling Method/Recruitment Process

Hartford Public School (HPS) nurses will identify students with and without asthma in their school eligible to participate in the study. School nurses will call families of children to introduce the study and determine interest in participation. If interested, school nurses will consent and enroll the family (depending on HPS IRB approval) and administer the questionnaires or will document and obtain a permission to contact form to allow study staff to consent and enroll.

Study Retention/Withdrawal

After enrollment/permission is obtained from caregivers/parents, school nurses or study staff will call families and administer the questionnaire collecting child demographics, asthma history, exposures, and level of impairment.The questionnaire will have the same study ID linked to the bag labeled with the same study ID, for a particular individual. The study ID will link the questionnaire data as well as the future mask sample results.

Participation is voluntary, and participants can withdraw from the study at any time. Losses to follow-up and withdrawals will be tracked.

Study Procedures

Surgical masks in paper/plastic bags will be provided to school nurses by Research personnel. After consent and enrollment, school nurses will provide participants with a mask and bag labeled with the study ID at the beginning of the in-person school day. The participant will wear the same mask throughout the duration of the school day. At the end of the school day, participants will remove their masks and place them into the labeled paper/plastic bag and store in a designated place at room temperature. School nurses will help ensure that masks are collected at the end of the school day. Participants will re-wear the same mask for one full day or up to 5 days at school during the in-person education days.

Microbiome sequencing.

To detect the bacterial composition on masks, the inner and outer layers will be carefully separated and soaked in PBS. DNA will be extracted from the soaked PBS, followed by PCR amplification of the V4 region of the 16S rRNA gene. V4 amplicons will be quantified using QIAxcel (Qiagen) and pooled at equimolar concentrations using a QIAgility (Qiagen). The PCR products will be purified using QIAquick spin columns (Qiagen). Paired-end sequencing (2x250) will be performed on an Illumina MiSeq system. Individual paired-end R1 and R2 reads will be trimmed to remove Illumina indices and low quality base calls and then overlapped to improve quality prior to being mapped against the most recent Greengenes 16S rRNA gene database (http://greengenes.lbl.gov) using Qiime version 1.9.0.

Respiratory viruses will be identified by multiplex qPCR and partial sequencing to identify viral species.

Aeroallergens will be identified via enzyme-linked immunosorbent assay (ELISA). Aeroallergens will be extracted from masks (or sections of masks) in a solution of PBS-Tween (10%) for 4 hours with agitation. Samples will be stored at -20°C in 200µL aliquots. Samples will be sent to Indoor Biotechnologies to test for indoor and outdoor aeroallergens common to New England: MARIA® analysis service for testing any FIFTEEN of the following allergens: Amb a 1 (ragweed), Der p 1 (dust mite), Der f 1 (dust mite), Der p 23 (dust mite), Mite Group 2

(dust mite), Blo t 5 (dust mite), Fel d 1 (cat), Can f 1 (dog), Can s 3 (dog), Mus m 1 (mouse), Rat n 1 (rat), Bla g 2 (cockroach), Alt a 1 (mold), Asp f 1 (mold), Bet v 1 EP (birch pollen) or Phl p 5 (timothy grass).

(This part will be the methods of this specific study)

The three layers of the surgical masks will be separated from each other. Small, equally-sized squares from the middle of each layer will then be cut up into smaller pieces and placed into 1.5mL microcentrifuge tubes. Then, each layer will separately go through the DNA/RNA extraction process. After extraction, the nucleic acids will be quantified using Photometry (UV-Vis),. The concentration of nucleic acids will be documented for each mask layer, by individual participants, and documented for statistical analyses. This will then finally result in the data necessary for analysis. The analysis will use parametric testing to properly conclude if there is a statistical difference between what is inside those with masks and those without.

Research team

These are the individuals who are credited for the work done in the first part of the methods section. As of now, I will be analyzing the data provided by other members of the research team.

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IRB Note

This research has already been approved by the Connecticut Children's Hospital IRB.

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