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# Factors influencing bacterial viability on face masks and bactericidal effect of disinfection methods

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**Face masks are contaminated with bacteria during daily use and storage. Several studies have already been conducted on face mask decontamination and bacterial survival during the pandemic. However, there are limited reports on methods of decontamination that can be implemented in daily life. In this study, we inoculated commercially available nonwoven face masks with *Staphylococcus aureus* and/or *Staphylococcus epidermidis* which are the main indigenous bacteria of human skin to obtain knowledge on the prevention of bacterial infection when wearing face masks in daily life, and investigated factors affecting bacterial viability on face masks and the bactericidal effect of different disinfection methods. Commercially available face masks were inoculated with two bacterial species and the effects of temperature, humidity, washing, contamination (sweat, sebum, saliva, protein, and cosmetics), and initial bacterial count and density on the viability of adherent bacteria were examined. After each bacterium was collected from the face mask, colonies were measured by the agar plate dilution method and the number of viable colonies. Various experiments were also performed to determine how to disinfect bacteria on face masks. Bacterial survival of face masks increased in the presence of artificial saliva and 1% peptone. By sealing the face mask after 70% ethanol spraying, the number of bacteria was reduced by 6 log order of magnitude and no colonies were detected. However, the presence of contaminants on the face mask weakened the bactericidal effect. Furthermore, direct exposure of the face masks to sunlight was suggested as a useful method for bacterial disinfection. Our findings revealed factors affecting bacterial viability on face masks, providing data that can be applied for the effective household disinfection of face masks.**

**Keywords** Disinfection, Microbial viability, Peptones, Staphylococcal infections, Ultraviolet rays

Coronavirus disease 2019 became endemic worldwide in 2020. Subsequently, the use of face masks was strongly recommended[1,2] and has been established to prevent infection, leading to the routine use of disposable nonwoven face masks[3,4]. Although face masks reduce the risk of viral infection[5], they can be a source of bacterial contamination, increasing the risk of transfer to food and other products.

Staphylococci, primarily *Staphylococcus epidermidis* (*S. epidermidis*) and *Staphylococcus aureus* (*S. aureus*), are present on human facial skin[6]. *S. aureus* is the most virulent *Staphylococcus* species, causing pyogenic skin diseases, inflammatory diseases (e.g., pneumonia and meningitis), and toxic shock syndrome in humans[7,8]. *S. aureus* also produces heat-resistant staphylococcal enterotoxins when growing in food, causing food poisoning[9–11]. These indigenous skin bacteria adhere to face masks and were reported to alter the diversity of the nasal and buccal microbiota[12]. Face masks can be kept hygienic using ultraviolet (UV) irradiation, autoclaving, and treatment with ethylene oxide or hydrogen peroxide[13]. However, these methods are difficult to implement in households. Although face mask condition affects bacterial viability, there are few data-based reports on the hygienic use of face masks in households.

In addition, the mass use of disposable face masks has raised new concerns about hygiene and safety as well as the environment[14]. It has been reported that if every British citizen used one disposable face mask every day

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for one year, a total of 124,000 tons of waste would be generated, of which 66,000 tons would be contaminated plastic waste that cannot be recycled[15]. Keeping face masks hygienic in a manner that can be implemented at home reduces the amount that is disposed of and reduces the impact on the environment. However, there is still insufficient data to determine what conditions of face mask-wearing use increase the frequency of bacterial contamination of face masks in the general household and what methods are most effective in controlling bacterial contamination. Identifying factors involved in bacterial contamination of face masks in the general household and ways to keep face masks hygienic is important to reduce the risk of bacterial contamination of face masks.

Thus, this study aimed to collect data on the hygienic use of face masks that could be effectively applied in households in order to maintain masks in a hygienic condition for longer periods of time. We inoculated commercially available nonwoven face masks with *S. aureus* and/or *S. epidermidis* and then investigated the effects of temperature, humidity, washing, contamination (by sweat, sebum, saliva, protein, and cosmetics), and the initial bacteria count and density on the viability of bacteria adhering to masks. We also investigated the efficacy of household methods of keeping face masks hygienic (e.g., heat treatment, spraying with 70% ethanol, and sunlight exposure inside and outside a car).

## Methods

### Face masks used in this study

A commercially available triple-layered nonwoven face mask (Artec Co., Ltd., Osaka, Japan)with a particle filtration efficiency (PFE) > 98% (as determined by a domestic third-party testing organization) was used in our study. The masks were cut into pieces (approximately 4 × 4 cm) for use in our experiments (Supplementary Fig. S1A).

### Inoculum preparation and face mask inoculation

*S. aureus* C-29 (human hand isolate[16]) and *S. epidermidis* ATCC 14990 were cultured in brain heart infusion (BHI) broth (Kanto Chemical Co., Inc., Tokyo, Japan) overnight at 37 °C with shaking, and inoculated 30 μL of each species into 3 mL of BHI broth (Kanto Chemical Co., Inc., Tokyo, Japan) for incubation at 37 °C for 18 h with shaking (main culture). Then 0.5 mL of the main culture of each species was transferred to microtubes and centrifuged (4 °C, 11,600 ×*g*, 5 min). Following supernatant removal, 1.0 mL of sterile PBS was added, mixed, and centrifuged as before. Finally, the supernatant was removed, and 0.5 mL of sterile PBS was added to the pellet. This suspension was used as the inoculum.

The face mask pieces were placed in sterile Petri dishes. Then 2 μL of the prepared inoculum was spotted at five different locations on the inner layer (side in contact with the skin) or the outer layer (side in contact with the environment), and the Petri dishes were closed with lids (Supplementary Fig. S1B). The number of bacteria on the inoculated face mask pieces was determined following exposure to different conditions. The outer layer of the face mask was only used to evaluate the effect of temperature on the viability of the inoculated bacteria. As controls, 10 μL of bacterial suspension was added to PBS in microtubes, which were sealed in separate zippered plastic bags and treated under the same conditions as the inoculated face mask pieces.

### Evaluation of temperature and humidity on the viability of bacteria on face masks

To examine the effects of temperature on the viability of *S. aureus* or *S. epidermidis*, inoculated face mask pieces were placed in Petri dishes. The dishes were closed with a lid and sealed in zippered plastic bags. As controls, microtubes containing the inoculum solution were sealed in separate zippered plastic bags. The zippered plastic bags were incubated at 37 °C, 45 °C, or 55 °C (60% humidity) for 7 h. To examine the effect of humidity on bacterial viability, face mask pieces inoculated with *S. aureus* were placed in Petri dishes, closed with a lid, and placed in incubators at 37 °C with 60% (vats filled with water in incubator) or 95% (in 5% $CO_2$ incubator) humidity for 7 and 24 h. As a control, the inoculum solution was placed directly in a Petri dish and closed with a lid.

### Evaluation of the effects of a mixed culture of *S. aureus* and *S. epidermidis* on the viability of bacteria on face masks.

Equal amounts of *S. aureus* and *S. epidermidis* suspensions were mixed and used to inoculate face mask pieces in Petri dishes. The dishes were closed with a lid, stored in zippered plastic bags, and incubated at 37 °C for 24 h (Day 1) or 96 h (Day 4). Unincubated samples were designated as Day 0.

### Evaluation of bacterial viability on face masks after washing

The face masks were placed in a commercially available laundry net for face masks (Daiso, Hiroshima, Japan). Approximately 25 g (25 mL, the amount specified in the instruction manual) of laundry detergent (TOP Clear Liquid; Lion Corporation, Tokyo, Japan) was added to 30 L of water, and the face masks were washed in the dry cycle of a washing machine. Then the face masks were removed from the laundry net, air-dried at room temperature, and cut into pieces (approximately 4 × 4 cm). These were used as the washed face mask pieces in subsequent experiments. The inner layer (side in contact with the skin) or the outer layer (side in contact with the environment) of the washed face mask pieces was inoculated with *S. aureus*, placed in a sterile Petri dish, closed with a lid, sealed in a zippered plastic bag, and incubated at 37 °C for 24 h (Day 1). Unincubated samples were designated as Day 0.

### Evaluation of bacterial viability on face mask surfaces treated with contaminants

Artificial sweat solution (Japanese Industrial Standards [JIS] L0848), artificial sebum solution (JIS K2246), artificial saliva (JIS T9010), 1% peptone water (buffered peptone water; Kanto Chemical Co., Inc.), and cosmetic

materials (10 mg/mL foundation powder and 10% squalene) were used as face mask contaminant solutions to which *S. aureus* or *S. epidermidis* was added instead of sterile PBS. These solutions were used to inoculate face mask pieces in Petri dishes. The dishes were closed with lids and sealed in zippered plastic bags for incubation at 37 °C for 24 h (Day 1). Unincubated samples were designated as Day 0.

### Evaluation of the viability of bacteria on contaminant-treated face masks under high-temperature conditions
Artificial saliva and 1% peptone water were used as face mask contaminant solutions to which *S. aureus* or *S. epidermidis* was added instead of sterile PBS. These solutions were used to inoculate face mask pieces in Petri dishes. The dishes were closed with lids and sealed in zippered plastic bags for incubation at 65 °C for 3 h. Unincubated samples were designated as 0 h.

### Evaluation of viability of bacteria with varying initial counts and adherence densities on face masks treated with contaminants
Inoculum solutions of *S. aureus* or *S. epidermidis* were prepared at concentrations of $10^7$ and $10^4$ CFU/mask. Artificial saliva, 1% peptone water, and a mixture of both were used as face mask contaminant solutions to which the prepared inoculum solutions were added instead of sterile PBS. Then each prepared solution was spotted on the back of the face mask pieces (five spots each of 2 µL or one spot of 10 µL) in Petri dishes. The dishes were closed with lids and sealed in zippered plastic bags for incubation at 65 °C for 3 h. Unincubated samples were designated as 0 h.

### Evaluation of the viability of bacteria on face masks sprayed with 70% ethanol and stored either open or closed
*S. aureus*-inoculated face mask pieces were placed in sterile Petri dishes and squirted twice (400 µL) from 15 cm directly above each piece with sterile water, PBS, or 70% ethanol using a spray bottle (Daiso). The dishes were closed with lids and stored at 23 °C for 21 days. In a second experiment, the inoculated masks were placed in Petri dishes, sprayed with 70% ethanol as described, and kept either closed or open at 23 °C for 30 min. In a third experiment, after inoculating the face mask pieces with *S. aureus*, they were fixed at a height of approximately 5 mm from the bottom of the Petri dish, keeping the inoculated surface face-down. Then we sprayed 70% ethanol from 15 cm directly above the face mask pieces (Supplementary Fig. S1C). After spraying, the Petri dishes were placed either closed or open at 23 °C for 30 min (Supplementary Fig. S2A). In the final experiment, after inoculating the face mask pieces with *S. aureus*, they were fixed in the same manner as mentioned above, with the inoculated surface facing up (Supplementary Fig. S2B) or down (Supplementary Fig. S2C), and 70% ethanol was sprayed from 15 cm directly above the mask pieces. The Petri dishes were either kept open or were closed for 0, 5, 15, and 30 min at 23 °C after spraying.

### Evaluation of the viability of bacteria on face mask surfaces treated with contaminants and then sprayed with 70% ethanol
Artificial saliva, 1% peptone water (protein stain), and 10% squalane (cosmetic stain) were used as face mask contaminant solutions to which *S. aureus* or *S. epidermidis* was added instead of sterile PBS and then inoculated onto face mask pieces. The inoculated surfaces were sprayed with 70% ethanol from 15 cm directly above the pieces. In another experiment, the inoculated face mask pieces were fixed at a height of approximately 5 mm from the bottom of the Petri dish to keep the inoculated surface face-down. Then 70% ethanol was sprayed on the reverse side of the inoculated surface from 15 cm directly above each piece. The Petri dishes were closed with lids, sealed in zippered plastic bags, and stored at 23 °C for 0, 5, and 15 min.

### Evaluation of bacterial viability on face mask surfaces following exposure to sunlight inside and outside a car
Face mask pieces were inoculated with *S. epidermidis* and placed in sterile Petri dishes with the inoculated surface face-side up. Additionally, face mask pieces inoculated with *S. epidermidis* were fixed at a height of approximately 5 mm from the bottom of the Petri dish keeping the inoculated surface face-side down. All Petri dishes were closed with lids, sealed in zippered plastic bags, and placed at various locations inside and outside a car: on the hood in direct sunlight, on the hood in a shade box (i.e., protected from sunlight), and inside the car either inside or outside the sunshade (Supplementary Fig. S3). For comparison, samples were also placed in an incubator at 55 °C for 1 h. Microtubes containing the bacterial suspension were used as the control. The temperatures at each location were measured at 13:00 (experiment start) and 14:00 (experiment end) using a TEMPERATURE HiTESTER (model 3441; Hioki, Nagano, Japan). The experiments were performed in July and November. The maximum and minimum temperatures in Japan in July (summer) 2023 were 33.9 °C and 24.7 °C, respectively, and the monthly average of the UV index (observation site Tsukuba), which is the maximum among hourly observations per day, was 8.2. The maximum and minimum temperatures in Japan in November (autumn) 2023 were 19.2 °C and 10.3 °C, respectively, and the average UV index (observation site Tsukuba) was 2.3.

### Bacteria recovery and detection
Figure S1B shows the bacteria recovery and detection methods. The treated face mask pieces or 10 µL of the inoculum solution that had been placed in microtubes for the various experiments were added to 30 mL of 1% peptone water (AccuDia™ Buffered Peptone Water, Shimadzu Diagnostics Corporation, Tokyo, Japan) dispensed into 50 mL tubes and stirred at maximum speed for 1 min. Then the face mask pieces were removed if applicable, and the tubes were centrifuged (3,500 × *g*, 10 min). The supernatant was removed, and the pellet was resuspended in 1 mL of PBS. Finally, 0.1 mL of the diluted bacterial solution was applied to mannitol salt agar

(Eiken Chemical Co., Ltd., Tokyo, Japan) with a bacterial cell spreader, and the number of colonies was counted following incubation at 37 °C for 2 days.

### SEM observation

The surface of the inner layer (side in contact with the skin) of face mask pieces that had been washed, treated with heat, or sprayed with 70% ethanol was coated with 5 nm of osmium by an OPC 40 osmium plasma coater (Filgen, Inc., Nagoya, Japan), and observed using SEM (JSM-7001 F; JEOL Ltd., Tokyo, Japan). We also observed the outer layer (side in contact with the environment) of the face mask pieces treated with 70% ethanol because the alcohol solution was considered to have penetrated through the mask onto the inside surface.

### Water repellency of masks

Five spots of sterile water (2 µL) were applied to washed face masks or 70% ethanol-treated face masks and left for 15 min. Then the water repellency was observed using an SZX10 Research Stereomicroscope System (Olympus Corp., Tokyo, Japan).

### Particle capture capability

We used an HT9600 particle counter (Shenzhen Yibai Network Technology Co., Ltd., Shenzhen, China) to first measure the number of 0.3, 2.5, and 10 µm particles (piece/L; number per liter) on each treated mask and in the environment of the measurement site without masks (control). Because particles measuring 2.5 µm and 10 µm were not detected in the indoor environment, we targeted particles measuring 0.3 µm in our assessment of the particle capture capability of face masks.

### Statistical analysis

Outcomes were presented as mean ± standard deviation (SD). Data were analyzed as means and SDs for each group. Statistically significant differences were analyzed using Dunnet test and Tukey–Kramer test. All tests were two-tailed, and $p$-values < 0.05 were considered statistically significant. All statistical analyses were performed using Microsoft Excel 2019 (Microsoft Corp, Redmond, WA, USA).

## Results

### Effects of temperature and humidity on the viability of bacteria on face masks

The effect of temperature on the viability of bacteria inoculated on the inner and outer layers of the face masks was compared after 7 h of incubation at various temperatures (Fig. 1A–D). There was no difference in the effect of temperature between the inner and outer face mask layers (Comparison of Fig. 1A and B, and Fig. 1C and D). At 37 °C, there was no significant difference in the number of bacteria in the control (phosphate-buffered saline; PBS) at 0 and 7 h ($p = 0.36$; Fig. 1A, $p = 0.15$; Fig. 1B, $p = 0.44$; Fig. 1C, $p = 0.56$; Fig. 1D), but the number of bacteria on the face masks tended to decrease by approximately 2 log orders for both strains. At 45 °C, the number of bacteria decreased between 0 and 7 h in the control and on the face masks (inner and outer layers) inoculated with *S. aureus* (Fig. 1A and B) and *S. epidermidis* (Fig. 1C and D). At 55 °C, both *S. aureus* (Fig. 1A and B) and *S. epidermidis* (Fig. 1C and D) were killed in the control but were not completely killed on the upper and lower layers of the face masks, surviving 2–3 log orders. There was no difference in bacterial viability in controls incubated at 60% and 90% humidity (Fig. 1E). For bacteria inoculated on the inner layer of the face mask, the higher the humidity resulted in 2 log and 3 log orders of the greater the viability of the bacteria after incubation at 37 °C for both 7 and 24 h, respectively.

### Effect of a mixed culture of S. aureus and S. epidermidis on the viability of bacteria on face masks

A mixture of *S. aureus* and *S. epidermidis* was inoculated on the face masks to determine the effect of the coexistence of bacterial species on the viability of bacteria on face masks. There were no significant changes in viability between inoculations with single and mixed species (Fig. 2A). However, the colonies and the change in the color of mannitol salt agar demonstrated that *S. aureus* was predominant in the control, suggesting that the two species coexisted on the face mask pieces (Fig. 2B).

### Effect of washing on the viability of bacteria on face masks

The effect of washing on the viability of bacteria inoculated on the inner and outer layers of the face masks was compared. Washing did not affect bacterial viability on either layer (Supplementary Fig.S4).

### Effects of contamination on the viability of bacteria on face masks

The effects of contaminants (artificial sweat solution, artificial sebum solution, artificial saliva, 1% peptone water [protein stain], and cosmetic ingredients [10 mg/mL foundation powder, 10% squalene]) on the viability of bacteria on face masks were examined using sterile PBS as a control (Fig. 3A–F). Compared with sterile PBS, differences in the viability of *S. aureus* (Fig. 3A) or *S. epidermidis* (Fig. 3B) were not significant following the application of artificial sweat solution and artificial sebum solution. Bacterial viability increased by more than 1 log order for *S. aureus* (Fig. 3C) and less than 1 log order for *S. epidermidis* (Fig. 3D) with the following the application of artificial saliva and 1% peptone water. Bacterial viability was also decreased by 2 log orders with the following the application of foundation powder for both of the two strains, but was unchanged with the application of 10% squalene (Fig. 3E and F).

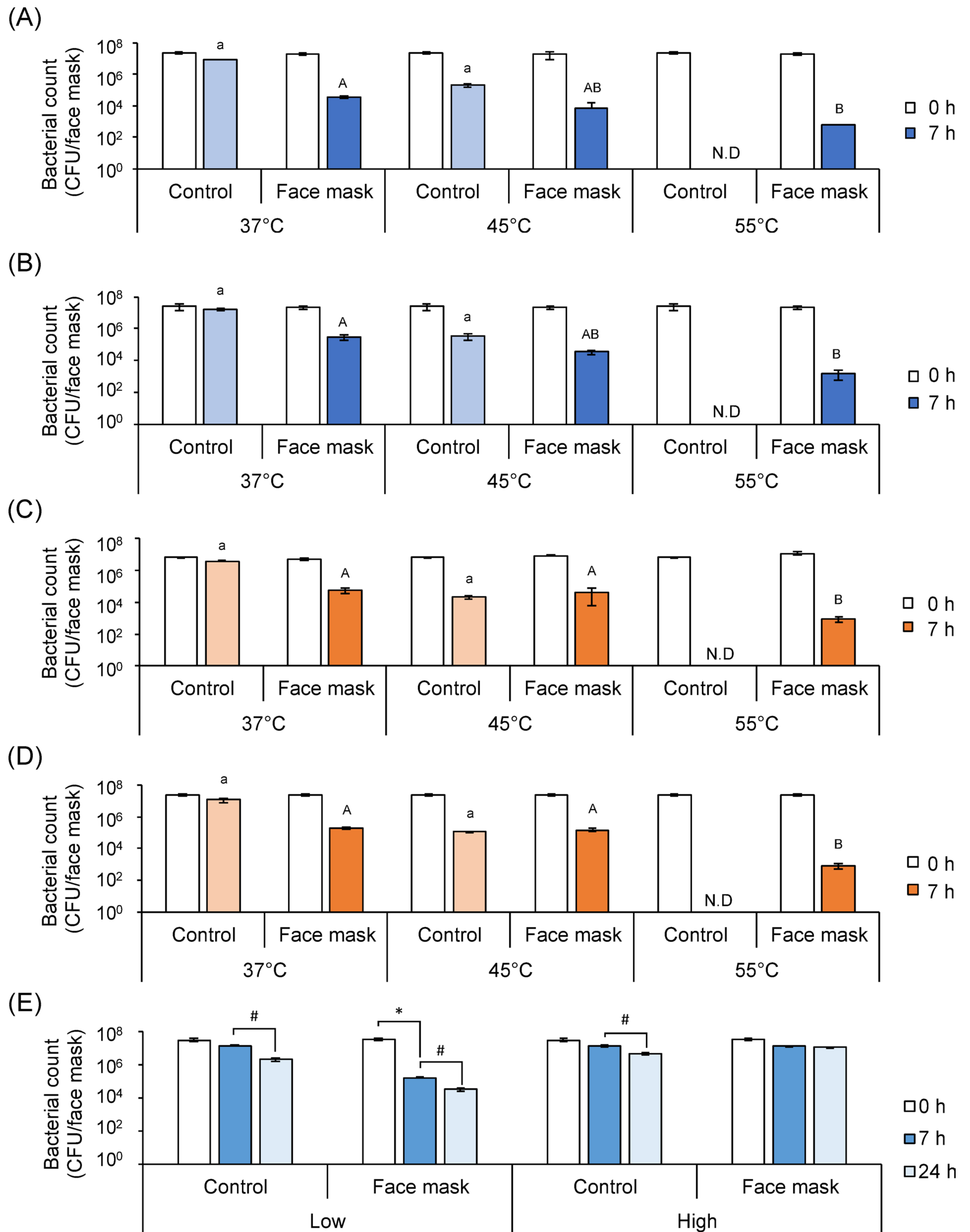


**Fig. 1**. Effects of temperature and humidity on the viability of bacteria inoculated on the face masks. Effects of temperature on bacterial viability on the (**A**) inner layer and (**B**) outer layer of face mask pieces inoculated with *Staphylococcus aureus* and on the (**C**) inner layer and (**D**) outer layer of the face mask pieces inoculated with *S. epidermidis* at 37 °C, 45 °C, and 55 °C (60% humidity) for 7 h. (**E**) Effect of humidity on the viability of *S. aureus* inoculated on the inner layer of face mask pieces and incubated at 37 °C at 60% humidity (low) or 95% humidity (high) for 7 and 24 h. N.D.: not detected. Control: inoculum suspended in phosphate-buffered saline in microtubes. Tukey–Kramer test, $p < 0.05$ (significant difference between upper- and lowercase letters). *$p < 0.05$ vs. 0 h and #$p < 0.05$ vs. 7 h. CFU: colony-forming unit.

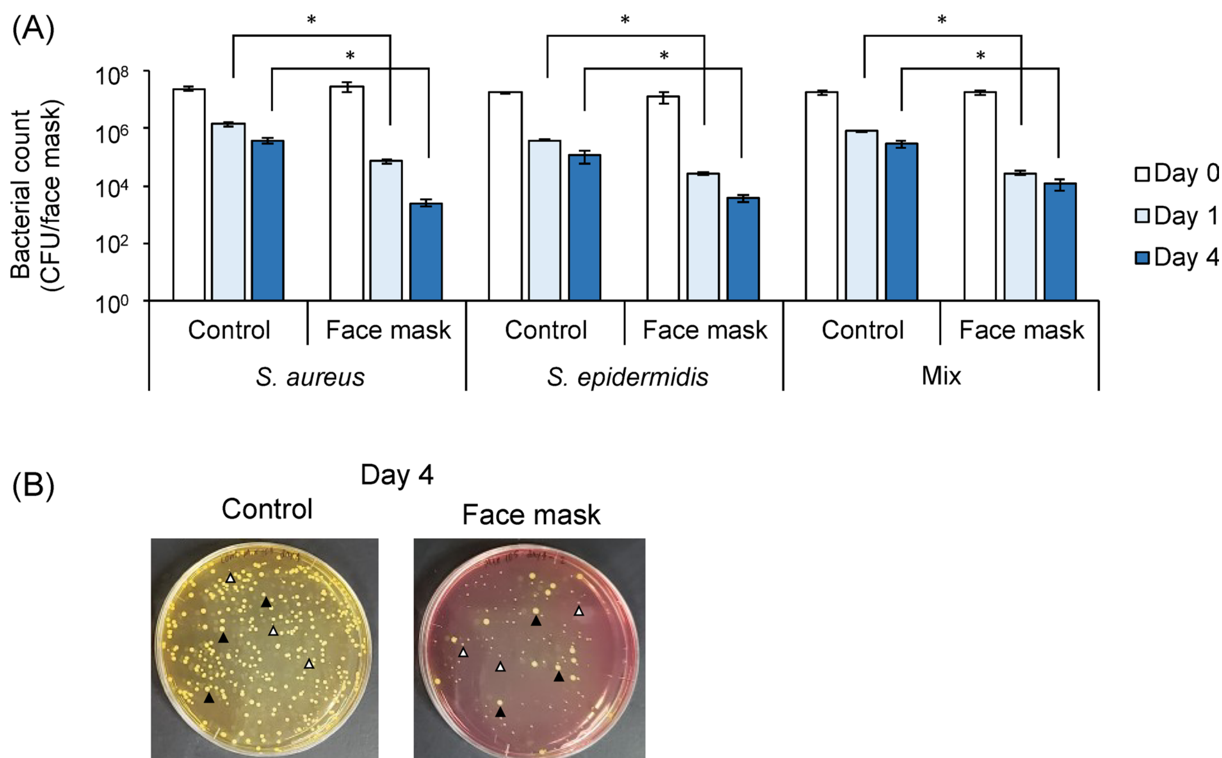


**Fig. 2**. Effect of a mixed culture of *S. aureus* and *S. epidermidis* on the viability of bacteria on face masks. (**A**) Bacterial viability on the face mask pieces inoculated with a mixture of *Staphylococcus. aureus* and *S. epidermidis* at 37 °C for 1 and 4 days. Control: inoculum suspended in phosphate-buffered saline (PBS). Mix: mixed inoculum of *S. aureus* and *S. epidermidis*. $^*p < 0.05$ vs. Control. (**B**) Photographs of colonies after 4 days. Control: inoculum suspended in PBS in microtubes; black triangles: *S. aureus* colonies; white triangles: *S. epidermidis* colonies. CFU: colony-forming unit.

## Effect of contamination on the viability of bacteria on face masks under high-temperature conditions

The effect of face mask contamination with artificial saliva and 1% peptone water on the viability of bacteria on face masks under high-temperature conditions (65 °C, 3 h) was examined. Compared with PBS, the viability of *S. aureus* (Supplementary Fig. S5A) and *S. epidermidis* (Supplementary Fig. S5B) on face masks was increased by approximately 2 log orders in the presence of both contaminants.

## Effect of initial bacterial count and adherence density on the viability of bacteria on contaminant-treated face masks

The effect of the effects of differences in the initial bacterial count ($10^7$ vs. $10^4$ CFU/face mask) and densities (one spot vs. 5 spots; 10 µL total) on the viability of bacteria on contaminant-treated face masks was examined (Fig. 4). For both *S. aureus* and *S. epidermidis*, the greater the adhesion density (1 spot), the greater the bacterial viability on the face mask by approximately one log order, regardless of the initial bacteria and the type of contaminants (Fig. 4A and C). Additionally, the PBS results in Fig. 4B and D show that the higher the initial bacteria, the higher the bacterial viability on the face mask, as no bacteria survived at low adhesion densities (5 spots).

## Effects of the spray side of 70% ethanol and open or closed storage on the viability of bacteria on face masks

The effect of spraying 70% ethanol on the viability of bacteria on face masks was examined. Although bacterial viability decreased over time after spraying with PBS and sterile water by approximately 2 log orders, viable bacteria were still present after 3 weeks (Fig. 5A). In contrast, bacteria were confirmed as viable immediately after spraying with 70% ethanol, $8.3 \times 10^3$ CFU/mask of viable bacteria were observed, but 6 log order reduction in the number of bacteria and no colonies detected after 1 day (Fig. 5A), indicating that the sterilization effect of 70% ethanol spraying was not immediate. Thus, further investigations were performed in which face mask pieces sprayed with 70% ethanol were immediately placed in Petri dishes and allowed them to stand, either closed with a lid or open, at 23 °C for 30 min before testing bacterial viability. Additionally, to investigate the penetration of ethanol into the masks, 70% ethanol was sprayed on the inoculated surface of the mask or the back side of the inoculated surface. When 70% ethanol was sprayed on the inoculated surface and the back side of the inoculated surface of the face mask pieces, the bacteria count was approximately $2.9 \times 10^4$ and $4.1 \times 10^6$ CFU/mask, respectively (Fig. 5B), a difference of 2 log orders of magnitude between the front and back sides. No bacteria were detected on either surface when the sprayed face mask pieces were stored in closed Petri dishes for

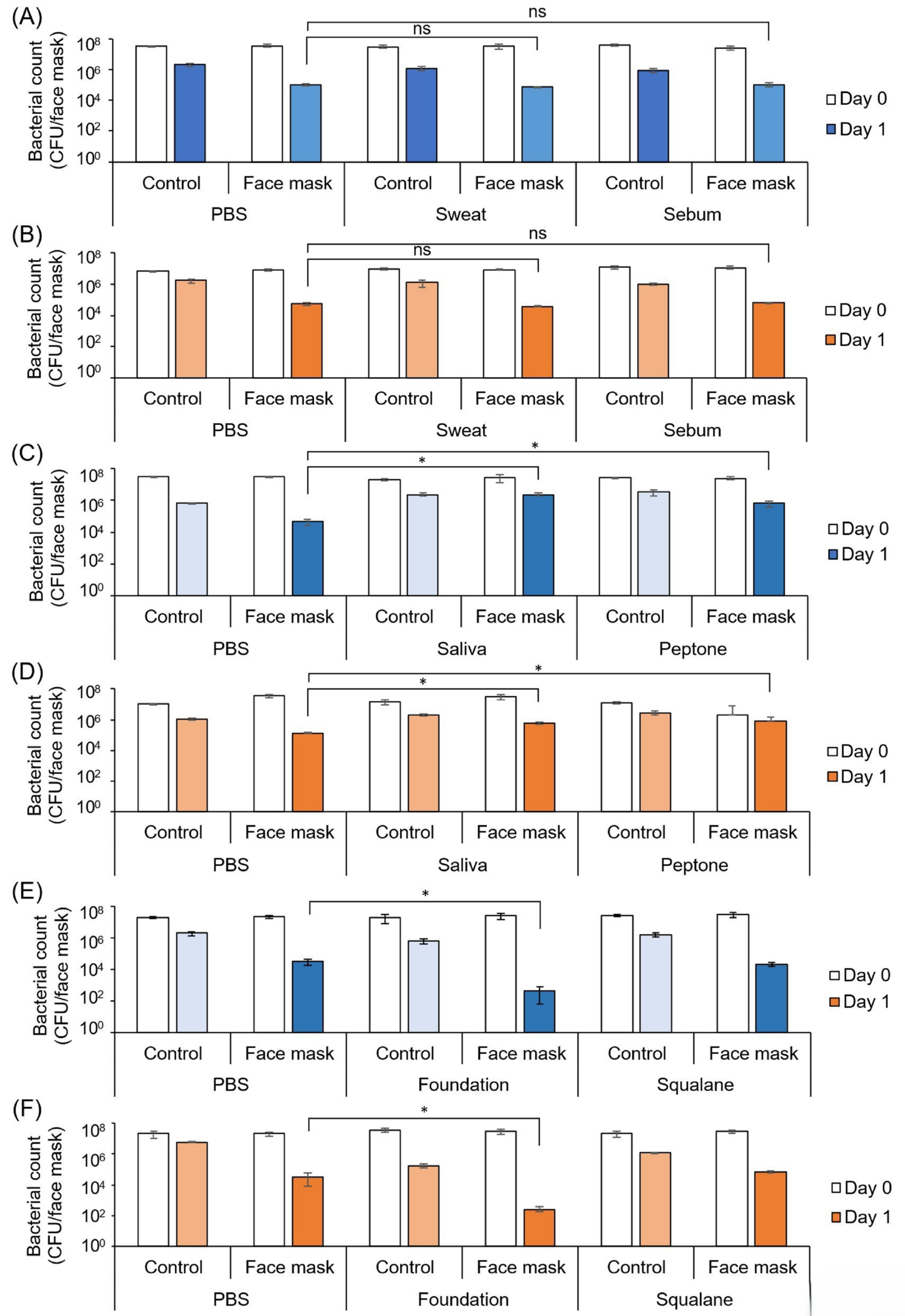


**Fig. 3**. Effect of contamination on the viability of bacteria inoculated on face mask pieces. Bacteria were inoculated on face mask pieces in the presence of contaminants and incubated at 37 °C for 1 day. (**A**) Artificial sweat and artificial sebum solutions, (**C**) artificial saliva and peptone water, and (**E**) foundation powder and squalene were used to contaminate *Staphylococcus aureus* inoculated on face mask pieces. (**B**) Artificial sweat and artificial sebum solutions, (**D**) artificial saliva and peptone water, and (**F**) foundation powder and squalene were used to contaminate *S. epidermidis* on face mask pieces. PBS: phosphate-buffered saline. Control: inoculum suspended in PBS in microtubes. *$p < 0.05$ vs. PBS. CFU: colony-forming unit, n.s.: no significant difference.

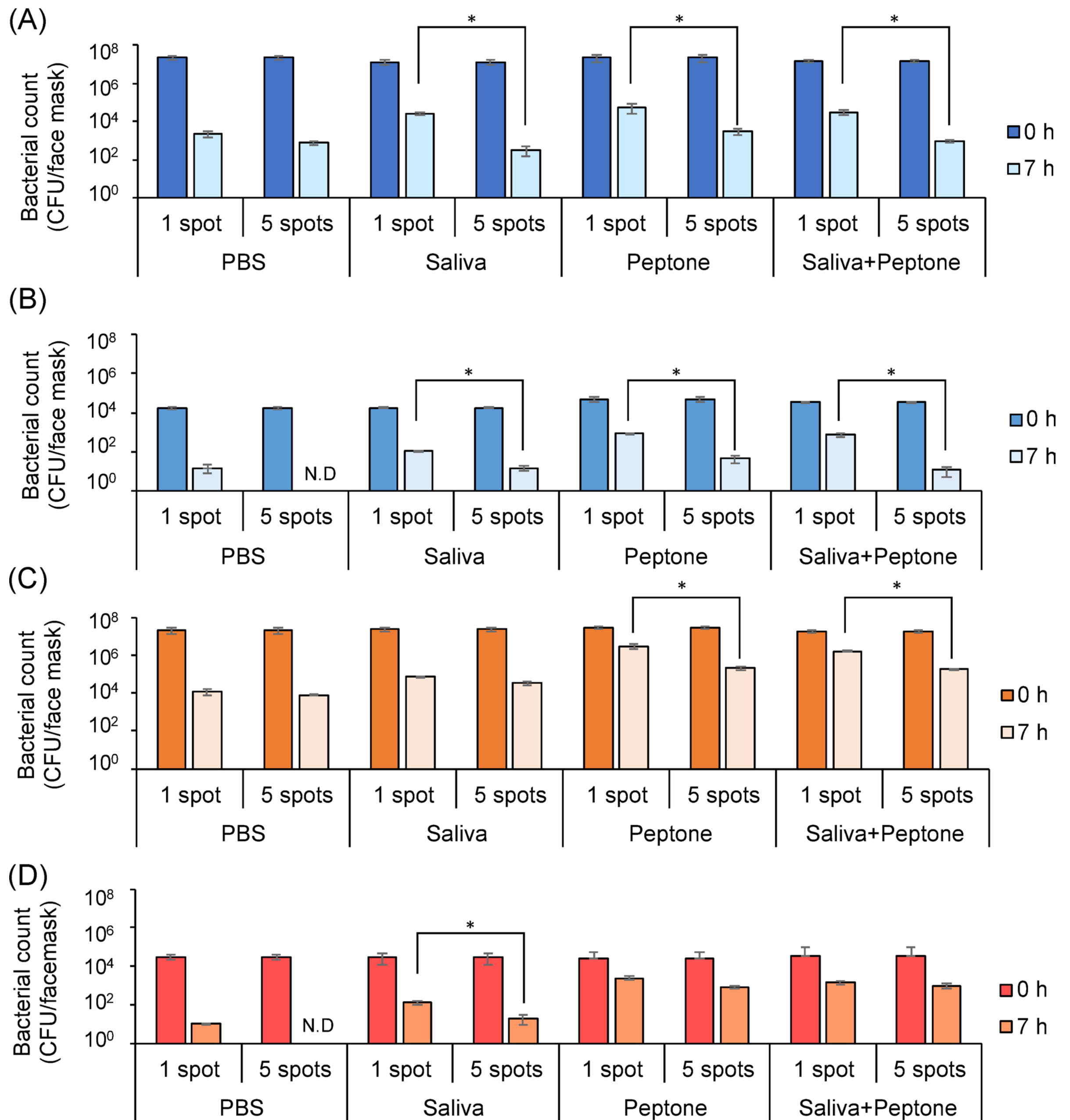


**Fig. 4**. Effect of initial bacterial count and adherence density on the viability of bacteria on contaminant-treated face masks. (**A**) $10^7$ CFU/mask and (**B**) $10^4$ CFU/mask of *Staphylococcus aureus*. (**C**) $10^7$ CFU/mask and (**D**) $10^4$ CFU/mask of *S. epidermidis*. The bacteria were suspended in phosphate-buffered saline (PBS; control), artificial saliva, peptone water, or artificial saliva + peptone water. Then 10 µL of each inoculum solution was spotted on face masks as either one spot of 10 µL (1 spot) or five spots of 2 µL each (5 spots) and incubated at 55 °C for 7 h. *$p < 0.05$ vs. 1 spot. CFU: colony-forming unit, N.D.: not detected.

30 min after spraying with 70% ethanol (Fig. 5B). There were no viable bacteria on masks in dishes closed 5 min after spraying, either on the inoculated surface or on the back side of the inoculated surface (Fig. 5C and D).

#### Effect of 70% ethanol spray on the viability of bacteria on contaminant-treated face masks

We found that the viability of bacteria on masks increased in the presence of artificial saliva and 1% peptone water and decreased in the presence of cosmetic material (squalene) (Fig. 3C–F). Thus, the influence of these contaminants on the sterilizing effect of 70% ethanol was investigated. No bacteria were detected on the face mask pieces inoculated with bacterial suspensions prepared with PBS or each contaminant solution when the Petri dishes were closed for 5 min after spraying the inoculated surface with 70% ethanol (Fig. 6A–C). In contrast, when the face mask pieces inoculated with bacterial suspensions prepared with each contaminant solution were sprayed with 70% ethanol on the back side of the inoculated surface and the Petri dishes were closed for 5 min,

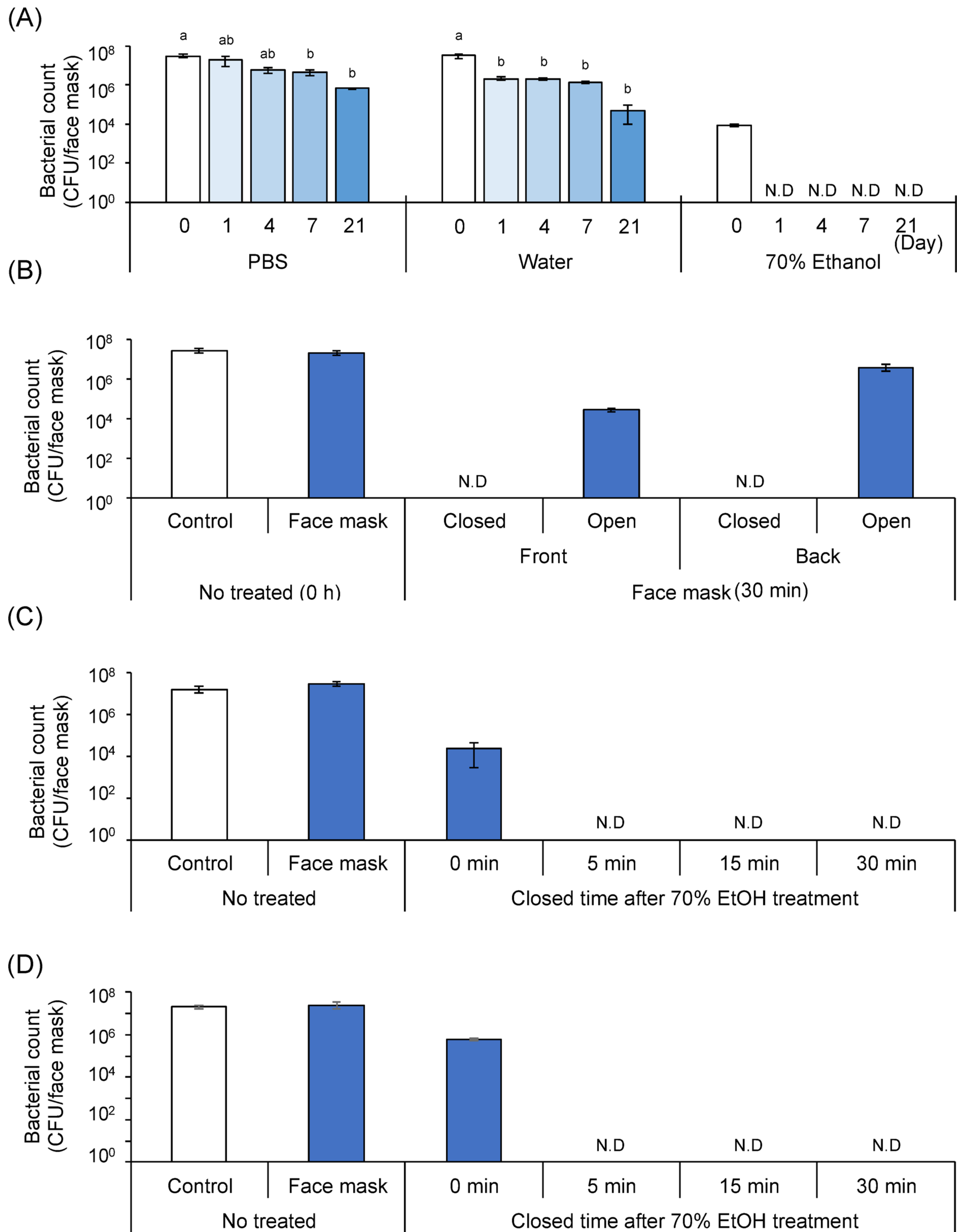


**Fig. 5**. Effects of the spray side of 70% ethanol and open or closed storage on the viability of bacteria inoculated on face masks. (**A**) Effect of 70% ethanol on the viability of bacteria inoculated on face mask pieces. Face masks were inoculated with *Staphylococcus aureus* and then sprayed with phosphate-buffered saline (PBS), sterile water, or 70% ethanol and stored in closed Petri dishes at 23 °C for 21 days. (**B**) Effect of closing on the viability of bacteria on face mask pieces sprayed with 70% ethanol on the inoculated surface (Front) or the back side of the inoculated surface (Back) and allowed to stand for 30 min either closed with a lid or open. (C and D) Effect of closing for 0, 5, 15, and 30 min on bacterial viability after spraying 70% ethanol on (**C**) the inoculated surface and (**D**) the back side of the inoculated surface. Control: inoculum suspended in PBS in microtubes. Tukey–Kramer test, $p < 0.05$ (significant difference between upper- and lowercase letters). CFU: colony-forming unit, N.D.: not detected, EtOH: ethanol.

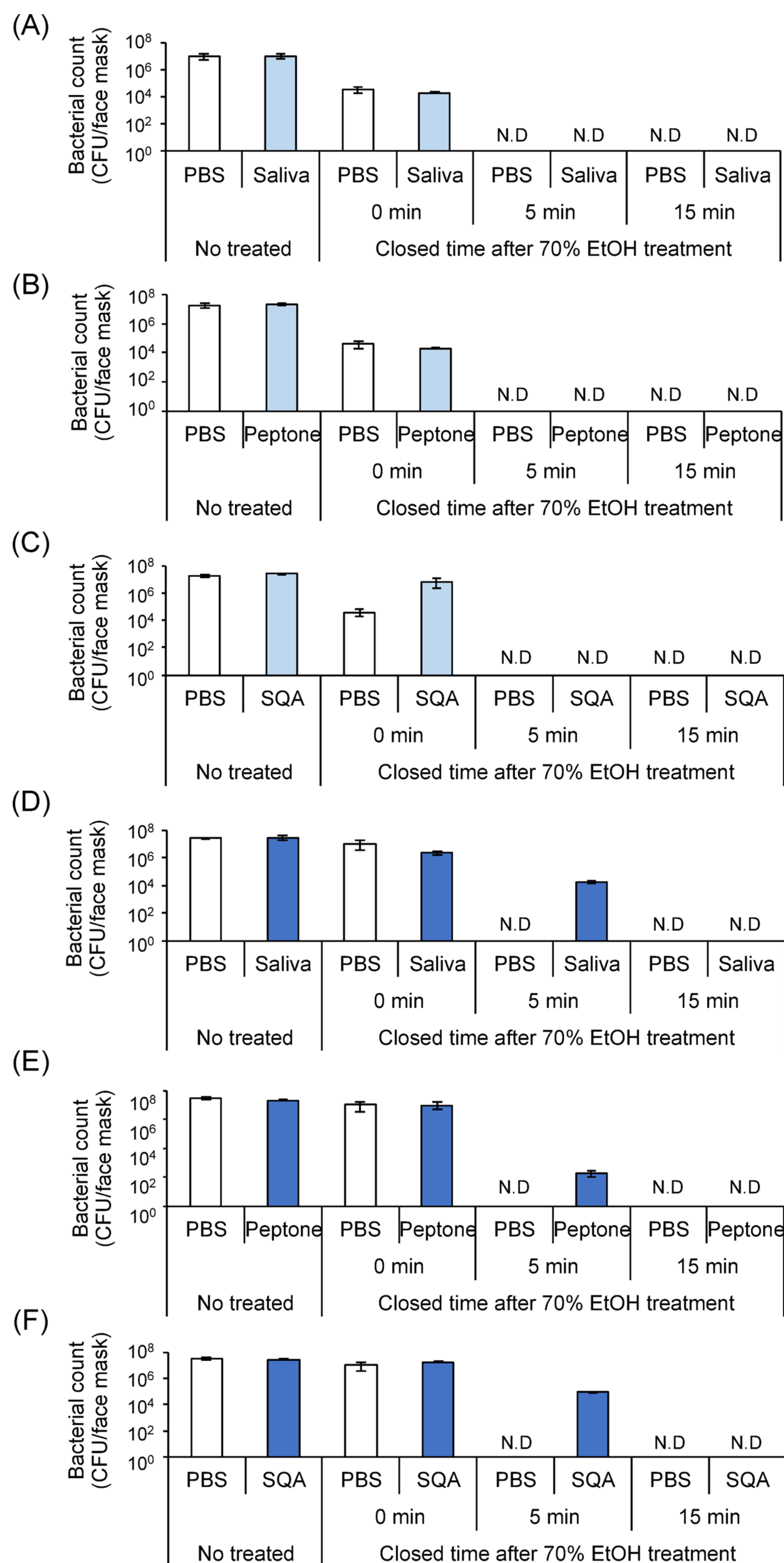


**Fig. 6**. Effect of 70% ethanol spray on the viability of bacteria on contaminant-treated face mask pieces. The inoculated surface of face mask pieces was sprayed with 70% ethanol in the presence of (**A**) 25% artificial saliva, (**B**) 1% peptone water, and (**C**) 10% squalane solution and placed in Petri dishes. The back side of the inoculated surface of face mask pieces was sprayed with 70% ethanol in the presence of (**D**) 25% artificial saliva, (**E**) 1% peptone water, and (**F**) 10% squalane solution and placed in Petri dishes. The dishes were allowed to stand for 0, 5, and 15 min before closing with a lid. CFU: colony-forming unit, PBS: phosphate-buffered saline, N.D.: not detected, SQA, 10% squalene solution, EtOH: ethanol.

the number of bacteria decreased by 2 log orders with artificial saliva, 5 log orders with 1% peptone water, and 3 log orders with squalane, but viable bacteria were detected (Fig. 6D–F). No bacteria were observed after closing the dishes with lids for 15 min after spraying with 70% ethanol.

### Effect of sunlight exposure inside and outside a car on the viability of bacteria on face mask surfaces

The bacteria-contaminated surface of face mask pieces was placed face-up or face-down in sterile Petri dishes and left inside and outside a car for 1 h on a sunny July day (average temperature, 55.0 °C outside the car [on the hood]) and 59.3 °C inside the car [outside the sunshade]; average UVA, 37 $w/m^2$; and average UVB, 0.95 $w/m^2$) (Supplementary Fig. S3B and C) and a cloudy July day (average temperature, 44.6 °C outside the car [on the hood]) and 57.7 °C inside the car [outside the sunshade]; average UVA, 28 $w/m^2$; average UVB, 0.70 $w/m^2$) (Supplementary Fig. S3D). On the sunny July day, the bacteria on the face mask pieces placed with the inoculated surface face-side up and left on the hood inside a shade box (average 39.4 °C) and inside the car inside the sunshade (average 39.4 °C) for 1 h were still remained almost the same as at 0 h, with the bacteria count maintained at approximately $10^6$ CFU/mask. However, no bacteria survived under direct sunlight on the hood (average 55.0 °C) and outside the sunshade in the car (average 59.3 °C) (Fig. 7A and Fig. S3). For the microtubes containing the bacterial suspension as a control, the results were similar to those of the face mask (Fig. 7A). For comparison, we determined the bacterial viability after placing the inoculated face mask pieces in an incubator at 55 °C for 1 h. The bacteria on the inoculated face mask pieces that were placed in an incubator at 55 °C for 1 h were decreased by 3 log orders, but remained viable. On a cloudy July day, the bacteria inoculated on the face mask pieces placed outside the sunshade inside the car (average 55.0 °C) and on the hood (average 42.6 °C) were killed, regardless of which side was facing up (Fig. 7B and Fig. S3). For the face mask pieces placed in an incubator at 55 °C and the microtubes containing the bacterial suspension as a control, the results were similar to those obtained on the sunny July day (Fig. 7B). When we performed a similar experiment on a November day, the bacteria inoculated on the face mask pieces were not killed when they were left outside the sunshade in the car (average 55.0 °C) and on the hood (average 42.6 °C) for 1 h (Fig. 7C and Fig. S3).

### Scanning electron microscopy (SEM) observation of the face mask surfaces treated under various conditions

To evaluate the performance of the nonwoven face masks used in this study following various treatments, we used SEM to observe the fibers on the inner layer surface (side in contact with the skin). The untreated (control) masks had random fibers with square areas, showing evidence of adhesive treatment performed as part of the manufacturing process. In contrast, the adhesive on the washed and heat-treated face masks appeared to have partially dissolved. There was little difference in the fiber structure of the masks in each treatment group compared with the untreated masks (Fig. 8A).

### Evaluation of water repellency of face masks by microscopic observation

Microscopic observation of the water-repellency effect of face masks revealed that washing weakened water repellency. In contrast, treatment with 70% ethanol did not affect water repellency (Fig. 8B).

### Measurement of particle capture capability of face masks

We used a particle counter to measure the number of 0.3 µm particles (piece/L; number per liter) passing through the face mask following various treatments. The fewer the number of particles passing through the face mask, the stronger its filtering effect. The particle count in the indoor environment was $6{,}490 \pm 749$ particles/L. The number of particles that passed through the untreated face mask was $186 \pm 63$ and $195 \pm 88$ particles/L for the inner and outer layer sides, respectively, both of which suppressed 97% of 0.3 µm particles, with no significant difference between layers ($p = 0.91$). The face masks washed or left outside the car in direct sunlight showed similar results compared with the untreated face masks. The number of particles was increased on face masks treated with 70% ethanol, those treated with 70% ethanol after washing, and those treated with heat (65 °C, 3 h) compared with untreated face masks (Fig. 8C). Although these treatments increased the particle permeability of the face masks, the particle capture rate remained > 80% (Fig. 8C).

## Discussion

Face masks become contaminated with bacteria during daily use and storage. The general public has not yet been properly educated on mask handling. An observational checklist of 1,500 participants recruited in Hong Kong showed that 91.5% did not perform hand hygiene before putting on the mask, 97.3% did not perform hand hygiene when removing the mask, and few were able to perform all the steps necessary for proper mask use[17]. Improper use of face masks may increase the risk of infection and spread by viral and bacterial pathogens. Although *S. aureus* is part of the microflora of the skin and mucous membranes, it also causes a variety of infections. Therefore, in this study, various experiments were conducted with the aim of providing information on the factors involved in bacterial growth of face masks and effective methods for disinfecting contaminated face masks.

Our investigation of the effects of temperature on bacterial viability revealed that there was no difference in the viability of *S. aureus* or *S. epidermidis* inoculated on the inner or outer layers of commercially available nonwoven face masks (Fig. 1A–D). A comparison of the number of colonies on each side of face masks (inner vs. outer layer) worn by individuals reported that the mean number of colonies was 13.4 times higher on the inner layer[18], suggesting that facial bacteria adhered to the face mask regardless of the face mask's structure. In our study, the viability of *S. aureus* and *S. epidermidis* was lower at higher temperatures. Increased temperature and humidity of skin covered by face masks is thought to have a significant effect on the function of face masks,

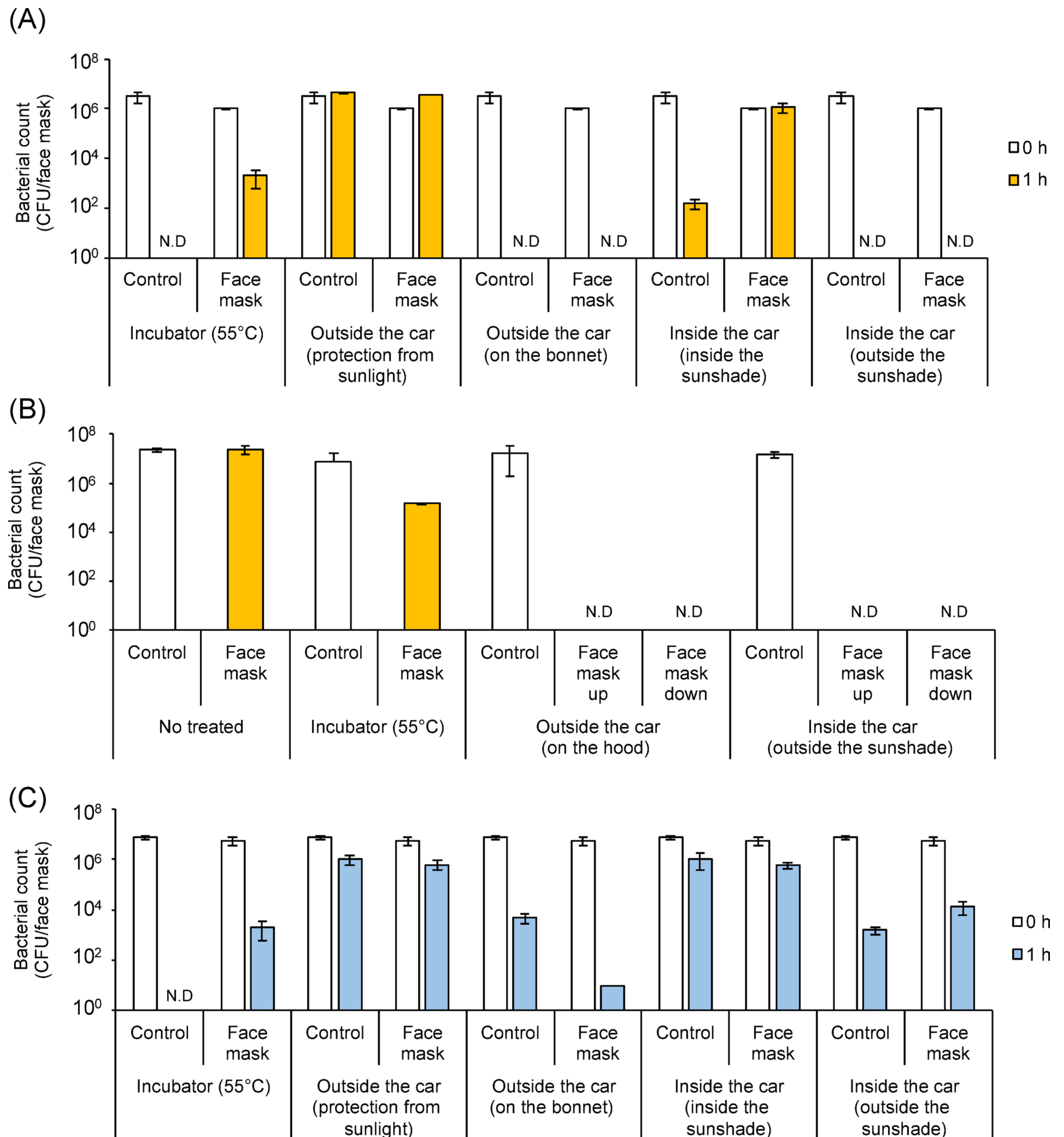


**Fig. 7**. Effect of sunlight exposure on the viability of bacteria inoculated on face masks placed inside and outside a car. (**A**) and (**B**) tests conducted in July on a sunny and cloudy day, respectively. (**C**) Test conducted in November. (**A**) Face mask pieces were inoculated with *Staphylococcus epidermidis*, placed in Petri dishes face-side up, closed with lids, and sealed in zippered plastic bags. As a comparison, face mask pieces inoculated with *S. epidermidis* were placed in an incubator at 55 °C for 1 h. The samples were placed on the hood in direct sunlight, on the hood in a shade box, and inside the car either inside or outside the sunshade for 1 h. (**B** and **C**) Face mask pieces were inoculated with *Staphylococcus epidermidis*, placed in Petri dishes face-side up or face-side down (fixed at a height of approximately 5 mm from the bottom of the Petri dish to keep the inoculated surface face-down), closed with lids, and sealed in zippered plastic bags. The samples were placed on the hood in direct sunlight, on the hood in a shade box, and inside the car either inside or outside the sunshade for 1 h. As a comparison, face mask pieces inoculated with *S. epidermidis* were placed in an incubator at 55 °C for 1 h. Control: inoculum suspended in phosphate-buffered saline in microtubes. Temperatures were measured at each location at 13:00 (experiment start) and 14:00 (experiment end). CFU: colony-forming unit, N.D.: not detected.

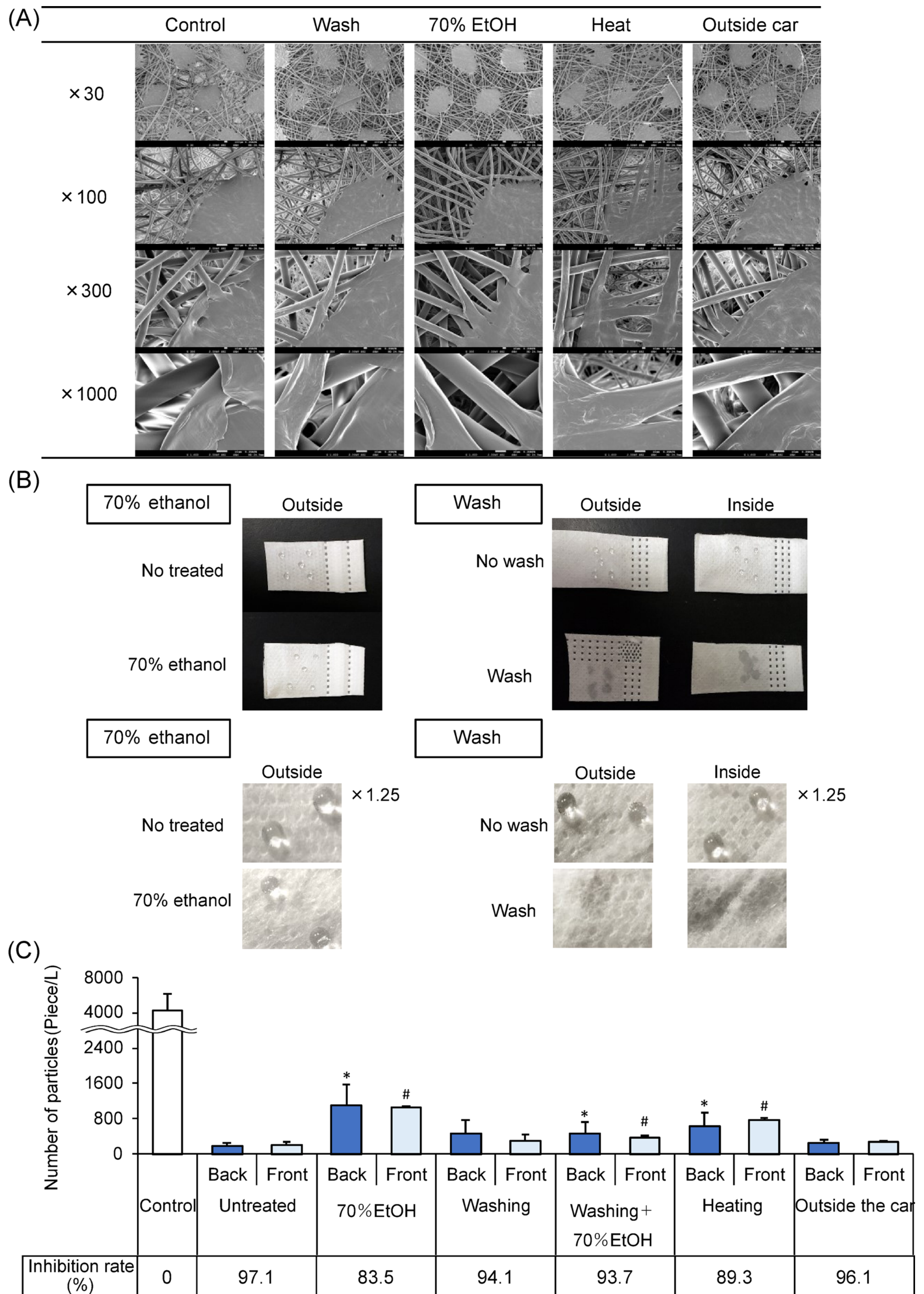


**Fig. 8**. Performance of face masks after various sterilization treatments. (**A**) Scanning electron microscopy of the inner layer surface (side in contact with the skin) of masks following various treatments. Magnification: 30, 100, 300, and 1,000×. (**B**) Water repellency following various treatments. Magnification: 1.25×. (**C**) Particle capture capability determined using a particle counter. $^{*}p < 0.05$ vs. Back untreated, $\#p < 0.05$ vs. Front untreated. EtOH, ethanol.

and temperature and humidity may also have a significant effect on the composition of the skin microflora[19]. Bacteria on face masks were suggested to be more susceptible at temperatures where bacteria are more likely to grow. In our study, when microtubes containing suspensions of *S. aureus* or *S. epidermidis* in PBS (control) were incubated at 55 °C, both species were killed, but bacteria attached to the inner and outer layers of the face mask pieces survived (Fig. 1A–D). Dry heating at 70 °C for 30 min in an electric oven was reported as insufficient to kill 99.9% of *S. aureus*[20]. Furthermore, dry heating at 82 °C for 20 min in an industrial washer inactivated < 90% of *S. aureus*[21]. A bactericidal efficiency > 99.9999% against *S. aureus* was achieved using a longer heating time of 3 h at 100 °C[22]. The fiber network of the face mask and the insulation properties of the polypropylene material may protect bacteria from heat. Taking all this into account, we inferred that effective heat sterilization of bacteria on face masks would be difficult for the average household to perform.

Our investigation of the effect of humidity on bacterial viability revealed that the higher the humidity, the higher the bacterial viability (Fig. 1E). This suggests that the local humidity of face masks favors bacterial survival. Continuous use of face masks without regular replacement can lead to the risk of pathogen spread because temperature and humidity induce humidity, which in turn induces microbial colony formation, and improper use can lead to the risk of pathogen spread[23,24]. Thus, when storing face masks before and after use, measures should be taken to avoid areas of high humidity (e.g., near water or in poorly ventilated areas).

We compared the viability of a mixture of *S. aureus* and *S. epidermidis* with the viability of each species alone following inoculation onto face mask pieces. Culture results suggested that *S. aureus* was predominant in PBS in microtubes inoculated with the mixture as a control, although the two species of bacteria showed the same growth rate on the face masks (Fig. 2B). A comparison of the colony counts on the inner and outer layers of face masks worn by individuals reported a higher detection rate of *S. epidermidis* compared with *S. aureus*[18]. This suggests that to some extent, the bacteria species status on face masks reflects the proportion of indigenous skin bacteria and that bacterial viability on face mask surfaces was different from that in liquid (PBS).

There was no difference in bacterial viability between unwashed and washed face masks (Supplementary Fig. S4). It was reported that the face mask structure was unaffected after a single wash[25], suggesting that washing under conditions where the structure of the face mask remains unchanged does not affect bacteria viability.

Studies on the effectiveness of masks generally do not take into account the fact that microorganisms in human saliva and exhaled breath can form a biological safety concern, especially if masks are worn for extended periods of time, not stored properly, or reused without proper disinfection[12,26]. In addition, possible factors affecting *S. aureus* viability include proteins in food and saliva[17] and cosmetics applied directly to the face. Therefore, we investigated the effects of artificial sweat solution, artificial sebum solution, artificial saliva, protein (peptone), foundation powder, and squalane on the viability of bacteria adhering to the face mask pieces. The viability of bacteria on face masks decreased in the presence of the foundation powder used in our study because it contained zinc chloride and chlorphenesin as bactericidal and antimicrobial agents, suggesting that these ingredients were responsible for inhibiting bacterial viability. The viability of bacteria inoculated on face mask pieces contaminated with artificial saliva and peptone was increased compared with PBS at both 37 °C for 24 h and high-temperature conditions of 65 °C for 3 h (Supplementary Fig. S5). Peptone is a protein that is broken down to amino acids and peptides following enzymatic digestion. Peptone utilization by bacteria leads to a shorter culture induction period. Peptone has also been reported to restore bacterial damage due to food processing and other activities[27,28]. The amount of serum albumin in saliva is 0.09–0.44 mg/mL[29]. Thus, we added 0.25 mg/mL of serum albumin to the artificial saliva used in this study. The addition of 0.01–1.0% serum albumin to synthetic media was reported to increase the doubling time and maximum viability levels[30]. Furthermore, serum has been reported to increase polysaccharide production and surface protein expression in *S. aureus* and to cause antibiotic resistance[31]. These reports suggest that the addition of serum albumin to artificial saliva enhanced bacterial viability on the face mask pieces in our study.

On contaminant-treated face masks, bacterial viability was higher when the adherence density was high, regardless of differences in the initial bacterial count. In contrast, the viability of bacteria in PBS was higher when the initial bacterial count was high (Fig. 4). *S. aureus* promotes the expression of surface proteins, such as adhesion factors, when bacterial density is low. When bacterial density is high, extracellular secreted proteins, such as toxins and degrading enzymes, are promoted[32]. Thus, adherence density affects the number of bacteria adhering to the face mask, suggesting that local contamination increases bacterial viability and pathogenicity.

Because pathogenic bacteria can remain on face mask surfaces for long periods[18,33,34], effective disinfection methods are crucial. With the support of government agencies, research institutions, and medical institutions worldwide, several efforts have been made to investigate the effectiveness of common disinfection methods for disposable face masks, including UVC irradiation, dry heating, and wet heating[35–40]. However, some of these methods are costly and require highly trained personnel, which may not be used to disinfect masks in the home. In industry, chemicals are widely used for killing bacteria in food handling facilities. Bacteria are also killed using various types of disinfectants, and chlorine compounds, surfactants, and hydrogen peroxide are used in food factories. In contrast, households use alcohol-based products, such as disinfectant ethanol and wet wipes. Disinfectants are classified as those with high, medium, and low levels of disinfection. Ethanol is classified as a medium-level disinfectant because it is effective against microorganisms and viruses except bacterial spores. Its bactericidal effect is not necessarily proportional to its concentration, and a concentration of 60–90% is considered appropriate for general use as a biological disinfectant[41]. Anhydrous ethanol may not be readily available to the average household, but could be applied in alcohol for disinfection and spray-type hand sanitizers. In Japan and other countries, anhydrous ethanol can be purchased at drugstores, home centers, and through Internet sales.

In our study, when 70% ethanol was sprayed on face masks as the method for their hygienic use, bacteria were detected immediately after spraying (0 h) (Fig. 5A). A previous study reported that spraying face masks with 70% ethanol resulted in a bacterial viability rate of 73% ± 5% immediately after spraying, decreasing to 22% ± 8%

after 24 h[42]. These findings suggest that disinfection of face masks with 70% ethanol requires adequate time for the ethanol to show its full bactericidal effect. The bactericidal effects of ethanol are due to the disruption and denaturation of bacterial cell membranes and proteins[42]. Our findings demonstrated that spraying face masks with 70% ethanol followed by sealing them in zippered plastic bags for 5 min enhanced its bactericidal effect.

We evaluated the effect of spraying with 70% ethanol on the viability of bacteria inoculated on face mask pieces contaminated with artificial saliva, peptone, and squalene. Collectively, our findings suggest that the bactericidal effect of 70% ethanol spray on contaminated surfaces is maintained, even in cases of face mask contamination with dietary proteins, saliva, cosmetics, and other contaminants. Furthermore, the bactericidal effect also depends on the surfaces being sprayed.

We investigated the effect of sunlight on the viability of bacteria inoculated on face mask pieces placed at various locations inside or outside a car for 1 h. On the cloudy July day, the face mask pieces placed with the inoculated side face-up and face-down on the hood and outside the sunshade in the car did not survive the direct exposure to sunlight. When the same experiment was performed in November, viable bacteria were detected on the face mask pieces under all conditions (Fig. 7). The bacteria on the inoculated face mask pieces that were placed in an incubator at 55 °C for 1 h remained viable. In the cloudy July experiments, the bactericidal effect was observed even on the hood at a temperature lower than 55 °C (average 44.6 °C), suggesting that the UV rays contained in the sunlight reduced bacterial viability. It was reported that 10 min of UVC irradiation with a UV disinfection device on the surface of a surgical facemask infected with *S aureus* showed a bactericidal efficiency > 99.9999% (> 6 log)[22]. Similarly, when the back side of the facemask was exposed to UVC irradiation, it penetrated the mesh of the face mask to reach hidden bacteria[22], and these results are supported by our study findings. UV irradiation has no significant effect on the physical properties of the filter layer of face masks and is effective for face mask reuse[43]. This suggests that the face masks can be disinfected by exposure to strong UV rays in sunlight during summer.

Our assessment of face masks after washing showed that the water repellency was weakened. Washing face masks made from nonwoven fabrics not only weakens the water-repellent effect but also removes static electricity from the fibers[44]. Therefore, after being washed, the nonwoven layer can only filter particles by mechanical interaction. Since the passage of bacteria is inhibited by the meltblown nonwoven fabric in the center of the face mask[45], the particle collection efficiency of the face mask as well as its structure is critical. Furthermore, it was reported that spraying with 70% ethanol for 10 min until saturated or heating at 70 °C for 60 min reduced the particle collection efficiency of face masks[36]. Our measurement of the number of 0.3 µm particles (piece/L; number of particles per liter) that penetrated the treated face masks revealed that washing, 70% ethanol spraying, washing + 70% ethanol spraying, heat treatment, and leaving face masks outside the car in direct sunlight blocked the passage of 0.3 µm particles through the mask by > 80% (Fig. 8C), suggesting that these methods are effective for using the face masks under hygienic conditions. These results suggest that sealing masks after 70% ethanol spraying and direct exposure to sunlight are useful methods for bacterial disinfection and can be applied to effective face mask disinfection at home.

## Conclusion

Face masks are contaminated by bacteria during use or storage in daily life. In this study, we performed various experiments to explore the factors involved in bacterial growth on face masks and the disinfection methods for bacteria attached to the face masks. *S. aureu*s and/or *S. epidermidis* were inoculated on nonwoven face masks and treated under various conditions to evaluate changes in bacterial viability and bactericidal efficacy. The bacteria survived on the face masks under high-temperature conditions, and *S. aureu*s was found to coexist with *S. epidermidis* on the face masks. Bacterial viability increased in the presence of artificial saliva and 1% peptone as contaminants. The bactericidal effect of 70% ethanol spraying was maintained by closing up the mask after spraying but was attenuated on the contaminated face masks. Furthermore, direct exposure of the face masks to sunlight was suggested as a useful method for bacterial disinfection. Our findings clarified the behavior and survival of bacteria contaminating face masks, and new observations were made regarding methods used for their disinfection. These findings are important for decreasing the risk of bacterial contamination of masks in developing countries, where infectious diseases are becoming problematic due to the lack of masks. We plan to extend this area of research to develop better sterilization techniques, such as combining various treatment methods.

## Data availability

All data generated or analyzed in this study are included in this published article and its supplementary information file.

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## References

1. Singhal, T. A review of coronavirus disease-2019 (COVID-19). *Indian J. Pediatr.* **87**, 281–286. https://doi.org/10.1007/s12098-020-03263-6 (2020).
2. MacIntyre, C. R. & Wang, Q. Physical distancing, face masks, and eye protection for prevention of COVID-19. *Lancet* **395**, 1950–1951. https://doi.org/10.1016/S0140-6736(20)31142-9 (2020).
3. Cowling, B. J. & Leung, G. M. Epidemiological research priorities for public health control of the ongoing global novel coronavirus (2019-nCoV) outbreak. *Eurosurveillance* **25**, 2000110. https://doi.org/10.2807/1560-7917.ES.2020.25.6.2000110 (2020).
4. Leung, N. H. et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. *Nat. Med.* **26**, 676–680. https://doi.org/10.1038/s41591-020-0843-2 (2020).

5. Liang, M. et al. Efficacy of face mask in preventing respiratory virus transmission: A systematic review and meta-analysis. *Travel Med. Infect. Dis.* **36**, 101751. https://doi.org/10.1016/j.tmaid.2020.101751 (2020).
6. Krishna, S. & Miller, L. S. Host–pathogen interactions between the skin and *Staphylococcus aureus*. *Curr. Opin. Microbiol.* **15**, 28–35. https://doi.org/10.1016/j.mib.2011.11.003 (2012).
7. WHO. Coronavirus disease (COVID-19) advice for the public: When and how to use masks & WHO., 12 (2021). https://www.who.int/emergencies/diseases/novel-coronavirus-2019/advice-for-public/when-and-how-to-use-masks (2021).
8. Jefferson, T. et al. Physical interventions to interrupt or reduce the spread of respiratory viruses: Systematic review. *BMJ* **336**, 77–80. https://doi.org/10.1136/bmj.b3675 (2008).
9. Saunders-Hastings, P., Crispo, J. A., Sikora, L. & Krewski, D. Effectiveness of personal protective measures in reducing pandemic influenza transmission: A systematic review and meta-analysis. *Epidemics* **20**, 1–20. https://doi.org/10.1016/j.epidem.2017.04.003 (2017).
10. Christensen, G. J. M. & Brüggemann, H. Bacterial skin commensals and their role as host guardians. *Benef Microbes*. **5**, 201–215. https://doi.org/10.3920/BM2012.0062 (2014).
11. Marrack, P. & Kappler, J. The Staphylococcal enterotoxins and their relatives. *Science* **248**, 705–711. https://doi.org/10.1126/science.2185544 (1990).
12. Delanghe, L. et al. Cotton and surgical face masks in community settings: Bacterial contamination and face mask hygiene. *Front. Med.* **8**, 732047. https://doi.org/10.3389/fmed.2021.732047 (2021).
13. Peters, A. et al. Decontaminating N95/FFP2 masks for reuse during the COVID-19 epidemic: A systematic review. *Antimicrob. Resist. Infect. Control*. **10**, 1–25. https://doi.org/10.1186/s13756-021-00993-w (2021).
14. Roberts, K. P. et al. Increased personal protective equipment litter as a result of COVID-19 measures. *Nat. Sustain.* **5**, 272–279. https://doi.org/10.1038/s41893-021-00824-1 (2022).
15. Allison, A. L. et al. The impact and effectiveness of the general public wearing masks to reduce the spread of pandemics in the UK: A multidisciplinary comparison of single-use masks versus reusable face masks. *UCL Open. Environ.* **3**, e022. https://doi.org/10.14324/111.444/ucloe.000022 (2021).
16. Shimamura, Y., Kidokoro, S. & Murata, M. Survey and properties of *Staphylococcus aureus* isolated from Japanese-style desserts. *Biosci. Biotechnol. Biochem.* **70**, 1571–1577. https://doi.org/10.1271/bbb.50617 (2006).
17. Lee, L. Y. K. et al. Practice and technique of using face mask amongst adults in the community: A cross-sectional descriptive study. *BMC Public. Health* **20**, 948. https://doi.org/10.1186/s12889-020-09087-5 (2020).
18. Park, A. M. et al. Bacterial and fungal isolation from face masks under the COVID-19 pandemic. *Sci. Rep.* **12**, 11361. https://doi.org/10.1038/s41598-022-15409-x (2022).
19. Spigariolo, C., Giacalone, B., Nazzaro, S. & Maskne, G. The epidemic within the pandemic: From diagnosis to therapy. *J. Clin. Med.* **11**, 618. https://doi.org/10.1016/j.ijantimicag.2020.105955 (2022).
20. Pascoe, M. J. et al. Dry heat and microwave-generated steam protocols for the rapid decontamination of respiratory personal protective equipment in response to COVID-19-related shortages. *J. Hosp. Infect.* **106**, 10–19. https://doi.org/10.1016/j.jhin.2020.07.008 (2020).
21. Wigginton, K. R. et al. Validation of N95 filtering facepiece respirator decontamination methods available at a large university hospital. *Open. Forum Infect. Dis.* **8**, ofaa610. https://doi.org/10.1093/ofid/ofaa610 (2021).
22. Teo, J. Y. et al. Exploring reusability of disposable face masks: Effects of disinfection methods on filtration efficiency, breathability, and fluid resistance. *Glob Chall.* **5**, 2100030–2100030. https://doi.org/10.1002/gch2.202100030 (2021).
23. Margarucci, L. M. et al. Photocatalytic treatments for personal protective equipment: Experimental microbiological investigations and perspectives for the enhancement of antimicrobial activity by micrometric $TiO_2$. *Int. J. Environ. Res. Public. Health*. **18**, 8662. https://doi.org/10.3390/ijerph18168662 (2021).
24. Jones, R. M. & Rempel, D. Standards for surgical respirators and masks: Relevance for protecting healthcare workers and the public during pandemics. *Ann. Work Expo Health*. **65**, 495–504. https://doi.org/10.1093/annweh/wxab008 (2021).
25. Jang, E. S. & Kang, C. W. Do face masks become worthless after only one use in the COVID-19 pandemic? *Infect. Chemother.* **52**, 583. https://doi.org/10.3947/ic.2020.52.4.583 (2020).
26. Zhang, R., Li, Y., Zhang, A. L., Wang, Y. & Molina, M. J. Identifying airborne transmission as the dominant route for the spread of COVID-19. *Proc. Natl. Acad. Sci. USA*. **117**, 14857–14863. https://doi.org/10.1073/pnas.2009637117 (2020).
27. Andrews, W. H. Methods for recovering injured classical enteric pathogenic bacteria (*Salmonella*, *Shigella*, and enteropathogenic *Escherichia coli*) from foods. Injured Index and Pathogenic Bacteria. 55–113 (CRC, 2024).
28. Shao, L., Zhao, Y., Zou, B., Li, X. & Dai, R. Recovery and virulence factors of sublethally injured *Staphylococcus aureus* after ohmic heating. *Food Microbiol.* **102**, 103899. https://doi.org/10.1016/j.fm.2021.103899 (2022).
29. Shaila, M., Pai, G. P. & Shetty, P. Salivary protein concentration, flow rate, buffer capacity and pH estimation: A comparative study among young and elderly subjects, both normal and with gingivitis and periodontitis. *J. Indian Soc. Periodontol.* **17**, 42–46. https://doi.org/10.4103/0972-124X.107473 (2013).
30. Dekio, S. & Jidoi, J. Effect of serum albumin on growth of *Staphylococcus aureus* in synthetic tissue culture medium. *J. Dermatol.* **10**, 505–508. https://doi.org/10.1111/j.1346-8138.1983.tb01172.x (1983).
31. Ledger, E. V., Mesnage, S. & Edwards, A. M. Human serum triggers antibiotic tolerance in *Staphylococcus aureus*. *Nat. Commun.* **13**, 2041. https://doi.org/10.1038/s41564-019-0627-y (2022).
32. Wang, X., Liu, M., Yu, C., Li, J. & Zhou, X. Biofilm formation: Mechanistic insights and therapeutic targets. *Mol. Biomed.* **4**, 49. https://doi.org/10.1186/s43556-023-00164-w (2023).
33. Ghosh, S. et al. Permanent, antimicrobial coating to rapidly kill and prevent transmission of bacteria, fungi, influenza, and SARS-CoV-2. *ACS Appl. Mater. Interfaces* **14**, 42483–42493. https://doi.org/10.1021/acsami.2c11915 (2022).
34. Wißmann, J. E. et al. Persistence of pathogens on inanimate surfaces: A narrative review. *Microorganisms* **9**, 343. https://doi.org/10.3390/microorganisms9020343 (2021).
35. Lowe, J. J. et al. N95 filtering facepiece respirator ultraviolet germicidal irradiation (UVGI) process for decontamination and reuse. *Nebraska Medicine* **19** (2020). https://www.nebraskamed.com/sites/default/files/documents/covid-19/n-95-decon-process.pdf.
36. Fischer, R. J. et al. Effectiveness of N95 respirator decontamination and reuse against SARS-CoV-2 virus. *Emerg. Infect. Dis.* **26**, 2253. https://doi.org/10.3201/eid2609.201524 (2020).
37. Xiang, Y., Song, Q. & Gu, W. Decontamination of surgical face masks and N95 respirators by dry heat pasteurization for one hour at 70°C. *Am. J. Infect. Control* **48**, 880. https://doi.org/10.1016/j.ajic.2020.05.026 (2020).
38. Zulauf, K. E. et al. Microwave-generated steam decontamination of N95 respirators utilizing universally accessible materials. *mBio* **11**, 00997. https://doi.org/10.1128/mBio.00997-20 (2020).
39. Ma, Q. X. et al. Decontamination of face masks with steam for mask reuse in fighting the pandemic COVID-19: Experimental supports. *J. Med. Virol.* **92**, 1971. https://doi.org/10.1002/jmv.25921 (2020).
40. Li, D. F. et al. Steam treatment for rapid decontamination of N95 respirators and medical face masks. *Am. J. Infect. Control* **48**, 855–857. https://doi.org/10.1016/j.ajic.2020.05.009 (2020).
41. Sahu, P. K. et al. Surface sterilization for isolation of endophytes: Ensuring what (not) to grow. *J. Basic. Microbiol.* **62**, 647–668. https://doi.org/10.1002/jobm.202100462 (2022).
42. Lin, T. H., Tang, F. C., Hung, P. C., Hua, Z. C. & Lai, C. Y. Relative survival of *Bacillus subtilis* spores loaded on filtering facepiece respirators after five decontamination methods. *Indoor Air* **28**, 754–762. https://doi.org/10.1111/ina.12475 (2018).

43. Ye, S. et al. Fast and deep disinfection for face masks recycle using vacuum ultraviolet irradiation. *J. Clean. Prod.* **368**, 133221. https://doi.org/10.1016/j.jclepro.2022.133221 (2022).
44. Cheng, S., Hao, W., Wang, Y., Wang, Y. & Yang, S. Commercial janus fabrics as reusable facemask materials: A balance of water repellency, filtration efficiency, breathability, and reusability. *ACS Appl. Mater. Interfaces* **14**, 32579–32589. https://doi.org/10.1021/acsami.2c09544 (2022).
45. Jeong, S. B. et al. Evaluation of survival rates of airborne microorganisms on the filter layers of commercial face masks. *Indoor Air* **31**, 1134–1143. https://doi.org/10.1111/ina.12816 (2021).

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## Author contributions
YS, HT, and SM designed the experiments. YS, MO, MS, RO, MK and KS conducted the experiments. YS and SM wrote the manuscript. All authors contributed to data analysis and the review of the manuscript.

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## Declarations

## Competing interests
Although HT is an employee of Chubu Electric Power Co., Inc., which provided funding for this work, this study was conducted for nonprofit purposes. No inappropriate data have been created based on the existence of a financial relationship with the company. Other author have no competing interests.

## Additional information
**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-09204-7.

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