Changes in the Facial Microbiome in Healthy Women After Wearing Masks during COVID-2019

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Abstract

Wearing masks has become a new normal in our daily lives because of the global outbreak of COVID-2019 (COVID-19). To compare the differences in the facial microbiota of healthy women before and after wearing masks and to further explore the probable effect of the habits of regularly wearing masks on the facial microbiome, we re-enrolled the same 19 healthy female participants in our previous study and detected the microbial composition of facial DNA samples using 16S rRNA gene amplicon sequencing. Both alpha and beta diversity, and the abundance and function of facial microflora of recruited healthy women changed remarkably before and after wearing masks. The destination with different air quality indices, which ever was the strongest factor on microbial composition before wearing masks, no longer contributed to both microbiota composition and pathway after wearing masks. Sufficient sleep protected skin from sensitivity and apoptosis, which might be related to \textit{Prevotella} expression and the function of cytochrome c. Maskne was the common complication of wearing masks. Suitable mask-wearing habits should be recommended to avoid facial skin problems.

Introduction

The COVID-19 pandemic has caused profound impact on public health and health policies. Wearing masks, which is an appropriate nonpharmaceutical intervention, is the most effective measure to prevent and control the spread of COVID-19 among the public\textsuperscript{1}. The State Council of China issued the "Guidelines for the Public to Wear Masks Scientifiically (Revised Edition)", which stated that wearing masks in public areas is required to effectively prevent COVID-19 during the epidemic outbreak\textsuperscript{2}. In China, with the optimization of epidemic prevention policies, wearing masks will continue to be one of the leading measures for the prevention and control of COVID-19 and a prominent public habit for a long time in the future.

However, facial skin problems should be considered when wearing masks. Not only among medical personnel but also the public, the use of masks has promoted the occurrence of facial skin problems during COVID-19\textsuperscript{3,4}. The occurrence of facial skin problems caused by regularly wearing masks may affect the maintenance of the habit of wearing masks by the public. In our previous cross-sectional survey involving 4385 participants, we found that having a history of facial skin problems, facial skin allergies, daily face washing frequency $\geq$ 3 times, daily sleep time < 8 hours, and average time spent wearing a mask > 6 hours were high-risk factors associated with facial skin problems\textsuperscript{5}. Among these participants, 19 participants were recruited in our 2019 destination-AQI(Air Quality Index, AQI)-microbiome study\textsuperscript{6}.

Therefore, in this study, we intended to analyze and compare the differences in the facial microbiome before (2019) and after (2020) outbreak of COVID-19 to explore the probable causes of skin problems caused by wearing masks, and expected to determine how to minimize facial skin problems by adjusting the habit of wearing masks and daily lifestyle.
Results

Characteristics of the Participants. Finally, 9 participants from HZ (Hangzhou city in Zhejiang Province, HZ) and 10 participants from YH (Yunhe city in Zhejiang Province, YH) were included for further analysis. The detailed characteristics of all individuals after wearing masks were shown in Table 1. Among the 19 participants, 5 (26.32%) participants reported acne occurrence after wearing masks.

Table 1: Demographic of participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ± SD)</td>
<td>3 ± 30.21 ± 4.78</td>
</tr>
<tr>
<td>Region</td>
<td>HZ (Hangzhou) 9</td>
</tr>
<tr>
<td></td>
<td>YH (Yunhe) 10</td>
</tr>
<tr>
<td>Skin type (According to sebaceous gland secretion)</td>
<td>Combination 6 31.58%</td>
</tr>
<tr>
<td></td>
<td>Oily 4 21.05%</td>
</tr>
<tr>
<td></td>
<td>Dry 1 5.26%</td>
</tr>
<tr>
<td></td>
<td>Neutral 7 36.84%</td>
</tr>
<tr>
<td>Acne occurred after wearing masks</td>
<td>Yes 5 26.32%</td>
</tr>
<tr>
<td></td>
<td>No 14 73.68%</td>
</tr>
<tr>
<td>Daily sleep time during wearing mask</td>
<td>4–8 hours 13 68.42%</td>
</tr>
<tr>
<td></td>
<td>8–12 hours 6 31.58%</td>
</tr>
<tr>
<td>Whether or not to use medical protective mask, N95</td>
<td>Yes 10 52.63%</td>
</tr>
<tr>
<td></td>
<td>No 9 47.37%</td>
</tr>
<tr>
<td>The average cumulative time of wearing masks per day</td>
<td>3–6 hours 11 57.89%</td>
</tr>
<tr>
<td></td>
<td>≥ 6 hours 8 42.11%</td>
</tr>
<tr>
<td>Average time of wearing mask per time</td>
<td>&lt; 2 hours 7 36.84%</td>
</tr>
<tr>
<td></td>
<td>2–4 hours 4 21.05%</td>
</tr>
<tr>
<td></td>
<td>≥ 4 hours 8 42.11%</td>
</tr>
<tr>
<td>Whether or not to change mask when a mask was getting damp</td>
<td>Yes 14 73.68%</td>
</tr>
<tr>
<td></td>
<td>No 5 26.32%</td>
</tr>
</tbody>
</table>

Facial Microbial Diversity Changes Before and After Wearing masks. In this study, we used principal coordinate analysis (PCoA) to assess beta diversity metrics (intersample diversity). In the PCoA plot for
genus, principal coordinate 1 (PC1) represented 46.5% of the variation, and principal coordinate 2 (PC2) represented 8.4% of the variation. In the PCoA plot for pathway, principal coordinate 1 (PC1) represented 49.9% variation, and principal coordinate 2 (PC2) represented 23.9%. Each dot in Fig. 1a and 1b represented a sample from the facial oily (triangle) or dry (circle) zone of participants before (red) or after (green) wearing masks. Here, we observed that red dots and green dots separated from each other, which indicated that wearing masks changed the facial microbiota composition and pathways.

**Differences in Taxonomic Composition of Facial Microbiota Before and After Wearing Masks.** As shown in Fig. 2a-2c, the phyla of Proteobacteria (25.91%), Firmicutes (22.67%), Actinobacteria (10.74%), and Bacteroidetes (7.62%) still dominated the facial microbiomes, similar to the results before wearing masks in 20196. At the genus level after wearing masks in 2020, the top nine genera were *Staphylococcus* (18.24%), *Acinetobacter* (4.21%), *Streptococcus* (3.92%), *Corynebacterium* (3.72%), *Enhydrobacter* (3.36%), *Pseudomonas* (2.60%), *Deinococcus* (1.76%), *Xanthomonas* (1.67%) and *Propionibacterium* (1.60%), as shown in Fig. 2d-2f.

Then the changes in taxonomic profiles in paired samples were next investigated. We compared the relative abundance (logX + 1e-6) of the respective genera before or after wearing masks using the the paired Wilcoxon rank-sum test and drew a heatmap (Fig. 3). In paired samples before and after wearing masks, *Faecalibacterium, Blautia, Bacteroides*, and *Bifidobacterium* were significantly reduced after wearing masks, while *Staphylococcus, Corynebacterium, Acinetobacter, Streptococcus, Corynebacterium, Enhydrobacter, Pseudomonas, Deinococcus* and *Xanthomonas* were markedly increased (Supplementary Figure S1 a-b, and Figure S2 a-l). Notably, *Faecalibacterium*, listed as the top 1 before, dropped out of the top 10, while *Staphylococcus* established dominance as the No. 1 after wearing masks.

**Effect of variables on microbiota composition and pathways.** To assess the effect of factors of wearing masks as predictors of microbiota composition and pathways, we partitioned the explained variability (R2) attributable to each factor (relative importance analysis). For microbiota composition (Fig. 4a), the contributing variables ($P < 0.05$) included repeated use of masks, average duration per instance of wearing the mask or total duration per day of wearing masks, the amount of sleep, whether to replace a damp mask or use medical masks. Furthermore, the physiological state of individuals, including skin type, BMI (Body mass index, BMI), and age, also influences the facial microbiota composition. As shown in Fig. 4b, the average duration per time of wearing masks, age, sleep, whether to replace a damp mask, and mask time contributed to the microbiota pathway. It was worth noting that destination, which ever was the strongest factor affecting microbial composition in our previous study[^6], did not play a role in either microbiota composition or pathway after wearing masks.

Focusing on the top 20 genera of facial bacterial microbiota, we analyzed the relative abundance of specific genera among groups with different wearing habits. As shown in Fig. 5, the abundance of *Prevotella* was higher in the group with 8–12 hours of sleeping time than in the group with 4–8 hours of sleeping time per day ($P = 0.0066$, Fig. 5a), especially in the oily zone ($P = 0.0357$, Fig. 5b). Moreover, analysis of the top 20 pathways of microbiomes revealed a decreased expression PWY-
In addition, we also compared the relative abundance of the top 10 genera of facial bacterial microbiota between the maskne (acne due to masks) group and the normal group (non-maskne). Figure 6a-d showed that the relative abundance of Staphylococcus, Propionibacterium and Pseudomonas in the maskne group was much higher than that in the non-maskne group, while that of Paracoccus in the maskne group decreased compared to the non-maskne group. Furthermore, the abundance of Staphylococcus and Propionibacterium displayed a positive relationship between them ($R^2 = 0.317$).

Discussion

Masks are a core component of personal protective equipment that can significantly reduce the incidence of respiratory diseases, including COVID-19, and wearing masks are becoming a new normal in our daily life. The incidence of facial skin problems was found to be between 30% and 45% among medical staff wearing personal protective equipment against COVID-19\textsuperscript{7,8}; however, data on the incidence of facial skin problems caused by wearing masks among the general population are lacking. In our previous cross-sectional survey, approximately one-third of participants reported skin problems due to wearing masks\textsuperscript{5}. In another study in 2019, we reported that the difference in AQI between two destinations (HZ and YH) resulted in the difference in the abundance and diversity of facial microbiota of healthy women\textsuperscript{6}. In 2020, we re-enrolled the same 19 participants and collected microbial samples from facial oily and dry zones under similar conditions. Intriguingly, it was shown that the distance of the genus and pathway between HZ and YH caused by differential AQI decreased remarkably after wearing masks\textsuperscript{5}. This result suggested that wearing masks might affect facial microbiota composition and pathways and further result in facial skin problems. Therefore, in this study, we aimed to deeply explore the probable influence of the habits of wearing masks on the facial microbiome.

First, in addition to alpha diversity\textsuperscript{5}, the beta diversity of the microbiome genus and pathway showed a discrete distribution of points indicating before or after wearing masks, with a considerable change of the genera composition. A multivariable analysis revealed that COVID-19 precautions in hospitalized patients alter the gut microbiota, thereby mediating pathogen susceptibility and nosocomial infections\textsuperscript{9}. This might explain why in our present study, the gut microbiota of Faecalibacterium, Blautia, Bacteroides and Bifidobacterium on participants' faces dropped significantly. In contrast, the relative abundance of several respiratory microbiota, including Staphylococcus\textsuperscript{10}, Streptococcus\textsuperscript{11} and Pseudomonas\textsuperscript{12}, increased after wearing masks. In a healthy population after sexual maturity, the cheek microbiota are dominated by Propionibacterium and Staphylococcus\textsuperscript{13}. The diversity and composition of the skin microbiota in elderly individuals are affected largely by oral bacteria\textsuperscript{14}. Furthermore, analyses of the microbial community on disposal surgical masks or non-woven fabric masks of healthy volunteers declared masks mainly contained Streptococcus and Staphylococcus\textsuperscript{15}, or Propionibacterium acnes and Staphylococcus epidermidis/aureus\textsuperscript{16}. Thus, we speculated that the change of microbial community after wearing masks...
might be due to microflora carried in masks, an increased effect of oral or respiratory bacteria, and the critical role of masks in protecting person-to-person spread and in controlling outward aerosol particle emissions\textsuperscript{17,18}.

Second, we found that many factors related to wearing masks were positively correlated with facial microbiota composition and pathways. At the genus level, we revealed an increase in \textit{Prevotella} with prolonged sleep time. \textit{Prevotella} are common and abundant microbial communities in humans and inhabit various body sites. However, whether \textit{Prevotella} are benefit or detrimental to human health depends on the sites of colonization and the specific strain involved\textsuperscript{19}. On one hand, \textit{Prevotella} sometimes were considered as pro-inflammatory factors and were reported to be upregulated significantly in lesions of atopic dermatitis and psoriasis\textsuperscript{20,21}. On the other hand, the maternal carriage of \textit{Prevotella copri} during pregnancy can protect offspring from food allergies, by accelerating the postnatal transition from a Th2- to Th1- and Th17-dominant immune phenotype indirectly\textsuperscript{22,23}. In our present study, we did observe the increase of \textit{Prevotella} along with the prolongation of sleep time. However, which specific strains of \textit{Prevotella} are involved, and how about their function, are not revealed. Future studies using metagenomic analyses and functional experiments are encouraged to identify the specific strains and to evaluate mechanistic links between the facial microbiome with sleep.

In the functional analysis, PWY-3781(aerobic aspiration I) became a dominant pathway after wearing masks. Aerobic aspiration I is associated with cytochrome c, which not only plays a role in cell apoptosis but also amplifies signals generated by other apoptotic pathways and participates in certain nonapoptotic functions\textsuperscript{24}. After cytochrome c is released into the cytosol, it binds to APAF-1 to activate pro-caspase 9 and trigger an enzymatic cascade leading to cell death\textsuperscript{25}. Our data indicated that enough sleep might downregulate the function of cytochrome c to protect skin from sensitivity or apoptosis.

Every coin has two sides, so does wearing masks. In our online survey, it was found that the top 3 types of facial skin problems after wearing were indentation/crush, allergic dermatitis, and acne/worsening of acne\textsuperscript{26}. Maskne, a new term coined during the COVID-19 pandemic, refers to acne caused or worsened by the use of masks\textsuperscript{26}. In our 19 recruited healthy women, 5 cases developed acne after wearing masks. We further found that in the maskne group, the relative abundance of \textit{Staphylococcus}, \textit{Propionibacterium} and \textit{Pseudomonas} was much higher than that in the normal group without skin problems. The causal role of \textit{Propionibacterium acnes} in the pathogenesis of acne has been well demonstrated\textsuperscript{27}, while the effect of \textit{Staphylococcus} (especially \textit{Staphylococcus epidermidis}) on acne pathogenesis remained contradictory\textsuperscript{28}. On one hand, \textit{Staphylococcus epidermidis} induced the activation of inflammation-related markers, even had a more important role than \textit{Propionibacterium acnes} on the induction of IL-6\textsuperscript{29}. On the other hand, \textit{Staphylococcus epidermidis} limited \textit{Propionibacterium acnes} over-colonization and inflammation via the release of succinic acid homeostasis\textsuperscript{30}. Under physiological conditions, \textit{Staphylococcus epidermidis} and \textit{Propionibacterium acnes} check each other in a state of dynamic equilibrium\textsuperscript{28}. However, in our results, their expression showed a positive correlation after wearing masks, especially both highly expressed in the maskne group. Which specific species is dominant in up-
regulated bacteria after wearing masks, and whether the combination of high abundance of *Staphylococcus* and *Propionibacterium* indicates vulnerability to maskne, needs to be further investigated.

There are some limitations to this study. The number of re-enrolled participants was small. The present study is an extension of our previous study, in which the data from 29 participants (18 from HZ and 11 from YH) were eventually used for further analysis to compare the facial microbiome in healthy women between HZ and YH two districts with different AQI. In the present study, after wearing masks as a normal lifestyle, we re-enrolled the same 29 subjected and only 19 participants agreed or met the inclusion criteria. Secondly, it’s better to set up a group without wearing masks as a control. However, with the optimization of epidemic prevention policies in China, wearing masks is still one of the most important measures for the prevention and control of COVID-19. It’s difficult to recruit participants who don’t wear masks under the present condition. It might be feasible to design and perform a larger-scale and blank-controlled prospective study in the near future.

**Methods**

**Participants.** The Institutional Review Board of the First Affiliated Hospital, School of Medicine of Zhejiang University approved the experiments, including any relevant details, under protocol numbers IIT20200252 and 2017-866. All experiments were performed in accordance with relevant guidelines and regulations. Written informed consent, was obtained from all participants. A total of 19 young healthy females aged 25-35 y, 9 from HZ and 10 from YH were recruited again in this study (recruitment process as shown in Fig. 7). They were the same participants as in our previous study for comparison of the facial microbiome between two districts in Zhejiang Province with different air quality indices (AQIs). After the outbreak of COVID-19, all of them wore masks daily for more than 3 months. Participants would be excluded if they answered "yes" to any of the exclusion criteria as shown in Supplementary Table S1. To minimize the impact of cosmetics, they only used the provided cleanser and lotions for 2 weeks prior to sampling. Their facial DNA samples were also collected in May and under the same conditions as in a previous study in 2019. In addition, they completed a cross-sectional questionnaire about facial skin problems after wearing masks during the COVID-19 pandemic.

**Facial DNA Collection, Processing and Bioinformatics Analysis.** We performed DNA extraction, PCR amplification, sequence processing, and bioinformatics analysis according to the protocols described in a previous study. In brief, samples were collected from the oily and dry zones of the face with sterile Catch-All sample collections swabs (QEC091H, Epicenter, USA) and stored at -80°C within 15 min. A PSP Spin DNA plus kit (Stratec, Berlin, Germany) was used to extract DNA. Specific primers 341F 5'-CCTAYGGGRBGCASCAG-3' and 806R 5'-GGACTACNNGGGTATCTAAT-3' with barcodes were used for PCR amplification of the V3-V4 region of 16S rRNA. All PCRs were carried out in 30 µL reactions, and the PCR product was sequenced on an Illumina MiSeq sequencer. Raw Illumina read data were deposited in the EMBL database (study accession number PRJEB42523). We used the same bioinformatics analysis flow from our previous study.
Statistics Analysis. Statistical analyses were performed in R version 3.6.1. The Wilcoxon rank-sum test was used to perform hypothesis tests of alpha diversity and principal components (PCs). For paired samples (two samples from the same participant), we used the $P$ value estimated by the paired Wilcoxon rank-sum test. A $P$ value $< 0.05$ was considered significant.

Declarations

Data Availability Statement

Raw data for 16S rRNA amplicon sequencing in this study are available in the EMBL database (study accession number PRJEB42523).

Author contributions statement

X.L. and S.Y. conceived the study. Q.S., C.H., and X.L. performed the literature search and wrote the protocols. Y.W. and Y.Z. recruited the participants and collected the specimens. A.L. performed the laboratory analyses and analyzed the data. X.L., A.L., and S.Y. interpreted the data. All authors contribute to the writing and editing of the report and approved the final version.

Ethics Statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of the First Affiliated Hospital, School of Medicine of Zhejiang University. The patients/participants provided their written informed consent to participate in this study.

Fundings

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References


Figure 1

The bacterial community beta diversity of facial microbiota before and after wearing masks. Oily represented samples from the facial oily zone and dry represented samples from the facial dry zone. Each dot represented a sample.
Figure 2

Taxonomy resolution of facial microbiota in healthy females after wearing masks in 2020. (a-c) Relative abundance levels of the phyla presented in samples from the HZ and YH healthy females after wearing masks in 2020. (d-f) Relative abundance levels of genera presented in samples from the HZ and YH females after wearing masks. *H1 indicated facial oily zone data in the HZ district, H2 indicated facial dry
zone data in the HZ district; Y1 indicated facial oily zone data in the YH district, Y2 indicated facial dry zone data in the YH district.

**Figure 3**

Heatmap of the most dominant genera of the facial microbiota before or after wearing masks. *H1 indicated oily zone data in the HZ district, H2 indicated dry zone data in the HZ district; Y1 indicated oily zone data in the YH district, Y2 indicated dry zone data in the YH district.
Figure 4

Effect size of variables on facial microbiota composition (a) and pathways (b). Variables were found to be significantly correlated with facial microbiota composition and pathways, sorted by their relative importance (% of R2). R2 values represented the fractions of microbiota composition and pathways explained by the variables in each category. * +, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$.

![Figure 4](image)

Figure 5

The effects of sleep on specific genera. (a-c) The relative abundance of Prevotella between groups with different sleeping time. (d-f) The relative abundance of the pathway of PWY-3781 between groups with different sleeping time. * Oily indicated facial oily-zone data, Dry indicated facial dry zone data. Significance determined in Wilcoxon rank sum test with one-sided alternatives.

![Figure 5](image)
Figure 6

The relative abundance of specific genera between the maskne group and the normal group. (a-d) The relative abundance of *Staphylococcus*, *Propionibacterium*, *Pseudomonas* and *Paracoccus* between the maskne group and the normal group. (e) The positive linear correlation of the abundance between *Staphylococcus* and *Propionibacterium*. Significance determined in Wilcoxon rank sum test with one-sided alternatives.
Figure 7

The recruitment process. AQI, air quality index.

Supplementary Files

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