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Microbiome alterations from volatile organic compounds (VOC) exposures among workers in salons primarily serving women of color

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A R T I C L E I N F O

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A B S T R A C T

Salon workers, especially those serving an ethnically and racially diverse clientele (i.e., Black/Latina), may experience disparately high levels of workplace exposures to respiratory irritants, including volatile organic compounds (VOCs). Salon workers are also reported to have a greater risk of developing respiratory conditions compared to the general population. Emerging evidence suggests that occupational chemical exposures may alter the human microbiome and that these alterations may be an important mechanism by which workplace VOC exposures adversely impact respiratory health. This preliminary research investigated the potential effects of 28 VOC urinary biomarkers on the 16S rRNA nasal microbiome in 40 workers from salons primarily serving women of color (Black and Dominican salons) compared to office workers. Our exploratory analysis revealed significant differences in microbial composition by worker group; namely dissimilar levels of *Staphylococcus* species (*S. epidermidis* and *S. aureus*, specifically) in salon workers compared to office workers, and higher alpha diversity levels in workers in Dominican salons compared to workers in Black salons. Within-sample alpha diversity levels tended to be decreased with higher VOC urinary biomarker concentrations, significantly for carbon disulfide, acrolein, acrylonitrile, crotonaldehyde, and vinyl chloride biomarkers. Our research highlights that occupational exposures, particularly to chemicals like VOCs, can impact the respiratory microbiome in the vulnerable salon worker group. Further understanding of the potential effects of chemical mixtures on microbial composition may provide key insights to respiratory health and other adverse health outcomes, as well as direct prevention efforts in this largely historically understudied occupational population.

1. Introduction

Salon workers are exposed to many chemicals that are present, formed or emitted from products used as part of their daily work, resulting in both acute and chronic exposures to a myriad of chemicals. In the U.S. alone, there are over 600,000 workers employed in salons, the majority of whom are women (U.S. Bureau of Labor Statistics, 2020). Data from several studies, including our own work, show that some of the chemicals of concern present in or emitted from salon products include volatile organic compounds (VOCs) (Chang et al., 2018; Durgam and Page, 2011; Labrèche et al., 2003; Louis et al., 2021; Pierce et al., 2011). Findings from our prior pilot work in hair salons also indicate that salon workers servicing a predominantly Black and Latinx clientele are at an increased risk of exposures to several of these agents (Louis

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et al., 2021). Inhalation of VOCs may increase risk of respiratory irritation and respiratory illnesses (Bahadar et al., 2014; Mendes et al., 2011; Nemer et al., 2015; Takkouche et al., 2009). Limited studies indicate that salon workers have a greater risk of developing respiratory and allergic conditions, like asthma, compared to the general population (Hollund et al., 2001; Lysdal et al., 2014; Nemer et al., 2015; Quiros-Alcala et al., 2019; Skoufi et al., 2013). Still, the exact mechanisms by which VOCs could impact respiratory health have not been clearly elucidated though some mechanisms have been postulated, including immune modulation (Kwon et al., 2018; Wang et al., 2013; Antonelli et al., 2020).

A growing body of literature links alterations in respiratory microbial communities to respiratory outcomes, including those that colonize the nose, both microbial populations generally and respiratory pathogens specifically. The nasal microbiota exists at the interface between the exterior environment and the interior of the human body and can undergo modification due to external environmental factors. As a gatekeeper to respiratory health, the nasal microbiota is implicated in the maintenance of homeostatic functions in the airway (Ho Man et al. 2017) and epidemiological studies have revealed that disturbances in the respiratory microbiota play a role in the pathogenesis of respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), and bronchitis (Dickson et al., 2016; Marsland et al., 2013).

Emerging evidence suggests interactions between chemicals and the microbiome may affect host health. Exposure to chemicals, including those commonly found in hair and beauty products such as parabens, phthalates, and VOCs, has been preliminarily shown to disrupt the microbiome (Gálvez-Ontiveros et al., 2020; Rosenfeld, 2017; Yang et al., 2019). This resulting microbial dysbiosis could potentially impact host health and development of diseases, thus mediating the pathway between VOC exposures and health. In addition, microbial dysbiosis can result in unique compositions of microbial enzymes, which can cause differential metabolism of chemicals, thereby modifying the potential toxicity to the host and associated health outcomes (Rosenfeld, 2017). However, the influences of chemicals on respiratory microbial communities and its contributions to the progression of respiratory diseases remain largely understudied and may be important to identify uniquely vulnerable populations and potential mechanisms by which occupational exposures may impact worker health.

Given continual exposures to potentially harmful VOCs among salon workers and the increased risk for adverse respiratory conditions, there is a critical need to elucidate the potential role of the respiratory microbiome on these pathophysiological pathways. In the present pilot study, we compared the nasal microbiota of female salon workers compared to office workers and investigated the effects of 28 unique VOC urinary biomarkers on the nasal microbiome. We focused our subsample exclusively on Black and Latina women due to emerging evidence that use of hair care products marketed to this demographic may be of concern, given the high chemical exposures among this occupational group compared to the general public (Louis et al., 2021). This foundational work specifically found that VOC biomarkers were detected in all salon workers with higher concentrations observed among workers serving a predominantly Black versus Latino clientele, and among salon workers overall versus office workers and women in the U.S. general population, specifically for acrolein, 1,3-butadiene, xylene, and 1-bromopropane, which highlights the importance to comprehend consequential pathophysiological disruptions from increased chemical exposures (Louis et al., 2021).

2. Methods

2.1. Participant recruitment

Our study population and recruitment strategy have been described elsewhere (Louis et al., 2021). Briefly, 23 licensed female workers were recruited from six salons (three salons primarily serving Black women and three salons primarily serving Latinxs) in Maryland and the Washington D.C. metropolitan area. Salons primarily serving a Black/African American clientele will be referred to herein as "Black" salons, and salons primarily serving a Latino clientele will be referred to as "Dominican" salons. Salon workers were women of color (Black/African American or Latinas originally from Central America or the Caribbean). To serve as a comparison group, an additional convenience sample of 17 female office workers from the University of Maryland, College Park were recruited via email and word of mouth, for a total enrollment of 40 participants. Eligibility criteria for all participants included women who were ≥18 years of age, willing to complete two interviewer-administered questionnaires and provide a urine biospecimen. Additionally, salon worker participants had to be licensed to work in a salon and had to report working in a salon for at least one year prior to study enrollment. Participation in the study was voluntary for all study participants and all study protocols were reviewed and approved by the University of Maryland's Institutional Review Board (IRB). Written informed consent was obtained from salon workers and office workers prior to any data and sample collection.

2.2. Data and biospecimen collection

An initial baseline questionnaire elicited information on participant demographics, health-related information (e.g., respiratory and reproductive health), and lifestyle and workplace behaviors (e.g., use of personal protective equipment (PPE), personal care products, and cleaning products at home and work, etc.). Except for salon-specific questions, office workers were asked the same questions as salon workers. One post shift spot urine sample was collected from each study participant. Urine samples were collected in polypropylene, metal-free urine collection cups. Additionally, nasal swabs were collected for microbiome analysis from the anterior nares of each participant at the time of urine collection using sterile flocked swabs (Puritan, Guilford, ME, USA), as previously described (Dalton et al., 2021).

2.3. Laboratory analysis

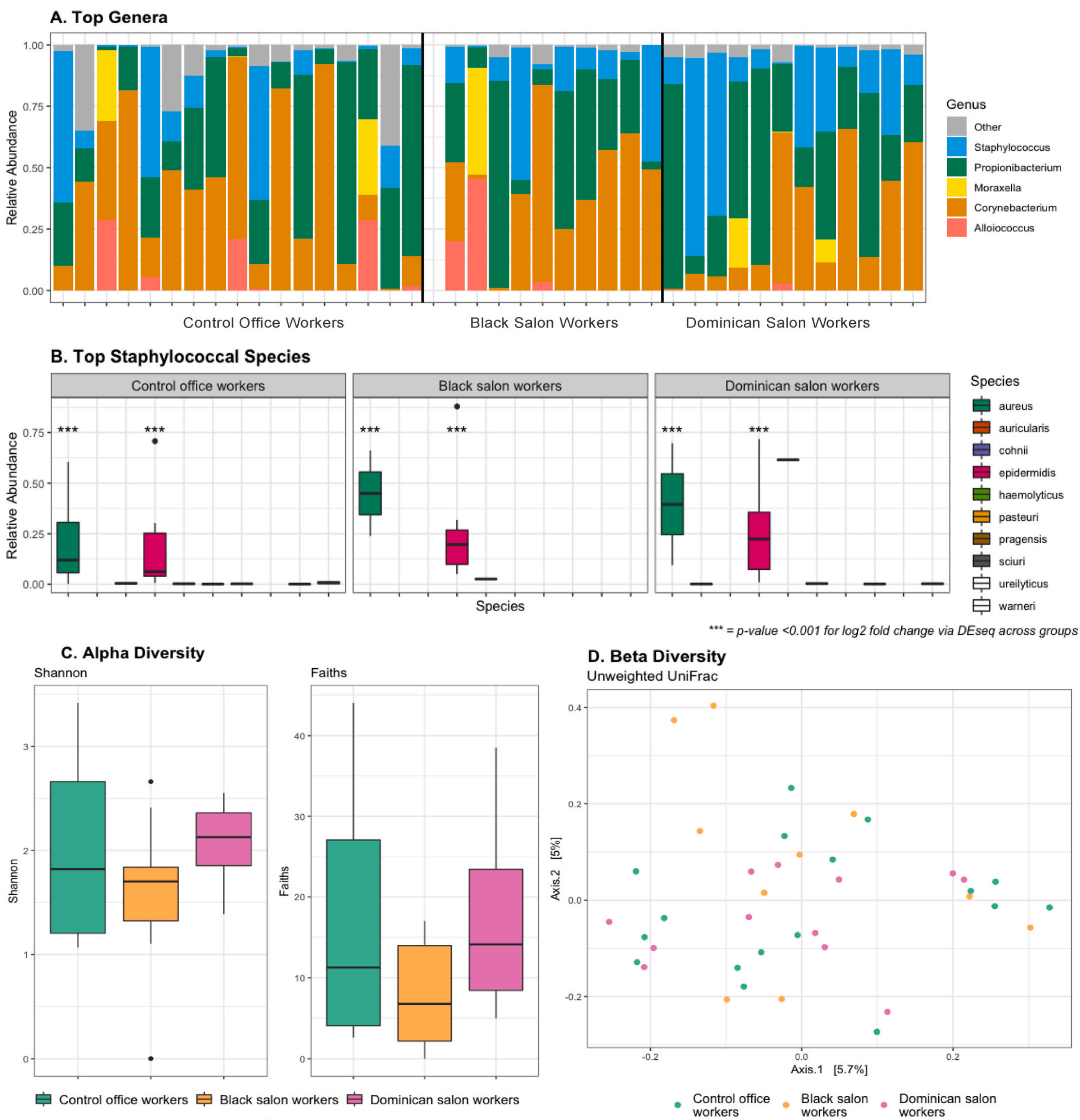
2.3.1. VOC urinary biomarkers

Briefly, all samples were transferred to the lab in an ice chest with ice packs, aliquoted into 2 mL cryovials, and stored at −80 °C within an hour of collection. Samples remained at −80 °C until shipment on dry ice to the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, for laboratory analysis of 28 VOC biomarkers representing exposure to 21 parent VOCs (refer to Fig. 2 for full VOC biomarker list) using a validated laboratory method (Alwis et al., 2012; Louis et al., 2021). Selection of urinary VOC biomarkers was based on availability of a validated analytical protocol and potential presence of the VOC in salons and salon products. Limits of detection for VOC urinary biomarkers ranged from 0.3 ng/mL to 64.4 ng/mL and were corrected for specific gravity to account for urinary dilution (Louis et al., 2021). Specific gravity was measured for each individual urine sample using a handheld refractometer (ATAGO™3741, Tokyo, Japan). Additional details on data and biospecimen collection, and processing of the VOC urinary biomarkers in this study population have been described elsewhere (Louis et al., 2021).

2.3.2. 16S rRNA sequencing and bioinformatics for microbiome

Nasal swab samples were thawed prior to DNA extraction. DNA sequencing was performed as previously described (Dalton et al., 2021; Misic et al., 2015). For each set of extractions, one blank swab exposed to laboratory air was processed as a negative laboratory control. The V1-3 region of the 16S rRNA gene was amplified using barcoded primers

(27F, 534R) for the Illumina platform as previously described (Fadrosh et al., 2014) via the MiSeq instrument (Illumina, San Diego, CA) using 300 base paired-end chemistry at the University of Pennsylvania Next Generation Sequencing Core. QIIMEv2.7 was used for paired-end read assembly and quality filtering for the sequences from all samples (Bolyen et al., 2019). The DADA2 plug-in for QIIME2.7 was used to remove chimeric sequences and sequences greater than 300bp in length, and cluster sequences into amplicon sequence variants (ASVs) (Callahan et al., 2016), and matched to taxonomy with Greengenes13.8 99% OTU match (McDonald et al., 2012).



Alpha Linear models,
Coefficient (p-value)

	Shannon	Faiths
Salon vs Office Workers	-0.127 (0.555)	-3.902 (0.335)
Dominican vs Black Salon workers	*0.46 (0.065)*	**9.622 (0.024)**

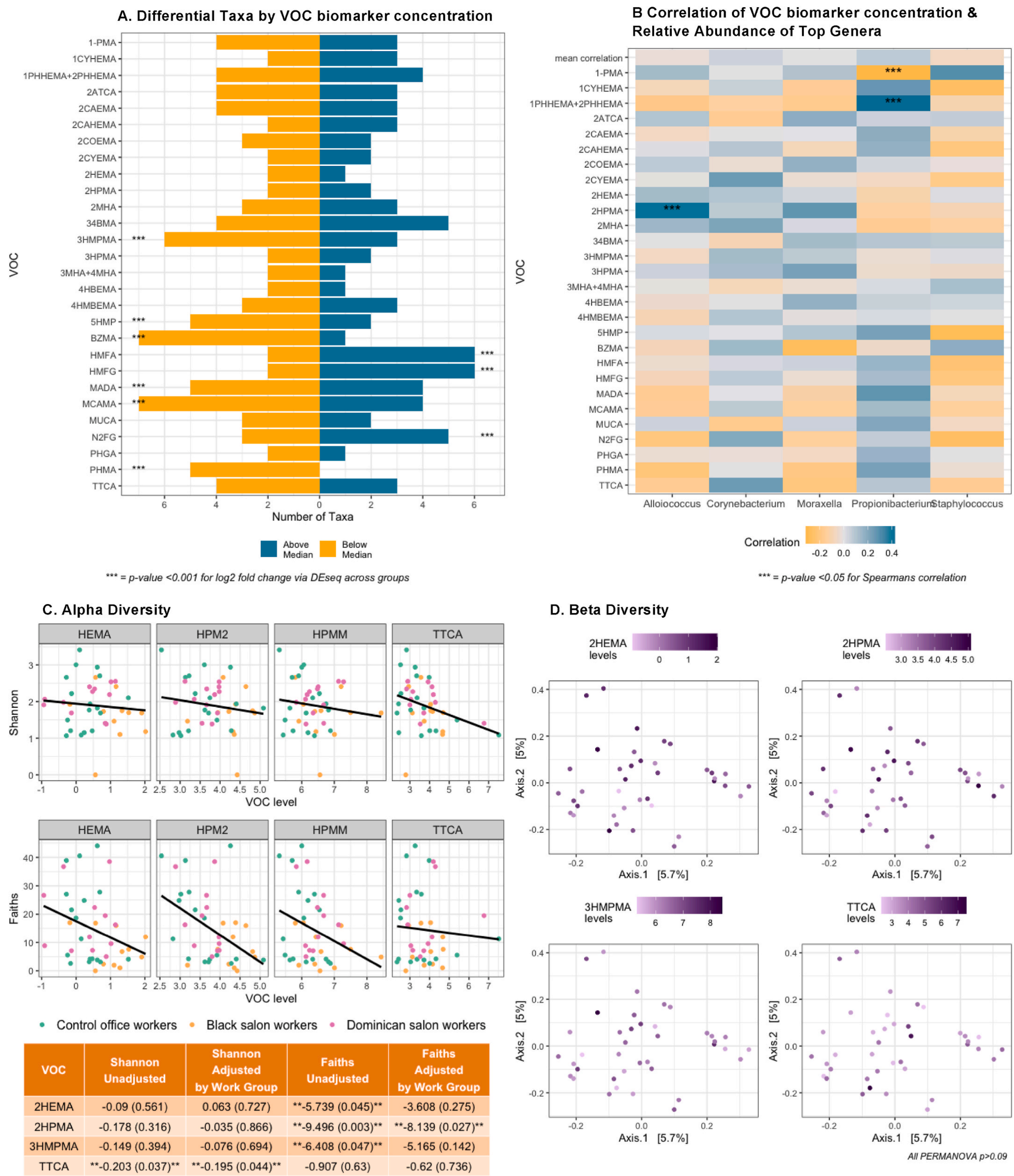
PERMANOVA models

	R2	F (p-value)
Salon vs Office Workers	0.027	1.029 (0.345)
Dominican vs Black Salon workers	0.047	0.997 (0.482)

Fig. 1. Microbiome composition and diversity by worker group.

2.4. Statistical analysis

Statistical analysis was performed in RStudio v1.1.423 (R Development Core Team, 2010). Taxa tables, and matching phylogeny and taxonomy, were analyzed using the phyloseq pipeline to calculate alpha and beta diversity metrics (McMurdie and Holmes, 2013). The primary analysis was the change in microbial composition by worker type (salon vs. office workers) and by salon type (workers in Dominican vs. Black salons). Secondary analysis was the change in composition by personal VOC urinary biomarker concentrations. VOC urinary biomarker



VOC	Shannon Unadjusted	Shannon Adjusted by Work Group	Faiths Unadjusted	Faiths Adjusted by Work Group
2HEMA	-0.09 (0.561)	0.063 (0.727)	**-5.739 (0.045)**	-3.608 (0.275)
2HPMA	-0.178 (0.316)	-0.035 (0.866)	**-9.496 (0.003)**	**-8.139 (0.027)**
3HMPMA	-0.149 (0.394)	-0.076 (0.694)	**-6.408 (0.047)**	-5.165 (0.142)
TTCA	**-0.203 (0.037)**	**-0.195 (0.044)**	-0.907 (0.63)	-0.62 (0.736)

Fig. 2. Microbiome Composition and Diversity by VOC biomarker concentration.

concentrations were log-transformed to control for skewedness and treated as a continuous variable, except for DESeq2 analysis where it was treated as a binary variable with a median cutoff. Differential abundance of specific taxa between groups were analyzed using DESeq2 (Love et al., 2014), which tests for differences in read counts for each taxa, as well as Spearman's correlation with VOC urinary biomarker concentration and abundance of top taxa. Within-sample alpha diversity was measured with the Shannon index (accounts for species richness and evenness) and Faith's Phylogenetic distance (accounts for richness, evenness, and phylogenetic similarity) metrics. The Kruskal-Wallis nonparametric one-way analysis of variance test was used to examine differential alpha diversity across all groups, and the Wilcoxon rank-sum test was used for pair-wise comparisons within groups; both tests were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) correction. Linear regression models were used to assess the dependent and independent effect of salon type and VOC urinary biomarker concentration on alpha diversity, controlling for work group. Beta diversity was assessed using the weighted and unweighted UniFrac metric (Lozupone and Knight, 2005) principal coordinate analysis (accounts for phylogenetic distance between sets of taxa), and the non-parametric permutational multivariate analysis of variance (PERMANOVA) model tested which factors were most important in determining microbial composition overall taxa relative abundance (Anderson, 2017).

3. Results

3.1. Study population

Demographic characteristics for our study population have been described in detail elsewhere. (Boyle et al., 2021; Louis et al., 2021). Briefly, most salon workers (96%) self-identified as either Non-Hispanic (NH) Black (47.8%) or Hispanic/Latina (47.8%) and were non-smokers (82.6%). Similar to salon workers, that majority of office workers (82.4%) self-identified as either NH-Black (41.2%) or Hispanic/Latina (41.2%) but tended to be younger than salon workers (office workers: mean age: 33.6 years; SD = 7.9 vs. salon workers: mean age: 40.2 years; SD = 10.6). Additionally, personal use of cosmetics and personal care products outside the workplace did not significantly differ between salon workers and office workers. Lastly salon workers from Black salons reported serving more clients per week (32.7 clients/week vs. 18.7 clients/week; $p < 0.05$) than those working in Dominican salons.

3.2. Microbial compositional and diversity by worker groups

The percent relative abundance of the top 5 most abundant genera represented 90% of all taxa (Fig. 1A). The dominant five genera in descending order were *Corynebacterium*, *Propionibacterium*, *Staphylococcus*, *Moraxella*, and *Alloiococcus*. *Corynebacterium* was the most dominant within all samples, with a mean relative abundance of 0.349 (SD 0.271, median 0.368, Fig. 1A). Kruskal-Wallis chi-squared tests did not identify statistical differences in abundance of top genera when comparing across all groups, nor between salon workers compared to office workers, and workers in Dominican compared to Black salons (Supplemental Table 1).

When comparing across all taxa using DESeq analysis, *Staphylococcus* was differentially abundant across workers. Due to its clinical significance, *Staphylococcus* was analyzed at the species level (Fig. 1B). *S. aureus* and *S. epidermidis* were the dominant two species, both of which were significantly higher in salon workers compared to office workers (DESeq log-fold change 23.02, FDR-adj $p < 0.0001$ for *S. epidermidis*, DESeq log-fold change 24.19, FDR-adj $p < 0.0001$ for *S. aureus*).

Alpha diversity did not differ between salon workers overall and office workers, as measured by Shannon and Faith's Phylogenetic metrics (Fig. 1C). However, a difference within salon workers was observed, with Dominican salon workers having a higher alpha diversity than Black salon workers (Shannon: median 2.13 Dominican salon workers, 1.71 Black salon workers, Wilcoxon rank-sum $p = 0.093$; Faiths: median 14.12 Dominican salon workers, 6.79 Black salon workers, Wilcoxon rank-sum $p = 0.027$). Linear models showed an effect for both Shannon (difference 0.46, $p = 0.065$) and Faiths (difference 9.62, $p = 0.024$). When accounting for other covariates, race was a confounder on the association between salon type and alpha diversity, as evidenced by the null associations ($p > 0.09$) in the stratified analysis shown in Supplemental Table 2.

The overall distribution of samples in principal coordinate analysis plots for unweighted UniFrac beta diversity metric by worker group, shows that the axes accounted for a maximum of 5.7% variation (Fig. 1D, Fig. S2). There was no difference in microbial composition beta diversity by worker group (PERMANOVA models $p > 0.30$, Fig. 1D).

3.3. Microbial compositional and diversity by VOC urinary biomarker concentration

The microbial composition and diversity differences across the 28 VOC biomarkers evaluated in this study shows nine VOC urinary biomarkers that have differential abundance of taxa when comparing if samples were above and below the median for each VOC biomarker using DESeq analysis (Fig. 2A). PHMA (N-Acetyl-S-(phenyl)-L-cysteine), MADA (mandelic acid), 3HMPMA (N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine), 5HMP (5-Hydroxy-N-methylpyrrolidone), BZMA (N-acetyl-S-(benzyl)-L-cysteine), and MCAMA (N-Acetyl-S-(N-methylcarbamoyl)-L-cysteine) were all shown to have more significantly different taxa for samples below the median compared to above, while N2FG (N-2-Furoylglycine), HMFG (5-Hydroxymethyl-2-furoylglycine), and HMFA (5-Hydroxymethyl-2-furoic acid) had more significantly different taxa for samples above the median compared to below. When examining correlations in VOC urinary biomarker concentrations by the relative abundance of top genera (Fig. 2B), overall *Corynebacterium* and *Propionibacterium* tended to be increased in abundance with higher VOC urinary biomarker concentrations, while *Staphylococcus* had an inverse correlation with mean VOC biomarker concentration. *Propionibacterium* was significantly and positively correlated with 1PHHEMA+2 PHHEMA (N-Acetyl-S-(1-phenyl-2-hydroxyethyl)-L-cysteine + N-Acetyl-S-(2-phenyl-2-hydroxyethyl)-L-cysteine) (Spearman's correlation = 0.424, $p = 0.007$) and negatively correlated with 1-PMA (N-Acetyl-S-(n-propyl)-L-cysteine) (Spearman's correlation = −0.317, $p = 0.049$), while *Alloiococcus* was significantly and positively correlated with 2HPMA (N-Acetyl-S-(2-hydroxypropyl)-L-cysteine) (Spearman's correlation = 0.407, $p = 0.01$).

In general, alpha diversity tended to be decreased with increased VOC urinary biomarker concentrations (Supplemental Fig. 1). VOCs that were significantly associated with alpha diversity (Fig. 2C). A significant inverse relationship was observed between 2HEMA (N-Acetyl-S-(2-hydroxyethyl)-L-cysteine) and 3HMPMA concentrations with Faiths phylogenetic diversity in the unadjusted linear models (−5.739, $p = 0.045$ and −6.408, $p = 0.047$, respectively), but not when controlled for work group (office workers, Black salon workers, or Dominican salon workers), although there was still a negative trend (−3.608 and −5.165, respectively). An inverse association was observed between Faiths alpha diversity and 2HPMA concentrations in both the unadjusted (−9.496, $p = 0.003$) and adjusted models (−8.139, $p = 0.027$). Neither were significantly associated with Shannon's alpha diversity, though they did have a negative relationship. Conversely, TTCA (2-Thioxothiazolidine-4-carboxylic acid) concentrations had an inverse trend with Shannon's diversity in the unadjusted and adjusted models (unadjusted −0.203, $p = 0.037$, and adjusted by work group −0.195, $p = 0.044$).

The overall distribution of samples in principal coordinate analysis plots for unweighted UniFrac beta diversity metric by the four VOC biomarkers were significantly associated with alpha diversity levels (Fig. 2D, Fig. S3). There was no difference in microbial composition beta diversity across any of the VOC biomarkers (PERMANOVA models $p > 0.09$).

4. Discussion

Our pilot study is a novel contribution to the literature by beginning to describe associations between VOC exposures and nasal microbiome composition and diversity among a population of salon workers and office workers. Our exploratory analysis revealed significant differences in microbial composition by worker group; namely lowered alpha diversity levels in Black salon workers compared to Dominican salon workers, and dissimilar levels of *Staphylococcus* species (*S. epidermidis* and *S. aureus*, specifically) in salon workers compared to office workers. We also found an association between select VOC urinary biomarker concentrations and microbial composition, in that *Corynebacterium* and *Propionibacterium* levels were generally positively correlated with VOC urinary biomarker concentrations, while inverse correlations were observed with *Staphylococcus*, generally. Within-sample alpha diversity levels tended to be inversely related to VOC urinary biomarker concentrations, specifically for TTCA, 2HEMA, 2HPMA and 3HMPMA. Taken together, this suggests that occupational exposures, particularly to select VOCs such as carbon disulfide, acrolein, and vinyl chloride, could impact the respiratory microbiome in this vulnerable worker group. This is critical as prior work in this cohort has shown higher exposure to VOCs in salon workers compared to office workers and the general public, including acrolein where the median concentration in salon workers was more than twice as high compared to office workers, and four times higher compared to women in the general U.S. population (Louis et al., 2021).

Our results show some differences in the microbial composition based on occupation. Levels of staphylococcal species appeared to be in higher abundance in salon workers compared to office workers. *Staphylococcus* may be less susceptible to damage from chemical exposure compared to other bacteria, as it is known to be a hardy, resilient bacteria that is resistant to environmental degradation (Weese and van Duijkeren, 2010). *Staphylococci* are dominant bacteria in the human microbiome of the skin and mucosal surfaces (The Human Microbiome Project Consortium et al., 2012; Wilson and Hamilos, 2014). *S. aureus* is found in 30% of the US population via culture-identification and is typically a commensal organism (Graham et al., 2006; Liu et al., 2015), but can become an opportunistic pathogen associated with adverse respiratory conditions (Davis et al., 2018, 2015; Kim et al., 2019). Alternatively, the commensal *S. epidermidis* has been shown to be protective against colonization from bacterial and viral pathogens (Chen et al., 2016; Liu et al., 2020). Numerous occupational studies have found higher staphylococcal levels in workers' nasal microbiome compared to the general public, including among workers in occupations with chemical exposures such as pesticide applicators and product manufactures (Pushalkar et al., 2020; Stanaway et al., 2017; Wong et al., 1991). Another noteworthy finding was the difference in within-sample alpha diversity levels between workers serving Black/African American clientele to those serving Dominican/Latino clientele, with Black salon workers having lower diversity than Dominican salon workers. The two types of salons were shown to perform different services and to use different hair products, resulting in different levels of exposure to chemicals and other product components that may alter the microbiome, specifically higher VOC exposures among Black compared to Dominican salon workers in general (Louis et al., 2021). However, when stratifying results by participant race, no difference in microbial diversity was observed between Black and Dominican salon workers. This could be due to other lifestyle factors, which, in addition to occupational exposures, play a significant component in microbial diversity, as demonstrated in previous studies evaluating differences in the microbiome by race/ethnicity (Bassis et al., 2015; Dowd and Renson, 2018; Renson et al., 2019). However, these results should be interpreted with caution given our small sample size. This study is the first to observe this trend within salon workers, an occupational cohort reported to have high prevalence of adverse health outcomes including respiratory conditions (Kronholm Diab et al., 2014; Quiros-Alcala et al., 2019).

The primary finding from this research is the association between occupational chemical exposure to select VOCs and the nasal microbiome. There was an inverse relationship between higher concentrations of VOC urinary biomarkers and lowered alpha diversity, significantly for 2HEMA, 3HMPMA, 2HPMA, and TTCA. As lower within-sample diversity level in nasal microbiota has been correlated with various adverse respiratory outcomes, this suggests that the microbiome may play an important role in the detrimental respiratory effects from VOC exposure. 2HEMA, or N-Acetyl-S-(2-hydroxyethyl)-L-cysteine, is the metabolic byproduct from both acrylonitrile and vinyl chloride, which can be found in hairspray, hair wigs and extensions, and perfume, in addition to tobacco smoke (DeLima Associates, 2022; Kanny and Mohan, 2017; National Institute of Occupational Safety and Health, 2020). 3HMPMA, or N-Acetyl-S-(3-hydroxypropyl-1-methyl)-L-cysteine, is the metabolite of crotonaldehyde which is found in perfumes (CosmeticsInfo.org, 2022). 2HPMA, or N-Acetyl-S-(2-hydroxypropyl)-L-cysteine, is the metabolite of acrolein and is found in hair fixatives and artificial nail components (DeLima Associates, 2022; Environmental Working Group, 2022). TTCA, or 2-Thioxothiazolidine-4-carboxylic acid, is the metabolite from carbon disulfide, which is found in tobacco smoke, but not in common salon products (Agency for Toxic Substances and Disease Registry, 2021; Newhook et al., 2002; Rodgman and Perfetti, 2013). Acrylonitrile, vinyl chloride, crotonaldehyde, acrolein, and carbon disulfide have all been associated with various respiratory conditions, from inflammatory outcomes such as asthma and COPD, to respiratory cancers (Bein and Leikauf, 2011; Jang et al., 2007; Li and Holian, 1998; Li et al., 2017; Newhook et al., 2002; Ng et al., 1991; Nurmatov et al., 2015; Pappas et al., 2000). Previous research on the association between environmental chemicals and microbiome has focused primarily on gut microbiome and/or chemicals other than VOCS, including phthalates, bisphenol A, polychlorinated biphenyls, and pesticides (Gálvez-Ontiveros et al., 2020; Rosenfeld, 2017; Yang et al., 2019). Much of this research includes controlled laboratory studies in animal models, which, while helpful in better illustrating the association between chemical exposures and microbiome changes, may not reflect real-life chemical exposures and the effects on complex human microbial communities.

In the present pilot study, we focused on a novel, yet vulnerable population of salon workers serving a racially diverse clientele previously shown to have higher VOC exposures than women in the general U.S. population (Louis et al., 2021). To our knowledge, this is the first study to begin to explore the associations between a robust suite of VOC biomarkers and the nasal microbiome in this highly exposed worker group. However, our study has several limitations, most notably our small sample size. Limited resources prevented us from characterizing VOC exposures in a larger and more racially/ethnically diverse sample of salon workers, and it also restricted our ability to collect more than one urine and nasal sample per participant. Therefore, it was not possible to assess VOC exposure and microbial composition variability and temporality, which may vary within and between individuals due to episodic exposures and variations in bioavailability (Ho Man et al., 2017). This potentially resulted in the low accounted variability observed in our beta diversity principal coordinate analysis plot (maximum of 5.7% variation accounted). We were also not able to differentiate whether respiratory exposures to VOCs via inhalation affected microbial communities directly, or impacted host immune response with indirect impacts on nasal microbiota. Still, the primary objective of this research was to collect data to assess the feasibility of testing our central hypothesis that VOC urinary biomarker concentration could be associated with differential composition and diversity in the nasal microbiome, rather than to examine causality. Future studies should aim to collect multiple samples to better characterize occupational VOC exposures and nasal microbiota in this population.

Additional insight could be gained in future work by targeted measurement via real-time quantitative PCR of the 16S gene overall, and key microbes of clinical importance, such as *Staphylococcal* species.

In summary, our exploratory findings suggest that occupational VOC exposure may alter host nasal microbiota. This may have important implications to respiratory health and other adverse health outcomes, as microbial dysbiosis can mediate or modify the relationship between occupational chemical exposures and health outcomes (Rosenfeld, 2017). These findings add to the existing, but limited body of evidence on the link between environmental chemical exposures and the microbiome, specifically in salon workers who are exposed to a myriad of chemicals that may pose unique health risks (Gaston et al., 2020; Helm et al., 2018). Our preliminary findings from this pilot study support further research in this field to better depict the complex relationships between occupational chemical exposures, the microbiome, and various health outcomes among this and other understudied populations, and is critical to the larger goal of reducing exposures that are known to be disproportionate (Quiros-Alcala et al., 2019). As few studies have assessed the impact of chemical exposures on host microbial compositions in highly exposed populations, this pilot work will inform future studies with larger sample sizes and repeated samples within individuals, given microbiome variability between people and over time. Our work can be used to inform and design future studies in larger and more racially/ethnically diverse population of salon workers, or other occupational cohorts, to identify modifiable exposure factors and potential risk disparities arising from exposures to chemical mixtures of potential concern. Such studies could also help inform exposure mitigation strategies and regulations of potentially harmful chemical ingredients that are particularly disruptive to the microbiome. Overall, this study represents an important first step toward investigating the potential role of VOCs on the nasal microbiome in an understudied and occupational group.

Credit author statement

Kathryn R. Dalton, Ph.D., V.M.D., M.P.H: - Original Draft, Formal analysis, Data Curation, Writing - Review & Editing. Lydia M. Louis, Ph. D., M.S., M.P.H: Methodology, Investigation, Data Curation, Writing - Review & Editing. Magdalena Fandiño-Del-Rio, Ph.D., M.S: Methodology, Investigation, Data Curation, Writing - Review & Editing. Ana M. Rule, Ph.D: Investigation, Writing - Review & Editing. Walkiria Pool: Supervision, Writing - Review & Editing. Katrina Randolph: Supervision, Writing - Review & Editing. Stephen Thomas: Supervision, Writing - Review & Editing. Meghan F. Davis, Ph.D., D.V.M., M.P.H: Conceptualization, Methodology, Investigation, Supervision, Project administration, Funding acquisition, Writing - Review & Editing. Lesliam Quirós-Alcalá Ph.D., M.S: Conceptualization, Methodology, Investigation, Supervision, Project administration, Funding acquisition, Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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