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Microbiological contamination of nebulizers used by cystic fibrosis patients: an underestimated problem

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ABSTRACT

Objective: Home nebulizers are routinely used in the treatment of patients with cystic fibrosis (CF). This study aims to evaluate the contamination of nebulizers used for CF patients, that are chronically colonized by *Pseudomonas aeruginosa*, and the association of nebulizer contamination with cleaning, decontamination and drying practices. **Methods:** A cross-sectional, observational, multicenter study was conducted in seven CF reference centers in Brazil to obtain data from medical records, structured interviews with patients/caregivers were performed, and nebulizer's parts (interface and cup) were collected for microbiological culture. **Results:** overall, 77 CF patients were included. The frequency of nebulizer contamination was 71.6%. *Candida* spp. (52.9%), *Stenotrophomonas maltophilia* (11.9%), non-mucoid *P. aeruginosa* (4.8%), *Staphylococcus aureus* (4.8%) and *Burkholderia cepacia* complex (2.4%) were the most common isolated pathogens. The frequency of nebulizers' hygiene was 97.4%, and 70.3% of patients reported cleaning, disinfection and drying the nebulizers. The use of tap water in cleaning method and outdoor drying of the parts significantly increased (9.10 times) the chance of nebulizers' contamination. **Conclusion:** Despite the high frequency hygiene of the nebulizers reported, the cleaning and disinfection methods used were often inadequate. A significant proportion of nebulizers was contaminated with potentially pathogenic microorganisms for CF patients. These findings support the need to include patients/caregivers in educational programs and / or new strategies for delivering inhaled antibiotics.

Keywords: Cystic fibrosis; *Pseudomonas aeruginosa*; Nebulizers and vaporizers; Equipment contamination; Decontamination.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease, which predominantly affects Caucasians and is potentially fatal.[1,2] Brazilian incidence estimates vary across the country, from 1/1,587-1/32,258 live births.[3] Chronic respiratory infections are the leading cause of death among these patients and *Pseudomonas aeruginosa* is the pathogen most frequently associated with clinical deterioration.[4]

Home nebulizers are widely used by CF patients as part of their treatment, to deliver mucolytics and antibiotics directly to the lungs.[5,6] Epidemiological studies reported the use of inhaled treatment among 35.8%-82.1% of CF patients, depending on the type of medication.[7] Several studies which assessed contamination of the equipment and frequency of at least one pathogen reported a high rate of nebulizer contamination, around 60%.[8-14] Home nebulizer use was associated with a 28.5-fold greater

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chance of bacterial contamination.[15] Nebulizers might be the primary source of colonization for some patients,[14] since proper cleaning instructions are not adequately followed.[10] Therefore, instead of acting as an auxiliary tool for the treatment of CF, nebulizers can become a harmful device if not properly managed.

International guidelines and, recently, the CF Brazilian guideline point to the importance of proper care with nebulizers.[16,17] Cultural, socioeconomic, and even climatic differences can interfere with the quality of care with nebulizers and consequently their contamination.[12,14] In this way, knowledge of regional particularities is essential, since few studies about the contamination profile of home nebulizers are available in developing countries, mainly in Brazil. This study aims to evaluate the contamination of nebulizers used for CF patients chronically colonized by *P. aeruginosa* and its association with cleaning, decontamination and drying practices.

METHODS

Ethics approval

The study was approved by the independent Ethics Committees of each participating site. Informed consent (assent, for those <18 years old) was signed for each patient before any study procedures.

Study design

Cross-sectional, observational, multicenter study was conducted in seven CF Brazilian reference centers. Data collection was performed from January 2013 to December 2014. Data were obtained from three sources (swab samples from nebulizers, medical records and interviews). Patients were asked to carry the nebulizer to the center during a routine visit. At this point, they were not informed about sample collection to avoid an information bias caused by unusual cleaning of the equipment. Swab samples for culture were collected from interface (mouthpiece/mask) and cup to evaluate nebulizers' contamination. Medical records were revised to collect CF data about diagnosis, Shwachman-Kulczycki score, pulmonary function, age, gender, ethnic groups and patients' body mass index (BMI). During a face-to-face structured interview, patient/caregiver (depending on who was responsible for cleaning the device) answered questions about aspects related to nebulizer hygiene routine, nebulizer use and sociodemographic characteristics. For this study, the nebulizer hygiene process was considered as the following steps: cleaning, disinfection and drying.

Eligibility criteria

Eligible patients were those ≥ 6 years old; diagnosed with CF confirmed by sweat chloride test above 60 mEq/dl or evidence of at least two CF causing mutations and on inhaled antibiotics therapy due to chronic airway colonization by *P. aeruginosa*. Patients should be using nebulizer of the brand PARI® and compressor PRONEB® for at least 3 consecutive months. Patients who did not use the nebulizer for inhaled antibiotic therapy for more than 30 days; who shared the nebulizer with other people; had participated of a similar study in the last 12 months and currently participating in a clinical study were excluded.

Sample collection and laboratory testing

Nebulizer assessment was performed between Day 21 and Day 28 from the OFF period of inhaled antibiotic therapy. Samples were collected via swab using aseptic technique described in laboratory's manual and shipped in Amies culture media, which is a liquid used to maintain the viability of microorganisms during transport.[18] Samples were shipped to a central laboratory and analyzed for the presence of pathogens in CF such as *P. aeruginosa* (mucoid and non-mucoid strains), *B. cepacia* complex, *Stenotrophomonas maltophilia*, *Staphylococcus aureus* (sensitive and resistant to methicillin), *Acinetobacter* sp., *Chromobacter* sp. and fungus. Culture mediums used for bacteria isolation were blood agar[19] and chocolate agar[20] and selective agar to *B. cepacia* complex. Bacterial identification was performed on Vitek 2 or mass spectrometry (Vitek MS), both automated systems.[21-23] Antibiotic susceptibility testing was performed on Vitek 2, using a manual confirmation when applicable in accordance with guidelines from Clinical and Laboratory Standards Institute. The culture mediums used to isolate fungus were sabouraud and mycosel agar. These culture mediums had been previously used in CF.[24,25] Non-fermenting Gram-negative bacilli not identified in the culture mediums were analyzed and identified through molecular biology.[26]

Statistical analysis

Considering the contamination risk of 63%,[12] a sample size of 80 patients would provide a 95% confidence interval (CI) with a margin of error of ± 10.5% to assess the study primary endpoint. However, due to recruitment difficulties, the study was interrupted with 77 patients, which provided a 95% CI with a margin of error of ± 10.7% (still lower than the reference study, which CI was 14.3%, considering the same contamination risk of 63%).

Descriptive analysis was performed through measures of central tendency, measures of dependency and measures of dispersion to quantitative variables, and frequency to qualitative variables. To determine the association among variables were estimated p-value by Pearson's Chi-square test and the odds ratio by binary logistic regression. Data were analyzed using the statistic software Stata MP11 and R Project 2.13.1, using a 95% CI and p-value ≤0.05 as significant.

RESULTS

Patients' demographic and disease characteristics

Demographic and disease characteristics of included patients are shown in Table 1.

Nebulizers' contamination profile

Microbiological contamination profile of home nebulizers was grouped in accordance with nebulizer's part (interface, cup or any part of the device) and pathogen contaminant (bacteria, fungus or any contamination) - Table 2. Assessing any nebulizers' parts there was a prevalence of 71.6% (95%CI = 61.3- 81.9) pathogen contamination in the study. According to nebulizer part, frequency observed was 60.8% (95%CI = 49.7-71.9) in interface and 62.2% (95%CI = 51.2-73.2) in cup.

Bacterial contamination was observed in 56.8% (95%CI = 45.5-68.1) of the cases and fungal contamination in 45.9% (95%CI = 34.5-57.3). Among those with bacterial contamination, Gram-negative bacteria was the most commonly found pathogen (85.7%; 95%CI = 75.1-96.3). The most frequently observed Gram-negative bacterial species were *Pseudomonas* spp. (31.0%; 95%CI = 17.0-45.0) and *Acinetobacter* spp. (21.4%; 95%CI=9.0-33.8). *Staphylococcus* spp. (21.4%; 95%CI = 9.0-33.8) and *Micrococcus* spp. (14.3%; 95%CI = 3.7-24.9) were the most frequent Gram-positive bacterial species. *Candida* spp. was the most frequently observed fungus (52.9%; 95%CI = 36.1-69.7), followed by environmental contaminant fungus (26.5%; 95%CI = 11.7-41.3). Other pathogens of interest and with an important role in clinical practice were also isolated: non-mucoid *P. aeruginosa* (4.8%; 95%CI = 0.0-11.3), *B. cepacia* complex (2.4%; 95%CI = 0.0- 7.0), *S. maltophilia* (11.9%; 95%CI=2.1-21.7) and *S. aureus* (4.8%; 95%CI = 0.0-11.3) - Table 2.

Table 1. Cystic fibrosis patients' profile.

Characteristics	N (%)
Age (years)	15.8 ± 6.5
Gender	
Male	44 (57.9)
Female	32 (42.1)
Ethnic groups	
Caucasian	51 (66.2)
Afro-descendent	11 (14.3)
Mixed (*Pardo*)	13 (17.1)
No information	2 (2.6)
Educational level of the responsible for cleaning the nebulizer	
Never been to school	--
Incomplete elementary school	19 (25.0)
Complete elementary school	6 (7.9)
Incomplete high school	7 (9.2)
Complete high school	25 (32.9)
Incomplete graduation	7 (9.2)
Complete graduation	12 (15.8)
Monthly Family Income (BRL$)	2,972.3 ± 2,975.4
Number of people who cohabit	3.9 ± 1.4
Siblings living in the same residence	0.9 ± 1.0
Rooms in the house	3.7 ± 1.6
Distance between the household and the treatment center (km)	56.3 ± 92.2
BMI (kg/m^2)	18.7 ± 3.65
Height (cm)	155.0 ± 17.2
Weight (kg)	49.7 ± 16.1
Time since CF diagnosis (years)	10.2 ± 5.68
Shwachman-Kulczycki Score	
Excellent	4 (5.2)
Good	10 (13.0)
Medium	11 (14.3)
Moderate	6 (7.8)
Severe	1 (1.3)
Pulmonary function test	
FEV_1 (%)	61.3 ± 22.9
FVC (%)	75.5 ± 24.1
FEV_1/FVC (%)	76.8 ± 19.4

Values are presented as mean ± SD or number (%). BMI: Body mass index; FEV_1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; FEV_1/FVC: ratio of forced expiratory volume in 1 second to forced vital capacity.

Table 2. Contamination profile of home nebulizers of cystic fibrosis patients: type of fungus and bacteria according to place of contamination.

Characteristics	Interface		Cup		Any part of the device	
	N (%)	95%CI	N (%)	95%CI	N (%)	95%CI
Any contamination	45 (60.8)	49.7 - 71.9	46 (62.2)	51.2 - 73.2	53 (71.6)	61.3 - 81.9
Bacterial contamination	35 (47.3)	35.9 - 58.7	37 (50.0)	38.6 - 61.4	42 (56.8)	45.5 - 68.1
Gram-negative bacteria[a]	25 (71.4)	56.4 - 86.4	23 (62.2)	46.6 - 77.8	36 (85.7)	75.1 - 96.3
Pseudomonas spp.[a]	5 (14.3)	2.7 - 25.9	9 (24.3)	10.5 - 38.1	13 (31.0)	17.0 - 45.0
Non-mucoid *Pseudomonas aeruginosa*[a]	--	--	2 (5.4)	0.0 - 12.7	2 (4.8)	0.0 - 11.3
Mucoid *Pseudomonas aeruginosa*[a]	--	--	--	--	--	--
Other *Pseudomonas*[a]	5 (14.3)	2.7 - 25.9	7 (18.9)	6.3 - 31.5	10 (23.8)	10.9 - 36.7
Acinetobacter spp.[a]	8 (22.9)	9.0 - 36.8	7 (18.9)	6.3 - 31.5	9 (21.4)	9.0 - 33.8
Stenotrophomonas spp.[a]	5 (14.3)	2.7 - 25.9	4 (10.8)	0.8 - 20.8	5 (11.9)	2.1 - 21.7
Stenotrophomonas maltophilia[a]	5 (14.3)	2.7 - 25.9	4 (10.8)	0.8 - 20.8	5 (11.9)	2.1 - 21.7
Enterobacter spp.[a]	3 (8.6)	0.0 - 17.9	4 (10.8)	0.8 - 20.8	4 (11.9)	2.1 - 21.7
Klebsiella spp.[a]	1 (2.9)	0.0 - 8.5	4 (10.8)	0.8 - 20.8	4 (9.5)	0.6 - 18.4
Sphingobacterium spp.[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	2 (4.8)	0.0 - 11.3
Delftia spp.[a]	1 (2.9)	0.0 - 8.5	2 (5.4)	0.0 - 12.7	2 (4.8)	0.0 - 11.3
Burkholderia spp.[a]	--	--	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 -7.0
Burkholderia cepacia complex[a]	--	--	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 -7.0
Other[a]	12 (34.3)	18.6 - 50.0	9 (24.3)	10.5 - 38.1	15 (35.7)	21.2 - 50.2
Chryseobacterium indologenes[a]	5 (14.3)	2.7 - 25.9	4 (10.8)	0.8 - 20.8	5 (11.9)	2.1 - 21.7
Sphingomonas paucimobilis[a]	2 (5.7)	0.0 - 13.4	1 (2.7)	0.0 - 7.9	3 (7.1)	0.0 - 14.9
Pantoea agglomerans[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	2 (4.8)	0.0 - 11.3
Aeromonas hydrophila[a]	1 (2.9)	0.0 - 8.5	--	--	1 (2.4)	0.0 - 7.0
Comamonas testosteron[a]	--	--	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Moraxella osloensis[a]	1 (2.9)	0.0 - 8.5	--	--	1 (2.4)	0.0 - 7.0
Rhizobium radiobacter[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Serratia marcescens[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Gram-positive bacteria[a]	10 (28.6)	13.6 - 43.6	10 (27.0)	12.7 - 41.3	17 (40.5)	25.7 - 55.3
Staphylococcus spp.[a]	6 (17.1)	4.6 - 29.6	7 (18.9)	6.3 - 31.5	9 (21.4)	9.0 - 33.8
Staphylococcus aureus[a]	2 (5.7)	0.0 - 13.4	2 (5.4)	0.0 - 12.7	2 (4.8)	0.0 - 11.3
Oxacillin-resistant coagulase-negative *Staphylococcus aureus*[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Other *Staphylococcus*[a]	3 (8.6)	0.0 - 17.9	4 (10.8)	0.8 - 20.8	6 (14.3)	3.7 - 24.9
Micrococcus spp.[a]	4 (11.4)	0.9 - 21.9	4 (10.8)	0.8 - 20.8	6 (14.3)	3.7 - 24.9
Bacillus spp.[a]	3 (8.6)	0.0 - 17.9	4 (10.8)	0.8 - 20.8	5 (11.9)	2.1 - 21.7
Streptococcus spp.[a]	--	--	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Fungal contamination	20 (27.0)	16.9 - 37.1	28 (37.8)	26.8 - 48.8	34 (45.9)	34.5 - 57.3
Candida spp.[b]	11 (55.0)	33.2 - 76.8	14 (50.0)	31.5 - 68.5	18 (52.9)	36.1 - 69.7
Non-*albicans Candida* spp.[b]	9 (45.0)	23.2 - 66.8	14 (50.0)	31.5 - 68.5	16 (47.1)	30.3 - 63.9
Candida albicans[b]	1 (5.0)	0.0 - 14.6	--	--	1 (2.9)	0.0 - 8.5
Candida spp.[b]	1 (5.0)	0.0 - 14.6	--	--	1 (2.9)	0.0 - 8.5
Environmental contaminant fungus[b]	4 (20.0)	2.5 - 37.5	8 (28.6)	11.9 - 45.3	9 (26.5)	11.7 - 41.3
Other[b]	7 (35.0)	14.1 - 55.9	7 (25.0)	9.0 - 41.0	10 (29.4)	14.1 - 44.7
Cladosporium sp.[b]	3 (15.0)	0.0 - 30.6	3 (10.7)	0.0 - 22.1	4 (11.8)	1.0 - 22.6
Rhodotorula spp.[b]	3 (15.0)	0.0 - 30.6	3 (10.7)	0.0 - 22.1	4 (11.8)	1.0 - 22.6
Aspergillus niger[b]	--	--	1 (3.6)	0.0 - 10.5	1 (2.9)	0.0 - 8.5
Penicillium sp.[b]	1 (5.0)	0.0 - 14.6	--	--	1 (2.9)	0.0 - 8.5

[a]Proportion calculated among number of interfaces (N=35), cups (N=37) and any part of nebulizer (N=42) with bacterial contamination. [b]Proportion calculated among number of interfaces (N=20), cups (N=28) and any part of nebulizer (N=34) with bacterial contamination.

Characteristics of nebulizers´ hygiene

Considering characteristics of nebulizers use, frequency of nebulizer hygiene and method employed, 76 patients answered the interview questions. Patients reported the use of the following medications in nebulizer: dornase alfa (N = 72; 94.7%), tobramycin

inhaler solution (N = 64; 84.2%), hypertonic saline solution (N = 17; 22.4%), colistin (N = 15; 19.7%), bronchodilator (N = 5; 6.6%) and isotonic saline solution (N = 1; 1.3%). All patients reported to use only one drug during each nebulization.

Regarding the nebulizers' parts replacement, most patients had not performed it in the analyzed equipment (N = 48; 63.2%) and the reported reasons were: recommended interval to switch had not been reached (N = 29; 60.4%); lack of knowledge about the necessity (N = 12; 25.0%); forgetfulness (N = 2; 4.2%); and other reasons (N = 7; 14.6%). For those who reported to replace at least one part, cup was replaced in 85.7% (N = 24), hose in 64.3% (N = 18), interface and filter in 60.7% (N = 17) of the cases, other parts in 7.1% (N = 2) and all parts in 21.4% (N = 6). For patients who replaced all parts, half did it after more than six months of use.

Regular nebulizer hygiene was reported by 97.4% of the cases. Among those who reported regular nebulizer hygiene, the cup was the most cited part (N = 74; 100.0%), followed by the interface 79.7% (N = 59), hose 50.0% (N = 37) and filter 12.2% (N = 9). Most patients (71.1%) reported to perform nebulizer hygiene process after each nebulizer's use -Table 3.

Considering each step of nebulizer hygiene, 64 (86.5%) patients performed the cleaning process, 62 (83.8%) patients performed the disinfection process, and 73 (96.0%) patients performed the drying process. Most frequent cleaning process observed was lather and rinse under tap water (N = 49; 76.6%). A wide variety of disinfection methods were reported and the most frequent were immersion in boiling water (24.2%) and immersion in hypochlorite solution (21.0%). The entire process of nebulizer hygiene, using at least one cleaning, disinfection and drying method was reported by most of the study sample (70.3%) -Table 3.

Also, as a secondary objective of the study, the association between educational level and demographic data from patients and/or caregivers and the frequency of nebulizers cleaning was assessed and no significant differences were observed (data not shown).

Relation between nebulizers' cleaning and pathogen contamination

Bivariate analysis of the association between the nebulizers' cleaning and a positive culture for bacteria and/or fungus in the analyzed pieces are described in Table 4. A statistically significant difference in the frequency of contamination was observed for cleaning method (Only tap water = 92.9% of contamination vs. Lather and rinse under tap water = 66.0% of contamination; p = 0.049), performing or not disinfection (Yes = 66.7% of contamination *vs*. No = 100.0% of contamination; p = 0.015) and drying method (With a cloth, paper towels, fan/dryer or compressor/compressed air = 60.5% of contamination *vs*. Only outdoors = 84.4% of contamination; p = 0.028). A multivariate analysis by binary logistic regression for the factors associated to the positivity of culture was performed using the stepwise backward strategy. For this analysis, any contamination in nebulizer and the variables reported in Table 4 were included. The use only of tap water as a cleaning method increased 9-fold chance of contamination (OR = 9.10; 95%CI = 1.01-81.77; p = 0.049) when compared with the use of lather and rinse under tap water. Drying outdoors increased 4.87-fold chance of contamination (OR = 4.87; 95%CI = 1.10-21.61; p = 0.038) when compared with the use of some material, such as a cloth, paper towel, fan/dryer or the compressor/compressed air.

As nebulizer drying process performed outdoor is a recommended practice, the frequency of an inadequate cleaning (none or only tap water) or disinfection (none, sodium hypochlorite or vinegar solution) was assessed. An inadequate cleaning method was observed in 26% of the sample (N = 7), an inadequate disinfection in 40.7% (N = 11), both inadequate cleaning and disinfection methods in 7.4% (N=2) and inadequate cleaning or disinfection methods in 59.2% (N = 16).

DISCUSSION

In this multi-centric Brazilian study, a high prevalence of nebulizer contamination was observed among CF patients chronically colonized by *P. aeruginosa* under inhaled antibiotic on-off therapy. The role of home nebulizers as a source of contamination for patients with CF has been studied since 1987[8-14] but the amount of good and representative data within CF Brazilian patients is scarce. In addition, a high rate of nonconformities was observed in nebulizer use by patients and caregivers. This is an important issue as inadequate cleaning of the nebulizer has been associated with its contamination.[12]

Considering the prevalence of contamination in any part of the device, previous Brazilian studies found estimates from 25.0% to 57.5%, lower than those found in the current study.[10,13] This difference can be possibly attributed to distinct clinical characteristics between populations such as severity of lung function impairment and by patients' behavior. In the present study, adequate care with nebulizers was systematically analyzed and a low rate of appropriate management was observed.

Bacteria were the main pathogenic contaminants identified in the studied devices (56.8% of the patients), mainly the Gram-negative ones. Nevertheless, fungal contamination was also a relevant finding since 40.5% of patients were contaminated by large fungal species variety. Current literature, ranging several countries, has also shown a wide variety of bacterial specimens with heterogeneous results, varying the higher prevalence between Gram-positive and Gram-negative bacteria. *Acinetobacter* spp. and *Pseudomonas* spp. were the most frequently reported Gram-negative bacteria and *Staphylococcus* spp., was the most

Table 3. Hygienization profile of home nebulizers of cystic fibrosis patients.

Characteristics	N (%)
Nebulizer is regularly hygienized	
Yes	74 (97.4)
No	2 (2.6)
Nebulizer parts usually hygienized	
Interface	59 (79.7)
Cup	74 (100.0)
Hose	37 (50.0)
Filter	9 (12.2)
Other	1 (1.4)
Hygienization after each use	
Yes	54 (71.1)
No	20 (26.3)
No information	2 (2.6)
Length of each cleaning/disinfection	
Less than 15 minutes	44 (57.9)
More than 15 minutes	30 (39.5)
No information	2 (2.6)
Cleaning	64 (86.5)
Only tap water	15 (23.4)
Lather and rinse under tap water	49 (76.6)
Disinfection	62 (83.8)
Immersion in boiling water	15 (24.2)
Immersion in sodium hypochlorite solution	13(21.0)
Immersion in boiling water and Immersion in sodium hypochlorite solution	11 (17.7)
Immersion in vinegar solution	5 (8.1)
Immersion in boiling water and Immersion in alcohol	4 (6.5)
Immersion in boiling water and Immersion in alcohol and Immersion in vinegar solution	1 (1.6)
Immersion in boiling water and Immersion in vinegar solution	3 (4.8)
Immersion in alcohol	2 (3.2)
Immersion in sodium hypochlorite solution and Microwave	2 (3.2)
Immersion in boiling water and Immersion in sodium hypochlorite solution and Immersion in alcohol	2 (3.2)
Immersion in boiling water and Immersion in sodium hypochlorite solution and Immersion in vinegar solution	2 (3.2)
Immersion in boiling water and Immersion in sodium hypochlorite solution and Immersion in vinegar solution and Immersion in alcohol	1 (1.6)
Immersion in sodium hypochlorite solution and Immersion in alcohol	1 (1.6)
Drying	73 (98.6)
Only outdoors	32 (43.8)
With a cloth	20 (27.4)
With paper towel	19 (26.0)
With a fan/dryer	1 (1.4)
With the compressor/compressed air	1 (1.4)
Hygienization method	
Clean, disinfection and dry	52 (70.3)
Clean and dry	11 (14.9)
Disinfection and dry	10 (13.5)
Only clean	1 (1.4)

frequent Gram-positive bacteria.[8-14] Previous Brazilian studies found *Staphylococcus* spp. as the most frequent pathogen contaminating nebulizers.[10,13] In the present study, the assessment of nebulizer contamination was performed in a significantly larger population, although still pediatric, older than in previous studies. Furthermore, it is well described the relationship of increasing airway colonization by Gram-negative bacteria in CF patients with age increase, associated with a decrease by Gram-positive.[1] This behavior might interfere in the nebulizer contamination and may explain the different results in this field.

Table 4. Association between nebulizers' hygienization and positivity of culture.

Characteristics	Contamination by fungus or bacteria at least in one part of the nebulizer		p-value
	Yes N (%)	No N (%)	
Nebulizer parts usually hygienized[a]			
Interface	40 (70.2)	17 (29.8)	0.380
Cup	51 (71.8)	20 (28.2)	0.378
Hose	24 (66.7)	12 (33.3)	0.262
Filter	5 (55.6)	4 (44.4)	0.221
Other	1 (100.0)	0 (--)	0.536
Length of each hygienization			
Less than 15 minutes	31 (72.1)	12 (27.9)	0.951
More than 15 minutes	20 (71.4)	8 (28.6)	
Hygienization after each use			
Yes	23 (45.1)	28 (54.9)	0.710
No	8 (40.0)	12 (60.0)	
Cleaning			
Yes	44 (72.1)	17 (27.9)	0.839
No	9 (75.0)	3 (25.0)	
Cleaning method			
Only tap water	13 (92.9)	1 (7.1)	0.049
Lather and rinse under tap water	31 (66.0)	16 (34.0)	
Disinfection			
Yes	40 (66.7)	20 (33.3)	0.015
No	13 (100.0)	0 (--)	
Disinfection method			
Immersion in boiling water and/or alcohol and/or microwave only or in association with sodium hypochlorite and/or vinegar solution	30 (69.8)	13 (30.2)	0.595
Immersion in bleach and/or vinegar solution, without other methods	10 (62.5)	6 (37.5)	
Dry			
Yes	50 (71.4)	20 (28.6)	0.277
No	3 (100.0)	0 (--)	
Drying method			
With a cloth, paper towel, fan/dryer or compressor/compressed air	23 (60.5)	15 (39.5)	0.028
Only outdoors	27 (84.4)	5 (15.6)	
Hygienization method			
No hygienization	2 (100.0)	0 (--)	
Clean, disinfection and dry	33 (66.0)	17 (34.0)	
Only clean	1 (100.0)	0 (--)	0.197
Clean and dry	10 (100.0)	0 (--)	
Disinfection and dry	7 (70.0)	3 (30.0)	

[a]Since the answers were not mutually exclusive, each option was analyzed as a dichotomous variable generating different p-values.

Pulmonary infection is the leading cause of death in CF, being most cases associated to *P. aeruginosa* chronic infection. Several different sources can be implicated in airway colonization by *P. aeruginosa*, including nebulizers.[12,14] Despite the high estimates of *Pseudomonas* spp detected in the present study, most cases were related to species other than *P. aeruginosa*. The prevalence of *P. aeruginosa* in the literature ranges from 0%- 38%.[8-14] A low frequency of contamination by this pathogen in our data may probably be associated with the specific profile of our sample. Inclusion criteria definition allowed only patients with chronic colonization by *P. aeruginosa* in regular use of anti-Pseudomonas inhaled antibiotics that could interfere in bacterial growth even in the OFF month of treatment cycle.

Fungal contamination is less explored in available literature and specimens found were not clearly assessed in other studies.[8-14] In our sample, *Candida* spp. was the most common fungus found. Other

studies reported contamination by yeast, specifically by *Candida albicans* (14.0%), which was also observed in our sample (2.9%).[10,12,13] Peckham et al. also conducted a study to analyze specifically the fungal flora of nebulizers of CF adult patients and found a higher frequency of positivity (57.7%) than reported in our study (45.9%).[24]

We found a considerably higher frequency of patients who reported a regular hygiene of the nebulizer compared to other surveys.[5,9,10,13] National and international guidelines emphasizes the necessity to adequate care of nebulizers.[16,17] Cleaning steps must be performed with dish detergent soap and water, disinfection with boiling water, microwave, dishwasher, alcohol or hydrogen peroxide and lately air drying the equipment.[27] A high percentage of patients reported performing all the proposed steps. However, methods not recommended such as cleaning using only tap water, disinfection by sodium hypochlorite or vinegar solution and use of materials for drying were frequently reported. This discrepancy between the high frequency of contamination of nebulizers despite a high self-reported rate of adequate care of the devices points toward the need for better education of patients and caregivers. It is important to emphasize that self-reporting care with nebulizers does not necessarily translate into daily practice. However, in this study a high rate of not recommended nebulizer hygiene actions were observed, further reinforcing the need for improvement in the knowledge of this population. Because this is a multicenter study covering different regions of the country, we consider these data as highly relevant because it characterizes a problem found in all the centers studied and reflects a widespread problem.

A higher frequency of contamination among patients who clean the nebulizer only under tap water, do not disinfect it and dry outdoors was observed. Previous studies found that only the cleaning after each use had significant differences.[8,9] Hohenwarter et al. compared different steam disinfection and drying methods and found recontamination only among those equipment in which an active drying (such as paper or cotton towels) was performed.[6] A multivariate model including these characteristics was built and demonstrated that cleaning under tap water only and drying outdoors were the factors that increase the chance of contamination.

Drying outdoors is a recommended method as category II of evidence level (supported by suggestive clinical and epidemiologic studies). However, in the present study, it was associated with an increase of 4.87-fold chance of contamination. To verify if this association was related to cleaning and disinfection patterns, these frequencies among contaminated nebulizers that were dried outdoors were assessed and most patients reported at least one inadequate method of cleaning or disinfection (59.2%). This study was not designed to test a hypothesis and available recommendations are not based on the highest evidence level, which highlights the need for conducting more studies regarding each particular component of the nebulizer hygiene process. Another Brazilian study assessed the effect of a standardized instruction regarding nebulizers' cleaning and disinfection based on the international recommendations on the frequency of contamination[13,28] and after a single educational intervention, a significant impact was observed, reducing the frequency of contamination by 43%.[13] The proper cleaning of nebulizers can have clinical impact, since lack of cleanliness can reduce nebulizer performance and the equipment can become a potential source of contamination.[29]

There are some limitations in our study. Although this was a multicenter study with CF centers from different regions in Brazil, it was not possible to cover all states of the country. Another limitation refers to the request for patients to bring their nebulizers to assessment by the CF staff. Patients were not aware of the objective of the study before arriving in CF clinic, but we cannot exclude unusual cleaning before visit and information bias due to fear of reporting known misconduct acts to the study team. In addition, no viral agents were tested in this study although the relevance of transmission of this type of pathogen by nebulizer is yet not clear. Finally, data from patients' sputum culture was not assessed. Therefore, the relationship between airway and nebulizer contamination in the present study could not be determined.

In conclusion, high prevalence of contamination in CF nebulizers was observed despite the reports of elevated frequency of nebulizer hygiene. Most patients reported wrong cleaning techniques, emphasizing that CF team should be aware about this problem and intensify educational programs. Airway infection is one of the most important issues in CF management and several strategies should be stimulated to avoid it. The present study highlights that nebulizers are still a potential source of infection for CF patients.

Therefore, better knowledge about this area should be encouraged between patients and caregivers and/or new strategies for inhaled antibiotic delivery, such as dry powder formulations, should be implemented.

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