

# Case Report of Microbial Contamination on N95 Masks Used by Health Care Workers

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## 1. Abstract

The prevalence of pandemics, such as COVID-19 transmitted through the respiratory system, is a serious global public health concern. Microbial contamination of face masks used by healthcare workers may vary depending on the degree of exposure to bioaerosol in various healthcare environments; however, data on this topic are limited. Therefore, in this study, we analyzed the microbial contamination of N95 masks used in hospital offices, wards, and outpatient settings. Total indoor airborne bacteria were also measured in healthcare environments. The samples isolated from N95 masks worn for 2, 4, or 6 h were incubated at a temperature of 35–37 °C or 25–28 °C for 24 h or 3–7 days, and colony-forming units were counted in chocolate agar, tryptic soy agar, and Sabouraud dextrose agar plates. Finally, microbial species were identified using the morphological analysis with Gram staining. The results showed that the three types of environments did not deviate from maintenance of standard indoor air quality. The numbers of bacteria in the masks worn in each environment differed, while the degree of contamination increased with mask-wearing time ( $p < 0.05$ ). No differences were observed between the microbial species identified in the healthcare environment and mask contamination. However, care must be taken to avoid recontamination of masks due to improper use and exposed biological hazards in healthcare environments. Our results provide scientific evidence necessary for safe mask-wearing times. Further studies are warranted to establish guidelines for the safe use of face masks during epidemics of respiratory diseases.

## 2. Keywords:

Healthcare workers; N95 masks; microbial contamination; biological hazard

## 3. Introduction

Respiratory infectious diseases have constantly been emerging, while their related pandemics have adversely affected human health. Recently, due to the emergence of coronavirus disease-19 (COVID-19), mask wearing has become commonplace, however, due to the lack of specific guidelines for the use of masks, it is common for individuals to reuse them or use them for a long time. Several studies have reported on the reusability of masks as it can cause economic and environmental problems [1, 2, 3, 4]. However, concerns have been expressed regarding respiratory pathogens contaminating the surface of masks, which can cause infection through hands or skin. In particular, studies examining bacterial and viral contamination of masks worn in healthcare settings have shown that standards for mask-wearing and continuous mask use are needed [5, 6]. Personal protective equipment, such as respiratory protective masks, gloves, goggles, and face shields, can be used to prevent contact with pathogens in droplets transmitted by breathing, coughing, or aerosols produced during treatment or splashing of patient blood and body fluids [7]. The surface of a used mask can be contaminated with airborne respiratory pathogens [5]. Viruses such as influenza can be easily transmitted via long duration of mask-wearing and frequent contact with patients. The influenza virus can survive 24–48 h on hard surfaces, 8–12 h on clothing, and 5 min on hands. Indeed, previous studies [5, 8] have confirmed contamination with respiratory syncytial virus (RSV) RNA on cloths. Therefore, unclear guidance on the use of masks or continuous use of contaminated masks can lead to the spread of respiratory infections. Bioaerosols can be attached to the surface of a mask for a considerable period while high humidity and temperature are maintained due to the user's breathing, which increases the possibility that the contaminated particles contain bacteria and viruses.

Moreover, the contamination can interfere with the filtration mechanism of masks and accelerate their penetration, which leads to the potential for microbial species to invade the human body [9]. On the outer surface of the mask worn by dentists, streptococci, staphylococci, Gram-negative bacteria (GNB), and some viruses have been identified [5, 8]. Therefore, when using a mask in a healthcare environment where nosocomial pathogens exist, a threshold for mask-wearing time is required according to the environment and time. In addition, to develop a comprehensive policy on the use of masks, appropriate protocols for the duration and contact frequency should be implemented, reusing masks should be avoided, and the duration should be limited to  $< 6$  h. A previous study

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[10] has reported that skin problems can occur due to the use of masks for a long time in a medical environment, and the microbial contamination of masks in general environments is significantly different from the increase in mask-wearing time [11]. Multiple uses of contaminated masks in healthcare settings can affect not only users but also patients and potentially their families. Due to the recent COVID-19 pandemic that has increased the general population's interest in airborne microbial exposure, it is necessary to measure microbial contamination of masks used in healthcare settings where there is a high risk of exposure to pathogenic microbial species. Following the multi-facility indoor air quality standards [12], medical institutions must maintain  $\leq 800$  colony-forming units (CFU)/m<sup>3</sup> of total airborne bacteria and  $\leq 500$  CFU/m<sup>3</sup> of fungi. Of the approximately 20,000 microbial species transmitted through air, 200 types of fungi have been identified indoors, of which  $> 30$  of them have harmful effects on health. In this study, microbial species that may exist in healthcare environments were collected with a bio air sampler, and the degree of contamination of masks worn in healthcare settings was examined. We analyzed the differences in microbial contamination according to healthcare environments, determined whether there were differences in microbial contamination with mask-wearing duration, and compared the number of microbial species cultured on the inside and outside of masks. Finally, we determined whether the microbial species collected in the healthcare environment were similar to those cultured in the contaminated mask. Our findings suggest that masks worn for long periods in healthcare settings may cause health problems due to microbial contamination. Therefore, in Korea, there is still a tendency to reuse disposable masks for multiple days, so this study aims to raise awareness. The results of this study provide scientific evidence for safe mask-wearing times to protect public health from respiratory infections, and will help to facilitate the establishments of guidelines for proper mask use.

## 4. Materials and Methods

### Participants

Forty-five healthcare workers from medical institutions participated in this study. The participants were randomly selected among those who agreed for voluntary participation from the hospital office, outpatient department, and ward. The participant data were analyzed using the G\*Power 3.1.9.4 program (Dusseldorf, Germany) for statistical power. Our study was approved by the Institutional Review Board (approval number SHIRB-202103-HR-122-01) and participant information was anonymized. Informed consent was obtained from the participants.

### 5. Materials

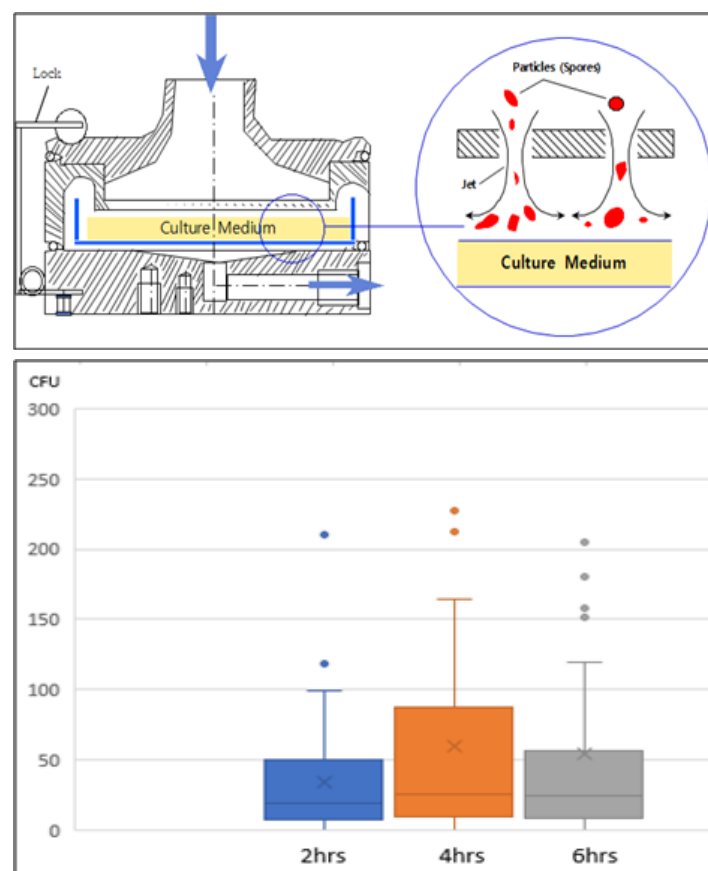
The type of mask used for the study was a National Institute for Occupational Safety and Health (NIOSH)-certified N95 mask manufactured in the Republic of Korea. The mask was a two-fold model with a width of 100 mm and a length of 155 mm. This model has nose support and a headband strap fastened on the wearer's head and behind the neck. This mask was produced in only one size regardless of the wearer's face size and age, and the outer material of mask was electrostatic non-woven polypropylene

fiber. The material of filter is a three-layer structure of melt-blown non-woven fabric. The mask-wearing time was set at 2, 4, and 6 h, as previously described [13]. The used masks were placed in sterile bags to prevent additional contamination, collected, and immediately inoculated in the laboratory.

## 6. Air sampling

Capturing of airborne microbial species was carried out in the office, outpatient, and ward settings in hospitals. Concentration measurements of airborne bacteria and fungi were conducted following the indoor air quality process test standards of the MOE of the Republic of Korea [14]. N6 Single-Stage Viable Andersen Cascade Impactor (model 10-800, Thermo Fisher Scientific, Waltham, MA, USA) was used to collect airborne microbes (Fig 1). The sampling was conducted at a flow rate of 16 L per min. The sampler was installed at a height of 120–150 cm on the sampling sites in office, outpatient, and ward settings. The tryptic soy agar (TSA) plate used as a medium for total airborne bacteria was placed in the bio air sampler and airborne bacteria were collected through 300–400 holes. Samples were continuously collected three times at 20-min intervals according to the standard test method. When the collection was complete, the Petri dish lid was closed, sealed with parafilm, and transported to the laboratory. All efforts have been made to minimize additional contamination.

**Fig 1:** Bio air sampling using an air sampler



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## 6.1. Assessment for microbial contamination

The microbial contamination experiment was conducted in a biological safety cabinet (1300 Series A2 Biological Safety Cabinet, Thermo Fisher Scientific). As soon as the participants' masks were collected, the medium was inoculated using the contact method with outer and inner surfaces of the masks. Three media were used to cultivate contaminated microorganisms: TSA, which is used to cultivate various bacterial strains; chocolate agar, which is used to cultivate respiratory bacteria; Sabouraud dextrose agar (SDA), which is used to cultivate fungi. TSA and chocolate agar plates were incubated at 35–37 °C in a 24-h incubator (Forced Convection Incubator, model C-INDF, Changshin Science, Seoul, Republic of Korea) and SDA plates were incubated at 25–28 °C for more than 24 h or 3–7 days. Microbial contamination isolated from TSA plates collected by air sampling and from participants' masks was measured by counting colonies after the culture was completed. Microbiology experts, blinded to the experimental groups, identified the strains through Gram staining and microscopy.

## 6.2 Statistical analysis

The degree of microbial contamination tested in the three environments in this study is presented as the mean and standard deviation (SD) of CFUs and was evaluated for its statistical significance. The mean difference in the number of cultured CFU between the outer and inner sides of the masks was compared using t-test, while the differences in environments and with time were tested using analysis of variance. Statistical significance was set at  $p < 0.05$  for all analysis results. Data analysis was performed using IBM SPSS Statistics for Windows, version 20 (IBM, Armonk, NY, USA).

## 7. Results

### 7.1 Participant characteristics

Among the total number of participants ( $n = 45$ ), 15.6% were male ( $n = 7$ ), and 84.4% were female ( $n = 38$ ). The distribution by age was 33.3% ( $n = 15$ ) in their 20s, 24.5% ( $n = 11$ ) in their 30s, 33.3% ( $n = 15$ ) in their 40s, and 8.9% ( $n = 4$ ) in their 50s; 33.3% ( $n = 15$ ) of the microbial contamination from masks worn in hospital office environments, 44.5% ( $n = 20$ ) in outpatient settings such as emergency rooms, and 22.2% ( $n = 10$ ) in ward environments were analyzed. Furthermore, 53.3% ( $n = 24$ ) of participants reported being exposed to blood and saliva at work and 46.7% ( $n = 21$ ) did not report such instances.

### 7.2 Concentrations of airborne microorganisms

Airborne microbial contamination in the healthcare environments was calculated using the average number of colonies measured three times according to the indoor air quality standard set by the MOE. Microbial concentration indoors  $C$  was calculated as the colony number divided by the adjusted amount of air.  $C = \text{CFU}/V_{(25\text{ }^{\circ}\text{C}, 1\text{ atm})} \times (10)^{\wedge}3$

Where,  $C$  : airborne microbial concentration indoors (CFU/m<sup>3</sup>), CFU : colony-forming units, and  $V$  (25°C, 1 atm) : adjusted air volume (l) According to the results of temperature and relative humidity measurements, the maximum temperature was 25.5 °C and the minimum temperature was 24.5 °C whereas the maximum relative humidity was

44.8% and the minimum relative humidity was 28.2%. The airborne microbes in the three types of healthcare environments are outlined in Table 1. In the environment of hospital office, calibrated CFU was 7 and collection air volume  $V$  was 189.3 m<sup>3</sup>; therefore, airborne microbial concentration indoors was 36 CFU/m<sup>3</sup>. Coagulase-negative staphylococci (CNS) and *Candida* spp. was isolated from the environment of hospital office. In the outpatient environment, CFU was 4 and  $V$  was 190.1 m<sup>3</sup>; therefore,  $C$  was 0.021 CFU/m<sup>3</sup>. Therefore, airborne microbial concentration in the outpatient environment was 21 CFU/m<sup>3</sup>. Gram-positive bacteria (GPB), CNS, *Bacillus subtilis*, and yeast were isolated from the cultured colonies. In the ward environment, CFU was 0,  $V$  was 189.5 m<sup>3</sup>; therefore,  $C$  was 0 CFU/m<sup>3</sup>, and no colonies could be isolated. Airborne microbial concentrations measured in the three environments of the hospitals were well below the limit of microbial contamination set by the MOE (bacteria of  $\leq 800$  CFU/m<sup>3</sup> and fungi of  $\leq 500$  CFU/m<sup>3</sup>).

**Table 1:** Airborne microbes in hospital environments.

Environment	Calibrated CFU	V	CFU/m <sup>3</sup>	Microbes
Hospital office	7	189.3	36	CNS, <i>Candida</i> spp.
Outpatient	4	190.1	21	GPB, CNS, <i>B. subtilis</i> , yeasts
Ward	0	189.5	< detection limit	-

**CNS:** coagulase-negative staphylococci; **GPB:** Gram-positive bacteria; **CFU:** colony-forming units(#); **V:** adjusted air volume(m<sup>3</sup>)

### 7.3 Comparison of microbial contamination

The microbial contamination of masks worn in the office, outpatient, and ward environments is shown in Table 2. Among the masks worn by participants working in office environments, such as infection control departments, emergency room and outpatient environments, and ward environments, contamination was the highest in the office environments with colony count mean (SD) of 123.8 (146.0) CFU/mask, followed by the outpatient environment at 50.4 (65.2) CFU/mask, and ward environment at 41.8 (66.8) CFU/mask ( $p < 0.001$ ).

**Table 2:** Differences in microbial contamination according to the healthcare environment.

Environment	Na	mean (SD) <sup>b</sup>	95% Confidence interval		p-value
			Lower	Upper	
Hospital office	90	123.8 (146.0)	93.2	154.4	0.001c
Outpatient	120	50.4 (63.2)	38.9	61.8	
Ward	60	41.8 (66.8)	24.6	59	

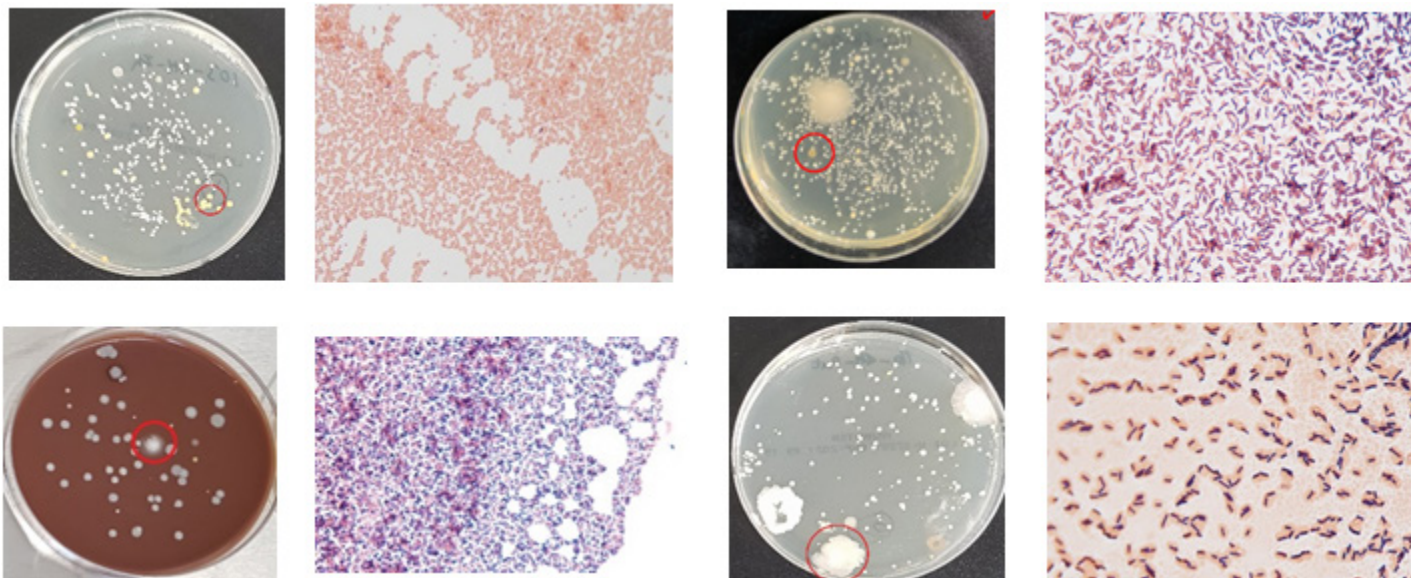


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$aN(270) = 45 \text{ participants} \times 3 \text{ times} \times 2 \text{ media bCFU/mask}$ ; SD: standard deviation cCalculated using Welch's post hoc test Analysis of the degree of mask contamination with mask-wearing time showed that wearing the mask for 4 or 6 h significantly increased the number of bacteria compared with that for 2 h (Fig 2). This indicates that the degree of microbial

contamination of the masks increases as the duration of mask-wearing increases ( $p < 0.05$ ).

**Fig 2:** Degree of contamination of the outside of masks with time: Levene's test ( $p = 0.032$ ).



CNS, GPB, Corynebacterium, B. anthracis, S. aureus, Lactobacillus, etc.

Microbes on the outside and inside of masks used by participants were cultured on the test medium to examine the degree of microbial contamination. In Table 3, the contamination of outer side of the mask worn in the outpatient and the ward environments was lower than the inner side of mask, while the difference between the outer and the inner sides of mask was significant ( $p < 0.05$ ). The contamination level was higher in the office environment than that in the outpatient and the ward environments. In particular, the contamination of the outer side of mask

was 3–4 times higher than that of other two environments. However, in the SDA plate where fungi culture could be identified, there was no difference among the environments in the contamination on the outside and the inside of masks ( $p > 0.05$ ).

**Table 3:** Differences in microbial contamination of the inside and the outside of used masks.

Environment	Na	TSA		Chocolate agar		SDA	
		mean(SD) <sup>b</sup>		mean(SD) <sup>b</sup>		mean(SD) <sup>b</sup>	
		Inside	Outside	Inside	Outside	Inside	Outside
Hospital office	270	147.6(168.9)	126.1(156.3)	162.46(160.7)	121.5(136.7)	1.5(2.2)	1.4(2.4)
		0.532		0.196		0.786	
Outpatient	360	110.6(123.7)	43.8(52.8)	101.8(118.9)	56.9(71.9)	0.7(1.3)	1.7(5.6)
		0.000c		0.014c		0.181	
Ward	180	125(147.7)	35.9(47.0)	128.7(133.7)	47.7(82.3)	1.0(1.4)	1.5(3.8)
		0.000c		0.001c		0.173	

TSA: tryptic soy agar SDA: Sabouraud dextrose agar

$aN(810) = 45 \text{ participants} \times 3 \text{ times} \times 3 \text{ media} \times 2 \text{ surfaces}$

bCFU/ mask; SD: standard deviation cSignificance calculated using t-test ( $p < 0.05$ )

Table 4 lists the result of identification of airborne microorganisms cultured from the healthcare environments and used masks. Microorganisms collected by the air sampler included bacteria such as CNS, GPB, B. subtilis and fungi such as Candida spp., among others. The microorganisms

isolated from the contaminated masks were CNS, GPB, GNB, B. subtilis, Corynebacterium, Staphylococcus aureus, Streptococcus, Enterococcus, Micrococcus spp., Bacillus spp., Lactobacillus, B. anthracis, Penicillium spp., and Candida albicans. Most were similar to those collected by air sampling. Bacteria is a normal flora that resides in the oral cavity of most people and was measured inside and outside the mask. However, masks contaminated with Penicillium spp., which was not found during collection with the air sampler, were also identified. These fungi were found inside as well as outside the masks.

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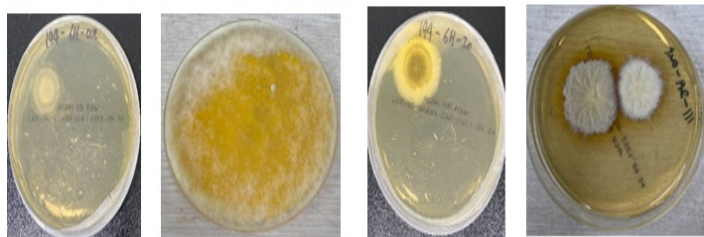
Various types of fungi and bacteria identified as contaminants, as presented in Fig 3, including *C. albicans*, an opportunistic pathogenic yeast, and *Corynebacterium*, found in normal human intestinal flora. These contaminants have low virulence, yet can cause infection.

**Table 4:** Types of contaminated microbial species isolated from healthcare environments

	Bacteria	Fungi
Air sampling	CNS, GPB, <i>B. subtilis</i>	Yeast including <i>Candida</i> spp.
Culture medium	CNS, GPB, GNB, <i>Bacillus subtilis</i> , <i>Corynebacterium</i> ,	<i>Penicillium</i> spp.
	<i>Staphylococcus aureus</i> , <i>Enterococcus</i> , <i>Micrococcus</i> spp., <i>Bacillus</i> spp., <i>Lactobacillus</i> , <i>B. anthracis</i>	Yeast including <i>Candida albicans</i>

CNS: coagulase-negative *Staphylococci*; GPB: Gram-positive bacilli; GNB: Gram-negative bacilli Agar plate and Gram stain

**Fig 3:** Types of microbial species cultured



*Penicillium* spp. Yeast spp. *Candida albicans*, etc.

CNS, GPB, *Corynebacterium*, *B. anthracis*, *S. aureus*, *Lactobacillus*, etc. *Penicillium* spp. Yeast spp. *Candida albicans*, etc.

## 8. Discussion

In the early period of the COVID-19 pandemic, various studies have attempted to determine whether masks could be disinfected and reused [13, 16], while other studies have been conducted on bioburdens that contaminate mask surfaces. In a previous study [17], the microbial cleanliness of masks was hypothesized to be associated with surgical site infections. The authors insisted that the CFU measured while speaking and wearing masks was significantly higher than that while not speaking. This study was conducted to develop guidelines for mask-wearing that can protect against biohazards such as bacteria and viruses. The topic of mask contamination in hospital settings has often been addressed in previous studies. However, studies on microbial contamination on masks used in general environments are limited. Studies of airborne microorganisms and microorganism exposure levels have been conducted for various industries [18, 19], such as occupational groups in agriculture [20], general urban air

environments [19], and airborne bacteria in hospitals [21]. Recently, the microbe studies on public health have been conducted in multi-purpose facilities and schools [11, 22]. Seo et al. [11] have reported that 86.7% of workers in offices, multi-purpose facilities, and schools replaced their mask with a new one once a day, while 84.5% of workers wore masks for more than 6 h and 55.6% for more than 8 or 10 h. Therefore, microbial contamination occurring while wearing a mask for more than 8 or 10 h at work could be significant. According to a previous study [17], CFUs increased on the mask's surface when the mask-wearing time was prolonged, especially when used for more than 4–6 h, similar to previous findings in healthcare and general environments, while the number of bacteria increased with mask-wearing time. This is similar to the results of this study, which showed that masks used for 4 or 6 h are more likely to be contaminated than those used for 2 h. Notably, microorganisms exist everywhere including healthcare and hospital settings, and contaminated airborne microbes can enter the respiratory system if healthcare workers come into contact with hands after wearing a mask for long time [6]. *Staphylococcus*, CNS,  $\alpha$ -hemolytic *Streptococcus*, and *Moraxella* cultivated on blood agar and placed 30 cm near the mouth without a mask, followed by mask use for 5–15 min showed that masks used for 15 min showed significantly higher levels of bacterial contamination than those worn for 5 min [23]. These studies demonstrate that used masks can be contaminated by various microorganisms present in everyday life.

However, the study results on bacterial contamination of masks used in healthcare settings are controversial and inconsistent. Although a study reported that bacterial contamination increased rapidly on masks worn for more than 2 h [17], bioaerosols can easily cause contamination since the filtration efficiency of masks decreases after 20–30 min of exposure to moisture [24]. While wearing masks, breathing inside masks can create a humid environment, which accelerates the spread of microorganisms. Since most microorganisms show the highest growth rate after approximately 5–6 h in a humid environment and at temperatures similar to human body temperature [25, 26], microbial contamination may increase or may be delayed depending on the humidity and temperature of the mask-wearing environment. In our study, microbial contamination significantly increased on masks used for 4 h but slightly higher in masks used for 6 h. There may be differences in the microbial growth rate since it was a controlled hospital environment with a humidity of 28.2–44.8% and a temperature of 24.5–25.5 °C. A previous study [27] has reported that the concentration of bacteria and fungi in the air according to the working conditions of aircraft cleaners was linked to weather conditions and increased humidity. However, it is necessary to determine whether the physical environment affects microbial contamination in future studies. In this study, airborne microorganisms from the office environment showed higher levels of contamination than those in ward and outpatient environments. Future studies should determine whether physical variables such as ventilation, desk placement, and partitions in the work environment influence microbial colonization. However, conditions of temperature and humidity were kept constant throughout the study. A previous study [6] measured the number of bacteria on the outside and the inside of masks in a hospital environment and found that the number of bacteria on the

outside of masks (166 CFU/mask) was higher than on the inside (47 CFU/mask), contrary to the results of this study. The main bacteria identified by the authors of that study were *S. aureus*, *Penicillium* spp., and *Aspergillus* spp, while in this study, we confirmed more diverse strains such as CNS, GPB, GNB, *B. subtilis*, *Corynebacterium*, *Streptococcus*, *Enterococcus*, *Micrococcus* spp., *Bacillus* spp., *Lactobacillus*, *B. anthracis* and yeasts such as *C. albicans*. However, Liu et al. [17] have reported that the number of bacteria is significantly higher on the inside than on the outside of the mask, which are consistent with the results of this study. The authors examined whether speaking while wearing a mask increases the bioburden and showed a significant increase in bacterial contamination while speaking compared with not speaking [17]. This observation is attributed to normal bacteria in the respiratory tract contaminating the inner surface of masks while speaking, suggesting that masks used for long hours may increase microbial contamination. These results are consistent with a previous study [28], which recommended that surgeons avoid wearing masks for long periods and talk as little as possible during surgery. Surgical site infection can occur in immunocompromised individuals due to CNS and  $\alpha$ -hemolytic *Streptococcus* released during speech; therefore, wearing a mask should be a priority when dealing with patients.

The bacteria and fungi identified in this study were similar to those of previous studies[28], although the use of surgical masks differed from ours. Studies have confirmed the presence of viruses on the mask surfaces, although these studies were only preliminary clinical studies. Chughtai et al. [5] confirmed high mask contamination with RSV, influenza virus, adenovirus, among others and suggested that the reuse of masks in hospital environments should be prohibited, and that the maximum continuous duration of use should be set as a protocol. The results of this study provide evidence for the safe use of masks as it found a significant increase when masks were used for 4 or 6 h compared with 2 h of mask-wearing. This study has some limitations. This study was conducted on a small scale, targeting only two healthcare institutions. In addition, since the study was conducted in an environment where temperature and humidity were constantly maintained, it was possible to influence the conditions for microorganism growth. Although these results cannot be regarded as a quantitative analysis of the degree of microbial contamination generalized to hospital environments, our findings were similar to previous studies. Though there are other several bioaerosol agents and airborne bacteria and fungi, this study evaluated exposure to only airborne bacteria and fungi. Thus, additional studies on various characteristics of bioaerosols remain warranted, such as the identification of airborne microorganisms and their potentially infectivity and toxicity in healthcare settings.

In addition, since indoor airborne microorganisms were not measured separately for each participant's environment, they could not be directly linked to the contamination of the masks used; therefore, we observed no correlation between airborne bacteria and healthcare environment. However, given that exposure to mold during work causes allergy-associated diseases [19], the safety of airborne microorganism exposure in healthcare settings should not be overlooked. Although this result did not deviate from the indoor air quality maintenance standard of multi-

use facilities such as medical institutions (revised on 11 November 2022), which states the total number of airborne bacteria must  $\leq 800$  CFU/m<sup>3</sup> and fungi  $\leq 500$  CFU/m<sup>3</sup>, if the management of biohazard exposure at medical institutions is neglected, it can be an obstacle to the health of medical personnel. Among indoor air pollutants, biological agents include irritants such as toxins, infectious bacteria, and allergens; therefore, the management of medical institutions is important in this respect [29]. Therefore, scientific data are needed to determine the appropriate duration of mask use with regards to microbial contamination. Further research on various organizations and diverse settings is required. Reasonable mask use standards will reduce adverse effects on healthcare workers, patients, and their caregivers, thus contributing to public health. The results of this study demonstrate that masks used for a long time in healthcare settings can cause health problems due to microbial contamination and will serve as a basis for establishing guidelines for safe use of masks.

## 9. Acknowledgments

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## Ethical approval

The study was approved by the Institutional Review Board of Shinhan University (approval ID: SHIRB 202103-HR-122-01).

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