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Original Article

An evaluation of different steam disinfection protocols for cystic fibrosis nebulizers

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Abstract

Background: Contamination is a key element in cystic fibrosis. For this reason, nebulizer hygiene is an important, but complex and time-consuming task for cystic fibrosis patients. The aim of this study was to compare different steam disinfection and drying protocols.
Methods: One hundred nebulizer parts were inoculated with cystic fibrosis-related bacteria in high concentrations (*Burkholderia multivorans* 3.9×10^{10}/ml, *Staphylococcus aureus* 8.9×10^{8}/ml and *Pseudomonas aeruginosa* 2.1×10^{9}/ml). Tubes with *Mycobacterium abscessus* complex were additionally tested. Six steam disinfectors were compared. Different methods of drying were examined.
Results: All tested bacteria were efficiently killed by the different steam disinfectors tested. The risk of contamination depended on the method of drying.
Conclusions: Steam disinfection is a safe disinfection method. It is better to leave the nebulizers wet after steam disinfection than to manipulate them by active drying, which seems to be a source of recontamination.

Keywords: Aerosol drug therapy; Equipment contamination; Infection control; Infection prevention; Inhalers; Medical devices; Nebulizer; Inhalation; Hygiene; Cleaning; Steam disinfection; Mycobacteria; Compliance; Cystic fibrosis; Bacteria; *Pseudomonas aeruginosa*

1. Introduction

Survival and quality of life in cystic fibrosis (CF) have improved in recent years. This improvement was, however, associated with more complex treatment, resulting in a substantial burden for the patients. [1–3]. CF involves many time-consuming high-maintenance treatments, including airway clearance and nebulization. The daily duration of these treatments can be long. Moreover, the time needed for device disinfection must also be considered. So it is crucial to make cleaning steps as simple as possible to achieve optimal compliance and to reduce barriers to effective home nebulizer therapy and hygiene [4].

Home nebulizers are in widespread use among patients with chronic pulmonary diseases such as cystic fibrosis. Contamination of these devices has been well-documented [5–8]. Even though nebulizer disinfection is routinely recommended [9], advice varies among countries, manufacturers and organisations. As in our CF centre, steam disinfection has become increasingly regularly recommended, followed by drying with clean, ironed cotton towels prior to storing [10]. Steam disinfection is a very potent method for killing bacteria [11–13]. It reduces bacterial populations more effectively and is less complicated than other methods [14,15]. In comparison, chemical disinfection requires preparation of a solution which is a risk, both for contamination and faulty measurement. Temperature has an influence on chemical reactions, and protein or soap related errors can also occur. The disinfectant has to be stored correctly. Disinfecting solutions can be contaminated by microorganisms [16,17]. There are further considerations regarding the safety of these

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products for humans, such as hypersensitivity [18–20]. Finally, the nebulizer has to be rinsed with clean water to eliminate residual chemical substances[21].

Steam disinfection denaturizes bacterial proteins and therefore reduces bacteria significantly; however, not all manufacturers recommend it for their nebulizers. A study showed that steam disinfection has no influence on the functionality of eFlow® devices (Pari, Germany) [22]. Despite the use of steam disinfection, some of our patients' devices showed multiple, bacterial contamination at their annual nebulizer quality check (personal unpublished data). As concordance between bacteria isolated from the nebulizer and from patient sputa was rarely verified [4,8,23,24], the source of bacteria contaminating the nebulizer parts is poorly understood.

The aim of this study was (1) to investigate different modalities of steam disinfection and (2) to compare the efficacy of different steam disinfectors on various nebulizer or airway clearance devices.

2. Material and methods

2.1. Protocols

Six different protocols (Table 1) for steam disinfection of nebulizer parts, mimicking situations that might occur if a patient has to handle steam disinfection alternating with dry inhalation, on a regular basis, were investigated after artificial inoculation of the nebulizer parts:

- Protocol 1: immediate steam disinfection, using tap water (pH 10, 7°dH, no bacteria) and instant drying with paper towels.
- Protocol 2: immediate steam disinfection, using tap water, with the nebulizer parts then left in the moist environment, defined by leaving it in the steam disinfector with the lid continuously closed for 96 h after steam disinfection.
- Protocol 3: air drying for one hour prior to steam disinfection using tap water, with the nebulizer parts then left in the moist environment of the steam disinfector for 24 h.
- Protocol 4: nebulizer stored in a box for four months before inoculation, extended drying time (48 h) before steam disinfection using tap water, followed by a long exposure (72 h) to the moist environment of the steam disinfector.
- Protocol 5: extended drying time (48 h) before steam disinfection using tap water contaminated with 31,000 CFU/ml, *Pseudomonas aeruginosa* and subsequent long exposure to the moist environment of the steam disinfector (48 h).
- Protocol 6: drying time of 96 h before steam disinfection using tap water contaminated with 31,000 CFU/ml *P. aeruginosa*, and subsequent extended exposure (four days) to the moist environment of the steam disinfector, followed by active drying with paper towels (6a) or no active drying (6b).
- CF bacteria control: immediate steam disinfection after use, using tap water, immediate resuspension and cultivation.
- Mycobacteria abscessus complex: immediate steam disinfection of mycobacterial mass with Petra 3 and Avent 3-in-1 disinfectors, using tap water, immediate resuspension and qualitative cultivation.
- Mycobacteria control: immediate steam disinfection of mycobacterial suspension with Petra 3 and Avent 3-in-1 disinfectors using tap water, immediate resuspension and quantitative cultivation.

For each of the six protocols, 100 parts of nebulizer and airway clearance devices [seven sets of eFlow®rapid (Pari, GE), two sets of LC plus (Pari, GE), three sets of RC-Cornet® (RC, GE), three sets of I-Neb® (Phillips, NL), three sets of VRP-Desitin (Tyco, GE), three sets of nasal douche (Pari, GE), two sets of PEP I (Pari, GE), four sets of Pep/RMT (AstraTech, SE) and four sets of Vortex (Pari, GE)] were contaminated using cotton swabs with a mix of 5 ml of each standard suspension, containing bacteria grown overnight on Columbia agar and inoculated into NaCl 0.9% at a density of 3.0 McFarland (Table 2) and additionally, 5 ml of anonymized liquefied patient sputa, containing bacteria (Table 3).

The CF bacteria controls consisted of three plastic tubes filled with 0.5 ml of three different standard suspensions, respectively (Table 2)

For *Mycobacterium abscessus* complex: one glass tube and one plastic tube, each filled with 60 µg of living bacterial mass of either (1) *M. abscessus abscessus* or (2) *M. abscessus massiliense* or (3) *M. abscessus bolletii* were investigated. The mycobacteria strains were patient isolates confirmed by the German National Reference Center for Mycobacteria (FZ-Borstel). Mycobacteria control: one glass and one plastic tube, each filled

Table 1
Duration of the different phases and the mode of sampling for each protocol.

Protocol	Phase 1: Air drying before disinfection	Disinfection	Phase 2: Moist storage after disinfection	Sampling	Phase 3: Active paper drying	Sampling
1	0 h	Yes	0 h	No	Yes	Yes
2	0 h	Yes	96 h	Yes	No	No
3	1 h	Yes	24 h	Yes	No	No
4	48 h	Yes	72 h	Yes	No	No
5	48 h	Yes	48 h	Yes	No	No
6a	96 h	Yes	96 h	No	Yes	Yes
6b	96 h	Yes	96 h	Yes	No	No
CF bacterial control	0 h	Yes	0 h	Yes	No	No
M. abscessus complex	0 h	Yes	0 h	Yes	No	No
Mycobacterial control	0 h	Yes	0 h	Yes	No	No

Table 2
Bacterial concentration in the standard suspensions applied to all device parts using a cotton swab.

Bacteria	Source	CFU/mL
P. aeruginosa	ATCC 35032	2.1×10^9
Staphylococcus aureus	ATCC 29213	8.9×10^8
Burkholderia multivorans	Clinical isolate *	3.84×10^{10}

* Clinical isolate, confirmed by Max von Pettenkofer-Institute, GE.

with 0.5 ml of one of three mycobacterial suspensions which consist of 60 µg of each mycobacterium in 4.5 ml NaCl 0.9% [(1) *M. abscessus abscessus* 8.4×10^{10} CFU/ml, (2) *M. abscessus massiliense* 3.2×10^9 CFU/ml, (3) *M. abscessus bolletii* 1.1×10^8 CFU/ml].

After contamination, six different experimental protocols (protocols 1–6) and a CF bacteria control, using six different steam disinfectors (2.2), were carried out. A *M. abscessus* protocol and a mycobacterial control were evaluated using two steam disinfectors to test the limits of this method (Table 1).

The experimental protocols were in three phases (Table 1). Phase 1: air drying of parts before disinfection in the opened steam disinfector. Phase 2: moist air drying of parts after disinfection in the steam disinfector. Phase 3: active drying of parts using paper towels and hands washed with soap. Nebulizer parts were cleaned and disinfected after each protocol using a validated washer disinfector.

2.2. Electric steam disinfectors

Steam disinfection was performed using the following electric steam disinfectors: Avent 3-in-1 electric steam sterilizer (Phillips, NL), Avent 4-in-1 electric steam sterilizer (Phillips, NL), NUK electric steam vaporiser 2-in-1 (Gerber, US) and 3 different models of Petra Di 6.00 Dampfdesinfektion (Petra Electric, GE) which seem to be the most widespread in Europe.

The performances of these devices were previously monitored by medical engineers using Thermologger (Ebro, GE) to check the efficacy of the steam disinfection. A threshold of A0 = 3000, which is a measurement of the expended energy (temperature/time) needed to kill microorganisms, and corresponds to a five minute exposure at 90 °C [13,25,26] in moist heat processes, was considered the cutoff value for determining efficacy.

A predetermined volume of tap water or contaminated water, depending on the protocol, was poured into the disinfector. All six steam disinfectors were additionally tested using Simicon EF (Simicon, GE) Bio Indicators (containing populations of 10^9 *Enterococcus faecium* ATCC 6057), which are designed for the validation and routine monitoring of cleaning and disinfecting (>35 °C) flexible endoscopes.

Nebulizer parts were randomly placed in the six steam disinfectors. They were massively overloaded and filled to their maximal capacity in order to mimic a worst case scenario that could occur in household routine. Parts were neither upright nor tidily positioned as they should have been for optimizing steam distribution and minimizing water residue.

Table 3
Bacterial mix from anonymized patients' sputa, liquefied by beads and counted by dilution series.

Bacteria	CFU/mL
P. aeruginosa	7×10^5
Mucoid *P. aeruginosa*	1×10^5
S. aureus	1×10^5
E. faecium	1×10^4
Candida albicans	3×10^5
Burkholderia cepacia complex	6×10^5
Haemophilus influenzae	1.6×10^6

2.3. Sampling and culturing methods

The method of sampling depended on the protocol:

- For protocols 1–6: sampling was performed at different times depending on the protocol. Sterile cotton swabs moistened with sterile NaCl 0.7 were wiped over the whole internal and external surface of each contaminated nebulizer part. The samples were then cultured in BHI Bouillon (Oxoid, UK) at 37 °C.
- For the CF bacteria control: resuspension of the standard suspensions using bouillon and direct plating.
- For *M. abscessus* complex: resuspension of the mycobacteria using MGIT.
- For the mycobacteria control: resuspension of the mycobacterial suspension using MGIT and direct plating.

A standardized procedure was used for culturing:

- For protocols 1–6: cultures were read every 24 h for five days to allow time for slow-growing and thermally weakened bacteria. If the bouillon was turbid (indicating bacterial growth), it was plated on Columbia + 5% sheep blood (Biomerieux, FR), Mac Conkey II Agar (Axonlab, DE), Haemophilus Agar (Biomerieux, FR) and BCSA (Biomerieux, FR) and incubated at 37 °C for a maximum of seven days (BCSA 32 °C for five days and two days at room temperature) for qualitative analysis. Growing bacteria were additionally determined using MALDI-TOF (Biomerieux, FR). If there was no turbidity within five days, this was considered qualitative proof of negative bacterial contamination.
- For the CF bacterial control: resuspension using BHI Bouillon (Oxoid, UK) for long-term growth and direct plating on BCSA Agar (Biomerieux, FR), Mac Conkey II Agar (Axonlab, CH) and Columbia + 5 % sheep blood (Biomerieux, FR) for counting at 37 °C. No growth within the five-day incubation period, except on the BCSA Agar, which was incubated for five days at 32 °C and two days at room temperature, was considered quantitative proof of negative bacterial contamination.
- For *M. abscessus* complex: resuspension and cultivation using BACTEC MGIT 960 (Becton Dickinson, US). No growth within eight weeks at 37 °C was considered qualitative proof of negative bacterial contamination.

- For the mycobacteria control: resuspension and cultivation using BACTEC MGIT 960 (Becton Dickinson, US) for eight weeks at 37 °C and direct cultivation on Middlebrook 7H10 (Becton Dickinson, US) for counting. No growth within eight weeks was considered quantitative proof of negative bacterial contamination.

3. Results

3.1. Performance of the disinfector

The general performances of the disinfectors were: Petra 1, 121,808; Petra 2, 105,465; Petra 3, 121,714; NUK, 161,690; Avent 3-in-1 upper storey, 14,900; Avent 3-in-1, 15,993; Avent 4-in-1 upper storey, 14,012; Avent 4-in-1, 17,286. All steam disinfectors demonstrated efficacy with an A-0 value higher than the A-0 value of 3000 required by ISO 18883-1. All six steam disinfectors tested negative for bacterial growth of *E. faecium* using the bioindicator.

3.2. Effectiveness of the different modalities of steam disinfection

There was no recovery of any CF bacteria after disinfection (Table 4).

In protocols 2, 3, 4, 5 and 6b, there was no recovery of CF bacteria on nebulizer parts (Table 4), nor was there a significant level of contamination by bacteria from the human skin or ambient surroundings (Table 5).

In protocols 1 and 6a, there was contamination on some parts by coagulase negative staphylococci from human skin (Table 5).

In protocol 4, there were some nebulizer parts contaminated by spore-forming bacteria. If colony morphology was not obvious, identification was done using MALDI-TOF. The results were *Bacillus pumilus* or *Bacillus cereus/Bacillus mycoides/Bacillus thuringiensis*. None of these three can be clearly distinguished from one another using MALDI-TOF (Table 5).

3.3. Comparison of the different steam disinfectors

There were no differences in the efficacy of the different steam disinfectors in killing CF bacteria and *M. abscessus* complex (Table 4) in either protocols 1–6, the *M. abscessus* complex protocol, or the control protocols.

4. Discussion

This study showed that steam disinfection is an efficient disinfection process for CF pathogens on nebulizers or airway clearance devices with no growth of CF bacteria.

Similarly to the Towle study [11], we showed that steam disinfection is an efficient and simple method to disinfect nebulizers or airway clearance devices in a household setting. We found no differences in the performance of the disinfection process regardless of the bacterial strain, the steam disinfection device or the contaminated nebulizer pieces used [25,27]. Spore-forming bacteria are not efficiently killed by any kind of disinfection. In the protocol with the largest number of spore-forming bacteria (protocol 4), these bacteria settled on nebulizer parts when stored for a long time without efficient dust protection [28]. These bacteria are not considered to be pathogenic for cystic fibrosis patients.

Protocol 1 corresponds to the guidelines of the Robert Koch Institute by employing active drying directly after disinfection, modified by using paper towels instead of cotton towels. In this protocol, skin bacteria were the most often recovered, contaminating agent. This was similar in protocol 6a. Both protocols are the only two including active paper drying, which leads to more manipulation and therefore more skin and towel contact. The recovered bacteria (coagulase negative staphylococci) belong to the resident flora of the human skin and are not typical pathogens for cystic fibrosis patients. These bacteria do, however, indicate the possibility of contamination by the transient skin bacteria of inefficiently washed hands, which could transmit Enterobacteriaceae, MRSA and other bacteria. In our laboratory setting with time, space, equipment and staff dedicated to the task, the risk for recontamination is very low in contrast to a household setting. However, in real life, this could be a source of contamination as previously demonstrated [29,30]. We can suppose that for each manipulation, such as drying nebulizer parts actively, the risk of contamination increases dramatically in an imperfect home settings with its ambient bacteria [31–33]. This is probably the explanation for the contamination of nebulizer parts by different bacteria that do not correspond with patient sputa, as described before in the literature [34,35] and also

Table 4
Recovery of CF bacteria after five days of incubation.

Steam disinfectors	P1	P2	P3	P4	P5	P6	C	M	MC
Total	0/100	0/100	0/100	0/100	0/100	0/100	0/24	0/12	0/12
Petra 1	NG	NG	NG	NG	NG	NG	NG	–	–
Petra 2	NG	NG	NG	NG	NG	NG	NG	–	–
Petra 3	NG	NG	NG	NG	NG	NG	NG	NG	NG
NUK	NG	NG	NG	NG	NG	NG	NG	–	–
Avent 3-in-1	NG	NG	NG	NG	NG	NG	NG	NG	NG
Avent 3-in-1, upper storey	NG	NG	NG	NG	NG	NG	NG	–	–
Avent 4-in-1	NG	NG	NG	NG	NG	NG	NG	–	–
Avent 4-in-1, upper storey	NG	NG	NG	NG	NG	NG	NG	–	–

NG: no growth.
CF bacteria (Tables 1 and 2): C, CF bacterial control; M: *M. abscessus* complex; MC, Mycobacterial control.

Table 5
Number of nebulizer parts contaminated by ambient or skin bacteria in protocols with contaminated parts.

	P1	P2	P3	P4	P5	P6a	P6b
Coagulase negative *Staphylococci*	53/100	2/100	2/100	1/100	3/100	22/50	1/50
Aerobic spore formers	1/100	2/100	4/100	75/100	3/100	0/50	2/50
Indeterminable ambient bacteria	5/100	0/100	0/100	0/100	2/100	0/50	0/50

P: protocol.

in our annual controls (unpublished data). Many cystic fibrosis association and CF centre guidelines emphasize the importance of drying nebulizer parts, as recommended by popular device manufacturers: air drying, forced air drying or drying using clean lint-free cotton or paper towels. Our results suggest that no active drying after steam disinfection is necessary. This limits recontamination opportunities and reduces cleaning time for the patients by simplifying the process which in turn, improves therapy and hygiene compliance. As was previously demonstrated, storing of the nebulizer between each use is not necessary if it is used again within the subsequent 24 h [23]. Steam disinfection has been shown to also work perfectly for assembled nebulizer parts [11]. Steam disinfection effectively kills bacteria, so no regrowth can be expected. This was confirmed by the results of all protocols in our study. To make the decision for patients of which steam disinfector to buy easier, producers should have to declare the A-0 value of the disinfector.

When active drying is required, the use of paper towels seems to be the best choice, in spite of the well-known bacterial pre-contamination of paper towels with a low count of mostly spore-forming bacteria [36,37]. Drying of nebulizers using clean cotton towels would be a logistic routine difficult to standardize in a household setting. Commercial laundries have strict regulations and a functional separation of soiled and clean areas to prevent contamination [38]. In the home, simple errors such as using the same storage bags for dirty and clean towels or storing clean towels in the proximity of waste disposal would contribute to the risks of laundry contamination [39]. Forced air drying requires the use of a hair dryer, which blows ambient air through an unclean filter. This is intuitively non-appropriate. Active drying, if done correctly, is a very time-consuming and complicated process and is therefore often left out [40,41].

Drying before disinfection had no influence on the disinfection process as seen in our study; nevertheless, nebulizer parts have to be washed after each use to remove drug residues and to avoid the accumulation of debris and biofilm formation [42].

The A-0 values of the steam disinfectors vary on a very high level influenced by their shape, leakage, volume, material and the heaters installed. Avent disinfectors, for instance, have two storeys and therefore present two places, the lid and the storey junction, for steam to escape.

Some limitations to our study must be mentioned. We did not quantify the residual amounts of water in the steam disinfector and on the nebulizer parts after disinfection. This water could lead to a dilution of the medicine subsequently applied with the nebulizer. As different amounts of residual water clung to different nebulizer parts, their placing in the steam disinfector could have influenced these results. In our analysis, we just discarded residual water by shaking the pieces. Optimal placement of parts in the steam disinfector could reduce the amount of residual water to a minimum. As already discussed, we did not use cotton towels because ours would have presented a reduced risk of contamination compared to household cotton towels. The fact that the disinfector was fully loaded might have influenced our results, but it probably mimics reality more precisely. For safety reasons, we did all mycobacterial testing in a BSL-3 Lab in a safety cabinet Class II, which meant we were limited to two steam disinfectors and disposable test tubes instead of nebulizer parts [43]. Finally, some materials are not heat-resistant enough for steam disinfection; therefore, all manufacturers of nebulizers or airway clearance devices should declare if steam disinfection is possible and choose materials suitable for steam disinfection. And manufacturers of steam disinfectors should declare the A-0 value of their device.

In conclusion, steam disinfection is a simple, effective and non–time-consuming method for disinfecting nebulizer or airway clearance devices. This method disinfects nebulizer parts perfectly. Manipulation such as, for example, active drying is the only cause of recontamination.

Authorship and conflicts

Kinga Hohenwarter, MD, MBA, Wolfgang Prammer, MD, Walter Aichinger, MD and Gregory Reychler, PhD have contributed sufficiently to the project to be included as authors, and all those who are qualified to be authors are listed in the author byline. The manuscript has been approved by all authors. To the best of our knowledge, no conflict of interest, financial or other, exists.

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Annex. Proposition for recommendations for effective steam disinfection

After every use:

1. Wash the assembled nebulizer with water, with or without dish-washing detergent.
2. Steam disinfect the assembled nebulizer using tap water.

3. Open the steam disinfector after disinfection only for a short time if it is desired to let some steam out; otherwise, leave the lid closed until the nebulizer is reused (a maximum of 24 h).
4. Wash hands and dry them with a clean paper towel (a) (e.g. the inner side of a leaf of kitchen roll) and place another clean paper towel (b) next to the steam disinfector.
5. Open steam disinfector and assemble the parts if dismantled.
6. If the parts are too wet, shake off the water, or tap it off on the clean paper towel (b).
7. Place the nebulizer only in the steam disinfector or on a clean paper towel (b)

At the end of the day: dismantle the nebulizer parts, wash them with or without dish-washing detergent and steam disinfect them. Leave them inside the steam disinfector overnight and assemble just before use.

Weekly: clean the area around the steam disinfector and the steam disinfector inside and out with a detergent and let it dry. Clean the steam disinfector outside with a disposable, singly packed, alcohol-based disinfecting wipe.

If more water than usual remains in the disinfector after the process, replace the steam disinfector with a new one.

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