Evaluation of the microbiota and integrity of respirators reused by health professionals in a hospital environment

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Abstract

The shortage of PFF2, N95, and KN95 respirators and their equivalents for the respiratory protection of the population and health professionals during COVID-19 pandemic has driven the adoption of alternative measures to address the lack of personal protective equipment (PPE). The use of surgical masks, handmade masks, and even the prolonged use of respirators were some of the measures adopted in response to the high demand for these products, and their consequent shortage. In this context, the present study evaluated the microbiota and integrity of reused PFF2 respirators in the central sterile services department of a hospital. Respirators that had been used for 0 h, 12 h, 24 h, and 36 h were sampled for the inoculation and cultivation of fungi and bacteria and the identification of their microbiota. To assess the integrity of the respirators, a filtration efficiency assessment test was conducted of the respirators used for 36 h. The results obtained showed that the microbiota of the respirators comprised commensal fungi and bacteria from the oral and nasal regions of human beings. It was also found that after 36 h of use, the respirators did not demonstrate a decrease in filtration efficiency; that is, they retained their 97% filtration efficiency. Considering the findings regarding the presence and pathogenicity of microorganisms, it is possible that the reuse of respirators for up to 36 h does not harm the health of immunocompetent users. In terms of PPE efficiency, no compromises were evidenced.

Keywords: respiratory protection; PPE; efficiency; pandemic; aerosol
1. INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has plagued the world since 2019 and has made health authorities aware of the vulnerability of frontline health workers combating the severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) (Allison et al., 2020). The virus is predominantly transmitted via droplets of saliva; however, it can also be aerosolized from procedures that use liquids, such as the reprocessing of medical and hospital materials in sterilization centers (Howard, 2020; Madeira et al., 2015).

The central sterile services department (CSSD) of a hospital is responsible for collecting and sterilizing dirty or contaminated materials that have been used in a hospital environment (Madeira et al., 2015). During the sterilization process, aerosols can be dispersed from brushing materials, thereby requiring personal protective equipment (PPE) for the respiratory protection of health professionals working in these departments (Howard, 2020).

The current state of the art shows that the use of N95 respirators and their equivalents (PFF2, KN95, FFP2, P2, Korea 1st Class, and DS) is essential for the respiratory protection of professionals exposed to aerosols (Plana et al., 2021). The high demand for PPE since the emergence of the COVID-19 pandemic generated a worldwide shortage of these products. Consequently, alternatives for respiratory protection have been adopted in several parts of the world (Shubhanshu and Singh, 2021).

In view of the shortage of respirators and the urgency imposed on its requirement by the COVID-19 pandemic, the reuse of PPE has become inevitable. However, the establishment of reuse protocols to minimize risks to the user is necessary.

The objective of the present study was to evaluate the microbiota and integrity of PFF2 respirators that were reused and exposed to aerosols generated during the sterilization of medical
and hospital materials in the CSSD of a large hospital located in the State of Pernambuco, Brazil.

Fragments of respirators reused by the research participants were inoculated into petri dishes to identify fungi and bacteria present therein (Al-Maghlouth et al., 2004). A part of the reused respirators was subjected to a filtration efficiency assessment test to verify whether the reuse of PPE causes a partial loss of its efficiency. The washing the PPE and decontaminating it by certain methods can inactivate the filter, thereby impairing its electrostatic capture and compromising filtration (Bergman et al., 2010).
2 METHODS

The present study is experimental with an analytical approach. It was conducted in a large public hospital in the State of Pernambuco, Brazil. The hospital specializes in urgent and emergency care.

Due to the COVID-19 pandemic, the hospital created a dedicated urgent, emergency, and infirmary wards to care for its patients. These wards as well as other sectors of the hospital employ various types of materials that need to be reprocessed and sterilized in the CSSD.

The CSSD centralizes all medical and hospital material reprocessing demands. It operates for 24 h a day, 7 days a week, and is classified as a biological risk 3 area. This sector was selected for conducting the research since it constantly exposes its workers to aerosols that are produced during the sterilization of reprocessed materials.

Professionals from the hospital’s CSSD nursing team were eligible for the voluntary use of PFF2 respirators (Figure 1). This type of respirator is used in Brazil and is equivalent to N95 (USA), KN95 (China), FFP2 (European Union), P2 (Australia/New Zealand), Korea 1st Class (South Korea), and DS (Japan) respirators.

Model 9920H PFF2 respirators (3M do Brasil, Brazil) were used in this study. This model is certified by the Brazilian Ministry of Labor and Social Security (Certificate of Approval No. 17611) and approved by the country’s National Health Surveillance Agency (ANVISA; registration No. 80284930200). Each respirator has a mean mass of 8 g, is collapsible (two panels), non-valved, and has a bacteriological filtration efficiency (BFE) of >99%. Notably, a BFE >99% in PFF2 respirators does not guarantee 94% virus filtration efficiency (ASTM F2101, 2019).

The durations of the use of the respirators adopted in the current study were 0h, 12 h (1 shift), 24 h (2 shifts with an interval of 60 h), or 36 h (3 shifts with 2 intervals of 60 h each). For
obtaining samples for microbiological analysis, first the respirators and extended use kit were
delivered. The kit comprised a sterile container, identification clip, and a portion of silica gel and
it was used to store the respirators during the intervals between their usages. The samples were
then manipulated in a laminar flow hood. A total of 49 fragments of respirators, each one 10 cm²
in area, were obtained from the mask’s contact area with the user’s nasal and oral regions. Overall,
3 samples of respirators were obtained for the 0-h, 8 samples for the 12-h group, 14 samples for
the 24-h group, and 22 samples for the 36-h group.

To examine the integrity of the respirators after being reused for 36 h, the filtration
efficiency of 30 samples of the PFF2 respirators was measured. Each sample was 176 cm² in area,
which is equivalent to one side of a mask.

2.1 Assessment of the microbiota of respirators

The bacteria and fungi present in the respirators were cultured from the 10 cm² respirator
fragments. The fragments were rubbed without dilution on petri dishes. All experiments were
performed in triplicate. For the cultivation of bacteria, petri dishes containing 9mL blood agar were
used (gelatin peptone: 5.7g/L, meat extract: 3g/L, bacteriological agar: 15g/L, blood: 1mL/pH=
6.9/T=25°C). The cultures were kept at 37°C for 72 h (Cullimore, 2000). Fungi were cultured in
petri dishes containing 9mL Sabouraud agar medium with chloramphenicol (meat peptone: 10g/L,
glucose: 20g/L, chloramphenicol: 0.5g/L, bacteriological agar: 15g/L). The fungal cultures were
also kept at 37°C for 72 h. After the incubation period, the colonies were identified on CHROMagar
Candida medium (Société CHROMagar, Paris, France) (St-Germain et al., 2010).

2.2 Assessment of the integrity of the reused respirators

The integrity of the respirators used for 36 h (3 shifts) was evaluated via measuring the
filtration efficiency of 30 samples of reused respirators and 32 samples of non-reused respirators.
The integrity of the reused respirators was assessed using the air filter test method. This method comprised an open system wherein the respirator sample to be tested was kept in constant contact with the ambient air. The atmospheric air contains thousands of particles, whether anthropic or natural. The air filter test method uses these naturally dispersed particles in the air having a size in the range of 0.3–10 µm to perform the PPE filtration efficiency test (Paiva et al., 2022).

The method was conducted at an air temperature of 0°C–50°C and a relative air humidity of 10%–90%, which is the operating range recommended by the particle counter’s manufacturer (Paiva et al., 2022).

The experimental apparatus used for the method (Figure 2) was positioned at a height of 1.3 m from the floor to match the mean height of the respiratory zone of a seated individual. Before initiating the process of evaluating each sample’s filtration efficiency, the possible sources of air currents in the environment were eliminated. The particle counter was calibrated, and then the sample was placed in the sample holder. With the aid of a vacuum pump, the incoming ambient air was let inside at a flow rate of 95 L/min. The selection of a flow rate of 95L/min for the respirator efficiency test is justified by the fact that the respirator used in the experiments (Respirator PFF2 - Brazil) was tested by the manufacturer through ABNT/NBR 13698:2011 before commercialization. The ABNT/NBR 13698:2011 certification standard uses a flow rate of 95L/min in the efficiency test. This flow rate is also the same used for certification of respirators in the European Union, Australia and South Korea. USA, China and Japan use a flow rate of 85L/min to test the efficiency of their respective respirators. Certain particles present in the air were captured by the respirator fibers, while others passed through. The latter were counted by a particle counter, which operated at a flow rate of 2.8 L/min for a fixed counting time of 10 min for each analyzed sample, totaling a volume of 28 L of air per sample tested. After each sample was tested, the same procedure was immediately performed again in the absence of the samples. After obtaining the number of particles
with a diameter of 0.3–10 µm that passed through the samples and the number of particles without
the samples, the filtration efficiency was calculated according to Equation 1 (Paiva et al., 2022):

\[ E(\%) = 100 - 100 \cdot \frac{PCWC}{PCNCW} \] (1),

where:

PCWC = number of particles counted with the sample;

PCNCW = number of particles counted without the sample.

### 2.2.1 Proof of method effectiveness

The evaluation of the effectiveness of the air filter test method for evaluating the efficiency (E) of PFF2 respirators was performed from the evaluation of 32 samples of PFF2 respirators with 0 h of use. These respirators have known efficiency (E≥94%). The EPI certification states that the product protects against dust, fumes and mists. The method is considered to be effective for evaluating the efficiency of PFF2 respirators, if the efficiency obtained by the method is the same as that of the product certification, that is, if E≥94%.

### 2.2.2 Stability monitoring during method execution

It is undeniable that the nature of the solid aerosol present in the air has differences according to each environment, however, the respirators used in the research are certified to protect against dust, fumes and mists of any nature, except oil aerosols. With the exception of this material, the respirator is capable of filtering with a minimum efficiency of 94% aerosol of diameters ≥0.3µm. Therefore, even if there is some kind of difference in the composition of the aerosol, the respirator provides the same level of protection.
The stability of the aerosol, that is, the variation in the availability of particles in the air, is an important parameter to be controlled. Aerosol stability control is maintained by avoiding the execution of experiments on rainy days and in environments with natural and artificial air currents.

The aerosol stability monitoring during the execution of the method was performed from the elaboration of the chart of individual efficiency values with 0 h and 36 h samples. The individual value chart is a statistical quality control tool used for the monitoring stability of individual values. According to the individual value chart, to consider that a given data set is statistically controlled, the values obtained must vary in the interval between the upper-level control (UCL) and the lower-level control (LCL) (Ishikawa, 2012). These values correspond to $-3\sigma$ and $+3\sigma$, respectively. If any value of the individual value chart exceeds the UCL or LCL, it means that there is significant instability in the availability of the aerosol, otherwise, there is no evidence that the aerosol has undergone relevant variations in the environment during the execution of the test.

To monitor possible failures in the execution of the method, a moving range chart was created from the average range of efficiency. The moving range chart is a widely used statistical quality control tool to monitor the stability of industrial processes.

According to the moving range chart, when the range of efficiency are between the UCL and the LCL, that is, when the data vary randomly around the central line, the process is understood to be stable. The UCL and LCL values are 0 and 3.267 CL, respectively, where CL is an average of the amplitude of the process under study (Ishikawa, 2012). Considering the method used in this research as a process, if the range of efficiency are between the UCL and LCL range, it means that there was no instability in the method during operation, otherwise it is necessary to correct the failures and perform new experiments.
2.2.3 Scanning electron microscopy

Scanning electron microscopy (SEM) of the samples was performed with the secondary electrons of the chemical cartridges using a Quanta 200 FEGSEM device (The FEI Company, Hillsboro, OR, USA) with an Everhart–Thornley detector. The images were obtained in high vacuum at a temperature of 21°C and relative humidity of 76%.

2.3 Statistical analysis

The statistical analysis of the microbiota evaluation of the PFF2 respirators used for 12 h, 24 h, and 36 h was conducted using chi-squared test for independence (α = 0.05) to determine the microorganisms associated with the prolonged use of PPE.

To evaluate the filtration efficiency data of the respirators reused for 36 h and compare them with the filtration efficiency of unused respirators (0 h). The student’s t-test (α = 0.05) was used from the normality verification by Shapiro–Wilk test (α = 0.05), assuming equality of variances, and performing Brown–Forsythe test (α = 0.05). Once the criteria described above were satisfied, the individual values chart and the moving range were applied.

To prove the efficiency of the method, a student’s t-test was performed for a level of 99% of hypothesis, significant as alternative hypothesis (H1) > 94.0 and null hypothesis (H0) $\bar{x}$=94.0.
3 RESULTS AND DISCUSSION

3.1 Microbiota of the respirators

The microbiota of the respirators used for 0h, 12 h, 24 h, and 36 h (Table 1) showed the presence of both gram-negative and -positive commensal bacteria belonging to the oral and nasal microbiota of human beings (Aas et al., 2005; Chen et al., 2019).

The presence of microorganisms in the respirator samples is consistent with the identification of dirt observed in the visual inspection performed after using the PPE (Figure 3) as well as with the SEM images (Figure 4).

It was noticeable that the respirator users faced difficulties in following the training guidelines due to the nature and demand of their work during the initial period of the COVID-19 pandemic. The fact that the users were participating in a study may have contributed to a better behavior during mask usage, indicating that in the hospital routine, the conditions of prolonged use of respirators can be more complex. No fungi were identified in 78% of the samples, yet only 2% of the samples did not show the presence of any microorganism.

Chi-squared test ($\alpha = 0.05$) applied to analyze the microbiota of the PFF2 respirators showed that the variable duration of PPE use was not associated with of gram-positive commensal bacteria, \textit{Staphylococcus aureus} and \textit{Streptococcus mitis} presence ($p > 0.05$).

Hence, it was inferred that these microorganisms were already present in the respirators from the first minutes of PPE use. However, the presence of commensal gram-negative bacteria from the nasal and oral regions of humans is associated with the prolonged use of respirators ($p < 0.05$). There is a tendency for respirators to become contaminated with these bacteria with reuse, and consequently, these bacteria present themselves as indicators of prolonged mask use.
*S. aureus* is a gram-positive bacterium that colonizes the nasal cavity of 20%–30% of the human population without causing any apparent disease (Yang et al., 2018). However, under specific conditions, this species of microorganism can become pathogenic. The factors that determine the difference between its commensal and pathogenic states still remain largely unknown. Some studies, however, have shown that nasal colonization as commensals is predominant in immunocompetent individuals (Jenkins et al., 2015; Peres et al., 2013; Round et al., 2011).

*S. mitis* is one of the earliest commensal colonizers of the human oral cavity. It resides in the oral mucosa from early childhood and remains throughout life. In immunocompetent people, *S. mitis* is rarely associated with pathogenicity, especially since it shows poor survival in the blood and is immediately eliminated (Engen et al., 2018). In immunocompromised patients, however, the commensal oral streptococcal species most often associated with endocarditis or septicemia (Bochud et al., 1994).

Despite no statistically significant relationship between the presence of the fungus *Candida* spp. and the prolonged use of PPE ($p = 0.06$), the presence of the microorganism was observed only in the samples of respirators with 36 h of use. The presence of *Candida* spp. observed in 22% of the samples after 36 h of use ($p = 0.06$) is related to the user’s oral health conditions (Jordan et al., 2002; Zölner and Jorge, 2003). *Candida* spp. may present as a commensal microorganism or opportunistic pathogen in the oral cavity. Its pathogenic action is usually associated with pre-existing problems involving the host’s immune system, such as acquired immunodeficiency syndrome. *Candida* spp. infections can range from non-life-threatening, superficial manifestations to mucocutaneous disorders involving multiple organs (Patil et al., 2015).
3.2 Integrity of respirators after prolonged use for 36 h

3.2.1 Evaluation of the effectiveness of the method

Data from the evaluation of the efficiency of PFF2 respirators with 0 h of use showed a normal distribution ($p = 0.528$) and values above 94% efficiency ($\bar{X} = 97.7\%; \sigma = 0.3$). From the verification of the normality of the data, a student’s t-test was performed for a level of 99% of hypothesis, significant as alternative hypothesis ($H_1 > 94.0$) and null hypothesis ($H_0 \bar{X}=94.0$) (Figure 5).

According to the result of the student’s t-test ($p<0.001$), the efficiency of the respirators evaluated by the air filter test method has an efficiency greater than 94%, this result is in line with the value of the PPE certification ($E \geq 94\%$). From this result, it is considered that the air filter test method is effective for evaluating the efficiency of PFF2 respirators.

3.2. Evaluation of stability during the execution of the method

The evaluation of the aerosol stability during the execution of the method was verified from the elaboration of the chart of individual values of the efficiency of the samples with 0 h and 36 h of use (Figure 6).

According to the individual value chart, the obtained efficiency values were between the upper-level control (UCL) and low-level control (LCL). Therefore, the results suggest a close to 100% probability of the mean efficiency values being within the range of $-3\sigma$ to $+3\sigma$ from the mean (Ishikawa, 2012). This result indicates that the average efficiency of the two groups (0h and 36h) is statistically controlled, that is, there is no evidence that the aerosol suffered relevant variations in the environment during the execution of the tests.
To monitor the stability of the method during the evaluation of the efficiency of the PFF2 respirators (0 h and 36 h) the moving range chart was created (Figure 7). The findings revealed no anomalous variations in the method of evaluating the efficiency of the respirators. The data vary randomly around the centerline and are between the UCL and LCL. In other words, no trends or patterns are present; hence, the variation in the efficiency assessment process is stable, that is, all components of the method worked correctly (Ishikawa, 2012).

3.2.2 Comparison of the efficiency of respirators with 0 h and 36 h of use

Table 2 shows the results of the filtration efficiency of the respirators used for 36 h and of those with 0 h of use. The efficiency data were tested for normality (p = 0.355) and for equality of variances (p = 0.400). Once these criteria were satisfied, student’s t-test was applied with a two-tailed confidence interval of 95% for the difference between the means: −0.309–0.0357.

There was no statistically significant difference between the filtration efficiency of respirators reused for 36 h and that of respirators with 0 h of use (p = 0.098). Hence, it is acceptable to reuse PFF2 respirators for up to 36 h (3 shifts of 12 h) on an emergency basis. However, it must be emphasized that these respirators and their equivalents were designed to be discarded immediately after their first use, especially those used in a hospital environment (Fischer et al., 2020).

Regarding the integrity of the respirators, no difference was observed between the filtration efficiency of respirators with 0 h and 36 h of use. Consequently, it was inferred that the mechanism of filtration in the respirators did not appear to be negatively affected by the duration of PPE use extending up to 36 h. Nevertheless, it is important to highlight that the use of certain chemical
substances can weaken the electrical field of the fibers and generate a decrease in the filtration efficiency of respirators.

In addition to the urgency of developing respiratory protection alternatives to contain the spread of COVID-19, several studies have been conducted with the objective of observing the efficiency of respirators after decontamination with different types of agents (Fischer and Shaffer, 2010; Heimbuch et al., 2011; Lin et al., 2018; Fischer et al., 2020). The findings of these surveys showed no loss of filtration efficiency of the respective respirators after one decontamination cycle. The results obtained in the current study are consistent with those observed in the literature since there was no contact between the polypropylene fibers of the respirators’ filter or any substance that could interfere with their electrostatic capture phenomenon.
4 CONCLUSION

The use of PFF2 respirators and their equivalents in several countries is recommended by many health agencies as a primary respiratory protection measure for individuals exposed to aerosols of biological origin. It is important to note that these respirators are disposable, and hence, their prolonged use is not recommended by the manufacturers.

However, under exceptional circumstances, such as the worldwide shortage of respirators experienced recently during the COVID-19 pandemic, it is possible to reuse PPE, preferably after performing some decontamination procedure. If decontamination is not possible, the results obtained in the present study can support the decision-making regarding the reuse of respirators in potential emergency scenarios.

It is necessary to monitor the prolonged use of respirators and maintain clean conditions, well-adjusted elastic bands, and satisfactory fit testing. However, it is considered that the reuse of respirators for up to 36 h does not pose great risks to the health of immunocompetent users. According to the findings of the present study, the efficiency of PPE is not compromised with reuse for up to 36 h, which is equivalent to 3 shifts of 12 h. However, it is necessary to analyze the context of the reuse conditions for better decision making.
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DISCLAIMER

Reference to all specific commercial companies or products does not constitute advertising or conflict of interest.
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Staphylococcus aureus Provokes Immunity to Protect against Skin Infection of Methicillin-
Table 1. Percentage of respirator samples wherein bacteria and fungi were identified after using for 0h, 12 h, 24 h, and 36 h and chi-squared test for association between the non-parametric variables (time of use and presence of the microorganism).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>36 h (n = 22)</th>
<th>24 h (n = 14)</th>
<th>12 h (n = 8)</th>
<th>0 h (n = 3)</th>
<th>Chi-squared test p-value (α = 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative bacteria</td>
<td>50%</td>
<td>21%</td>
<td>0%</td>
<td>0%</td>
<td>p = 0.020</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>95%</td>
<td>92%</td>
<td>100%</td>
<td>0%</td>
<td>p = 0.741</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>77%</td>
<td>78%</td>
<td>87%</td>
<td>0%</td>
<td>p = 0.823</td>
</tr>
<tr>
<td><em>Streptococcus mitis</em></td>
<td>90%</td>
<td>100%</td>
<td>75%</td>
<td>0%</td>
<td>p = 0.146</td>
</tr>
<tr>
<td><em>Candida</em> spp.</td>
<td>22%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>p = 0.060</td>
</tr>
</tbody>
</table>
Table 2. Descriptive statistics of the efficiency data of respirators with 0 h and 36 h of use in a hospital environment

<table>
<thead>
<tr>
<th>Time of use (h)</th>
<th>$\bar{x}$ (%)$^*$</th>
<th>$\sigma$$^{**}$</th>
<th>Maximum (%)</th>
<th>Minimum (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>97.7</td>
<td>0.3</td>
<td>98.2</td>
<td>96.9</td>
<td>32</td>
</tr>
<tr>
<td>36 h</td>
<td>97.5</td>
<td>0.4</td>
<td>98.2</td>
<td>96.6</td>
<td>30</td>
</tr>
</tbody>
</table>

$^*$Mean; $^{**}$Standard deviation.
Figure 1. PFF2 respirator (3M) used in this study
Figure 2. Scheme of the method used to evaluate the filtration efficiency of PFF2 respirators.
Figure 3. Conservation status of PFF2 respirators after prolonged reuse for 36 h
Figure 4. Scanning electron microscopy image of respirator fibers after 36 h of use. The detail of nanometric particles attracted by the fiber shows that the phenomenon of electrostatic capture was not harmed by the reuse of the personal protective equipment.
Figure 5. Histogram with null hypothesis (H₀) and 99% confidence interval. H₀: \( \bar{x} = 94.0 \). H₁: \( \bar{x} \geq 94.0 \).
Figure 6. Individual value chart. A. 0 h of use. B. 36 h of use.
Figure 7. Moving range chart. A. 0 h of use. B. 36 h of use.