Author’s response to reviews

Title: Bacterial Contamination of Home Nebulizers in Children with Cystic Fibrosis and Clinical Implication on the Number of Pulmonary Exacerbations

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Version: 1 Date: 16 May 2019

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REVIEWER 1

This study compares the bacterial contamination of 61 home nebulizers with the sputum of the 61 CF children under treatment with these nebulizers. The authors found a high rate of bacterial contamination and a strong correlation between nebulizers and sputum for Ps aeruginosa and St aureus. There was also a correlation between the number of exacerbations and the presence of nebulizer contamination. More information is needed regarding the types of nebulizer used (all with an air compressor? Jet or mesh nebulizer?). the data regarding home nebulizers were summarized in table 1

We need also to know if these nebulizers were all used for inhaled antibiotics or were some children only under bronchodilators or saline? This description was added to material session; all patients were on bronchodilator and hypertonic saline (mucolytic) but some patients were on inhaled antibiotics (table 1)
The authors acknowledge in the discussion that the correlation found between sputum microbiology and nebulizer contamination does not allow to identify the primary source of infection. Indeed it is likely to be a bidirectional process. Genetic identification of the bacterial strains was beyond the aim and the tools available for this study. However it would strengthen the relevance of the correlation if the antibiogram of the bacteria found in sputum and in the nebulizer was available and showed similarity.

Yes, as you have mentioned there was also no facility for genetic identification of the bacterial strains. We only performed the antibiogram test for pseudomonas spp. And as you have mentioned this in your comments we added the data regarding antibiograms in the results and discussion part.

On the clinical side, an important piece of information is the prior recommendation made to the patients and their parents for the use and cleaning of the nebulizers, both prior to this study and now according to the present results.

This was added to the method part that in our center, the CF Foundation’s recommended nebulizer care is followed. According to CF foundation nebulizers should be cleaned. Disinfected and air dried after each use. How these steps were done by patients/parents were summarized in table 4

The last paragraph on limitations of the study is not comprehensible: what do you mean by "many confounding factors that may cause mistakes in interpretation"?

It was added to the paragraph that The current study had some limitations. Small sample size, and many confounding possibly factors such as socioeconomic level, the degree of disease progression, comorbidities, treatment adherence, and care resources that may cause mistakes in interpretation were among the limitations of the study. There was also no facility for genetic identification of the bacterial strains.

Figure 1 & 2: the acronym CONS should be explained (presumably coagulase neg staph?).

It was explained in the abbreviations

Coli and Albicans should be written with small caps.

It was corrected in the revised manuscript
REQUESTED REVISIONS: Design - Power calculation should be included it was added to the method part. and the inclusion criteria more clearly defined with respect to frequency of nebuliser use amongst the cohort.

It was added that Inclusion criteria were a definite diagnosis of CF based on positive results of two sweat chloride tests, and administration of home nebulizers at least once a day. Patients were excluded if they were receiving intravenous (IV) antibiotics at the time of recruitment or used an inhaled antibiotic on the day of sample collection, as well as the ones whose parents refused to consent. The patients not using their home nebulizers daily were also excluded.

Execution - The rationale for considering &lt;10 CFU as "clean" should be clarified. How is this distinguished from low-level contamination? We used this cut-off according to one of previous similar studies which was cited in the method part.

The authors should state whether any examples of multi-species contamination were encountered. This piece of information was added to result part. Multiple species contamination was observed in six devices.

Decontamination/cleaning advice given to children and parents should be stated. It was added to the method session that in our center the CF Foundation’s recommended nebulizer care is followed. According to CF foundation nebulizers should be cleaned. The CF foundation guideline also was cited as reference number (16). According to this guideline, nebulizers should be cleaned, disinfected and dried after utilization. There are two disinfecting options: heat and cold methods. Heat methods include boiling for 5 minutes, microwave for 5 minutes, dishwasher at temperature &gt;158°F (70°C) for 30 minutes and electric steam sterilizer. Cold methods include soaking in 70% isopropyl alcohol for 5 minutes or in 3% hydrogen peroxide for 30 minutes. How these steps were done by patients/parents were summarized in table 4.

Results of questionnaire regarding hygiene should be included. The results are summarized in table 1 and 4.

Statistics - Power calculation should be included as stated above. Statistical session was revised in this way; Statistical analyses were performed using SPSS version 19. The general characteristics of the patients were analyzed by the Chi-square test and the results were expressed as mean, standard deviation, frequency, and percentage. Agreement with normal distribution was assessed by estimation of skewness and kurtosis. Normality of data was assessed using the Kolmogorov-Smirnov test. The Mann-Whitney U test was performed to compare the measurements. P values less than 0.05 were considered significant.
Interpretation - Authors should discuss potential confounders in the study. It was discussed in limitations session

ADDITIONAL REQUESTS/SUGGESTIONS: The report is well written but there are some areas where a review of spelling/grammar is needed. The manuscript was sent for English edit to Nedmedica and the certificate also attached while submission

REVIEWER 3

Abstract A total of 43 of the 61 nebulizers (70.5%) were contaminated; 31 mouthpieces, 21 reservoirs, and 11 connecting tubes. Could the authors provide also the percentages of mouthpieces, reservoirs and connecting tubes that are contaminated (31/43, etc) in the abstract? This changes were done.

There was a significant increase in CF exacerbations over 12 months in children whom pathogenic organisms were recovered from their home nebulizers (p&lt;0.001). Could the authors report the numbers of exacerbations and not only the p value? The requested data was added

Background The introduction section is well written. As regards the previous studies that the authors cited about the same topic, could they specify if these studies were cohort studies, randomized controlled trials or other? All previous studies were cohort. This was added to the text too.

Methods What is the calendar period of the current study? Is the study a retrospective or a prospective analysis? It was added in the method session that The current observational, cross sectional study was conducted on 61 children with CF routinely followed up at Mofid Children’s Hospital, Tehran, from September 2017 to March 2018.

Regarding inclusion/exclusion criteria, did the authors allow the use of oral antibiotics at the time of enrollment? Yes, only those who were on IV antibiotics at the time of study were excluded, and patients were allowed to continue their medications as before.

Did the study receive authorization of the local Ethics Committee? Yes and in the ethic and consent session it was written that Informed written consent was taken from parents and/or patients and the study was approved by the ethical committee of Shahid Beheshti University of Medical Sciences with the ethics code of IR.SBMU.RETECH.REC.1396.937.

Was there an informed consent? Yes.

Could the authors specify the procedure of cleaning of the nebulizers that was adopted by parents?
it was added to the method session that in our center the CF Foundation’s recommended nebulizer care is followed. According to CF foundation nebulizers should clean. The CF foundation guideline also was cited as reference number (16). According to this guideline, nebulizers should be cleaned, disinfected and dried after utilization. There are two disinfecting options: heat and cold methods. Heat methods include boiling for 5 minutes, microwave for 5 minutes, dishwasher at temperature &gt; 158°F (70°C) for 30 minutes and electric steam sterilizer. Cold methods include soaking in 70% isopropyl alcohol for 5 minutes or in 3% hydrogen peroxide for 30 minutes. How these steps were done by patients/parents were summarized in table 4

Was it standardized? Yes. CF foundation guideline

Was the questionnaire about nebulizers' hygiene and maintenance validated or already used in previous studies? It was a check list (this was corrected in the method session) and it was similar to the checklist used in reference number (13)

Did the authors follow up the patients till the last available visit to record the number of exacerbations? Or did they follow up the patients for one year after the enrollment? Is it possible to conduct a survival analysis? Are the data about follow up of the patients available? We used patients’ medical records 12 months prior to the study time for the number of exacerbations.

In the statistical analyses section, please provide a more detailed description of statistical analyses. How missing data from clinical records were managed? Statistical analyses were performed using SPSS version 19. The general characteristics of the patients were analyzed by the Chi-square test and the results were expressed as mean, standard deviation, frequency, and percentage. Agreement with normal distribution was assessed by estimation of skewness and kurtosis. Normality of data was assessed using the Kolmogorov-Smirnov test. The Mann-Whitney U test was performed to compare the measurements. P values less than 0.05 were considered significant.

In the current study, there were no missing data due to the referral policies for all outpatient and inpatients, even if they were in a county. Authors had access to the records of clinic and hospital.

Results The results section has to be revised after a better definition of methods and statistical analyses. Are the data about antibiotic susceptibility testing available? 2 tables and more data were added. We only had antibiotic susceptibility testing for pseudomonas spp. So that is why we had not reported it, but in revised manuscript it was added to the result session.

Discussion The results (such as OR and p values) could be reported with confidence intervals in the results section and not in the discussion. It was corrected in the revised manuscript.
Minor points: The manuscript needs some language corrections and style corrections. The manuscript was sent for English edit to Nedmedica and the certificate also attached while submission.