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Microbial Contamination of Nebulizers in Patients With Cystic Fibrosis

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What is already known on this topic?

- *Several studies reported that home nebulizers of patients with cystic fibrosis might be contaminated with pathogenic or nonpathogenic microorganisms.*
- *Nonpathogenic microorganisms may transfer antibiotic resistance genes to pathogenic microorganisms.*
- *Nebulizer hygiene procedures are highly important in order to avoid contamination of the nebulizer.*

What this study adds on this topic?

- *Nebulizers were mostly contaminated with environmental/floral microorganisms.*
- *Only a few caregivers were performing the recommended cleaning/disinfection practices correctly.*
- *Continuous and regular educational programs regarding nebulizer hygiene should be implemented in all Cystic Fibrosis Centers in order to improve correct hygiene practices.*

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ABSTRACT

Objective: Nebulizer contamination has potential harmful effects on the respiratory system. The aim was to investigate the contamination profile of the nebulizers in cystic fibrosis patients and evaluate the relationship between hygiene practices and microbial contamination.

Materials and Methods: Microbiological swab samples were taken from 3 different locations of the nebulizers of 102 patients. A questionnaire regarding nebulizer hygiene practices was applied to participants.

Results: Contamination rate was 40.2%, while chambers were the most contaminated area. The bacterial contamination rate was 37.3%, with gram-negative bacterial growth being predominant. The organisms identified were mostly environmental or floral. Only 3 of the patients were performing the whole steps correctly. This number was not sufficient to assess the relationship between nebulizer cleaning and disinfection practices and microbial growth from nebulizers. When the relationship between nebulizer cleaning/disinfection frequencies, methods, and storage locations was evaluated separately with microbial growth from nebulizers, no statistically significant relationship was found for all ($P > .05$ for all).

Conclusion: The nebulizer contamination rate with pathogenic microorganisms is low in the present study. Regular educational interventions regarding nebulizer hygiene practices should be implemented in all Cystic Fibrosis Centers.

Keywords: Contamination, cystic fibrosis, nebulizer

INTRODUCTION

Decreased mucociliary clearance as a result of abnormal hydration of mucus, airway obstruction, and excessive inflammatory response are some of the predisposing factors for respiratory tract infections in patients with cystic fibrosis (CF).[1] Nebulized therapies are in widespread use among patients with CF both for mucociliary clearance and for the management of chronic infections.[2] Home nebulizers, which are used for delivering nebulized therapies, are one of the most essential respiratory equipment for patients with CF. However, inhalation of aerosols generated from contaminated nebulizers may be a potential source of infection in the respiratory systems of vulnerable patients, such as CF.[3] A vicious cycle of nebulizer contamination and patient re-infection may eventually lead to the patient becoming chronically colonized with pathogens for CF, which is associated with an increased frequency of pulmonary exacerbations, decreased lung function tests, and higher mortality.[4]

Several studies reported a wide variety of bacterial and fungal growth in nebulizer swab samples of CF patients.[3,5–11] Some studies reported high contamination rates with CF pathogenic microorganisms,[3,8,10] while others reported high rates of environmental or oral flora microorganisms.[5–7] Although environmental organisms are not believed to be clinically

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pathogenic, such organisms may host antibiotic resistance gene determinants. So antibiotic resistance of pathogenic microorganisms can potentially increase in patients with CF who have microbiological growth in the nebulizer, even if the microorganism is not defined to be pathogenic.[12,13]

Nebulizer hygiene procedures are significantly important in order to avoid contamination in the nebulizers. Cystic Fibrosis Foundation (CFF) Infection Prevention and Control (IP&C) Guideline recommends 3 different steps for nebulizer hygiene after each use: clean, disinfect, and air-dry.[14] After cleaning the nebulizer with soap and water, the nebulizer should be disinfected with a cold or heat method. Before storage, the nebulizer should be air-dried and then stored in a closed, clean container.[5,14] There are several studies in the literature investigating the relationship between nebulizer hygiene practices and nebulizer contamination rate.[3,5-7,10,11] Even though several other factors may also affect the contamination rate, nebulizer cleaning, disinfection, and storage are highly important steps for nebulizer hygiene.

The primary aim of the study is to demonstrate the rate and profile of the microbiological contamination of home nebulizers of CF patients. The secondary aim is to evaluate the relationship between nebulizer hygiene procedures and the contamination rate of the nebulizers.

MATERIALS AND METHODS

The study is designed as a single-center cross-sectional study including 102 patients with CF who were followed up in the CF Center, and conducted between July 2019 and February 2020. The participants were selected sequentially from the patients who came to the outpatient clinic for routine follow-up in the last 6 months. Participants were asked by phone to bring their home-used nebulizers without being informed about the study and without a specific recommendation about the transporting procedure. One hundred twenty patients who were living in the same city as the study center were invited, and 102 accepted to participate to the study. All of the patients were using their nebulizers at the time of the study.

A multiple-choice questionnaire was given to the patients and caregivers initially, regarding nebulizer hygiene practices (frequency/methods of cleaning, disinfection, and storage). Microbiological swab cultures were taken from 3 different locations of the nebulizers, including inside the mouthpiece/mask, the air outlet of the nebulizer, and the chamber by using wet swabs with phosphate-buffered saline. Swabs were transferred to the microbiology laboratory within 2 hours from sampling. Aliquots of 100 µL were spread on blood agar and MacConkey agar plates and were incubated for 48 hours at 37°C. The incubation time of the culture plates of nebulizers was extended to 72 hours if no growth was observed after 48 hours. Total colony-forming unit counts were determined from the plates and predominant colonies were identified using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry technology (Vitek-MS, Biomerioux®, France). Figure 1 shows the study design.

The demographic and clinical characteristics of the patients were recorded, including age, sex, respiratory function tests, respiratory colonization, and the currently used drugs.

Respiratory sample cultures (sputum/cough swab cultures) of the patients within the last 12 months were obtained from medical records, and Leeds criteria was used to define chronic colonization for bacterial pathogens.[15,16]

Culture media used for respiratory samples were blood agar, chocolate agar, and selective chromogenic agar. Plates were incubated for 72 hours at 37 °C, and identification was performed using mass spectrometry (Vitek MS).

Ethical approval was obtained from Ethical Committee of University of Marmara (approval number: 09.2019.684, date: 26.07.2019). Each participant signed informed consent before the study.

Statistical Analysis

Statistical analysis was performed with The Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows (IBM Corp.; Armonk, NY, USA). Normality was assessed using normality plots and the Kolmogorov–Smirnov test. Continuous variables with normal distribution were presented as mean and SD, and data with non-normal distribution were presented as median and 25-75th percentiles. The association between positive cultures and the remaining categorical variables was investigated by the chi-square test. Continuous variables for 2 independent groups with non-normal distribution were assessed through the Mann–Whitney *U* test, whereas with normal distribution were assessed through the independent samples *t*-test. Data were presented with 95% CI, and the significance level was set at a *P*-value of .05.

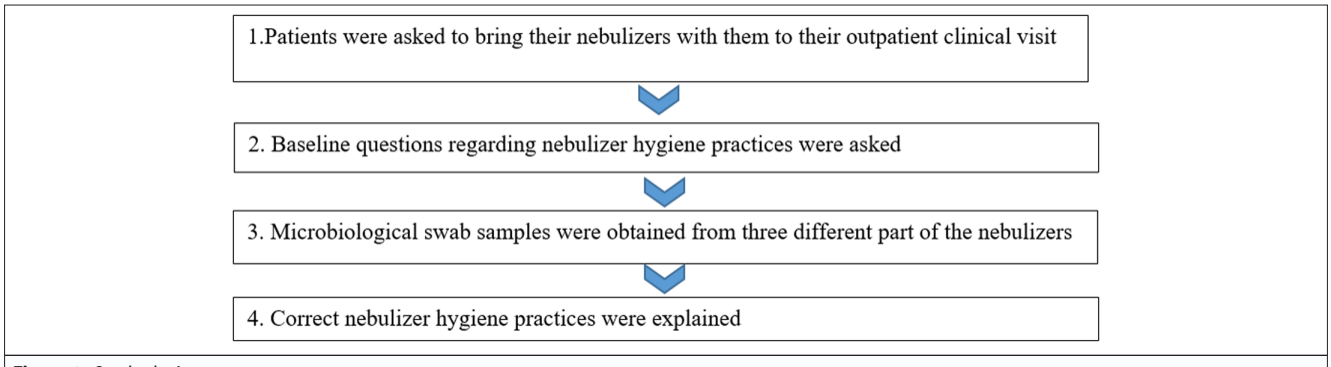

Figure 1. Study design.

RESULTS

Patient Characteristics

The study included 102 patients with CF (age range 6 months-30 years) who were using a home nebulizer for inhaler therapy. Table 1 shows the demographic and clinical characteristics of the patients. None of the patients were on cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapy during the study period. This eliminates CFTR modulator use as a potential confounding variable in bacterial recovery.

Microbial Contamination of the Nebulizers

Microbial swab samples revealed the presence of a wide variety of microorganisms; 40.2% (n = 41) of the nebulizer cultures were positive for at least 1 microorganism in at least one part of the nebulizer. Only 1 microorganism was identified in 18.6% (n = 19) of the 102 nebulizers, while 2 or more organisms were identified in 21.5% (n = 22). Bacterial growth, predominantly gram-negative bacteria, was found in 37.3% (n = 38) of the nebulizers. The highest microbial growth was found in the chambers of the nebulizers (32.4%). The most common microorganisms isolated from the nebulizer cultures are presented in Table 2. Other microorganisms were mostly environmental or floral microorganisms such as Enterobacter spp (*E. kobei*, *E. cloacae*), Bacillus spp (*B. clausii*, *B. altitudinis*, *B. flexus*), *Brevindumonas diminuta*, and *Rhodotorula mucilaginosa*.

Contamination rate of the nebulizers was not related with age, sex, inhaled medications, FEV % pred, pulmonary exacerbation rate in the last year, and the presence of a pathogenic microorganism in patients' respiratory samples. Similarly, there was no significant difference between groups in the last cleaning and disinfection time of the nebulizer and the last using time of the nebulizer. Table 3 shows the comparison of the patients whose nebulizers were contaminated or not.

Table 4 shows the frequency of the participants performing correct hygiene procedures including cleaning, disinfection, and storing according to CFF IP&C cleaning/disinfection guidelines. All of the patients were using heat methods for nebulizer disinfection. Fifty-eight patients were disinfecting their nebulizers; 54 of them were placing the nebulizer in continuously boiling water for 5 minutes, while 4 of them were using the dishwasher for disinfection.

Table 1. Demographic and Clinical Characteristics of the Patients (n = 102)

Age (month, median, 25-75th percentiles)	**113.5 (60-168)**
Sex (female/male), n (%)	45 (44.1)/57 (55.9)
FEV1 % pred (mean ± SD) (n = 74)	81.36 ± 2.59
Bacterial colonization in the respiratory samples†	(n, %)
No	25 (24.5%)
Yes	77 (75.5%)
MSSA	27 (26.4%)
MRSA	13 (12.7%)
Pseudomonas aeruginosa	17 (16.7%)
P. aeruginosa and MSSA	18 (17.6%)
P. aeruginosa and MRSA	2 (2%)
Other	8 (7.8%)
Patients receiving inhaled medications†† (n, %)	
Dornase alpha	100 (98%)
Short acting beta 2 agonist	77 (75.5%)
Hypertonic saline	32 (31.4%)
İnhaled antibiotics	28 (27.4%)
Inhaled corticosteroids	19 (18.6%)

†Some of the patients' respiratory samples are contaminated with multiple microorganisms.
††Some patients receive more than 1 inhaled medication.

Only 3 of the caregivers were performing the correct cleaning/disinfection steps according to the CFF IP&C cleaning/disinfection guideline. Two of these patients did not have contamination in their nebulizers. One of them had contamination with *Candida albicans* and *R. mucilaginosa* in only the mouthpiece of the nebulizer.

As only 3 of the caregivers were performing entire steps correctly, the number was not sufficient to assess the relationship between nebulizer cleaning and disinfection practices (cleaning/disinfection frequencies, methods, and storage locations) and microbial growth from nebulizers. However, each step was compared separately (Table 4). Patients who cleaned the nebulizer after each use were compared with patients who did not clean the nebulizer after each use (P = .831); patients who disinfected the nebulizer after each use with the patients who did

Table 2. The Contamination Rate of Microorganisms Isolated from the Nebulizers

Microorganism	**Mask/mouthpiece**	**Outlet**	**Chamber**	**Any Part of the Device**
	n (%)	n (%)	n (%)	n (%)
Any contamination‡	18 (17.6)	9 (8.8)	33(32.4)	41 (40.2)
Bacterial contamination‡	16 (15.7)	9 (8.8)	31 (30.4)	38 (37.3)
Gram-negative bacterial contamination‡	10 (9.8)	3 (2.9)	25 (24.5)	27 (26.5)
Acinetobacter spp (pittii, ursingii, johnsonii, junii)	2 (2)	3 (2.9)	5 (4.9)	8 (7.8)
Stenotrophomonas maltophilia	4 (3.9)	1 (1)	7 (6.9)	8(7.8)
Ochrobactrum spp (anthropi, intermedium)	3 (2.9)	–	5 (4.9)	5 (4.9)
Pseudomonas spp (putida, stutzeri)	–	–	3 (2.9)	3 (2.9)
Gram-positive bacterial contamination‡	7 (6.9)	5 (4.9)	14(13.7)	22 (21.6)
Staphylococcus spp (aureus/non-aureus)	5 (4.9)	3 (2.9)	5 (4.9)	9 (8.8)
Fungal contamination‡	4 (3.9)	–	2(2)	5 (4.9)
Candida spp	4 (3.9)	–	2(2)	5 (4.9)

‡Some of the nebulizers were contaminated with multiple microorganisms.

Table 3. Comparison of the Patients with and Without Nebulizer Contamination

	Not Contaminated (n = 61)	Contaminated (n = 41)	*P*
Sex, n (%)			.711*
Female	26 (42.6)	19 (46.3)	
Male	35 (57.4)	22 (53.7)	
Age (months, median, 25-75th *P*)	104 (52-156)	136 (74-171)	.202**
İnhaled antibiotic treatment, n (%)	19 (31.1)	9 (22)	.308*
İnhaled steroid treatment, n (%)	12 (19.7)	7 (17.1)	.741*
FEV1 % pred (mean ± SD)	83.24 ± 20.16	74.03 ± 24.83	.125***
Pulmonary exacerbation (mean ± SD) (last 12 months)	2.47 ± 2.24	2.73 ± 2.35	.885***
Last cleaning and disinfection time of the nebulizer (hours before) (median, 25-75th percentiles)	10.65 (7.65-15.00)	12 (9.30-15.30)	.347**
Last using time of the nebulizer (hours before) (median, 25-75th percentiles)	11.00 (7.50-14.50)	12.00 (10-14)	.915**
Respiratory colonization****, n (%)	46 (79.3)	31 (77.5)	.80*

*chi-square.
**Mann–Whitney *U* test.
***Independent samples *t* test.
****Valid percentages, 4 patients had insufficient number of respiratory samples to define colonization in the last year.

not disinfect the nebulizer after each use (*P* = .280); patients who cleaned the nebulizer with a recommended method or not (*P* = .234), and patients who stored the nebulizer in an appropriate place or not (*P* = .892). When the relationship between nebulizer cleaning/disinfection frequencies, methods, storage locations and microbial growth were evaluated from nebulizers separately, no statistically significant relationship was found (Table 4).

DISCUSSION

The current study revealed that microbial swab samples of the nebulizers were positive for a wide variety of floral/environmental microorganisms, while highest microbial growth was in the chambers. Only a few caregivers were performing the correct cleaning/disinfection steps according to the CFF IP&C cleaning/disinfection guidelines. Even though home nebulizers play a significant role in patients' treatments, potential harmful effects due to inappropriate cleaning and disinfection are barriers to optimal care. However, the data regarding nebulizer contamination and nebulizer hygiene practices of Turkish CF patients is scarce. The present study is important in demonstrating the contamination profile of the nebulizers and hygiene practices of Turkish CF patients.

Several studies reported a wide variety of bacterial growth in nebulizer swab samples of CF patients, with a contamination frequency between 20% and 75%.[3,5-11,17] Some studies have reported that pathogenic microbial contamination was predominant,[3,8,10] while others have reported that nonpathogenic (floral/environmental) microbial contamination was predominant in the nebulizers.[5-7] A recent review by Bell et al[2] reported

Table 4. Comparison of Nebulizer Contamination According to Hygiene Procedures

Hygiene Procedure	Contaminated (n = 41)	Not Contaminated (n = 61)	*P*
Cleaning the nebulizer after each use, n (%)			.831
Yes	24 (58.5)	37 (60.7)	
No	17 (41.5)	24 (39.3)	
Cleaning the nebulizer with a recommended method, n (%)*			.234
Yes	4 (9.8)	11 (18)	
No	37 (90.2)	49 (80.3)	
Disinfecting the nebulizer after each use, n(%)			.280
Yes	10 (24.4)	21 (34.4)	
No	31(75.6)	40 (65.6)	
Disinfecting the nebulizer with a recommended method, n (%)**			–
Yes	17 (41.5)	41 (67.2)	
No	0	0	
Storing the nebulizer in an appropriate place, n (%)			.893
Yes	25 (61)	38 (62.3)	
No	16 (39)	23 (37.7)	

Chi-square test was used for statistical analysis.
*One patient was not cleaning the nebulizer.
**44 patients were not disinfecting the nebulizer.

that a total of 35 bacterial genera, including 24 different gram-negative bacteria, 10 different gram-positive bacteria, and 16 different fungal genera, have been reported in the studies investigating nebulizer contamination up to the present time.

Although Tabatabaii et al[8] reported that 84.2% of Pseudomonas spp. contaminated nebulizers belonged to children whose respiratory cultures were positive for *P. aeruginosa*, a number of previous studies did not find a significant relationship between nebulizer cultures and concurrent respiratory samples of patients.[5,9,11] Even though simultaneous respiratory samples were not taken from the patients in the study, 36.3% of them were colonized with *P. aeruginosa* in their respiratory samples. However, *P. aeruginosa* growth was not observed in the nebulizer cultures in this study.

The microorganisms identified from the nebulizer cultures were mostly environmental or floral microorganisms. None of the isolated microorganisms, except for *Stenotrophomonas maltophilia* which was present in 7.8% of the nebulizers, are considered pathogens for CF patients.[18] The pathogenicity of these environmental/floral microorganisms was not clearly defined in patients with CF. For example, C. albicans is a floral microorganism but also has the potential role to be pathogenic in people with CF.[19] *R. mucilaginosa* is primarily an environmental yeast and part of human microbiota; however, this yeast may also be pathogenic for patients with chronic diseases.[20] The presence and interactions of microorganisms in the CF lung, other than major pathogens, are still unclear.

There are a few studies including fungal contamination in the nebulizers. Fungal contamination frequency was reported to be between 6.6% and 57.7% in the previous studies.[8,21] Candida spp (especially *Candida parapsilosis*) and *Aspergillus fumigatus* were reported to be the most frequently isolated fungi from the nebulizers.[6,21] In the present study, fungal contamination frequency was 4.9% with a predominancy of Candida spp. One of the highest fungal contamination rates was reported in a study by Peckham et al[21], which was specifically investigated fungal contamination in the nebulizers by using specific fungal cultures with a longer incubation period.

In the present study, 32.4% of the chambers were contaminated, mostly with gram-negative bacteria. Several studies revealed higher microbial growth in the chambers of the nebulizers similar to this study.[3,6-8,10,11] Since it is well known that a moist environment is a risk factor for bacterial growth—especially for gram-negative bacteria—chambers are at the highest risk of contamination, and hygiene of the nebulizer chambers requires extra care.[9,22]

Clinical characteristics of the patients, including age, severity of lung disease, presence of chronic colonization in the respiratory tract, and inhaled antibiotic usage, may also affect the contamination rate in nebulizers.[6] In this study, there was no significant difference in terms of age, sex, FEV % pred, pulmonary exacerbation rate in the last year, respiratory colonization rate, and inhaled antibiotic usage rate between the groups whose nebulizers were contaminated or not. Although several different patient-related and environmental factors may also affect the contamination ratio of nebulizers, it is highly important to perform all steps of nebulizer hygiene according to CFF IP&C cleaning/disinfection guideline.[14] Only 3 of the patients were performing all steps for nebulizer hygiene in the study, which makes it difficult to make an appropriate comparison for the effects of patient characteristics on the nebulizer contamination rate.

Nebulizer hygiene procedures are significantly important in order to avoid contamination in the nebulizers. Cystic Fibrosis Foundation (IP&C recommends cleaning, disinfecting, and air-drying the nebulizer parts after each use. In addition, storing the nebulizer in a clean, closed container is as important as hygiene procedures.[14] Even though several other factors such as improper handling during transport and disassembling the nebulizer parts before cleaning and drying procedures may affect the contamination rate, nebulizer cleaning and disinfection are important parts of nebulizer hygiene. In the current study, the frequency of nebulizer cleaning and disinfection after each use was 59.8% and 30.4%, respectively. The frequency of the participants performing correct methods for nebulizer cleaning and disinfection was 14.7% and 56.9%, respectively, while 61.8% of the participants were storing the nebulizer in an appropriate place. However, only 3 of the participants were performing the entire process correctly according to the guideline.

Studies investigating the relationship between cleaning/disinfection practices and microbiological contamination of the nebulizers have used different methods and reported a wide variety of results.[3,5-7,10,11] Some studies reported that inappropriate cleaning[3,6] and disinfection[5,6] practices are related with higher contamination rates in nebulizers. Since only 3 of the caregivers were performing the entire process (cleaning/disinfection frequencies/methods, storing) correctly, the relationship between nebulizer hygiene practices and microbiological growth could not be assessed properly in this study. However, when it was evaluated separately, no significant relationship was present between nebulizer cleaning/disinfection frequencies/methods, storing places, and microbiological growth.

One of the most important barriers to adherence to appropriate hygiene procedures is awareness of the current guidelines, both for healthcare providers and caregivers. Garber et al[23] reported that nearly 60% of healthcare providers were aware of the CFF IP&C guideline in their survey study, which included 522 healthcare providers working at CF centers. Zuana et al[7] reported that 2 months after the standardized instructions for nebulizer hygiene were given, the nebulizer contamination rate decreased from 57.5% to 25% in their study, which included 40 CF patients which indicates the importance of education on appropriate hygiene procedures for reducing the nebulizer contamination rate. In another study from the center, the effects of an educational intervention were also evaluated, showing that a single standardized training is highly effective in improving practices regarding nebulizer hygiene.[24] Regular training programs should be implemented to introduce current guidelines and appropriate hygiene procedures regarding nebulizer hygiene at CF centers.

The current study has several limitations. First, participants were asked to bring their nebulizers by the CF nurse without

any specific recommendation regarding transport procedures. Although participants were not aware of the objective of the study before arriving at the CF clinic, the authors cannot exclude unusual cleaning before the visit and information bias due to fear of reporting known misconduct acts to the study team. In addition, some of the patients who were invited did not participate to the study. The group participating in the study may be more interested in education, and this may be a potential source of volunteer bias. Another limitation: sputum or cough swabs were not taken from the patients on the same day, so simultaneous sputum/cough swab cultures with the cultures from the nebulizers could not be compared. Even though patients' respiratory tract colonization was assessed, as nonpathogenic microorganisms are not routinely identified in the patients' respiratory samples at the clinical microbiology laboratory, the authors could not compare if the nebulizer cultures are identical to patients' flora. Fungal specific cultures, which explains the low fungal contamination rate in the present study, were not performed. Even though the microorganisms in the nebulizers were primarily defined as environmental or floral microorganisms, most of them have a potential role in pathogenicity for patients with CF. Thus, the authors could not clearly define the microorganisms as pathogenic or nonpathogenic for CF and could not compare these groups. Additionally, in the study, the authors did not ask about drying methods. Drying nebulizer parts actively increases the risk of contamination with skin flora and may partly explain the contamination of nebulizer parts in the [study.] Lastly, as only 3 patients were performing all steps correctly for nebulizer hygiene according to CFF guidelines, the authors could not show the effect of correct hygiene practices on nebulizer contamination.

CONCLUSION

In conclusion, nebulizers have a potential risk for contamination and thereby may negatively affect the treatment and lung functions of CF patients. Even though the ratio of pathogenic microorganisms in the nebulizer cultures is low in the present study, the potential harmful effects of these microorganisms are highly possible. Nebulizers are cornerstones of CF treatment, and it is important to remember the potential harmful effects of nebulizer contamination. Continuous and regular education programs should be implemented in all CF centers in order to increase correct practices and decrease the contamination rate of the nebulizers.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: This study was approved by Ethics Committee of Marmara University School of Medicine University (Approval no:09.2019.684; Date: 26.07.2019).

Informed Consent: Verbal and written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – C.Y.Y., P.E, N.D., M.C.; Design – Y.G., E.E.E., A.K.Y., F.K., B.K.; Supervision – Y.G., E.E.E., F.K., B.K.; Resources – B.S.U., G.T., H.M.; Materials – C.Y.Y., B.S.U., G.T.; Data Collection and/or Processing – G.T., C.Y.Y., P.E., M.C., N.D., H.M.; Analysis and/or Interpretation – C.Y.Y., P.E., M.C., N.D.; Literature Search – C.Y.Y., Y.G., E.E.E., A.K.Y., F.K., B.K.; Writing – C.Y.Y., P.E., M.C., H.M.; Critical Review – Y.G., E.E.E., A.K.Y., F.K., B.K.

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