Nebulizers in cystic fibrosis: a source of bacterial contamination in cystic fibrosis patients?*

Nebulizadores: fonte de contaminação bacteriana em pacientes com fibrose cística?

Lorena Xavier Costa Brzezinski, Carlos Antônio Riedi, Paulo Kussek, Helena Homem de Melo de Souza, Nelson Rosário

Abstract

Objective: To determine whether nebulizers are a source of microbial contamination in patients with cystic fibrosis, as well as whether the technique and frequency of disinfection of these devices is appropriate. **Methods:** This was a cross-sectional, uncontrolled observational study. Samples were collected from 28 patients with cystic fibrosis. Samples were collected at the homes of the patients, who were not previously informed of the purpose of the visit. Three samples were collected from each patient: one from the nebulizer chamber, one from the mask/mouthpiece, and one from the patient (oropharyngeal swab/sputum). The samples were properly stored and taken for analyses. The patients, their parents, or their legal guardians completed a questionnaire regarding nebulizer cleaning and disinfecting methods. **Results:** We collected 84 samples from the 28 patients. Of those 28 patients, 15 (53.5%) were male. The median age of the patients was 11 years (range, 1-27 years). Of the 28 patients, 15 presented with positive oropharyngeal swab/sputum sample cultures. The most common bacterial isolates were *Staphylococcus aureus* (in 8 patients) and *Pseudomonas aeruginosa* (in 4 patients). Although the samples obtained from the nebulizers presented with various pathogens in culture, no specific species predominated. In 27 cases (96.7%), there were no associations between the samples obtained from the nebulizers and those obtained from the patients in terms of the results of the cultures. Cleaning and disinfection of nebulizers were inappropriate in 22 cases (78.6%). **Conclusions:** In this sample of patients, despite the inappropriate disinfection techniques, nebulizers were not found to be a source of microbial contamination.

Keywords: Cystic fibrosis; Nebulizers and vaporizers; Disinfection.

Resumo

Objetivo: Determinar se os nebulizadores de pacientes com fibrose cística são fonte de contaminação microbiana e verificar se a técnica e a frequência de desinfecção dos nebulizadores é apropriada. **Métodos:** Estudo de corte transversal observacional, sem grupo controle. Foram coletadas amostras de 28 pacientes com fibrose cística, no domicílio do paciente, sem aviso prévio sobre o motivo da visita. Foram colhidas três amostras por paciente: do reservatório do nebulizador, da máscara/bocal e do próprio paciente (*swab* da orofaringe/escarro). As amostras foram acondicionadas adequadamente e levadas para análise. Os pacientes, seus pais ou responsáveis preencheram um questionário sobre métodos de limpeza e desinfecção dos nebulizadores. **Resultados:** Foram obtidas 84 amostras dos 28 pacientes. Destes, 15 (53,5%) eram do gênero masculino. A mediana de idade foi de 11 anos (variação: 1-27 anos). Dos 28 pacientes, 15 apresentaram culturas de escarro/orofaringe positivas. As bactérias encontradas com maior frequencia foram *Streptococcus aureus* (8/15) e *Pseudomonas aeruginosa* (4/15). A cultura obtida dos nebulizadores identificou diversos patógenos, sem nenhum predominante. Não houve associações entre os resultados das culturas obtidas dos nebulizadores e aquelas dos pacientes em 27 casos (96,7%). A limpeza e a desinfecção não eram realizadas de forma adequada em 22 casos (78,6%). **Conclusões:** Nesta amostra de pacientes, apesar das técnicas de desinfecção inadequadas, os nebulizadores não foram uma fonte de contaminação microbiana.

Descritores: Fibrose cística; Nebulizadores e vaporizadores; Desinfecção.

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Introduction

Cystic fibrosis (CF) is an autosomal recessive hereditary disease that is common among White individuals and is life-threatening. In some Brazilian states, including the state of Paraná, a newborn screening test, popularly known as "the heel prick test", is available. Heel prick blood samples are submitted to laboratory tests that allow the diagnosis of and early medical intervention for certain diseases, including CF. The incidence of CF varies by country and region. In Brazil, the estimated incidence of CF is 1/10,000 live births and varies among the different states. The disease is more common in southern Brazil, where there is a greater concentration of European descendants.[1]

The disease is characterized by exocrine gland involvement due to changes in chloride transport in the cell membrane of the protein designated cystic fibrosis transmembrane conductance regulator (CFTR). The presence of two mutant alleles in the CF gene causes lack of activity or partial functioning of the CFTR protein. This reduces chloride excretion and increases intracellular electronegativity, resulting in greater sodium flux as a compensatory response in order to preserve the electrochemical equilibrium and, secondarily, as a result of an excess of intracellular ions and greater influx of water into the cell. Therefore, mucosal secretions dehydrate and become more viscous, which results in duct obstruction, inflammatory reaction, and a subsequent fibrotic process. Impaired mucociliary clearance results in mucus accumulation, duct obstruction, and airway infection, which typically worsens and becomes recurrent. Airway involvement occurs in over 95% of the patients, and the intensity of airway involvement determines the final prognosis. Approximately half of the patients who are diagnosed early and have access to appropriate multidisciplinary treatment survive until the third decade of life.[1] Initially, infections with *Staphylococcus aureus* and *Haemophilus influenzae* are common; subsequently, infections with other microorganisms can occur, generally in the following order: *Pseudomonas aeruginosa* infection, mucoid *P. aeruginosa* infection; and *Burkholderia cepacia* complex infection.[2]

The treatments that are available for CF patients include the use of inhaled drugs, such as bronchodilators, DNase, and inhaled antibiotics in 82%, 58%, and 61% of the patients, respectively.[3]

There is a concern that home nebulizers can be a source of bacterial infection of the lower airways.[4] Patients do not always use nebulizers correctly or disinfect them appropriately; this can decrease the performance of these devices and cause bacterial contamination.[5-8] The Cystic Fibrosis Foundation recommends that nebulizers be cleaned, disinfected, rinsed, and dried after their use in order to prevent bacterial contamination. Infection prevention and CF control measures are carried out in order to reduce the risk of exposure to pathogens. Nebulizer cleaning and disinfecting methods vary widely among hospitals and centers for the treatment of CF. Studies evaluating nebulizer cleaning and disinfecting methods concluded that disinfection with acetic acid is ineffective.[4] Various methods can be used in order to clean nebulizers. One effective method consists of using a solution of 1 L of water and 5 mL of sodium hypochlorite for 20 min daily, followed by the drying of the device.[4,9]

The objective of the present study was to determine whether nebulizers are contaminated by bacteria and correlate the contamination with the colonization of the airways of patients with CF, as well as to determine whether appropriate nebulizer disinfection techniques are being employed and whether the frequency of such disinfection is adequate.

Methods

This was a cross-sectional, uncontrolled observational study. The inclusion criteria were as follows: presenting with CF; being treated at the outpatient clinic of the *Hospital de Clínicas da Universidade Federal do Paraná* (HC-UFPR, Federal University of Paraná *Hospital de Clínicas*) or of the *Hospital Pequeno Príncipe* (HPP, Pequeno Príncipe Hospital); residing in the city or metropolitan area of Curitiba; and using inhaled medication on a regular basis. Patients who did not reside in the abovementioned areas were excluded because of the difficulty in collecting the material. Patients who did not use inhaled medication were also excluded, as were those whose parents or legal guardians refused to participate in the study.

The study was approved by the Research Ethics Committees of the HPP and HC-UFPR.

Patients were contacted by telephone in order to schedule a home visit. The purpose of the visit was not revealed, in an attempt to avoid changes in the attitudes of patients. At their homes, the patients, their parents, or their legal guardians gave written informed consent.

Three samples were collected from each CF patient: one from the nebulizer chamber, one from the mask or mouthpiece used by the patient, and one from the patient (oropharyngeal swab or sputum sample). The oropharyngeal material was collected from the tonsillar pillars and posterior pharynx using a sterile cotton swab. A single swab was used in order to collect material from all three points without touching the tongue or the lateral walls of the oral cavity. The material from the nebulizer chamber was collected by rubbing a swab moistened with PBS containing 0.1% gelatin (PBSG) against the bottom and corners of the chamber. The material from the mask/mouthpiece was collected by rubbing another swab moistened with PBSG against the inner part of the mask/mouthpiece, principally against grooves and depressions. The swabs were placed in tubes containing PBSG at room temperature, the excess swab stems were cut off, and the tubes were firmly capped. Subsequently, the samples were taken for analyses. The maximum time elapsed between sample collection and sample storage at the laboratory was 3 h.

The samples were seeded into or smeared onto bacterial culture media in accordance with the techniques routinely employed at the HC-UFPR bacteriology laboratory, as follows:

- Oropharyngeal samples: The tube containing a swab and PBSG was vortexed for 30 s, and the swab was subsequently discarded. By means of a 1:100 calibrated loop and the streaking technique, the material was seeded onto MacConkey agar, mannitol salt agar (MSA), and *B. cepacia* selective agar (BCSA) plates, and the plates were incubated at 35-37°C for up to 48 h (on MacConkey agar and MSA) or for up to 72 h (on BCSA) in room air. All of the colonies obtained were identified.
- Samples from the mask, mouthpiece, and nebulizer chamber: For the material obtained from the nebulizers, we also used the method described above.
- Sputum samples: Sputum was diluted at 10^{-4} and 10^{-6} and, by means of a 1:100 calibrated loop and the streaking technique, smeared onto MacConkey agar (10^{-4} and 10^{-6} dilutions), MSA (10^{-6} dilution), and BCSA (10^{-6} dilution) plates. The incubation and colony identification processes followed the protocol described above.

The patients, their parents, or their legal guardians completed a questionnaire regarding nebulizer cleaning and disinfecting methods, including the type of cleaning and disinfection, frequency/duration of use of the nebulizer and its parts, medications administered by the nebulizer, and the type of nebulizer used (Appendix 1).

After sample collection, the patients, their parents, or their legal guardians received a brochure with instructions on how to clean and disinfect nebulizers correctly.

For the statistical analysis, we used the test of proportions in order to determine whether there were correlations among the study variables, values of $p < 0.05$ being considered significant. It was impossible to determine the sample size because the values and variability of association between bacteria in sputum and the presence of bacteria in inhalers are unknown.

Results

A total of 34 patients were randomly selected. We excluded 6 patients because they did not use inhaled drugs. Of the patients evaluated, most used two or three types of inhaled drugs.

Of the 28 patients under study, 15 (53.5%) were male and 13 (46.5%) were female. The median age of the patients was 11 years (range, 1-27 years).

Of the 28 patients, 15 presented with positive oropharyngeal swab/sputum sample cultures. The most common bacterial isolates were *S. aureus* (in 8 patients), *P. aeruginosa* (in 4 patients), and *B. cepacia* complex (in 3 patients). In the nebulizers of 6 patients, there was growth of bacteria related to CF.

No specific bacterial strains predominated in the samples from the mouthpiece or in those from the mask, and the result was negative in samples from the nebulizers of 22 patients (Table 1).

Table 1 - Results of the cultures of the samples collected from 28 patients with cystic fibrosis.

Patients	Culture results by type of sample		
	Sputum/oropharynx	Nebulizer chamber	Mask/mouthpiece
1	*Staphylococcus aureus*	*Stenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*
2	*Staphylococcus aureus*	Negative	Negative
3	Negative	*Klebsiella pneumoniae*, coagulase-negative staphylococci	*Klebsiella pneumoniae*, coagulase-negative staphylococci
4	*Burkholderia cepacia*	Negative	Negative
5	*Staphylococcus aureus*	Negative	Yeasts
6	*Staphylococcus aureus* and *Pseudomonas aeruginosa*	Negative	Negative
7	*Burkholderia cepacia*	Negative	Negative
8	*Staphylococcus aureus*	*Serratia marcescens*	*Serratia marcescens*
9	Negative	*Klebsiella ozaenae*, coagulase-negative staphylococci, yeasts	Coagulase-negative staphylococci
10	Methicillin-resistant *Staphylococcus aureus*	Negative	Negative
11	*Pseudomonas aeruginosa*	Negative	Negative
12	Negative	Negative	Negative
13	Negative	Negative	Negative
14	*Pseudomonas aeruginosa*	Negative	Negative
15	Negative	Negative	Negative
16	Negative	Negative	Negative
17	Negative	Negative	Negative
18	*Pseudomonas aeruginosa* and Mucoid *Pseudomonas aeruginosa*	Negative	Negative
19	Negative	*Bacillus* sp.	Negative
20	*Staphylococcus aureus*	Negative	Negative
21	*Staphylococcus aureus*	*Achromobacter xylosoxidans*	Negative
22	*Pseudomonas aeruginosa*	Negative	Negative
23	*Burkholderia cepacia*	*Pseudomonas aeruginosa*	Negative
24	Negative	Negative	Negative
25	Negative	Negative	Negative
26	Negative	Negative	Negative
27	*Staphylococcus aureus*	Staphylococcus aureus	*Staphylococcus aureus*
28	Negative	Negative	Negative

No specific bacterial strains predominated in the material obtained from the nebulizer chamber.

In 27 cases (96.7%), there were no associations between the samples obtained from the nebulizers and those obtained from the patients in terms of the results of the cultures.

Of the 28 patients, 22 (78.6%) cleaned and disinfected their nebulizers incorrectly: with vinegar (1 patient); with alcohol (1 patient); with boiling water (6 patients); sporadically (6 patients); or not at all (8 patients). The mean duration of use for a single nebulizer was 3 years. In that period, 18 patients (64%) replaced nebulizer parts, such as the mouthpiece, the mask, and the chamber.

Discussion

The results allowed us to identify a variety of nebulizer cleaning techniques. This might be due to the fact that the patients had been referred from more than one treatment facility and had probably received different instructions.

Inhaled medication is essential in the treatment of patients with CF because it allows the administration of high concentrations of medication in the respiratory tract. The development of inhaled therapies is an advance

in the treatment of CF patients, and new inhaled therapies have been developed. Therefore, the risk of nebulizer contamination due to frequent use in CF patients colonized by communicable bacteria is higher.[7]

Two important aspects that constitute a cause for concern were observed in the present study: many of the patients had not changed their nebulizers frequently enough (mean duration of use, 3 years), and 50% rarely if ever disinfected their nebulizer. Prolonged use can create fissures in the nebulizer. These fissures can make nebulizer cleaning more difficult, which theoretically contributes to the development of bacteria. These results are similar to those of another study, in which the authors found that only 15% of the patients disinfected their nebulizers on a weekly basis.[7]

Regular cleaning and replacement of nebulizers can reduce bacterial contamination. Patients who follow the recommendations for the cleaning and subsequent drying of nebulizers are less likely to develop bacterial infections.[10] In the present study, three nebulizer disinfecting methods were observed. One group of authors evaluated five nebulizer disinfecting methods by artificially contaminating 160 masks and mouthpieces with 16 types of bacteria found in patients with CF. The only method that failed was disinfection with acetic acid (in 5.3%), which failed to eliminate a resistant strain of *S. aureus*.[4] Another group of authors reported that the level of nebulizer contamination can be as high as 55%. The recommendations for the cleaning of nebulizers generally depend on the type of nebulizer and vary among centers. In addition, these recommendations have not been validated. In daily practice, the proportion of patients with CF who follow the recommendations for the cleaning of nebulizers ranges from 3% to 98%.[11]

In the present study, most (78.6%) of the patients did not use the recommended nebulizer cleaning techniques, and 28.5% did not clean their nebulizer at all. However, we found no association between the presence of bacteria in the airways (as assessed by sputum culture) and bacterial contamination of the nebulizer parts (mask/mouthpiece or nebulizer chamber). These results corroborate those reported by another group of authors, who found nebulizer contamination to be low among patients with CF; however, of the patients analyzed in that study, 79% disinfected their nebulizers.[12] Although we found similar results in terms of bacterial contamination, 78% of the patients analyzed in the present study did not disinfect their nebulizers.

One study evaluated the bacterial contamination of 29 nebulizers used by patients with CF. Of the nebulizers that presented with bacterial growth, only 4 had been cleaned. *P. aeruginosa* was found in 10 nebulizers, and all of their users also presented with bacterial colonization by *P. aeruginosa*. However, no molecular study was conducted in order to determine whether the strain was the same.[13]

Despite the possibility of bacterial contamination, we found no association between the presence of bacteria in the patients and the presence of bacteria in the nebulizers in the present study. These results differ from those of a recent study, whose authors concluded that home nebulizers are often contaminated, principally when they are not cleaned properly, and can be a source of bacterial infection or reinfection. Such patients should be given written instructions regarding nebulizer cleaning techniques.[13]

We could have employed PCR or serology (or both) in the present study. The use of such techniques would probably have changed the result of the study because the use of PCR and serology in combination has been shown to be superior to the use of each method in isolation, to the combined use of PCR and culture, and to the combined use of culture and serology.[14]

The nebulizers used by the CF patients analyzed in the present study presented with a low rate of bacterial growth, although most of the patients did not often use disinfection procedures. There were no associations between the types of bacteria found in the sputum/oropharynx of patients and those found in the nebulizer parts (mask, mouthpiece, and chamber). This was probably due to the fact that we performed a point evaluation rather than a longitudinal evaluation, as well as to the fact that we evaluated only the culture of the samples. We found bacteria related to CF in the nebulizers of 6 of the 28 patients under study. This shows that nebulizers can be a source of contamination. Those bacteria probably came from the CF patients themselves. Therefore,

we conclude that nebulizers can be a source of bacterial infection, although no association was found in the present study. Studies involving a larger sample size and comparing different nebulizer cleaning techniques and frequencies, as well as other sample collection techniques (such as continuous collection), could produce results different from those obtained in the present study.

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Appendix 1 - Questionnaire administered to the patients with cystic fibrosis, their parents, or their legal guardians.

QUESTIONNAIRE
1) NAME:_____ 2) DATE OF BIRTH:___/___/___
3) QUESTIONNAIRE ANSWERED BY THE ( ) MOTHER ( ) FATHER ( ) LEGAL GUARDIAN
4) NEBULIZER TYPE/MODEL ( ) JET ( ) ULTRASONIC
DURATION OF USE:_____ NUMBER OF INHALATIONS/DAY:_____
5) DO YOU WASH YOUR NEBULIZER IMMEDIATELY AFTER HAVING USED IT? HOW?
6) HOW DO YOU CLEAN YOUR NEBULIZER? HOW OFTEN DO YOU CLEAN IT?
( ) running water ( ) every day
( ) warm water + soap + rinse ( ) once a week
( ) dishwasher ( ) once a month
( ) vinegar solution + rinse ( ) other:_____
( ) boiling water
( ) bleach solution: concentration: _____
( ) other:_____
7) WHICH OF THESE DRUGS DO YOU USE IN YOUR NEBULIZER?
( ) fenoterol (Berotec®) ( ) tobramycin ( ) amikacin ( ) other:
( ) DNase - Pulmozyme® ( ) colistin ( ) vancomycin
8) HAVE YOU EVER BEEN INSTRUCTED BY A PROFESSIONAL ON HOW TO CLEAN/DISINFECT YOUR NEBULIZER?
( ) no
( ) yes ( ) pulmonologist ( ) physical therapist ( ) pharmacist ( ) pediatrician ( ) other
9) DO YOU USE SALINE SOLUTION FOR INHALATION?
( ) no
( ) yes. How do you store it and for how long?_____
10) HOW OFTEN DO YOU REPLACE THE PARTS OF YOUR NEBULIZER (MOUTHPIECE, MASK, AND FILTER)?_____
HAVE YOU BEEN INSTRUCTED TO DO SO?_____