SEQUENCING THE SMOKE: The Unseen Microbial Hazards of Vaping
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I. Abstract
An e-cigarette epidemic is infecting America’s youth: 1 in 5 high school students now "vapes." The dangers of the aerosols are well-established, but the risks posed by e-cigarette cartridges - shared among peers and frequently cleaned - are unknown. The objective of this study was to analyze the diversity and potential virulence of bacteria isolated from e-cigarette cartridges, users’ noses, and a control group of non-users’ noses. Bacteriuria were isolated using selective and differential plates. The microanalysis of each sample was determined by 16S RNA sequencing.

II. Background

- Our study aimed to determine if e-cigarette cartridges and noses of users host diverse and pathogenic bacteria than those of non-users. We also sought to determine whether or not to biofilm could be exchanged between cartridge and nose. We expected to find major disparities between the microbial communities of users and non-users, as well as significant bacterial transmission between the cartridges and noses of users.

- E-cigarette aerosols weaken the immune system and increase bacterial virulence. Neutrophil and macrophage activity in mice exposed to aerosols and infected by Staphylococcus pneumoniae is significantly reduced (Huang et al., 2016). Furthermore, the aerosols can induce dormant methicillin-resistant Staphylococcus aureus to produce an acid secretion mechanism (Blanchet, 2017).

- The inherent health risks of vaping are exacerbated by the lack of user hygiene. Adolescents have been observed sharing e-cigarettes in groups, not cleaning them, and storing them unopened in pockets, backpacks, desks, and lockers. We observed this in our survey (Charts 1, 2, and 3). It is unknown whether these bad habits turn e-cigarettes into dangerous transmitters of microbes like viruses and bacteria.

III. Materials and Methods
We included an experimental group of e-cigarette users and a control group of non-e-cigarette users. The microorganisms of cartridges and e-cigarette users’ noses were compared to the normal microbial flora within non-users’ noses.

IV. Results
A total of 58 samples were collected and analyzed, including four unused control cartridges. Only 16 of the 22 non-users and 11 of the 16 users completed the questionnaire. 33 nasal colonies that grew on the agar plates were selected for Sanger sequencing: 19 were from non-users, and 13 were from users. 7 of the samples were S. aureus, a pathogenic strain of Staphylococcus: 6 out of those 7 were from users. 15 samples were S. epidermidis, a less pathogenic species of Staphylococcus: 10 came from non-users and 3 came from users. 3 non-users were positive for S. liquefaciens. S. warneri was found from a non-user sample and S. argenteus was found from a user sample. Only 1 sample from a non-user’s Candida plate was Pseudomonas aeruginosa and 1 sample from a user’s Staphylococcus plate was identified as Enterococcus faecalis.

V. Discussion
Our PCA plots disprove the hypothesis that cartridge users would harbor highly similar bacteria to those found in users and non-users’ noses (Figures 2 and 3). This demonstrates an exchange of bacteria between cartridges and noses is likely not occurring. However, a species diversity of bacteria in users’ and non-users’ noses is similar (Figure 5). The large amount of the environmental gram-negative Pseudomonas aeruginosa on cartridges compared to a large amount of gram-positive Staphylococcus on the nasal swabs is likely a result of the unfavorable storage conditions for e-cigarettes.

Sanger sequencing and culturing revealed a dearth of bacteria on the cartridges. S. aureus was present in the noses of e-cigarette users, and less pathogenic species of Staphylococcus were found in the noses of non-users. (Table 1). These findings agree with previous reports that e-cigarette aerosols prompt the growth of more virulent Staphylococcus (Huang et al., 2016).

In our pilot study, we recognize that our sample size was small, and that we did not process all of the samples we collected. Despite this, myriad new research directions lie ahead. The wide bacterial diversity revealed by Qiime suggests that other live pathogens may exist on cartridges that did not grow on the plates we selected. Furthermore, the link between exposure to e-cigarette aerosols and Staphylococcus virulence is now even stronger, highlighting the need for further investigations into the reservoir of other bacterial species.

VI. References

Table 1: Bacterial Cultures Results from Sampled Cartridges
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<tr>
<th>Cartridge</th>
<th>Non-user sample</th>
<th>User sample</th>
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