



# Microbiome and Mycobiome Analyses of Continuous Positive Airway Pressure Devices

Hyun Jin Min<sup>1,2,\*</sup> · Bo-Yun Choi<sup>3,\*</sup> · Woo Jun Sul<sup>3</sup> · Hyung-Ju Cho<sup>4,5</sup>

<sup>1</sup>Department of Otorhinolaryngology-Head and Neck Surgery, Chung-Ang University College of Medicine, Seoul, Korea

<sup>2</sup>Biomedical Research Institute, Chung-Ang University Hospital, Seoul, Korea

<sup>3</sup>Department of Systems Biotechnology, Chung-Ang University, Anseong, Korea

<sup>4</sup>Department of Otorhinolaryngology, Yonsei University College of Medicine, Seoul, Korea

<sup>5</sup>The Airway Mucus Institute, Yonsei University College of Medicine, Seoul, Korea

**Objectives.** Microorganisms are likely present in continuous positive airway pressure (CPAP) devices in daily use. Given the potential risk of infection among CPAP users, we aimed to compare the microbiomes of CPAP devices with those of nasal mucosa samples obtained from patients using these devices.

**Methods.** We conducted a prospective cohort study at multiple tertiary medical institutions. Samples were collected from the tubes and filters of CPAP devices and the nasal mucosa of device users. Microbiomes and mycobiomes were analyzed using 16S ribosomal RNA and internal transcribed spacer region sequencing. The results were compared according to sampling site and usage duration for each patient.

**Results.** Overall, 27 paired samples of human nasal mucosa and CPAP components were analyzed. Bacteria were detected in 7 of the 27 tubes (25.9%) and in 22 of the 27 filters (81.5%). Fungi were found in 2 tubes (7.4%) and 16 filters (59.3%). The most prevalent bacterial phyla across all samples were Actinobacteria and Firmicutes. Fungi were not detected in any nasal mucosa samples. However, fungi were identified in the CPAP filters and tubes, with the Basidiomycota and Ascomycota phyla predominating. No significant associations were identified according to sampling site or duration of CPAP use.

**Conclusion.** Some bacteria or fungi are detectable in CPAP samples, even after a short period of CPAP usage. However, the association between respiratory infections and these microbiomes or mycobiomes was not investigated. Further research is required to clarify the risk posed by CPAP devices as a microbial contamination source.

**Keywords.** Continuous Positive Airway Pressure Device; Microbiome; Mycobiome; Obstructive Sleep Apnea

## INTRODUCTION

Obstructive sleep apnea (OSA) is characterized by repeated reductions or cessations of airflow due to upper airway collapse during sleep [1]. OSA is highly prevalent, affecting approximately 38% of the general population [2]. Among older adults, its prevalence is estimated to be as high as 90% in men and 78% in women [1,2]. Typically, OSA is diagnosed based on polysomnography, subjective symptoms, and physical examination findings. Treatment options for OSA include surgical and nonsurgical approaches, with continuous positive airway pressure (CPAP) therapy being the most commonly employed.

• Received June 16, 2024  
Revised August 4, 2024  
Accepted August 12, 2024

• Corresponding author: **Hyung-Ju Cho**  
Department of Otorhinolaryngology, Yonsei University College of Medicine,  
50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea  
Tel: +82-2-2228-3605, Fax: +82-2-393-0580, Email: hyungjucho@yuhs.ac

• Co-Corresponding author: **Woo Jun Sul**  
Department of Systems Biotechnology, Chung-Ang University, 4726  
Seodong-daero, Daedeok-myeon, Anseong 17546, Korea  
Tel: +82-31-670-4707, Fax: +82-31-675-3108, Email: sulwj@cau.ac.kr

\*These authors contributed equally to this study as co-first authors.

© 2024 by Korean Society of Otorhinolaryngology-Head and Neck Surgery.

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

CPAP devices deliver continuous pressure to the airways. The pressure is maintained throughout the respiratory cycle, including both inspiration and expiration. These devices have been shown to alleviate daytime sleepiness and improve overall quality of life, while decreasing the risk of cardiovascular comorbidities [3,4]. For patients utilizing CPAP devices in outpatient settings, such as at home, it is crucial to wear them consistently during overnight sleep and daytime naps. Consequently, improving daily compliance with CPAP use is a key clinical objective.

The use of medical devices, particularly over extended periods, can confer an increased risk of microbial proliferation [5]. For instance, patients on ventilators face a high risk of pneumonia because the natural defenses of their upper airway are bypassed during intubation [5,6]. Ventilator-associated pneumonia affects approximately 20% of patients who undergo mechanical ventilation for more than 48 hours, resulting in longer hospital stays and higher costs. Bacteria can begin to colonize these devices within the first 24 hours of intubation, and these bacteria have been implicated in subsequent infections [7]. Similarly, the reported prevalence of infections, as recalled subjectively by patients, was considerably higher in patients with OSA who used a CPAP device compared to those who did not [8]. Specifically, the prevalence rate was about 43% among CPAP users, compared to 25% in non-users [8]. This disparity underscores the potential infection risk for individuals using CPAP devices [5,9]. Based on these findings, we hypothesized that microorganisms can colonize CPAP equipment through daily use and then enter the airways through the nasal cavity.

In this study, we aimed to evaluate the presence and distribution of the microbiome in CPAP devices, focusing on each component of the device. Additionally, we assessed the microbiome of the nasal airway, specifically the inferior turbinate, which is the initial site of airflow entry in patients with OSA using CPAP equipment. Furthermore, we sought to determine whether individual CPAP components remain free from microbial colonization and whether microorganisms from CPAP devices could be transmitted to patients through the nasal airway.

## H I G H L I G H T S

- The microbiomes and mycobiomes of continuous positive airway pressure (CPAP) devices and their users were analyzed.
- Bacteria were present in 7 of the 27 tubes (25.9%) and 22 of the 27 filters (81.5%).
- Fungi were present in 2 of the 27 tubes (7.4%) and 16 of the 27 filters (59.3%).
- Bacteria and fungi can accumulate in the components of CPAP devices, including tubes, filters, and masks.
- More research is required regarding CPAP devices as sources of microbial contamination.

## MATERIALS AND METHODS

### Ethics approval and consent to participate

This prospective multicenter cohort study was approved by the Institutional Review Boards of Chung-Ang University Hospital (No. 2050-006-419) and Yonsei University College of Medicine (No. 4-2020-0774). Informed consent was obtained from all participants.

### Study design and population

Patients who were diagnosed with OSA based on level 1 polysomnography and who were using nasal CPAP devices daily ( $\geq 4$  hours daily) were enrolled between March 2021 and June 2021. We initially provide our own education to patients on the proper maintenance protocol for the CPAP device and regularly monitor their condition. The hygiene protocol includes: washing the mask and humidifier of the CPAP device with a neutral detergent and air-drying them daily for a smooth fabric finish; washing the tube with a neutral detergent daily and letting it air-dry naturally; cleaning the main body of the CPAP device as needed; and wiping away any dust during use. Furthermore, we independently replace the tubes at least every 6 months and the filters at least every 3 months. Patient compliance with CPAP device cleaning was strictly monitored using a checklist throughout the duration of the study. The following patients were excluded: patients with a history of upper respiratory tract infections; those who had taken or were in the process of taking medications, including antibiotics and immunosuppressive drugs, during the past 1 month; those with a smoking history; those diagnosed with allergic rhinitis; those with inhalator usage; and those with a short total duration of CPAP device use ( $< 6$  months).

### Sample collection

Nasal mucosal swab samples from patients with OSA using CPAP devices as well as samples from their CPAP tubes and air filters were prospectively collected for microbiome analysis. Nasal swab samples were individually collected from inferior turbinate mucosa using sterile Copan culture swabs (Copan Italia S.P.A.) with a rigid 0° endoscope in an outpatient clinic according to a previously reported protocol [10]. Briefly, cotton swabs were inserted into the nasal cavities without touching the nostril and gently rotated in the anterior part of the inferior turbinate. CPAP samples were obtained from tubes and filters. Tube samples were obtained using sterile Copan swabs rotated at least 20 times around the area connected to the nasal mask. Air filter samples were inserted into the gel layer of the swab tubes, which were then closed and stored until analysis.

### Bacterial genomic DNA extraction and polymerase chain reaction

Bacterial genomic DNA was extracted from the CPAP filter samples and from swabs rubbed inside the CPAP tubes and pa-

tients' nostrils using the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's protocol.

### Microbiome and mycobiome analyses

Sample microbiomes and mycobiomes were analyzed using the QIIME 2 (Quantitative Insight into Microbial Ecology) pipeline (v.2019-7) [11]. Cutadapt analysis was performed using default settings to remove primer sequences from the bacterial sequences [12]. Paired-end sequence reads were merged, and the sequences were denoised using the DADA2 plugin [13]. Bacterial amplicon sequence variants (ASVs) were identified, and taxonomy was assigned using the Greengenes database via the feature-classifier classify-sklearn plugin, and mitochondrial and chloroplast ASVs were removed [14]. ASVs were aligned using the phylogeny align-to-tree-mafft-fasttree plugin; subsequently, alpha diversity (Shannon, Chao1, and Faith's phylogenetic diversity) and beta diversity were assessed using a rarefied depth of 43,000 reads per sample using the diversity plugin. Fungal sequence reads were merged, and primer sequences were removed using a method similar to the one used for bacterial sequences. Paired-end sequence reads were merged, and combined reads were trimmed based on a Q-score of 25 using VSEARCH with the quality-filter plugin. The remaining sequences were denoised using the DADA2 plugin, and fungal ASVs were identified. Taxonomy was assigned using the UNITE database. The ASVs were aligned using the methods described above, and diversity was determined at a rarefied depth of 76,600 reads per sample.

## RESULTS

### Patient characteristics

Of the 122 patients who agreed to participate in the study, 99 were excluded due to a history of smoking, a diagnosis of allergic rhinitis, prior inhaler use, or insufficient duration of CPAP usage. Consequently, 27 patients were enrolled, and samples from their nasal mucosa, as well as from their CPAP tubes and filters, were analyzed using 16S ribosomal RNA and internal transcribed spacer region sequencing (Supplementary Fig. 1). The mean age of the enrolled patients was  $58.29 \pm 14.70$  years, with a male-to-female ratio of 17:10. The mean apnea-hypopnea index was  $41.84 \pm 19.83$ , and the mean durations of device usage for the analyzed tubes and filters were  $8.00 \pm 3.84$  months and  $7.00 \pm 3.34$  months, respectively (Table 1).

Bacterial analysis indicated that bacteria were present in only seven of the 27 tube samples (25.9%) and in 22 of the 27 filter samples (81.5%). In comparison, fungal analysis indicated the presence of fungi in just 2 of the 27 tube samples (7.4%) and in 16 of the 27 filter samples (59.3%). The detected bacteria and fungi were analyzed. While bacteria were identified in all 27 nasal mucus samples, fungi were not found in any of these samples (Table 1).

**Table 1.** Characteristics of enrolled patients

Characteristic	Value
Total number of enrolled patients	27
Age (yr)	$58.29 \pm 14.70$
Male:female	17:10
Smoker	0
HTN	11
DM	3
AHI	$41.84 \pm 19.83$
Lowest O <sub>2</sub> saturation	$80.05 \pm 6.06$
Usage duration of tube (mo)	$8.00 \pm 3.84$
Usage duration of filter (mo)	$7.00 \pm 3.34$
Tube samples with bacteria detected	7
Filter samples with bacteria detected	22
Tube samples with fungi detected	2
Filter samples with fungi detected	16
Nasal mucosa samples with bacteria detected	27
Nasal mucosa samples with fungi detected	0

Values are presented as number or mean  $\pm$  standard deviation.

HTN, hypertension; DM, diabetes mellitus; AHI, apnea-hypopnea index.

### Microbiomes and mycobiomes at the sampling sites

We analyzed the bacterial composition of CPAP tubes, CPAP filters, and human nasal mucus samples. *Actinomycetota* and *Bacillota* were the predominant phyla for all three sample types. In contrast, *Pseudomonadota* was less abundant in the nasal mucosa samples than in the CPAP component samples (Fig. 1A). *Corynebacterium*, of the phylum Actinomycetota, and *Staphylococcus*, from the phylum Bacillota, were abundant in the nasal mucus. In the filter samples, *Corynebacterium* was the most abundant bacterium, followed by *Staphylococcus*; these were present in most samples, although their abundance varied between individuals. *Dorea*, a member of the phylum Bacillota, was detected more frequently in CPAP tube samples than in the other types (Fig. 1B).

Fungi were not detected in any of the nasal mucosa samples; however, fungi were found in CPAP device filters and tube samples, with Basidiomycota and Ascomycota being the predominant phyla (Fig. 2A). Variations in their abundance were noted across the samples. *Aspergillus penicillioides*, belonging to the phylum Ascomycota, was primarily identified in filter samples. Only two tube samples tested positive for fungi, one of which contained an abundant proportion of *Penicillium citrinum* (Fig. 2B).

We assessed the bacterial diversity at the sampling sites through alpha and beta diversity analyses (Fig. 3). Significant differences were observed in bacterial abundance (Fig. 3A) and phylogenetic diversity (Fig. 3C) between the CPAP filter and tube samples, as well as between the filter and mucosa samples. Notably, the nasal mucosa exhibited lower diversity compared to the CPAP tube and filter (Fig. 3A-C). Furthermore, the bacterial communities differed significantly between CPAP device sites (Fig. 3D-E).

The heatmap confirmed significant differences in bacterial diversity and communities between nasal mucosa and CPAP de-

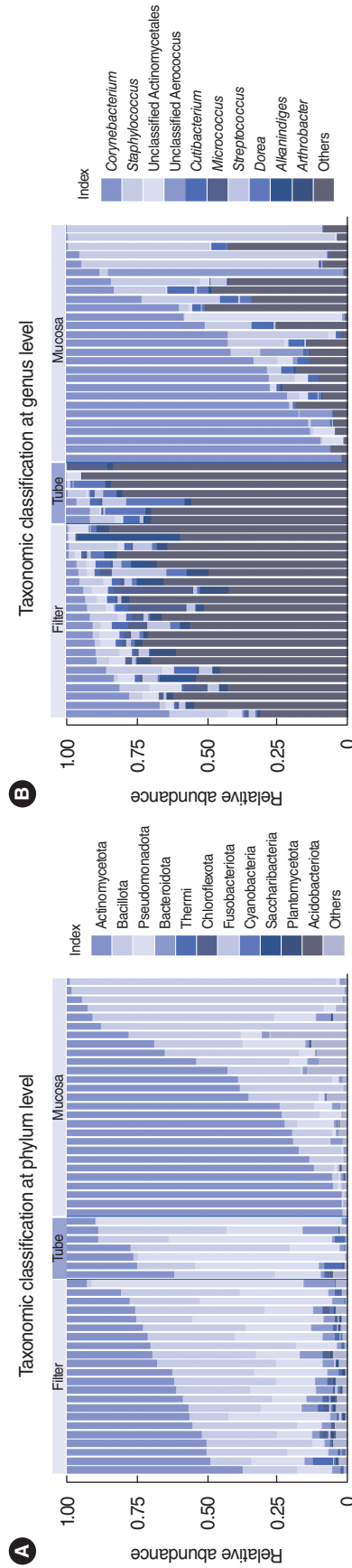


Fig. 1. Bar graph of taxa, illustrating the bacterial composition by sampling site. The graph is divided into continuous positive airway pressure (CPAP) filter, CPAP tube, and nasal mucosa samples. (A) At the phylum level, the graph displays the 11 most abundant bacteria. (B) At the genus level, the graph presents the 10 most abundant bacteria.

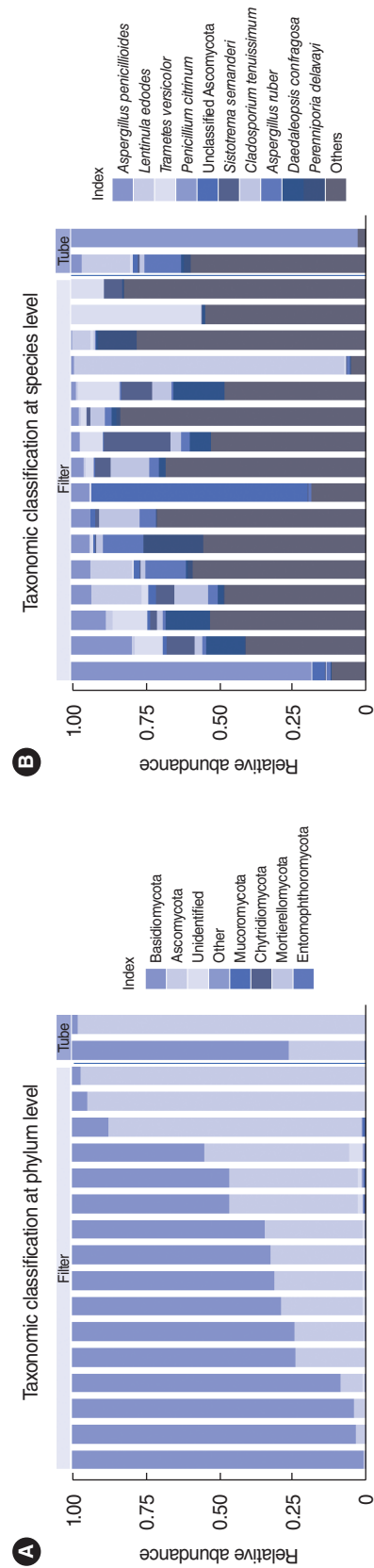
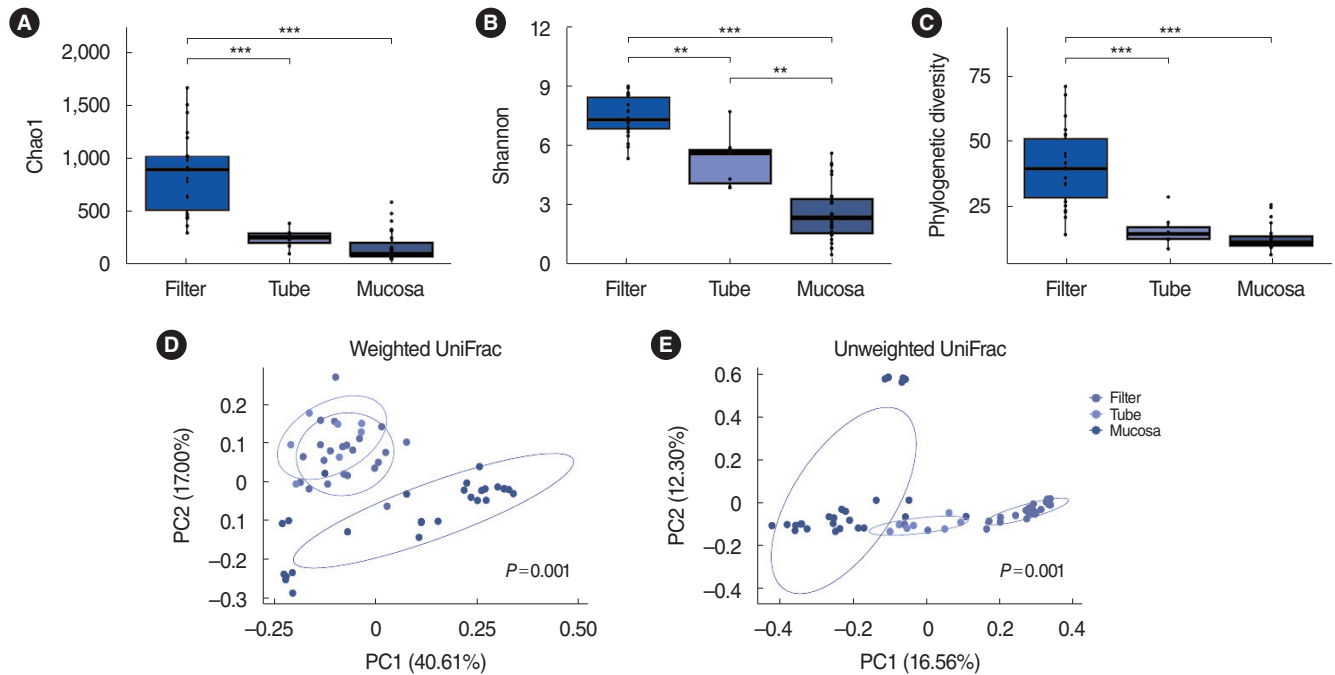


Fig. 2. Bar graph of taxa, illustrating the fungal composition by sampling site. The graph is divided into continuous positive airway pressure (CPAP) filter, CPAP tube, and nasal mucosa samples. (A) At the phylum level, the graph displays the 7 most abundant fungi. (B) At the species level, the graph presents the 10 most abundant fungi.





**Fig. 3.** Comparison of bacterial alpha and beta diversity levels according to continuous positive airway pressure (CPAP) sampling site. (A-C) Alpha diversity: (A) Chao1 index, (B) Shannon index, and (C) phylogenetic diversity (\*\* $P<0.01$  and \*\*\* $P<0.001$ ). (D, E) Principal coordinate analysis plots according to CPAP sampling site, based on weighted and unweighted UniFrac distances. (D) Weighted UniFrac distance. Significant differences were observed among the groups ( $P=0.001$ ; analysis of similarities [ANOSIM]). (E) Unweighted UniFrac distance. Significant differences were also noted among the groups ( $P=0.001$ ; ANOSIM).

vice samples, as previously mentioned (Supplementary Fig. 2). Analysis at the ASV level revealed distinct bacteria in the filter tube and nasal mucosa samples. In the nasal mucosa samples, ASV654 (*Staphylococcus saprophyticus*), ASV692 (*Corynebacterium aurimucosum*), ASV5021 (*S. saprophyticus*), ASV2018 (*C. aurimucosum*), and ASV506 (*Corynebacterium*) were detected. In the CPAP tube samples, ASV11068 (*Comamonadaceae*), ASV2527 (*Oxalobacteraceae*), ASV893 (*Thermaceae*), ASV762 (*Sphingobium estrogenivorans*), ASV14074 (*S. estrogenivorans*), and ASV11088 (*Arthrobacter*) were identified. The CPAP filter samples exhibited the greatest variety of ASVs, including ASV9290 (*Streptococcus agalactiae*), ASV3407 (*Albidovulum inexpectatum*), ASV12028 (*Clostridium ruminantium*), ASV9453 (*Methylobacterium mesophilicum*), ASV11380 (*S. agalactiae*), and ASV3013 (*Enhydrobacter aerosaccus*). Regarding fungal detection, CPAP tube samples contained ASV4298 (*Aspergillus ruber*), ASV3154 (*A. ruber*), ASV2742 (*Lentinula edodes*), ASV4355 (*A. ruber*), ASV5076 (*Xylodon flaviporus*), ASV5519 (*Aspergillus*), and ASV3834 (*P. citrinum*). One tube sample displayed the highest proportion of ASV3832 among all samples taken (*P. citrinum*) (Supplementary Fig. 3).

#### Effects of device usage duration

We compared the microbiome and mycobiome findings based on the duration of CPAP component usage, which was categorized as “long” when usage exceeded 3 months and “short” for

usage under 3 months. Specifically, we assessed alpha and beta diversity in relation to the duration of use. Regarding the filter samples, the diversity and composition of bacterial communities did not exhibit significant changes in this analysis, suggesting that the length of filter use did not influence bacterial diversity or community structure (Fig. 4A-E). However, at the ASV level, MaAsLin2 analysis revealed certain ASVs, such as *Micrococcus terreus* (ASV805), that displayed a significant increase in abundance with longer filter usage (Fig. 4F).

Among the CPAP tube samples, although graphical analysis revealed that the long-term usage group exhibited greater diversity than the short-term group, no significant differences were noted (Fig. 5A-C). Similarly, bacterial diversity levels exhibited no significant differences between long- and short-term usage (Fig. 5A-C). However, certain ASVs that significantly increased in abundance with longer CPAP tube use were identified using MaAsLin2. Notably, the relative abundance of *S. saprophyticus* (ASV3346) rose significantly with extended CPAP tube usage (Fig. 5D).

Using the same criteria employed for bacteria, we assessed the variations in fungal diversity and communities according to the duration of device component usage (Fig. 6). Like bacteria, fungi exhibited no significant differences in diversity, abundance, or phylogenetic diversity between samples associated with long-term and short-term usage. Similarly, no significant differences were evident in the composition of fungal communities. Howev-

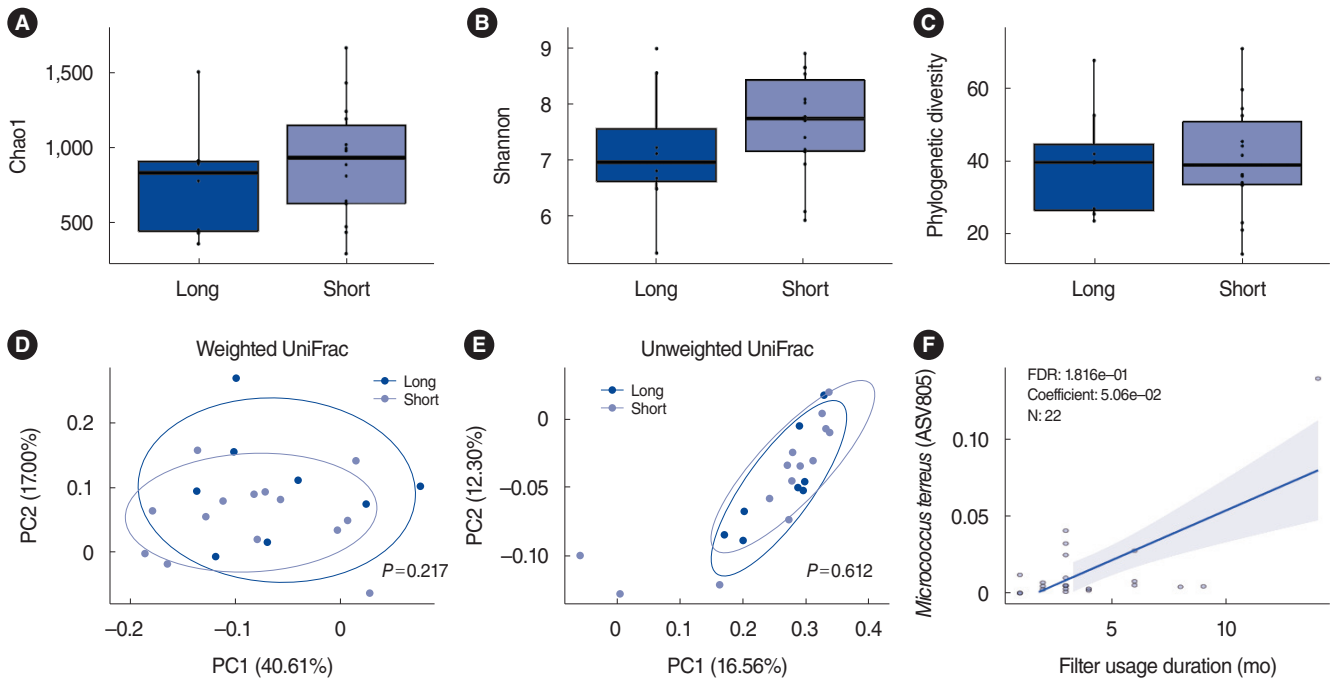


Fig. 4. Comparison of bacterial alpha and beta diversity levels according to filter usage duration and correlations between duration and microbial taxa (long, >3 months; short, ≤3 months). (A-C) Alpha diversity: (A) Chao1, (B) Shannon, and (C) phylogenetic diversity. (D, E) Principal coordinate analysis plots according to filter usage duration, based on weighted and unweighted UniFrac distances. (D) Weighted UniFrac distance. (E) Unweighted UniFrac distance. No significant difference was observed in either distance metric ( $P=0.217$ ,  $P=0.612$ ; analysis of similarities). (F) Amplicon sequence variants (ASVs) significantly correlated with filter usage duration, as determined using MaAsLin2 (false discovery rate [FDR] <0.05).

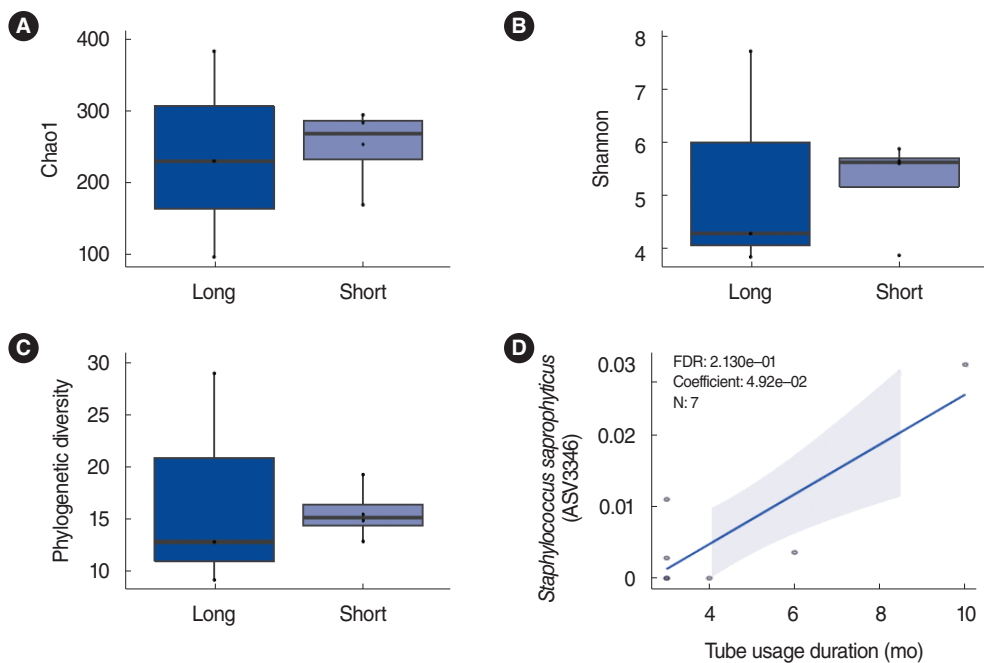


Fig. 5. Comparison of bacterial alpha diversity levels according to tube usage duration and correlations between tube usage duration and microbial taxa (long, >3 months; short, ≤3 months). (A-C) Alpha diversity: (A) Chao1, (B) Shannon, and (C) phylogenetic diversity. (D) Associations between tube usage duration and microbiome composition data, as determined using MaAsLin2. FDR, false discovery rate.

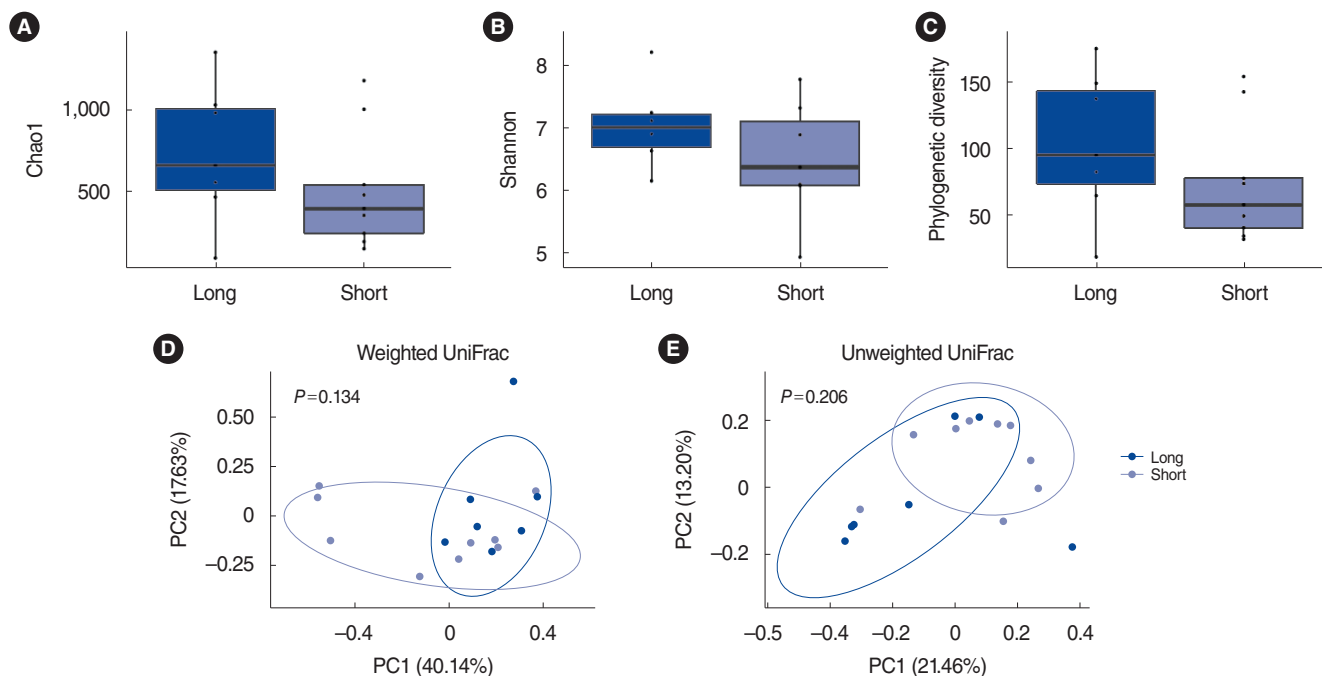


Fig. 6. Comparison of fungal alpha and beta diversity levels according to filter usage duration (long, >3 months; short, ≤3 months). (A-C) Alpha diversity: (A) Chao1, (B) Shannon, and (C) phylogenetic diversity. (D, E) Principal coordinate analysis plots according to filter usage duration, based on weighted and unweighted UniFrac distances. (D) Weighted UniFrac distance. (E) Unweighted UniFrac distance. No significant difference was observed in either distance metric ( $P=0.134$ ,  $P=0.206$ ; analysis of similarities).

er, at the ASV level, we observed that the relative abundance of fungi increased with the extended use of CPAP filters (Supplementary Fig. 4).

## DISCUSSION

Nasal CPAP therapy is a well-established treatment for OSA [8]. Common adverse effects of CPAP use include mask leakage; nasal congestion; dryness of the nose, mouth, and throat; and discomfort due to the air pressure applied. Additionally, the potential risk of infection associated with CPAP device usage cannot be overlooked. Therefore, caregivers and patients should remain vigilant about respiratory infections that may result from CPAP use. In a previous study, patients with OSA who were treated with CPAP devices exhibited an increased risk of upper airway infection compared to those not using these devices. However, the study relied on patients' subjective symptoms reported through questionnaire responses and did not elucidate the reasons for the reportedly higher infection rates among CPAP users [8]. Another study, employing participant responses to a questionnaire and conventional culture methods with charcoal swabs, indicated that CPAP device use did not significantly affect infection prevalence or the types of microorganisms isolated [15].

The primary finding of the present study is that some bacteria or fungi can be detected in CPAP samples, even after a short

period of equipment use. Microbial communities were found in 25.9% of the tube samples and 81.4% of the filter samples. In comparison, fungal communities were identified in 7.4% of tube and 59.2% of filter samples. Notably, the overall detection rate was less than 50%. While this detection rate may appear modest, a precise assessment of its clinical relevance is warranted. The microbiomes and mycobiomes of CPAP components were not significantly associated with the microbiomes of the nasal mucosa of CPAP users. Bacterial communities were detected more frequently than fungal communities. Additionally, both bacterial and fungal communities were found more often in samples from CPAP filters than in those from CPAP tubes.

In all patients with OSA, bacteria were detected in the nasal mucosa swab samples. The nose serves as a habitat for a variety of opportunistic and potentially harmful bacteria. The diversity of these bacteria is affected by factors such as temperature, humidity, and their location within the respiratory tract [16]. Consequently, while bacteria were detected in all nasal swab samples, only some of the CPAP filter and tube samples contained bacteria. Several factors may account for this discrepancy. First, microorganisms may not be present in the CPAP tubes and filters, or they might exist in quantities too small to detect. Second, the lack of a standardized method for bacterial detection in CPAP tubes and filters could have contributed to inconsistent detection rates due to varying sampling techniques. Third, the microbiome detection rate in the CPAP device may have been

influenced by our strict adherence to a cleaning protocol, which includes replacing the tubes every 6 months and the filters every 3 months. Finally, although no fungi were found in any nasal samples, they were present in some CPAP tube and filter samples. These findings suggest that while the risk of bacterial or fungal colonization may not be high, attention should be paid to the potential for bacterial or fungal passage from CPAP device into the patient's respiratory system.

In the present study, samples were collected from CPAP tubes and filters. Analysis regarding both bacteria and fungi revealed that detection frequencies were higher in the filter samples compared to the tube samples. This difference may be due to the design of CPAP devices. The filters of CPAP machines, which exist in direct contact with the external air for therapeutic purposes, are likely to capture airborne microorganisms, which are then detected. Consequently, the abundance of microorganisms may vary across CPAP components depending on their position within the device. Bacterial diversity was observed to increase progressively from the nasal mucosa to the CPAP tubes to the filters. The environmental microbiome has been shown to exhibit greater microbial diversity than the human microbiome [17]. This could have introduced comparatively high bacterial diversity near the filter samples, which are more exposed to the environment. Notably, Pseudomonadota species were more abundant in the filter and tube samples than in the nasal mucosa samples. Pseudomonadota species are found in various environments [18] and are considered the most abundant species in the human gut microbiota [19].

In the present study, we observed a significant increase in the abundance of one ASV in relation to the duration of CPAP tube use. However, we identified no significant time-based differences in bacterial diversity. Bacterial DNA was successfully detected in only seven tube samples. This suggests that either the patients in our study were effective in managing their CPAP tubes, or the tubes had lower bacterial DNA concentrations compared to the nasal mucosa and filter samples. Presumably, bacteria may not adhere to the material of the tubes as readily as to CPAP filters and the nasal mucosa. ASV3346 (*S. saprophyticus*) was the only bacterial ASV that increased in abundance with longer tube use. *Staphylococcus saprophyticus*, the habitats of which are not fully understood, is known to cause urinary tract infections [20]. Additionally, this species has been detected in various food products [9,21], polluted rivers [22], and wastewater treatment plants [23]. While one might assume that bacteria cannot efficiently attach to the walls of CPAP tubes, if the tubes are not regularly replaced by device users, bacteria could still adhere to the surfaces. Therefore, we suggest that CPAP users adhere to the recommended replacement schedule for these parts.

Our analyses of fungal diversity indicated that the abundance of ASVs tended to increase with the duration of filter usage. We could only conduct fungal composition analysis on tube samples; of these, we successfully extracted fungal DNA from just two

samples. Similar to the results of our bacterial analysis, no significant difference was evident in fungal diversity. However, we did detect fungal ASVs that exhibited significantly greater abundance with a prolonged duration of filter use. Among the ASVs was *Ceriporia lacerate*, a fungal species first isolated in 1994 from decaying wood in the Miyazaki Forest of Japan. This species is characterized by its distinctive white, dense, non-sporulating colonies [24]. Clinical reports have associated this fungus with a range of symptoms, from saprobic colonization to fungal pneumonia [25,26]. *Aspergillus* species can be spread through both indoor and outdoor air, with most people breathing in this fungus on a continuous basis. Those with healthy immune systems typically do not develop diseases caused by *Aspergillus* [27]. However, in individuals with compromised immune systems, *Aspergillus* can cause aspergillosis [28]. Furthermore, *A. penicillioides*, a common indoor fungal species found in damp environments and in filters, has been linked to allergic rhinitis [29,30]. The reproduction and activity of dust mites can be influenced by *A. penicillioides*, potentially harming human health [31]. In the present study, *Aspergillus* species were detected in samples collected from CPAP tubes. These findings suggest that prolonged use of CPAP filters and tubes without regular replacement may increase the risk of disease when an individual's immune system is weakened, thus adversely affecting respiratory health.

This study has several limitations. First, the design of the microbiome study necessitates cautious interpretation of the results. Notably, the presence of microorganisms in CPAP devices does not necessarily lead to infection [32]. Host factors should also be considered as potential contributing factors. Nonetheless, it is crucial to expand our understanding of the microbial communities in CPAP devices and their potential effects on human health. Second, the sample size was limited. While we collected multiple samples from CPAP components and the corresponding nasal mucosa, we only analyzed a subset to account for clinical factors that might influence the analysis. Consequently, further research with a larger number of samples is warranted. Third, we did not collect serial samples from participants to assess changes over time with CPAP device usage. In cases of ventilator-associated pneumonia, the abundance patterns of the oropharyngeal microbiome have been found to shift with the duration of mechanical ventilation [33]. The microbiomes of the nasal mucosa or CPAP components could also vary with device usage duration in a given individual. Moreover, our study did not include control samples from new, unused CPAP devices, which limits our ability to determine whether the findings are specific to the usage environment or reflective of microbes commonly present in CPAP equipment. Finally, we did not examine the distribution of respiratory viruses in the samples. The respiratory virome is an integral component of the human microbiome, and its characterization could improve our understanding of the microbiome in patients with OSA who use CPAP devices. Therefore, future research should focus on the respiratory vi-



rome in this patient population.

Our findings suggest that bacteria or fungi may be present in CPAP devices, even with a short period of use and regular cleaning of the components. We could not establish a correlation between the microorganisms found in the nasal mucosa of CPAP users and those detected in CPAP equipment samples. Therefore, additional research is needed to corroborate our results.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

## ACKNOWLEDGMENTS

This study was supported by the “Team Science Award” of Yonsei University College of Medicine (6-2021-0005) and the National Research Foundation of Korea (NRF) grants, funded by the Korean Government (MSIT) (No. RS-2023-00220853 and NRF2021R1A2C2010811) to HJC. This study was also supported (in part) by research grant from Biomedical Research Institute, Chung-Ang University Hospital (2022) to HJM.

## ORCID

Hyun Jin Min <https://orcid.org/0000-0003-3075-1350>

Bo-Yun Choi <https://orcid.org/0000-0003-0173-0016>

Woo Jun Sul <https://orcid.org/0000-0002-7016-1454>

Hyung-Ju Cho <https://orcid.org/0000-0002-2851-3225>

## AUTHOR CONTRIBUTIONS

Conceptualization: HJM, HJC. Methodology: all authors. Data curation: BYC, WJS. Visualization: HJM, BYC. Funding acquisition: HJM, HJC. Writing—original draft: all authors. Writing—review & editing: HJC. All authors read and agreed to the published version of the manuscript.

## SUPPLEMENTARY MATERIALS

Supplementary materials can be found online at <https://doi.org/10.21053/ceo.2024.00167>.

## REFERENCES

1. Cai Y, Juszczak HM, Cope EK, Goldberg AN. The microbiome in ob-

- structive sleep apnea. *Sleep*. 2021 Aug;44(8):zsab061.
2. Senaratna CV, Perret JL, Lodge CJ, Lowe AJ, Campbell BE, Matheson MC, et al. Prevalence of obstructive sleep apnea in the general population: a systematic review. *Sleep Med Rev*. 2017 Aug;34:70-81.
3. Pepperell JC, Ramdassingh-Dow S, Crosthwaite N, Mullins R, Jenkinson C, Stradling JR, et al. Ambulatory blood pressure after therapeutic and subtherapeutic nasal continuous positive airway pressure for obstructive sleep apnoea: a randomised parallel trial. *Lancet*. 2002 Jan;359(9302):204-10.
4. Laks L. Pulmonary arterial pressure in sleep apnea. *Sleep*. 1993 Dec; 16(8 Suppl):S41-3.
5. Ortolano GA, Schaffer J, McAlister MB, Stanchfield I, Hill E, Vandenburgh L, et al. Filters reduce the risk of bacterial transmission from contaminated heated humidifiers used with CPAP for obstructive sleep apnea. *J Clin Sleep Med*. 2007 Dec;3(7):700-5.
6. Ashraf M, Ostrosky-Zeichner L. Ventilator-associated pneumonia: a review. *Hosp Pract (1995)*. 2012 Feb;40(1):93-105.
7. Perkins SD, Woeltje KF, Angenent LT. Endotracheal tube biofilm inoculation of oral flora and subsequent colonization of opportunistic pathogens. *Int J Med Microbiol*. 2010 Nov;300(7):503-11.
8. Sanner BM, Fluerebrock N, Kleiber-Imbeck A, Mueller JB, Zidek W. Effect of continuous positive airway pressure therapy on infectious complications in patients with obstructive sleep apnea syndrome. *Respiration*. 2001;68(5):483-7.
9. Benhamou D, Cuvelier A, Muir JF. Prevention of infections transmitted by CPAP and noninvasive ventilation. *Rev Pneumol Clin*. 2001 Apr;57(2):73-8.
10. Kim HJ, Kim JH, Han SA, Kim W. Compositional alterations of the nasal microbiome and *Staphylococcus aureus*-characterized dysbiosis in the nasal mucosa of patients with allergic rhinitis. *Clin Exp Otorhinolaryngol*. 2022 Nov;15(4):335-45.
11. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. 2019 Aug; 37(8):852-7.
12. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J*. 2011 May;17(1):10-2.
13. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016 Jul;13(7):581-3.
14. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, et al. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J*. 2012 Mar;6(3):610-8.
15. Mercieca L, Pullicino R, Camilleri K, Abela R, Mangion SA, Cassar J, et al. Continuous positive airway pressure: is it a route for infection in those with obstructive sleep apnoea? *Sleep Sci*. 2017;10(1):28-34.
16. Rawls M, Ellis AK. The microbiome of the nose. *Ann Allergy Asthma Immunol*. 2019 Jan;122(1):17-24.
17. Panthee B, Gyawali S, Panthee P, Techato K. Environmental and human microbiome for health. *Life (Basel)*. 2022 Mar;12(3):456.
18. Bryant DA. Phototrophy and phototrophs. In: *Encyclopedia of microbiology*. 4th ed. Elsevier Science; 2019. p. 527-37.
19. Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A. Proteobacteria: a common factor in human diseases. *Biomed Res Int*. 2017; 2017:9351507.
20. Eriksson A, Giske CG, Ternhag A. The relative importance of *Staphylococcus saprophyticus* as a urinary tract pathogen: distribution of bacteria among urinary samples analysed during 1 year at a major Swedish laboratory. *APMIS*. 2013 Jan;121(1):72-8.
21. Soares JC, Marques MR, Tavarua FK, Pereira JO, Malcata FX, Pintado MM. Biodiversity and characterization of *Staphylococcus* species isolated from a small manufacturing dairy plant in Portugal. *Int J Food Microbiol*. 2011 Mar;146(2):123-9.

22. Basso AP, Martins PD, Nachtigall G, Van Der Sand S, De Moura TM, Frazzon AP. Antibiotic resistance and enterotoxin genes in *Staphylococcus* sp. isolates from polluted water in Southern Brazil. *An Acad Bras Cienc*. 2014 Dec;86(4):1813-20.
23. Faria C, Vaz-Moreira I, Serapicos E, Nunes OC, Manaia CM. Antibiotic resistance in coagulase negative staphylococci isolated from wastewater and drinking water. *Sci Total Environ*. 2009 Jun;407(12):3876-82.
24. Suhara H, Maekawa N, Kaneko S, Hattori T, Sakai K, Kondo R. A new species, *Ceriporia lacerata*, isolated from white-rotted wood. *Mycotaxon*. 2003 Apr;86:335-47.
25. Chowdhary A, Agarwal K, Kathuria S, Singh PK, Roy P, Gaur SN, et al. Clinical significance of filamentous basidiomycetes illustrated by isolates of the novel opportunist *Ceriporia lacerata* from the human respiratory tract. *J Clin Microbiol*. 2013 Feb;51(2):585-90.
26. Chowdhary A, Kathuria S, Agarwal K, Meis JF. Recognizing filamentous basidiomycetes as agents of human disease: a review. *Med Mycol*. 2014 Nov;52(8):782-97.
27. Hedayati MT, Mayahi S, Denning DW. A study on *Aspergillus* species in houses of asthmatic patients from Sari City, Iran and a brief review of the health effects of exposure to indoor *Aspergillus*. *Environ Monit Assess*. 2010 Sep;168(1-4):481-7.
28. Barnes PD, Marr KA. Aspergillosis: spectrum of disease, diagnosis, and treatment. *Infect Dis Clin North Am*. 2006 Sep;20(3):545-61.
29. Gonzalez De Leon J, Gonzalez Mendez R, Cadilla CL, Rivera-Mariani FE, Bolanos-Rosero B. Identification of immunoglobulin e-binding proteins of the xerophilic fungus *Aspergillus penicillioides* crude mycelial mat extract and serological reactivity assessment in subjects with different allergen reactivity profiles. *Int Arch Allergy Immunol*. 2018;175(3):147-59.
30. Zukiewicz-Sobczak WA. The role of fungi in allergic diseases. *Poy Dermatol Alergol*. 2013 Feb;30(1):42-5.
31. Hay DB, Hart BJ, Douglas AE. Effects of the fungus *Aspergillus penicillioides* on the house dust mite *Dermatophagoides pteronyssinus*: an experimental re-evaluation. *Med Vet Entomol*. 1993 Jul;7(3):271-4.
32. Lambotte O, Timsit JF, Garrouste-Orgeas M, Misset B, Benali A, Carlet J. The significance of distal bronchial samples with commensals in ventilator-associated pneumonia: colonizer or pathogen? *Chest*. 2002 Oct;122(4):1389-99.
33. Sommerstein R, Merz TM, Berger S, Kraemer JG, Marschall J, Hilty M. Patterns in the longitudinal oropharyngeal microbiome evolution related to ventilator-associated pneumonia. *Antimicrob Resist Infect Control*. 2019 May;8:81.