Effect of moist heat decontamination on methicillin-sensitive *S. aureus* for the reuse of N95 respirators in the COVID-19 emergency

Dmitry Gil, Keith K Wannomae, Rachel Connolly, Joseph A Gardecki, Hui-Min Leung, Ebru Oral, Orhun K Muratoglu

MGB COVID Innovation Center Re-use Working Group, Massachusetts General Hospital, Boston, MA

Background

Decontamination of N95 respirators has become critical to alleviate PPE shortages for healthcare workers in the current COVID-19 emergency. The factors that are considered for the effective reuse of these masks are the fit, the filter efficiency, the decontamination/disinfection level for SARS-COV2, which is the causative virus for COVID19, and other organisms of concern in the hospital environment such as Staphylococcus aureus or Clostridium difficile.

Here, we explored the efficacy of using moist heat as a decontamination method for an N95 mask on methicillin-sensitive *S. aureus*.

Methods

The efficacy of moist heat decontamination was assessed using an in-vitro method adapted from E. Pinho et al [1]. N95 respirators (3M 1860S, St. Paul, MN) were cut in 2 cm equilateral triangles and sterilized under UV in a biosafety cabinet for 20 minutes on each side. Antibacterial tests were conducted against laboratory strain of methicillin-sensitive *S. aureus* (MSSA, ATCC 12600).

Prior to conducting the experiments, bacteria were incubated until reaching log-phase. 100 μl of the bacterial suspension with an approximate concentration of 2×10⁸ CFU/ml was transferred onto the N95 respirator samples and spread evenly across the front (outer) surface. Samples were air dried for 10 minutes, placed in sterile 5 ml tubes, and decontaminated for 30 minutes at 60°C and 80% relative humidity. After treatment, 5 ml of sterile PBS was subsequently added, and the tubes were vortexed for 60 seconds to dislodge and resuspend bacterial inoculum. Bacterial concentration in the obtained suspension was determined using the spread-plate method outlined by Gil et al [2]. Appropriate control suspensions were also obtained from a) samples immediately after inoculation, b) samples that were inoculated and kept at RT for the duration of the decontamination procedure, and c) samples that were NOT inoculated and then decontaminated (negative control).

An n=3 samples were tested per group.

Results

The sensitivity level of our assay is 10² CFU. No colonies grew on any of the plates for the treated samples. The detailed results are shown in Table 1. The initial bacterial load on treated samples was 2*10⁷+/‐0.4*10⁷.
Table 1. Concentration of bacteria for untreated and moist heat treated samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Final bacterial load, CFU/sample</th>
<th>Log-reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control – no bacterial inoculation</td>
<td>&lt;10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>NA</td>
</tr>
<tr>
<td>Control – bacterial inoculation =&gt; no decontamination =&gt; immediate plating</td>
<td>1.6<em>10&lt;sup&gt;7&lt;/sup&gt; +/-0.4</em>10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.09</td>
</tr>
<tr>
<td>Control – bacterial inoculation =&gt; no decontamination =&gt; 30 min at RT</td>
<td>2.1<em>10&lt;sup&gt;7&lt;/sup&gt; +/-1.1</em>10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>-0.02</td>
</tr>
<tr>
<td>Sample inoculated =&gt; decontamination for 30 minutes</td>
<td>&lt;10&lt;sup&gt;2&lt;/sup&gt;</td>
<td>&gt;5.31</td>
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**Discussion**

The obtained results suggested that moist heat decontamination was highly effective against MSSA. Moist heat decontamination yielded at least a 5.3 log reduction of MSSA load on the N95 respirator sample.

The concentration and time of exposure in disinfection procedures are typically tested for sterility assurance level of 10<sup>-6</sup> (6-log reduction) with bacterial spores such as *Geobacillus stearothermophilus*. FDA guidance “Enforcement Policy for Face Masks and Respirators During the Coronavirus Disease (COVID-19) Public Health Emergency (Revised)” (April 2020) suggests that for determining the level of bioburden reduction using different decontamination methods, a ‘high level of disinfection’ requires ≥6-log reduction of bacterial spores. In this document, the FDA also recommends the demonstration of viricidal activity wherever possible (≥3-log).

Here, we showed that moist heat decontamination may be used to significantly reduce Gram-positive bacterial burden on N95 respirators before re-use.

While we present results for bacterial bioburden evaluation of this decontamination method for N95 masks, there are many other considerations such as other technical, financial, and logistical ones for any medical institution to decide on a decontamination method, if any, to utilize for this emergency use situation. The results presented here are not meant to constitute a stand-alone recommendation.

**References**