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Original Article

# Baby bottle steam sterilizers disinfect home nebulizers inoculated with bacterial respiratory pathogens

Dana Towle [a], Deborah A. Callan [a], Patricia A. Farrel [a], Marie E. Egan [b], Thomas S. Murray [a,c,d,*]

[a] Clinical Microbiology Laboratory, Yale New Haven Hospital, New Haven, CT 06510, United States
[b] Department of Pediatrics and Cellular and Molecular Physiology, Yale School of Medicine, New Haven CT 06510, United States
[c] Department of Medical Sciences, Frank H. Netter MD, School of Medicine at Quinnipiac University, Hamden, CT 06518, United States
[d] Department of Laboratory Medicine, Yale School of Medicine, New Haven CT 06510, United States

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**Abstract**

*Background:* Contaminated nebulizers are a potential source of bacterial infection but no single method is universally accepted for disinfection. We hypothesized that baby-bottle steam sterilizers effectively disinfect home nebulizers.
*Methods:* Home nebulizers were inoculated with the common CF respiratory pathogens methicillin resistant *Staphylococcus aureus*, *Burkholderia cepacia*, *Haemophilus influenzae*, mucoid and non mucoid *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*. The nebulizers were swabbed for bacterial growth, treated with either the AVENT (Philips), the NUK Quick & Ready (Gerber) or DRY-POD (Camera Baby) baby bottle steam sterilizer and reswabbed for bacterial growth.
*Results:* All steam sterilizers were effective at disinfecting all home nebulizers. Viable bacteria were not recovered from any inoculated site after steam treatment, under any conditions tested.
*Conclusions:* Steam treatment is an effective disinfection method. Additional studies are needed to confirm whether these results are applicable to the clinical setting.


*Keywords:* Disinfection; Nebulizers; Steam sterilization; Bacterial pathogens

## 1. Introduction

Home nebulizer therapy is an integral part of treatment regimens for patients with Cystic Fibrosis (CF). Benefits include the delivery of therapies such as antibiotics to the site of infection while reducing the systemic side effects. The risk of bacterial colonization of home nebulizers varies depending on the study but several studies report that home nebulizers used by asthmatics or CF patients may become colonized with bacteria [1–5]. This is not surprising as bacterial pathogens such as *Pseudomonas aeruginosa* survive in water and can colonize both plastic surfaces and human lungs via the formation of bacterial biofilms [6].

The recognition that bacterial colonization of home nebulizers is a potential risk for respiratory infection has led experts to examine many different methodologies for disinfection. These include cleaning with 2.0–3.5% acetic acid, soaking with water, washing with soap and water (either tap or sterile), 70–90% ethanol or isopropyl alcohol, 3% hydrogen peroxide, or 0.5% hypochlorite [7–11]. Ideally, the method used to clean and disinfect nebulizers, needs to be simple and efficient as to not add to the growing treatment burden that could significantly compromise patient adherence to therapy. Tai et al. reported that soaking a nebulizer for 10 min in water followed by a rinse was more effective at removing contaminated *Escherichia coli* than either soaking or rinsing alone [12]. Sterile water was not superior to tap water in this study and bacteria were recovered

* Corresponding author at: Department of Medical Sciences, Frank H. Netter MD, School of Medicine at Quinnipiac University, Hamden, CT 06518, United States. Tel.: +1 203 582 6475; fax: +1 203 582 1418.
*E-mail address:* Thomas.murray2@quinnipiac.edu (T.S. Murray).

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from most sites even after soaking and rinsing. Rosenfeld et al. found that aggressive tap water rinse sterilized 17/19 nebulizers inoculated with *Staphylococcus aureus* and mucoid and non-mucoid *P.aeruginosa* [7]. Reychler compared five methods of disinfection, hypochlorite solution, 3.5% acetic acid, 0.5% Hexanios, 0.5% washing detergent, and a dishwasher, using facemasks and mouthpieces inoculated with common CF pathogens (*S. aureus*, *P. aeruginosa*, *Stenotrophomonas maltophilia*, *Burkholderia cenocepacia*, and *Alcaligenes xylosoxydans*). The authors found that all were effective except acetic acid for the treatment of *S. aureus* [9]. In a separate study Reychler et al. found that environmental organisms but not the CF pathogens, methicillin sensitive *S. aureus* or *S. maltophilia*, were cleared from CF patient nebulizers with 0.5% hypochlorite [8]. Given that rinsing or soaking in tap water is efficacious in disinfecting home equipment in previous studies, it is likely that home steam-sterilizers, commonly used and sold for baby bottles, will also be effective. Brief exposure to steam (3 s) can effectively decontaminate a variety of surfaces and eradicate >99.5% of an existing bacterial biofilm [13]. Steam-sterilization is recommended to disinfect the Altera® nebulizer that delivers inhaled aztreonam (http://www.cff.org/treatments/Therapies/Respiratory/Cayston/). Importantly, repeated steam sterilization treatments do not impair the in vitro function of the eFlow® rapid nebulizer [14]. The procedure is fast, straightforward, and easy to perform making it an ideal method for disinfection. However, there is little published data on whether steam sterilization effectively disinfects home nebulizers. Therefore we sought to examine the effectiveness of three different commercially available baby-bottle steam sterilizers for their ability to disinfect nebulizers inoculated in vitro with respiratory pathogens commonly isolated from CF patients.

## 2. Methods

### 2.1. Bacterial strains and growth conditions

Table 1 lists the strains inoculated onto the nebulizers to test for disinfection. Bacteria were grown overnight on blood agar plates (Remel, Lenexa, KS), inoculated into Trypticase Soy Broth (TSB) (Remel, Lenexa, KS) at a density of 0.5 McFarland. Ten microliters of this suspension was used to inoculate the nebulizers. To determine the pre-exposure inoculum, the 0.5 McFarland suspension of each bacterial strain was serially diluted, the diluted bacterial suspensions inoculated on blood agar plates for 48 h at 37 °C, and the colony forming units recorded (Table 1).

Table 1
Bacterial strains tested for steam-treatment.

| Bacteria | Source | Estimated inoculum [a] |
|---|---|---|
| *Pseudomonas aeruginosa* | ATCC 27853 | $9.2\times10^5$ |
| Mucoid *Pseudomonas aeruginosa* | Clinical isolate | $5.7\times10^5$ |
| Methicillin resistant *Staphylococcus aureus* | ATCC 4330 | $6.5\times10^5$ |
| Methicillin susceptible *Staphylococcus aureus* | ATCC 25923 | $1\times10^7$ |
| *Haemophilus influenzae* | ATCC 10211 | $1.3\times10^6$ |
| *Burkholderia cepacia* | ATCC 25416 | $3.8\times10^5$ |
| *Stenotrophomonas maltophilia* | ATCC 51331 | $6.0\times10^5$ |

[a] Inoculum based on serial dilutions of 5.0–0.5 McFarland suspensions.

### 2.2. Nebulizer inoculation

For all conditions three different nebulizers, the Pari LC Plus®. eFlow® rapid, and eFlow Altera®, were inoculated with each of the above bacterial strains in three different locations for each individual experiment (Fig. 1). Initially, the disinfection of both unassembled and fully assembled nebulizers was compared. Assembled nebulizers were inoculated prior to assembly and then put together prior to steam treatment. Once we determined that there was no difference in bacterial recovery comparing assembled with the unassembled nebulizers (data not shown), all remaining experiments were performed on fully assembled nebulizers.

Three different conditions were tested: 1) *Dry samples*: The nebulizer inoculated with the 10 μl bacterial suspensions was air dried in a hood for 30 min and then was subjected to steam sterilization treatment. 2) *Wet samples*: The nebulizer with the 10 μl bacterial suspensions was immediately placed in the sterilizer. 3) *Sputum samples*: A pool of de-identified discarded sputum that had grown only normal flora recovered from three unknown CF patients was vortexed and 0.5 ml was transferred to a microfuge tube. Since the specimens were pooled de-identified sputum being discarded by the clinical microbiology laboratory, this study meets the criteria as being exempt from review by the Yale Human Investigations Committee. Ten microliters of 0.5 McFarland bacterial suspension was transferred to the sputum containing microfuge tube and 10 μl of each seeded sputum was inoculated to the three different sites on each nebulizer. An un-inoculated sputum sample was used as a control in these experiments. The sputum contained normal flora (Fig. 2) so the amount of bacteria recovered from the inoculated sputum was determined using the 4 quadrant semi-quantitative streaking method commonly used in clinical microbiology laboratories. With this method 1+ represents bacterial growth in the first quadrant only, 2+ in the first and second quadrant, 3+ the first three quandrants, and 4+ all streaked quadrants.

In a separate set of experiments we used mucoid *P. aeruginosa* and *S. aureus* with a 5.0 McFarland suspension, performed serial dilutions to quantitate the bacterial amount, and either inoculated the nebulizer directly or seeded 100 μl of sputum with 10 μl each of the higher bacterial inoculum and performed the experiments as described above. Additional experiments with 5.0 MacFarland mucoid *P. aeruginosa* and *S. aureus* included both 24 and 48 h bacterial incubation times to allow for potential biofilm formation prior to steam treatment. For 24 and 48 h incubation experiments, the water rinsing method of Rosenfeld et al. was performed as described to determine how steam treatment compared with a published disinfection protocol [7].