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Original article

In vitro evaluation of efficacy of 5 methods of disinfection on mouthpieces and facemasks contaminated by strains of cystic fibrosis patients☆

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Abstract

Introduction: Home-nebulizers are a potential source of bacterial infection of the respiratory tract in patients suffering from cystic fibrosis. Recommendations for disinfecting this equipment are often arbitrary and sometimes contradictory.
Objective: To assess in vitro the effectiveness of 5 methods of disinfecting this equipment.
Methods: 160 mouthpieces and 160 masks of nebulizers were artificially and massively contaminated with 16 strains of germs found in patients with cystic fibrosis (*Staphylococcus aureus, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Burkholderia cenocepacia, Alcaligenes xylosoxydans*). A controlled comparison was carried out of the five methods of disinfection (hypochlorite solution (0.02% active chlorine), acetic acid 3.5%, Hexanios 0.5%, washing-up detergent 0.5% and a dishwasher), tested with and without drying. Standardised bacteriological sampling took place 4 h after disinfecting.
Results: Following treatment, the disappearance of the germ was recorded in 84.1% of cases, and effective disinfecting (reduction>5 log CFU/mL) in another 10.6%. Disinfection failure (5.3%) was found almost only in the case of acetic acid against *Staphylococcus aureus*.
Conclusion: With the exception of acetic acid, the methods of disinfecting tested in this study appeared to be effective against common bacterial pathogens in cystic fibrosis.

Keywords: Contamination; Cystic fibrosis; Disinfection; In vitro; Nebulizer

1. Introduction

Most patients suffering from cystic fibrosis (CF) regularly use nebulizers. The substances most often delivered by this route are antibiotics, rhDNase, conventional mucolytics, saline and bronchodilators [1,2].

Bacterial infection plays a major role in the process leading to respiratory failure in this disease. CF respiratory pathogens are commonly isolated from used nebulizers so there is a concern that this equipment may be a source of bacterial infection of the lower airways [3–6]. Cleaning and sterilization or disinfection of respiratory therapy equipment is now considered essential to prevent infections of these patients [7].

There is a need to address nebulizer cleaning methods as in practice current recommendations appear to vary and sometimes to be contradictory [1]. In 2002, for example, the official site of the French Cystic Fibrosis Association

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Table 1
Numbers of strains used

Strain	No
Methicillin-resistant *Staphylococcus aureus* (MRSA)	2
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	2
Pseudomonas aeruginosa	8
-non-mucoid	(5)
-mucoid	(3)
Stenotrophomonas maltophilia	2
Alcaligenes xylosoxydans	1
Burkholderia cenocepacia	1

recommended the use of relatively concentrated solutions of hypochlorite (0.36% of active chlorine) while its German counterpart specifically warned against the use of this substance, considered to be an irritant. Some manufacturers recommend to use soap for cleaning and boiling water for disinfection.

2. Objectives of the study

The purpose of this study was to assess the in vitro efficacy of 5 methods of disinfection on mouthpieces and facemasks of nebulizers. The choice of the methods to be tested was based on CF specific data from the literature [3,8] and recommendations of national cystic fibrosis associations. Boiling water was not considered due to risks of burns.

3. Material and methods

160 polypropylene and PVC masks (1100E, Medic-Aid, Brussels) and 160 polypropylene mouthpieces (1605, Medic-Aid, Brussels) were contaminated in a massive and standardised way by strains from culture broths of pathogenic germs frequently found in CF (Table 1). Each of the sixteen strains contaminated 20 nebulizers (10 masks and 10 mouthpieces) of which 10 were disinfected with drying and 8 without. A mask and a mouthpiece per strain were not disinfected and were used as controls (Fig. 1).

Contamination was carried out with a germ culture broth (5 mL of BHI broth-incubation for 18 h at 37 °C-addition of 0.5 mL of bovine albumin at 30% to simulate the presence of organic debris). An equal quantity (0.1 mL) of this broth was spread on a particular surface of the nebulizer part, identical in each case. Dilutions of the broth were made to determine the concentration of germs present.

The five methods of disinfection tested were the use of a dishwasher (temperature: 70 °C) or immersion for 20 min in a litre of one of the 4 following solutions: Hexanios 0.5% (ANIOS Laboratories, Lille, France), shop-purchased hypochlorite solution (0.02% of active chlorine), acetic acid at 3.5%, hot water (40 °C)+washing-up detergent (SUN®) at 0.1%.

Apart from the dishwasher (where drying is automatically included in the programme), each technique was investigated with and without active drying. Following rinsing in tap water, the parts were either left to dry in the air or dried with a hairdryer.

A bacteriological assessment sample was taken at the place of contamination 4 h after this. For the masks, the surface contaminated was flattened on a count-tac plate. For the mouthpieces, a cotton swab soaked in a Letheen broth was used to rub the interior and exterior surfaces, then cultured on a Columbia blood agar. The result for the sample was read for the first time after 24 h of incubation at 37 °C. The sample was allowed to remain at ambient temperature for 6 days, after which the result was re-read, and compared to the initial load. The number of colonies present was expressed as a common logarithm.

Disinfection was defined by a reduction in the bacterial load greater than or equal to 5 log CFU/mL [9].

4. Results

The initial concentrations of germs in the culture broths used for contamination were the following: 7.8 to 8.1 log CFU/mL for *Staphylococcus aureus*, 7.3 to 8.1 log CFU/mL for *Pseudomonas aeruginosa*, 7.7 log CFU/mL for *Achromobacter xylosoxydans*, 7.3 to 8.2 log CFU/mL for

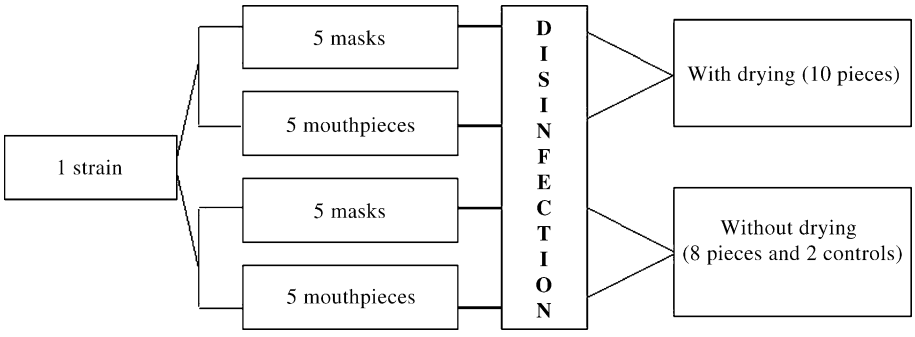

Fig. 1. Scheme of the experimental protocol.

Table 2
Initial bacterial load (log CFU/mL) versus after disinfection bacterial load (log CFU/mL) (corresponding to the smallest reduction of bacterial load) obtained at the end of incubation (6 days) for the 5 methods

	MSSA		MRSA		Ps. aer		S. malt		A. xylo		B. ceno		
	1	2	1	2	1	2	1	2	1	2	1	2	
Hexanios 0.5%	8.1	1	7.8	0.6	7.1	/	7.3	/	7.7	/	5.2	/	With drying
Hypochlorite solution (0.02% active chlorine)	8.1	0.3	7.8	1.2	7.1	/	7.3	/	7.7	/	5.2	/	
Hot water and 0.1% detergent	8.1	1.1	7.8	1.7	7.1	/	7.3	/	7.7	/	5.2	/	
3.5% acetic acid	8.1	6.1	8.1	6.1	7.1	/	7.3	0.5	7.7	/	5.2	2.6	
Dishwasher	8.1	0.7	7.8	0.7	7.1	/	7.3	/	7.7	/	5.2	/	
Hexanios 0.5%	8.1	/	7.8	/	7.1	/	7.3	/	7.7	/	5.2	/	Without drying
Hypochlorite solution (0.02% active chlorine)	8.1	/	7.8	0.3	7.1	/	7.3	/	7.7	/	5.2	/	
Hot water and 0.1% detergent	8.1	1.5	7.8	5.8	7.1	/	8	1.2	7.7	/	5.2	/	
3.5% acetic acid	8.1	8.1	8.1	6.1	8	2	7.3	/	7.7	/	5.2	/	

1 : before disinfection.
2 : at the end of incubation (6 days after disinfection).
/ : total disappearance of the germs.
: reduction <5 log CFU/mL=failure of disinfection.

Stenotrophomonas maltophilia, 5.2 log CFU/mL for *Burkholderia cenocepacia*. The results obtained after implementation of the disinfection methods are summarized in Table 2.

Acetic acid was not effective against *Staphylococcus aureus* (7 failures out of 8), but the other methods tested all resulted in effective disinfecting or even disappearance of this bacterium.

All the methods tested, whether followed by drying or not, were effective against *Pseudomonas aeruginosa, Stenotrophomonas maltophilia* and *Achromobacter xylosoxydans*. With drying, all the methods brought about the total disappearance of these bacteria, with the exception of acetic acid in the case of *Stenotrophomonas maltophilia*. Without drying, acetic acid and hot water with detergent did not completely get rid of, respectively, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*.

With drying, acetic acid was ineffective against *Burkholderia cenocepacia* (reduction of the minimal concentration of only 2.6 log), while all other methods caused this bacterium to disappear completely.

The methods tested proved to be equally effective with the mask and the mouthpiece, even though the surfaces and materials of which they are made differ.

5. Discussion

A recent European survey showed that in 95% of 54 CF centers, treatment with nebulizers is prescribed in at least half of patients from 9 to 19 years [1].

Nebulizers are considered by the CDC to be semi-critical elements in the prevention of nosocomial infections [10]. The risk of nebulizer equipment contamination has been documented not only in patients with cystic fibrosis [4–6], but also in asthmatics [11,12], immunodeficient patients, in intensive care units [13,14] and in units for serious burn victims [15].

With regard to cystic fibrosis, this risk is all the greater as the patients are infected [6]. In reality it probably remains relatively low, as is shown by the lack of concordance between the samples taken from the nebulizer equipment and the sputum [4,5], but it is recurrent, because of the often daily recourse of such patients to this method of treatment.

There is a consensus that the disinfecting of this equipment should be preceded by cleaning and followed by rinsing [16]. Complete cleaning prior to disinfection is required to remove all organic and inorganic debris and also helps to maintain the effectiveness of the nebulizers and the quality of the nebulization [17].

With regard to disinfecting this material at home, practices remain very disparate [1]. Studies are few and recommendations various, sometimes even contradictory. Jakobsson recommended daily steeping for an hour in a vinegar solution (2%) or for some minutes in boiling water and showed that observing precise recommendations could limit the risk of contamination of the nebulizer equipment [8,18]. The use of hypochlorite solution has been proposed by others but at very variable frequencies and, above all, very variable concentrations of active chlorine. Hutchinson et al. [3] reported weekly disinfection with a solution of 0.0125% of active chlorine, without specifying the steeping time. Recent recommendations of the French Cystic Fibrosis Association (AFLM) mention solutions of 0.36% and then 0.08% of active chlorine with a duration of immersion from 15 to 30 min; those of the American

Cystic Fibrosis Foundation a solution of 0.13% of active chlorine with an immersion of 3 min. The German Association cautions against hypochlorite use and recommends boiling water. Rosenfeld actually trivialises the various recommendations by reporting that after nebulization of a cocktail of *Staphylococcus aureus* and mucoid and non-mucoid *Pseudomonas aeruginosa*, simple rinsing of the material for a minute in running water, allowing it to dry in the air, led to the complete disappearance of the germs in 89% of cases [19].

In the conditions of our study, with the exception of acetic acid, the methods tested all proved effective against the principal pathogens encountered in cystic fibrosis. Acetic acid does not guarantee disinfection for *Staphylococcus aureus*, nor for *Burkholderia cenocepacia* and in fact its use must be considered inadequate [16]. The effectiveness of the dishwasher has probably to be relativised because it was studied during a cycle without any other item, which would appear impractical and expensive for daily use, and because the maximum temperature reached by some machines (<70 °C) may constitute another limiting factor. At the low concentration used, the household bleach (5 ml of bleach (3.61% of active chlorine) + 995 ml of water) is odourless and does not stain clothes. Its low cost makes it particularly attractive.

Several potential limitations of this work may be discussed.

The localised contamination was achieved on the basis of a highly concentrated germ broth (10^8 bacteria/mL) [6], but only a small quantity of this broth (0.1 mL) was used each time. In addition, the items were contaminated by only one strain at a time.

The material used was new. Wear and tear can make the surfaces more irregular and so more difficult to disinfect.

The study does not show the benefit of drying. But neither does it call into question an advantage which is now recognised by all, all the more so as several of the most pathogens in cystic fibrosis (*Pseudomonas aeruginosa* and *Burkholderia cenocepacia* in particular) are hydrophilic. Drying is a highly placed priority for disinfection and cleaning [3]. In fact, the surfaces studied here were very accessible and dried very well, conceivably even in the air, within a few hours.

The effectiveness of the methods was only tested 4 h after being applied. In a quite similar study, Vassal et al. did not however show any difference between the results of samples taken at 0, 6 and 24 h [6].

Finally, these results were obtained in laboratory conditions and it still necessary to validate them by an in vivo study.

6. Conclusion

In the conditions of this in vitro study, with the exception of acetic acid, the disinfection methods tested proved efficient against the principal pathogens found in cystic fibrosis. This was particularly the case with a hypochlorite solution at a very low concentration (0.02% of active chlorine). In practice, we recommend to clean dismanteled parts of nebulizer with water and detergent after each nebulization. Then pieces must be rinsed with tap water, dried actively and stored in a clean towel. Once a day, disinfection is recommended after cleaning . The pieces are putted for 20 min in an hypochlorite solution (5 mL of hypochlorite diluted in 1 L of water). This solution must be renewed each day.

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References

[1] Borsje P, de Jongste JC, Mouton JW, Tiddens HA. Aerosol therapy in cystic fibrosis: a survey of 54 CF centers. Pediatr Pulmonol 2000; 30(5):368–76.
[2] Huet F, Nivelon JL. Aerosol therapy and cystic fibrosis: a national survey. Rev Pneumol Clin 1997;53(2):91–7.
[3] Hutchinson GR, Parker S, Pryor JA, Duncan-Skingle F, Hoffman PN, Hodson ME, et al. Home-use nebulizers: a potential primary source of Burkholderia cepacia and other colistin-resistant, gram-negative bacteria in patients with cystic fibrosis. J Clin Microbiol 1996;34(3):584–7.
[4] Pitchford KC, Corey M, Highsmith AK, Perlman R, Bannatyne R, Gold R, et al. Pseudomonas species contamination of cystic fibrosis patients' home inhalation equipment. J Pediatr 1987;111(2):212–6.
[5] Rosenfeld M, Emerson J, Astley S, Joy P, Williams-Warren J, Standaert TA, et al. Home nebulizer use among patients with cystic fibrosis. J Pediatr 1998;132(1):125–31.
[6] Vassal S, Taamma R, Marty N, Sardet A, d'athis P, Bremont F, et al. Microbiologic contamination study of nebulizers after aerosol therapy in patients with cystic fibrosis. Am J Infect Control 2000;28(5):347–51.
[7] Saiman L, Siegel J. Infection control in cystic fibrosis. Clin Microbiol Rev 2004;17(1):57–71.
[8] Jakobsson BM, Onnered AB, Hjelte L, Nystrom B. Low bacterial contamination of nebulizers in home treatment of cystic fibrosis patients. J Hosp Infect 1997;36(3):201–7.
[9] Gilbert P, Brown MR, Costerton JW. Inocula for antimicrobial sensitivity testing: a critical review. J Antimicrob Chemother 1987; 20(2):147–54.
[10] Centers for Disease Control. Guideline for prevention of nosocomial pneumonia and prevention. Respir Care 1994;39(12):1191–236.
[11] Barnes KL, Clifford R, Holgate ST, Murphy D, Comber P, Bell E. Bacterial contamination of home nebuliser. Br Med J 1987;295(6602): 812 [Clin Res Ed].
[12] Wexler MR, Rhame FS, Blumenthal MN, Cameron SB, Juni BA, Fish LA. Transmission of gram-negative bacilli to asthmatic children via home nebulizers. Ann Allergy 1991;66(3):267–71.
[13] Koss JA, Conine TA, Eitzen HE, LoSasso AM. Bacterial contamination potential of sterile, prefilled humidifiers and nebulizer reservoirs. Heart Lung 1979;8(6):1117–21.
[14] Craven DE, Lichtenberg DA, Goularte TA, Make BJ, McCabe WR. Contaminated medication nebulizers in mechanical ventilator circuits. Source of bacterial aerosols. Am J Med 1984;77(5):834–8.

[15] Morris AH. Nebulizer contamination in a burn unit. Am Rev Respir Dis 1973;107(5):802–8.
[16] Saiman L, Siegel J. Infection control recommendations for patients with cystic fibrosis: microbiology, important pathogens, and infection control practices to prevent patient-to-patient transmission. Am J Infect Control 2003;31(3):S6–62.
[17] Standaert TA, Morlin GL, Williams-Warren J, Joy P, Pepe MS, Weber A, et al. Effects of repetitive use and cleaning techniques of disposable jet nebulizers on aerosol generation. Chest 1998;114(2):577–86.
[18] Jakobsson B, Hjelte L, Nystrom B. Low level of bacterial contamination of mist tents used in home treatment of cystic fibrosis patients. J Hosp Infect 2000;44(1):37–41.
[19] Rosenfeld M, Joy P, Nguyen CD, Krzewinski J, Burns JL. Cleaning home nebulizers used by patients with cystic fibrosis: is rinsing with tap water enough? J Hosp Infect 2001;49(3):229–30.