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 "vape." The dangers of the aerosols are well-established, but the risks posed by e-cigarette cartridges–shared among peers and infrequently 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. Bacteria were isolated using selective and differential agar plates. The microbiome of each sample was also determined by 16S rRNA sequencing. Few colonies were isolated from the cartridges, but several colonies were observed on agar plates from user and non-user noses. Bioinformatic analyses revealed that the nasal bacteria of users and non-users were similar to each other but distinct from the microbiomes of the cartridges, implying that e-cigarette surfaces may not contribute to bacterial transmission.

II. Background

- Our study aimed to determine if e-cigarette cartridges and noses of users host more diverse and pathogenic bacteria than noses of non-users. We also sought to determine whether or not microbes could be exchanged between cartridge and nose. We expected to find major disparities between the nasal microbiomes of users and non-users, as well as significant bacterial transmission between the cartridges and noses of users.
- E-cigarette prevalence in high school communities has soared from just 220,000 users in 2011 to more than 3 million in 2018, reaching "epidemic proportions" in the United States (Simon, 2018).
- E-cigarette aerosols weaken the immune system and increase bacterial virulence. Neutrophil and macrophage activity in mice exposed to aerosols and infected by *Streptococcus pneumoniae* is significantly reduced (Hwang et al., 2016). Furthermore, the aerosols can induce dormant methicillin-resistant *Staphylococcus aureus* to produce an acid defense mechanism (Buschman, 2016).
- 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 uncapped 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.

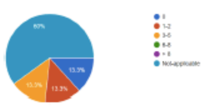


Chart 1: How many people do you share your e-cigarette with? From 30 total responses, 66% of e-cigarette users (red, orange, and dark blue) share their devices with one or more people. ←

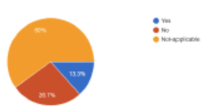


Chart 2: Do you clean your e-cigarette? Out of e-cigarette users (red and dark blue), only one-third of them regularly clean their e-cigarettes. →

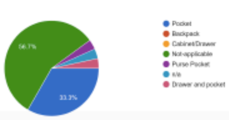


Chart 3: Where do you store your e-cigarette? Most of the e-cigarette users (dark blue, light blue, pink, and purple) store their devices in their pockets, known hotspots for bacteria. ←

III. Materials and Methods

We included an experimental group of e-cigarette users and a control group of e-cigarette non-users. The microbiomes of cartridges and e-cigarette users' noses were compared to the normal microbial flora within non-users' noses.

IRB approval was sought for permission to work with human samples. Posters calling for participants were tacked onto bulletins around campus. Outreach activities to raise awareness about environmental hazards were also run.

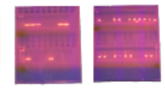
Each participant was given a sample kit consisting of an eSwab with Amies Solution, nasal swabbing instructions, a link to a questionnaire about e-cigarette usage, and a cartridge collection tube.

Bacteria were isolated from nasal and cartridge swabs to be cultured on selective and differential plates for Staphylococci, methicillin-resistant *Staphylococcus aureus*, Candida, *Streptococcus pneumoniae*, and *Escherichia coli*.

Colonies that were grew were stored at -80°C. DNA was isolated using the QIAGEN DNeasy UltraClean Microbial Kit.

PCR amplification was performed with primers for *tuf* and *mecA*: *Staphylococci* gene markers.

Agarose gel electrophoresis of the amplified DNA identified the samples positive for *tuf* or *mecA* to be sent for Sanger sequencing, allowing for further identification of pathogenic and nonpathogenic *Staphylococci*.



DNA was isolated directly from the cartridge, user, and non-user swabs using the QIAGEN DNeasy PowerSoil Kit.

The 16S rRNA gene was amplified using PCR, and appropriate barcodes were added to the amplicons to enable pooled sequencing on the New School iSeq 100.

Patterns in bacterial diversity across samples were visualized using QIIME and STAMP analysis in collaboration with Saptarsi Basu from the New York City College of Technology.

IV. Results

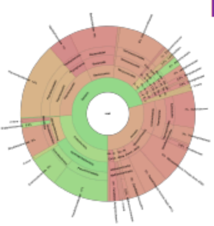


Figure 1: Geneious analysis for a cartridge sample. No *Staphylococcus* or *Streptococcus* were found. 11% of the reads were *Pseudomonadaceae*.

These two Geneious analysis charts represent 22 total iSeq results.

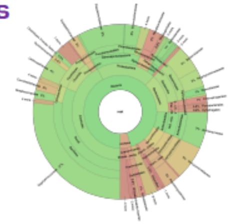


Figure 2: Geneious analysis for a swab sample. 27% of the found bacteria were from the genus *Staphylococcus*, 2% from *Streptococcus*, and 9% *Pseudomonadaceae*.

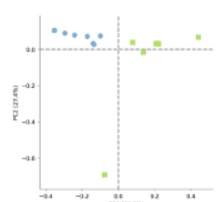


Figure 3: Cartridge and user nasal samples. This PCA analysis was made from 15 iSeq samples; sequence results found in the noses of 7 e-cigarette users (blue) and on 8 cartridges (green).

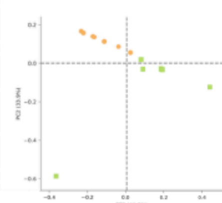


Figure 4: Cartridge and non-user nasal samples. This PCA graph was made from 15 iSeq results; the sequences found on 8 cartridges (green) and in the noses of 7 non-users (orange).

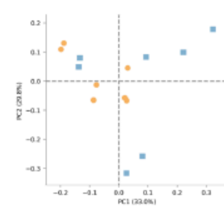


Figure 5: Non-user and user nasal samples. This PCA graph compares the iSeq results of the total DNA found in the noses of 14 samples; 7 samples from the noses of non-users (orange) and 7 samples from the noses of users (blue).

Table 1: Bacterial Culture Results from Samples Received

	Number of Plates Exhibiting Growth				
	Candida	*Streptococcus*	*Escherichia coli*	*Staphylococcus* (Mannitol plate)	*Staph. aureus*/MRSA
Non-user nasal swabs	2	9	3	20	6
User nasal swabs	1	4	5	11	1
Cartridges	0	0	0	3	0

Table 1: In addition to the iSeq analysis, we isolated colonies on selective agar plates that were later sent for Sanger sequencing. These are our results of the number of growths on agar plates.

IV. Results

A total of 58 samples were received and analyzed, including four unused control cartridges. Only 16 of the 22 non-users and 11 of the 16 users completed the questionnaire. 32 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 came from users. 13 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. saprophyticus*. *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 *Pseudomonadaceae* and 1 sample from a user's *Streptococcus* plate was identified to be *Enterococcus faecium*.

V. Discussion

- Our PCA plots disprove the hypothesis that cartridges would harbor highly similar bacteria to those found in users' and non-users' noses (Figures 3 and 4). This demonstrates an exchange of bacteria between cartridges and noses is likely not occurring. However, species diversity of bacteria in users' and non-users' noses are similar (Figure 5). The large amount of the environmental gram-negative *Pseudomonadaceae* on cartridges in comparison to a large amount of gram-positive *Staphylococcus* from the nasal swabs is likely a result of the unhygienic storage of the 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 *Staphylococci* were found in the noses of non-users (Table 1). These findings agree with previous reports that e-cigarette aerosols promote the growth of more virulent *Staphylococci* (Hwang 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 *Staphylococci* virulence is now even stronger, highlighting the need for further investigations into the responses of other bacterial species.

VI. References

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VII. Acknowledgments

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