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Effect of five decontamination methods on face masks and filtering facepiece respirators contaminated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract

Introduction. In the context of the global pandemic due to SARS-CoV-2, procurement of personal protective equipment during the crisis was problematic. The idea of reusing and decontaminating personal surgical masks in facilities was explored in order to avoid the accumulation of waste and overcome the lack of equipment.

Hypothesis. Our hypothesis is that this work will show the decontamination methods assessed are effective for bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Aim. We aim to provide information about the effects of five decontamination procedures (UV treatment, dry heat, vaporized H_2O_2, ethanol treatment and blue methylene treatment) on *S. aureus* and *P. aeruginosa*. These bacteria are the main secondary bacterial pathogens responsible for lung infections in the hospital environment.

Methodology. The surgical masks and the filtering facepiece respirators were inoculated with two bacterial strains (*S. aureus* ATCC 29213 and *P. aeruginosa* S0599) and submitted to five decontamination treatments: vaporized H_2O_2 (VHP), UV irradiation, dry heat treatment, ethanol bath treatment and blue methylene treatment. Direct and indirect microbiology assessments were performed on three positive controls, five treated masks and one negative control.

Results. The five decontaminations showed significant ($P<0.05$) but different degrees of reductions of *S. aureus* and *P. aeruginosa*. VHP, dry heat treatment and ethanol treatment adequately reduced the initial contamination. The 4 min UV treatment allowed only a reduction to five orders of magnitude for face mask respirators. The methylene blue treatment induced a reduction to two orders of magnitude.

Conclusions. The three methods that showed a $\log_{10}$ reduction factor of 6 were the dry heat method, VHP and ethanol bath treatment. These methods are effective and their establishment in the medical field are easy but require economic investment.

INTRODUCTION

Due to the global severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, one of the most essential measures has been the implementation of wearing of masks in public places. During the lockdown, the stock of masks quickly ran out. Many scientists have tried to find solutions to the reuse of masks [N95 filtering face mask respirators (FFRs) and surgical masks

(SMs)]. The consequences of medical waste due to face masks and filtering facepiece respirators is now considerable [1]. Just for Belgium, daily face mask use is 6804547 and annual use is 2484 million [2].

During the pandemic, the primary focus of mask disinfection was removal of SARS-CoV-2; however, in the medical field, many opportunistic bacteria remain and can be dangerous if disinfection is not done carefully. In the hospital environment, it is important to inactivate viruses, but opportunistic bacteria must also be considered. The two most common bacteria in the hospital environment are *Staphylococcus aureus* and *Pseudomonas aeruginosa* [3, 4]. These bacteria are classified as class 2 pathogens for humans and animals. *P. aeruginosa* is responsible for nosocomial infections in immunocompromised or critically ill patients [5]. *S. aureus* is responsible for skin infections, bacteraemia, endocarditis, pneumonia and food poisoning [6]. Multiple antibiotic resistance is progressively becoming more common in *S. aureus*. Methicillin-resistant *S. aureus* (MRSA) causes outbreaks in hospitals [6]. Moreover, *S. aureus* shows resistance to disinfectants, for example quaternary ammonium formulations [7]. Moreover, *S. aureus* can persist for 7 days to 7 months on dry surfaces [7]. *P. aeruginosa* shows intrinsic antibiotic resistance because of the low permeability of its outer membrane [5].

According to ISO 11138, in order to validate a mask decontamination method, it must reduce the biological indicator by a factor of 10^6 [8, 9]. By validating and proving this reduction factor of 10^6, this is indirectly enough to validate a sufficient reduction in the biological indicator given that SARS-CoV-2 is much less resistant to disinfection methods [8].

Several sterilization methods have been studied and validated on viruses in recent years including H1N1, H5N1, SARS-CoV-2, MERS-CoV and SARS-CoV [10–14].

Regarding UV-C decontamination, this treatment for 1 min reduced *S. aureus* by a factor of 3.2×10^6 and *P. aeruginosa* by a factor of 2.5×10^5 on a pre-contaminated surface [15].

Hydrogen peroxide (H_2O_2) treatment involves the use of an oxidizing agent which produces reactive hydroxyl radicals which damage DNA, membrane lipids and other cell components [16, 17]. Two systems exist: aerosolized hydrogen peroxide and H_2O_2 vapour systems. Aerosolized hydrogen peroxide treatment led to a reduction in *S. aureus* of $>10^4$ using a carrier test in a hospital room [16]. H_2O_2 vapour systems showed a $\log_{10}$ reduction factor of 6 with *Geobacillus stearothermophilus* and by a factor of 3.2×10^4 with MRSA [16, 18].

The dry heat method consists of applying heat treatment in an oven for a certain time and at a certain temperature. Dry heat treatment was effective in culture and hydrated biofilm as a function of temperature and time (reduction from 1.7×10^6 to 2.0×10^8 CFU per control coupon of *S. aureus*) but was not effective on a dry surface biofilm (a $\log_{10}$ reduction by a factor <2) [19].

A fourth decontamination method is the utilization of ethanol 70%, which is often used for disinfection of thermometers or medical endoscopes [20]. After exposure to ethanol 85% for 30 s, a reduction of *P. aeruginosa* by a factor of 10^6 and of *S. aureus* of 2.0×10^5 was observed [21].

Blue methylene is used in the medical field as an antiseptic in the livestock and aquaculture industries and as a treatment for several diseases [22]. No studies have been published on its effects on contaminated surfaces and its effects on the bacteria.

To validate these five decontamination methods for SARS-CoV-2 on SMs and FFRs, a study was carried out in parallel for the same project at the University of Liege with porcine respiratory coronavirus with a dry heat method, UV method and vaporized H_2O_2 (VHP) [23] and methylene blue photodynamic treatment [24]. Another study was carried out in parallel using the same protocols of decontamination and using filtration efficiency tests and breathability tests after reusing the mask [25].

This study, carried out in parallel with the previous ones [23–25], made it possible to evaluate the efficiency of five decontamination methods (UV decontamination, VHP, methylene blue treatment, dry heat treatment and ethanol treatment) on two opportunistic bacteria, one Gram-positive strain, *S. aureus* ATCC 29213, and one Gram-negative strain, *P. aeruginosa* S0599, commonly present in the medical environment.

METHODS

Bacterial strains

Two bacterial strains were used for this study, *S. aureus* ATCC 29213 and *P. aeruginosa* S0599 (internal reference). These bacteria were stored at −80 °C in glycerol-BHI (VWR 24388.295; Brain Heart Infusion, Oxoid CM1032). Bacterial strains were revived by inoculating 10 µl into BHI broth (Oxoid) and incubated at 37 °C overnight and under agitation (Unimax 2010; Heidolph) to obtain 10^8–10^9 CFU ml^{-1}. Ten microlitres of the solution was spread on plate count agar (PCA, 3564475; Bio-Rad) to control the purity of the bacterial strains and the absence of contamination.

Surgical masks and face mask filtering respirators

All FFRs and SMs, commonly used by the healthcare community in Belgium at the time of the study, were supplied by the Department of the Hospital Pharmacy, University Hospital Centre of Liege (Sart-Tilman): KN95 FFR – Guangzhou Sunjoy Auto Supplies (2020 N 26202002240270) and surgical mask (Type II) – Hangzhou Sunten Textile (SuninCare, Protect Plus). SMs and FFRs were verified to be from the same respective manufacturing lot to minimize any lot-to-lot variation and to ensure consistency during future respirability and filtration performance testing. FFRs consisted of four layers of polypropylene, specifically two outer spunbound structures, an intermediate meltblown layer and an inner spunbound layer. SMs were composed of three layers of polypropylene, with an outer and inner layer of spunbound polypropylene encasing a meltblown polypropylene barrier. Supplementary elements were described by Ludwig-Begall and colleagues [23].

Inoculation of SMs and FFRs

Before inoculation, SMs and FFRs were marked with a pencil on two squares of $4 \, cm^2$ on each mask, one on the left and the other on the right. For each decontamination method, several masks were used: one treatment control SM and FFR (not contaminated but treated, $n=1$), five treated SMs and FFRs (contaminated and treated, $n=5$), three positive control SMs and FFRs (contaminated and not treated, $n=3$) and one negative control SM and FFR (not contaminated and not treated, $n=1$). In total, $100 \, \mu l$ of the suspension at $10^8–10^9 \, CFU \, ml^{-1}$ of prepared bacteria as described above was injected in the middle of the square coupons between the layers using an insulin syringe and needle (BD Medical). The masks were dried for 20 min and then individually packaged in appropriate sterile containers. All masks, including those not treated in decontamination facilities, were transported in an icebox and the temperature was maintained at $4 \, °C$. The suppliers handled the masks with sterile instruments before and after decontamination to avoid potential bacterial contamination.

UV germicidal irradiation

SMs and FFRs were individually irradiated using an LS-AT-M1 device (LASEA) equipped with four UV-C lamps of 5.5 W (@ UV-C). Hung vertically on a metal frame, SMs and FFRs were inserted into a safety enclosure. A 2 min UV-C treatment (SMs and FFRs) led to a fluence of $2.6 \, J \, cm^{-2}$ per mask ($1.3 \, J \, cm^{-2}$ per side), while 4 min UV-C irradiation (SMs and FFRs) led to a fluence of $5.2 \, J \, cm^{-2}$ per mask. Following irradiation, SMs and FFRs were unloaded and placed in individual bags [23]. This treatment did not alter the filtration capacity of the mask [1, 7, 15].

Vaporized H$_2$O$_2$

SMs, FFRs and a biological indicator (3M Attest) were placed in individual Mylar/Tyvek pouches. VHP treatment was performed with a V-PRO Max Sterilizer (Steris) which uses 59% liquid H_2O_2 to generate hydrogen peroxide vapour. A 28 min non-lumen cycle was used, consisting of 2 min 40 s conditioning ($5 \, g \, min^{-1}$), 19 min 47 s decontamination ($2.2 \, g \, min^{-1}$) and 7 min 46 s aeration. Peak VHP concentration was 750 ppm [23]. After 50 VHP cycles, this treatment did not alter the filtration capacity [10, 17]

Dry heat

SMs and FFRs, hung horizontally on a metal frame, were inserted into an electrically heated 20 EM01 V1.4 vessel (MSteryl; AMB Ecosteryl) for treatment at temperatures of $102 \, °C$ for 60 min. Temperatures inside the heated vessel were recorded throughout to ensure correct exposure conditions. After termination of the treatment cycle, SMs and FFRs were allowed to cool for 15 min, then individually bagged and placed in a cold box.

Methylene blue treatment

Methylene blue was obtained from Sigma (M9140) or ThermoFisher Scientific (J60823). Stock solutions were prepared using ultrapure distilled water. After bacterial inoculation and 20 min of drying, methylene blue (MB) treatment was applied. Intact inoculated masks were sprayed with 7–8 ml of $10 \, \mu M$ MB and allowed to dry for 30 min protected from light before exposure to 12500 lux of red light for 30 min. We applied 12500 lux of red light, broad-spectrum light and red-light sources, based on the local laboratory's method for administering adequate light energy for MB activation. A light box of our own design containing horticultural lamps was used for this study. Luminescence was verified using light meters in all laboratories (Cooke; Model CK-CL400) [24].

Ethanol bath treatment

After the masks were received, they were placed in an ultrasonic bath filled with 70% ethanol and 30% sterile distilled water. Two studies were done, the first one with 30 min of contact and the second with 5 min of contact. After 1 h of drying, the masks were placed inside the new sterile packaging.

Data analysis and statistics

Statistical analysis of differences between contaminated and treated masks with the five decontamination methods were performed using GraphPad Software Prism 8 and *P*-values were calculated by using a valid Mann–Whitney non-parametric test to compare all masks before and after treatment. The limit of significance was *P<0.05.

Microbiological enumerations

Upon receipt of decontaminated masks, both coupons were cut to fit the designated areas of the mask. For direct enumeration, one coupon was placed into a tube containing 3 ml of BHI broth, at room temperature for 10 min and vortexed every 3 min. Suspensions from contaminated treated masks were spread on three agar types: PCA, Rapid *Escherichia coli* (Bio-Rad; 3555299) and Baird Parker (Bio-Rad; 3564814) with a spiral inoculator (Easyspiral; Interscience). The 1000× dilution of the untreated contaminated was also spread on PCA, Rapid *E. coli* and Bair Parker. The Petri dishes were then incubated at 37 °C for 24 h. For indirect enrichment, the other coupon was placed in a tube containing 10 ml BHI broth. After 24 h at 37 °C, if there was no growth of the bacteria that we were looking for on Petri dishes, the indirect enrichment solutions were spread to detect low levels of contamination (up to 0.25 CFU cm^{-2}). The treatment efficacy was calculated by dividing the average level of contamination of the masks after decontamination by the level obtained for the untreated contaminated masks.

RESULTS

Mean positive control concentrations (*n*=21 of each mask contaminated not treated) were 2.1×10^7 CFU 4 cm^{-2} in SMs and 2.0×10^7 CFU 4 cm^{-2} in FFRs for *P. aeruginosa*. Mean positive control concentrations (*n*=21 of each mask contaminated not treated) were 2.9×10^7 CFU 4 cm^{-2} in SMs and 4.0×10^7 CFU 4 cm^{-2} in FFRs for *S. aureus*. The detection limit of direct enumeration was 30 CFU 4 cm^{-2} (LOD2) and the detection limit of enrichment was 1 CFU 4 cm^{-2} (LOD1). If no bacteria were detected in the Petri dish after direct enumeration and the enrichment was positive, the concentration of these results was below the LOD2. If no bacteria were detected in the Petri dish after enrichment, the concentration of these results was below LOD1 (see raw data in Supplementary Material S1, available in the online version of this article).

All treatments showed significant reductions (see Fig. 1) and showed a different ratio of reductions (see Table 1).

UV germicidal irradiation

In the first experiment with 2 min of UV-C treatment, we observed a reduction to less than three orders of magnitude for SM (9.6×10^2 for *P. aeruginosa* and 3.5×10^2 for *S. aureus*) and a reduction to five orders of magnitude for FFRs (8.0×10^4 for *P. aeruginosa* and 1.7×10^5 for *S. aureus*) (see Fig. 1, Table 1). Regarding the second experiment with 4 min of UV-C treatment, a reduction to less than six orders of magnitude (4.5×10^5 for SMs and 2.7×10^5 for FFRs) was observed (see Fig. 1, Table 1). The 4 min treatment allowed a reduction to five orders of magnitude for FFRs, but this was lower than the sterilization requirements (see Table 1, Fig. 1).

Vaporized H$_2$O$_2$

VHP treatment induced a significant reduction to six orders of magnitude (8.1×10^6 and 1.3×10^7) for *P. aeruginosa* and a significant reduction to seven orders of magnitude (3.2×10^7 and 1.0×10^7) for *S. aureus* in SMs and FFRs (see Table 1, Fig. 1). This technical treatment was sufficient to reduce the initial contamination and to achieve the six orders of magnitude reduction (see Table 1, Fig. 1).

Dry heat

With this time–temperature pair, a reduction to six orders of magnitude for *P. aeruginosa* (5.0×10^6 for SM and 2.8×10^6 for FFRs) and a reduction to six orders of magnitude for *S. aureus* (1.0×10^6 for SM and FFRs) were observed. This treatment was sufficient to reduce the initial contamination and achieve a log$_{10}$ reduction by a factor 6 (see Table 1, Fig. 1).

Methylene blue treatment

Methylene blue treatment induced a reduction to two orders of magnitude (1.3×10^2 for *P. aeruginosa* and 2.1×10^2 for *S. aureus*) (see Table 1, Fig. 1).

Ethanol bath treatment

Concerning the efficacy of ethanol bath treatment for 30 min on *P. aeruginosa*, a reduction to six orders of magnitude (3.8×10^6 for SMs and for FFRs) was observed. Regarding the reduction of *S. aureus*, a reduction to six orders of magnitude (5.0×10^6 and 1.3×10^7 for SMs and for FFRs) was observed (see Table 1, Fig. 1). Treatment for 5 min led to a decrease to seven orders of magnitude. These treatments achieved a log$_{10}$ reduction by a factor of 6.
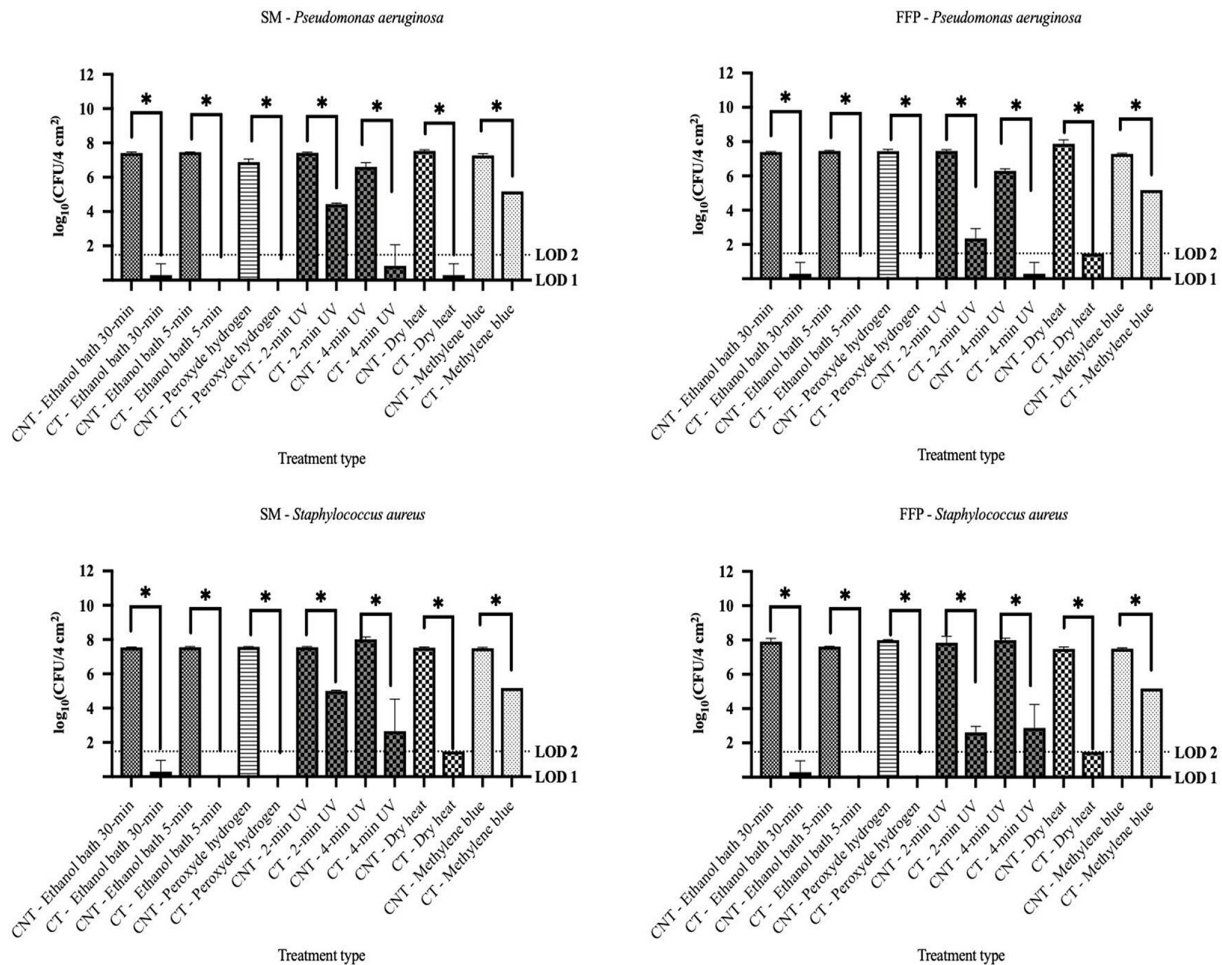
Fig. 1. Concentration of *P. aeruginosa* and *S. aureus* before and after treatment in SMs and FFRs expressed in $\log_{10}$ (CFU $4\,cm^{-2}$). *P*-values were calculated by using a valid Mann–Whitney non-parametric test to compare all the treatments before and after treatment where *$P<0.05$. LOD1: limit of detection fixed at 1 CFU $4\,cm^{-2}$ and LOD2: limit of detection fixed at 10 CFU $4\,cm^{-2}$. SM: surgical mask, FFR: filtering facepiece respiratory.

Data analysis and statistics

P-values calculated by a valid Mann–Whitney non-parametric test to compare all masks before and after treatment are shown in Fig. 1 and Table 2.

No statistical analysis was performed to compare the decontamination treatments because the initial bacterial contamination was different between the experiments. Descriptive analyses are shown in Table 1.

DISCUSSION

All five decontaminations showed significant but different degrees of reductions of *S. aureus* and *P. aeruginosa*. The three methods that demonstrated the required 10^6 reduction factor were the dry heat method, ethanol bath treatment and VHP. These methods are effective and their establishment in the medical field are straightforward but require economic investment. Several studies have shown that after VHP treatment or dry heat treatment, masks can be reused, but contact with ethanol alters the fabric and the filtration capacity [10, 17, 26, 27]. Other methods used are blue methylene photodynamic treatment and UV treatment. They showed significant reductions, but not the 10^6 reduction required for a sterilization method in the medical field.

Dry heat treatment showed a significant reduction in all experiments. With the time–temperature couple, a reduction to more than six orders of magnitude (10^6) was observed with both types of mask against *P. aeruginosa* and *S. aureus*. This result was similar to the results reported by Ludwig-Begall and colleagues on porcine respiratory coronavirus, a $\log_{10}$ reduction factor of 4 ($TCID_{50}\,ml^{-1}$) [23]. Treatment of 102 °C for 60 min was sufficient to decontaminate bacteria and a virus in the medical field or in industry. Xiang *et al.* applied this treatment at 60 and 70 °C for 1 h on masks (SMs and FFRs) previously contaminated with seven

Table 1. Ratio of decontamination [$\log_{10}$ (CFU 4 cm^{-2})] in five decontamination methods on FFRs and SMs

The ratio of decontamination was calculated by dividing the mean of CNT (mask contaminated not treated, *n*=3) and the mean of CT (mask contaminated treated, *n*=5)

Treatments	Bacteria	Mean CNT ($\log_{10}$ CFU 4 cm^{-2})		Ratio of decontamination ($\log_{10}$ reduction)		Mean CT ($\log_{10}$ CFU 4 cm^{-2})	
		SMs (*n*=3)	FFRs (*n*=3)	SMs	FFRs	SMs (*n*=5)	FFRs (*n*=5)
Ethanol bath 30 min treatment	*P. aeruginosa*	7.4±0.05	7.4±0.03	6.6	6.6	0.3±0.66	0.3±0.66
	S. aureus	7.5±0.02	7.9±0.20	6.7	7.1	0.3±0.66	0.3±0.66
Ethanol bath 5 min treatment	*P. aeruginosa*	7.5±0.01	7.55±0.02	7.5	7.6	0±0.00	0±0.00
	S. aureus	7.5±0.04	7.6±0.03	7.5	7.6	0±0.00	0±0.00
Hydrogen peroxide treatment	*P. aeruginosa*	6.9±0.18	7.5±0.09	6.9	7.5	0±0.00	0±0.00
	S. aureus	7.6±0.03	8.0±0.03	7.6	8.0	0±0.00	0±0.00
2 min UV treatment	*P. aeruginosa*	7.4±0.03	7.4±0.08	3.0	4.9	4.4±0.06	2.3±0.56
	S. aureus	7.5±0.04	7.8±0.37	2.5	5.2	5.0±0.03	2.6±0.35
4 min UV treatment	*P. aeruginosa*	6.6±0.26	6.3±0.12	4.6	5.5	0.8±1.23	0.3±0.66
	S. aureus	8.0±0.13	8.0±0.10	4.7	5.4	2.6±0.12	2.9±0.12
Dry heat	*P. aeruginosa*	7.5±0.08	7.5±0.22	6.7	6.4	0.3±1.86	1.47±1.37
	S. aureus	7.6±0.04	8.0±0.11	6	6	1.47±0.00	1.47±0.00
Blue methylene treatment	*P. aeruginosa*	7.3±0.10	7.3±0.04	2.1	2.1	5.2±0.00	5.2±0.00
	S. aureus	7.5±0.05	7.5±0.05	2.3	2.3	5.2±0.00	5.2±0.00

bacteria: *E. coli*, *S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Corynebacterium pseudodiphteriae* and *Candida albicans*. This treatment was effective on these bacteria [11]. Reusing masks after five cycles of dry heat treatment did not negatively affect the efficiency of bacterial filtration [25]. This treatment showed decreased efficiency in respirator filtration after one or three cycles of decontamination [25]. With regard to visual appearance, this treatment showed a brown discoloration of FFR elastic straps and detachment of the metal noseband [25]. This method has been validated for reuse of decontaminated N95 FFRs [27].

The VHP treatment showed a significant reduction in all experiments. The reductions were greater than six orders of magnitude with both bacteria. After VHP treatment, a reduction to five orders of magnitude (TCID$_{50}$ ml^{-1}) on porcine respiratory coronavirus was observed by Ludwig-Begall and colleagues [23]. Their study demonstrated that VHP is efficient for this virus and bacteria. In another study, N95 FFRs were inoculated with heat-resistant *Geobacillus stearothermophilus* spores and VHP was applied. No bacteria were found after 4 days of enrichment [17]. This treatment was sufficient to decontaminate *G. stearothermophilus* spores [10, 17, 18]. This study used the most heat-resistant bacteria and showed that the treatment reduced the biological indicator by a factor of 10^6 in a contaminated mask. In another study, FFRs contaminated with SARS-CoV-2 and *S. aureus* were subjected to treatment with hydrogen peroxide plasma and, after this treatment, there was no trace of virus or bacteria [28]. This study demonstrated that VHP is efficient with regard to SARS-CoV-2 and on *S. aureus*. Twenty cycles exhibited no visible degradation of masks [17]. Hydrogen peroxide did not alter the physical appearance of the N95 FFR [29]. Reusing the masks after five cycles of VHP treatment did not negatively influence the efficiency of bacterial filtration or the visual appearance of the mask but showed a decrease in respirator filtration efficiency after one or three cycles of decontamination [25]. These studies show that VHP can be used in the long term. However, the impact of peroxide on sensitive skin needs further study.

The 2 min UV treatment indicated a significant but different degrees of reduction. The UV treatment showed a reduction to five orders of magnitude (1.0×10^5) on FFRs and four orders of magnitude (1.0×10^4) on SMs with the 4 min treatment. Previous studies showed a similar $\log_{10}$ reduction (of 5 TCID$_{50}$ ml^{-1}) when subjecting porcine respiratory coronavirus to 2 min of UV treatment on SMs and a similar $\log_{10}$ reduction (4 TCID$_{50}$ ml^{-1}) when subjecting coronavirus to 4 min of UV treatment on FFRs [23]. This treatment showed significant results, but below the required $\log_{10}$ reduction factor of 6. After UV exposure for 5 min, an N95 FFR that had previously been contaminated with *B. subtilis* exhibited no colonies on Petri dishes [10, 30]. Cadnum and colleagues exhibited that when three different kinds of FFRs were submitted to UV-C treatment, the initial contamination was reduced by a factor of 10^6 on MRSA after 31 min or one cycle of 22 min treatment [31]. This study showed

Table 2. Statistical analysis of differences between contaminated masks and masks treated with five decontamination methods performed using GraphPad Prism 8 and *P*-values calculated by using a Mann–Whitney non-parametric test to compare all the treatments before and after treatment

Treatment	Bacteria	Mann–Whitney (*P*-value)		Mann–Whitney (*P*-value)
		SM	FFRs	
Ethanol bath 30 min treatment	*P. aeruginosa*	0.0179 *	0.0179 *	0.0286 *
	S. aureus	0.0179 *	0.0179 *	
Ethanol bath 5 min treatment	*P. aeruginosa*	0.0179 *	0.0179 *	0.0286 *
	S. aureus	0.0179 *	0.0179 *	
Hydrogen peroxide treatment	*P. aeruginosa*	0.0179 *	0.0179 *	0.0286 *
	S. aureus	0.0179 *	0.0179 *	
2 min UV treatment	*P. aeruginosa*	0.0357 *	0.0179 *	0.0286 *
	S. aureus	0.0357 *	0.0357 *	
4 min UV treatment	*P. aeruginosa*	0.0357 *	0.0179 *	0.0286 *
	S. aureus	0.0179 *	0.0357 *	
Dry heat	*P. aeruginosa*	0.0179 *	0.0179 *	0.0286 *
	S. aureus	0.0179 *	0.0179 *	
Blue methylene treatment	*P. aeruginosa*	0.0179 *	0.0179 *	0.0286 *
	S. aureus	0.0179 *	0.0179 *	

*$P<0.05$.

better results with a longer period of UV-C treatment. Reusing the masks after five cycles of UV treatment did not negatively affect the efficiency of bacterial filtration, respirator efficiency or visual appearance of the mask [25].

The ethanol batch treatment indicated a significant reduction of more than six orders of magnitude (3.2×10^6) in all experiments. This treatment needs further investigation before using it in the field. Studies have shown that contact with ethanol altered the fabric and filtration capacity [28]. This treatment should be used occasionally and not over the long term.

The methylene blue treatment showed a limited, but significant ($P<0.001$) reduction to two orders of magnitude (1.0×10^2). This treatment was sufficient to reduce 2–4 $\log_{10}$ viral titres of three coronavirus strains after 5 min of light exposure and complete inactivation after 30 min of light exposure [24].

In this study, the bacteria were inoculated inside the mask between the layers of the mask not just on the top of the mask. This mode of inoculation reflects the fact that saliva droplets are absorbed inside the mask. This is the worst-case scenario and does not represent reality because the bacterial concentrations are high. The longer that you wear a mask, the more important bacterial contamination becomes [32], but bacterial contamination remained below 10^6 CFU ml^{-1} in this study [33]. A more in-depth investigation is needed to determine the total floral concentration in a mask worn for 4 or 8 h.

This study shows has some limitations. It is only valid for one type of bacterial strain of *S. aureus* and *P. aeruginosa*. These analyses focused on one type of FFP and SM. The ratio of decontamination was underestimated because many samples were below LOD1 before treatment and the mean was used for calculation.

During the pandemic, attention towards mask disinfection has centred on the virus (SARS-CoV-2) but in the medical field, many opportunistic bacteria are still present and can be dangerous if disinfection is not performed carefully.

In conclusion, this study, carried out in parallel with previous ones [23], made it possible to evaluate the efficiency of five decontamination methods on two opportunistic bacteria, one Gram-positive strain, *S. aureus* ATCC 29213, and one Gram-negative strain, *P. aeruginosa* S0599, commonly present in the medical environment. The three methods that demonstrated the required 10^6 reduction factor were the dry heat method, ethanol bath treatment and VHP. Several studies have shown that after VHP treatment or dry heat method, masks can be reused, although contact with ethanol altered the fabric and the filtration capacity [10, 17, 26, 27]. The ethanol treatment could be used once but not in the long term. Other methods are blue methylene photodynamic treatment and UV treatment. They showed significant reductions, but not the 10^6 reduction

required for a sterilization method in the medical field. Another study identified efficacy on bacteria with a longer time under UV-C [31]. The impact of peroxide on sensitive skin needs to be studied further. Future work will investigate the effects of a longer test period for UV-C treatment. Regarding methylene blue treatment, better homogenization and consistent quantity of blue methylene, greater quantity of methylene blue and an association with low dry heat treatment (<75 °C) will be studied. Hydrogen peroxide treatment and dry heat treatment can already be used in the medical field to reuse masks but require economic investment.

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