
Contamination of nebulizers with environmental allergens

Mary E. Bollinger, DO*; Arlene Butz, PhD†; Kim Mudd, RN, MSN*; and Robert G. Hamilton, PhD‡

Background: A previous article described cockroach allergen in the nebulizer reservoir of an asthmatic patient who experienced a life-threatening exacerbation after nebulizer use.

Objective: To determine whether indoor allergens can be measured in home nebulizers.

Methods: As part of a large study examining nebulizer use in underserved asthmatic children, visiting nurses replaced nebulizer sets in patients' homes. Twenty used sets were randomly selected for analysis, without linkage to clinical or home environmental data. Nebulizer reservoirs and negative controls (buffer and albuterol) were extracted overnight with 2 mL of buffer. For positive controls, nebulizer sets were placed in homes with cats and dogs, and other reservoirs were intentionally contaminated with cat (Fel d 1), dog (Can f 1), cockroach (Bla g 1 and Bla g 2), and mouse (Mus m 1) skin test solutions. Extracts were tested for allergens in a masked manner using enzyme-linked immunosorbent assay.

Results: Of 17 reservoirs with adequate specimens for allergen detection, 5 (29%) had measurable levels for at least 1 of 5 allergens tested. One reservoir had measurable Can f 1, 2 had Bla g, 3 had Mus m 1, and none had Fel d 1 allergen. Two of 3 homes with cats where nebulizer setups were placed had measurable Fel d 1 in the reservoir, and 1 of 2 homes with dogs had measurable Can f 1. Reservoirs kept in sealed plastic bags had no detectable allergen.

Conclusions: Indoor allergens can be found in the nebulizer equipment of children with asthma, with the potential for adverse consequences. Storing nebulizer sets in sealed plastic bags may prevent contamination.

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INTRODUCTION

Nebulizers are commonly used by asthmatic patients, particularly children. It is estimated that approximately one third of children with asthma use a nebulizer for dispensing asthma medication.¹ Patients who use nebulizers have also been found to have higher asthma morbidity rates, with increased hospitalizations and emergency department visits for asthma.¹ Most nebulizers are used to deliver rescue β -agonist treatment for acute asthma symptoms; however, with the addition of nebulized budesonide to the market, some patients use nebulizers daily to dispense controller asthma medications as well. Physicians often have anecdotal reports of patients describing increased asthma symptoms after nebulizer treatments; however, there are few published reports of such exacerbations.² The proposed mechanisms for worsening symptoms with the use of nebulizers may include the development of tachyphylaxis or increased bronchial hyperresponsiveness due to β -agonist overuse or possibly adverse reactions to the medication or excipient. We previously described 2 patients with asthma who had severe life-threatening respi-

ratory deterioration after the use of a nebulizer for rescue albuterol treatment.² These exacerbations may have been related to contamination of their nebulizer equipment with cockroach allergen. In the present study, we sought to determine whether contamination of nebulizer equipment with allergens is a common finding.

METHODS

This study received an exemption from The Johns Hopkins University and the University of Maryland institutional review boards. As part of a study examining nebulizer use in inner-city children with asthma, visiting nurses went to the patients' homes and replaced their nebulizer sets, including the reservoir, mask, mouthpiece, and tubing. The nebulizer sets were not linked to any clinical or environmental data from the patient's home. The replaced sets were sent in a masked manner to The Johns Hopkins University Dermatology, Allergy, and Clinical Immunology Reference Laboratory (Baltimore, MD) for analysis. Each nebulizer set was evaluated for common indoor cockroach, cat, dog, and mouse allergens. The reservoir was extracted with 2 mL of phosphate-buffered saline solution containing 1% bovine serum albumin and 0.05% Tween 20, with overnight rotation. Albuterol nebulizer medication was used as 1 negative control. The solutions were tested for Bla g 1, Bla g 2, Fel d 1, Can f 1, and Mus m 1 using a monoclonal antibody-based immunoassay (Indoor Biotechnologies, Charlottesville, VA). The analytical detection limits of the assays were 0.01 U/mL for Bla g 1, 0.05 U/mL or 2 ng/mL for Bla g 2, 4 ng/mL for Fel d 1, 2 ng/mL for Can f 1, and 0.4 ng/mL for

* Division of Pediatric Pulmonology/Allergy, University of Maryland School of Medicine, Baltimore, Maryland.

† Division General Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland.

‡ Division of Allergy and Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, Maryland.

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Mus m 1. There is currently no conversion factor for Bla g 1 units to nanograms of allergen. Control samples were also obtained by placing nebulizer sets in homes with cats and dogs. Other nebulizer sets were intentionally contaminated with standard allergen skin test solutions for cat, dog, cockroach, and mouse allergen as masked positive controls. For each allergen, 0.05 mL of a stock solution of each allergen was added to a separate nebulizer reservoir containing 0.45 mL of albuterol solution. Protein levels of allergen extracts were as follows: cockroach, American-German mix, 1:10 wt/vol (Hollister-Stier, Spokane, WA); standardized cat hair, 10,000 BAU/mL (ALK-Abello, Round Rock, TX); mouse epithelia, 1:20 wt/vol (Greer Laboratories, Lenoir, NC); and dog, mixed breed, 1:20 wt/vol (Greer Laboratories). For the in-home measures, the nebulizer set (including the attached tubing) was primed with 0.25 mL of albuterol solution in the reservoir and was placed in homes containing cats, dogs, or both for 2 weeks in an open box in a common living area where the animals were also allowed. Four nebulizer sets were sealed in plastic bags and then placed in homes with animals. In addition, nebulizer reservoirs were contaminated with allergen solution for Bla g 1, Bla g 2, Fel d 1, Can f 1, and Mus m 1. All reservoirs were coded and sent in a masked manner to the Dermatology, Allergy, and Clinical Immunology Reference Laboratory for extraction and allergen analysis.

RESULTS

A total of 20 nebulizer sets were randomly selected from the clinical study for allergen content evaluation. Seventeen (16 for Mus m 1) of the reservoirs had adequate specimens after extraction for allergen detection. None of the nebulizer sets had measurable cat allergen, 1 had measurable dog allergen (7.3 ng/mL; mean, 0.43 ng/mL; range, 0–7.3 ng/mL), 2 had measurable cockroach allergen (Bla g 1 and Bla g 2 combined: 0.64 and 1.14 U/mL; Bla g 1: mean, 0.03 U/mL; range, 0–0.36 U/mL; Bla g 2: mean, 0.08 U/mL; range, 0–0.99 U/mL), and 3 had measurable mouse allergen (0.45, 1.03, and 2.6 ng/mL; mean, 0.24 ng/mL; range, 0–2.6 ng/mL). At least 1 of the allergens was detected in 5 (29%) of the 17 nebulizer reservoirs available for testing. Data on the individual home environments where the nebulizer sets originated were not available, and neither were clinical data such as asthma severity and allergen sensitivity. Of the 221 homes in the overall nebulizer study, 4.5% included children with intermittent asthma and 95.5% included children with persistent asthma. Overall, 41 homes (18.6%) in the general nebulizer study had cats, 33 (14.9%) had dogs, and 74 (33.5%) had both. Only 34 homes (15.4%) had cockroach infestation, and 66 (29.9%) had mice. However, these data could not be generalized to the subset of 20 homes that were randomly selected for nebulizer testing.

Measurable Fel d 1 was found in nebulizer equipment intentionally placed in 2 of 3 homes with cats, and Can f 1 was found in 1 of 2 homes with dogs. In these same homes,

when nebulizer equipment was placed in sealed plastic bags before home placement, no allergen was detected. No measurable animal allergen was found in the nebulizer sets placed in 1 home without a cat or dog. For the 3 homes with cats, the mean level of Fel d 1 was 20.04 ng/mL (range, <4–64 ng/mL). For the 2 homes with dogs, 2 measurable samples were obtained from 1 home, with Can f 1 levels of 3.4 and 7.8 ng/mL. There was no measurable Bla g 1, Bla g 2, or Mus m 1 in any of the homes where nebulizer sets were intentionally placed, although none of these homes were infested with these pests.

To validate the analysis system, positive control samples were prepared by placing known amounts of allergen extract in a nebulizer reservoir. These samples, when analyzed in a masked manner, revealed measurable allergen levels for the correct allergen that was added in all 4 samples. None of the negative control nebulizers receiving only saline or dust mite extract contained any detectable cat, dog, cockroach, or mouse allergen. The sample prepared with cat allergen extract (1:10 dilution of 10,000 BAU) yielded a level of 33.7 ng/mL, which is within the range found in 2 of the homes with cats (36.2 and 64.0 ng/mL). The sample prepared with dog allergen extract yielded a level of 7.74 ng/mL; cat, 33.7 ng/mL; cockroach, 0.03 U/mL (Bla g 1) and 0.08 U/mL (Bla g 2); and mouse, 0.8 ng/mL.

DISCUSSION

Nebulizers are used by many asthmatic patients,¹ primarily for administering rescue β -agonist medication, although they may also be used to deliver daily controller medications. Patients with asthma who use nebulizers regularly have been found to have higher rates of hospitalizations and emergency department visits than patients who do not use nebulizers.¹ Many physicians and health care providers have anecdotal reports of patients who experienced acute worsening of their asthma symptoms after use of a nebulized medication. Possible explanations of such nebulizer reactions include tachyphylaxis or increased bronchial hyperresponsiveness due to β -agonist overuse or allergic reactions to the medication or the excipient.

To our knowledge, there are no previously published reports of accidental nebulization of allergen, although clinical studies using nebulized allergens, such as cat, cockroach, ragweed, and grass, have been performed to characterize and study allergic asthmatic populations.^{3–5} We did not find measurable Fel d 1 in any of the 17 nebulizer reservoirs with adequate specimens for testing. This finding may be due to the lack of cats in the homes of the randomly selected patients; however, data on environmental exposures were not available. Bronchoprovocation studies using cockroach allergen can be performed to evaluate the asthmatic response; however, those studies reported Bla g 1 and Bla g 2 levels in protein nitrogen units, and there are no conversion factors to determine the clinical relevance of these units of Bla g found in the nebulizers in the study.^{5,6} Furthermore, to our knowledge,

there are no published reports of allergen bronchoprovocation studies using dog or mouse allergen in the same units used in this study to determine their clinical relevance. Without a known conversion factor, it is difficult to determine the clinical significance of these previously reported allergen levels, although it is conceivable that in sensitized individuals, exposure to such levels, particularly on a repeated basis, could have adverse consequences, as has been demonstrated in bronchoprovocation studies.^{7,8}

Although clinical data were not available for these patients, we previously described 2 patients with acute life-threatening exacerbations after using nebulizers.² In 1 case, cockroaches were visually observed in the reservoir, and in the other case, measurable cockroach allergen was found when the reservoir was tested. The levels of Bla g 1 and Bla g 2 in that case were 0.18 and 0.22 U/mL, which is in the range of the levels found in the present study (<0.06–0.99 U/mL). These levels are also similar to what was found when the nebulizer reservoir was intentionally contaminated with a 1:100 dilution of standard cockroach extract.

Levels of Bla g allergen of 0.05 to 2.0 U/g have been found in settled dust in the homes of children with asthma who experience increased wheezing episodes,⁹ and levels greater than 8.0 U/g are associated with increased asthma morbidity.¹⁰ It is conceivable that levels of Bla g in the range found in some of the nebulizer sets could have a clinical effect in susceptible individuals, particularly if they are inhaled directly into the lung. This may partly explain the increased morbidity reported in children with asthma who use nebulizers compared with children who do not use nebulizers.¹ Sears et al¹¹ reviewed 75 asthmatic deaths in New Zealand, all of whom had nebulizers at home for medication delivery. They did not find evidence of direct toxicity of high-dose β -agonist use, but details of electrolyte and electrocardiographic findings were not available on all patients to evaluate this possibility. Information about the home environment of the patients in their study was not provided.¹¹ Given the life-threatening nature of the 2 previously presented cases of nebulizer contamination, it is possible that similar exposures could lead to serious outcomes.

We simulated home allergen exposure to furred animals and determined the ability of allergens to contaminate nebulizer equipment in a few homes. Two of 3 nebulizer sets placed in homes with cats contained measurable Fel d 1, and 1 of 2 nebulizer sets placed in homes with dogs had measurable Can f 1. There was no measurable animal allergen when the nebulizer sets were kept in sealed plastic bags (controls).

Manufacturers' guidelines for the proper care of nebulizers provide instructions for cleaning and storing the nebulizer sets. One manufacturer (DeVilbiss/Sunrise Medical, Somerset, PA) and some asthma Web sites (eg, <http://www.univmedassoc.com/asthmaed/nebulizer.htm>) specifically recommend storing nebulizer equipment in zippered plastic bags. However, it is well known that many patients

do not receive specific instructions or do not follow manufacturer guidelines for proper cleaning of nebulizer equipment (A.B., unpublished data 2004). If proper cleaning of the nebulizer set is performed, potential contamination of the equipment with environmental allergens should be reduced, although this remains to be studied. In addition, although nebulizer equipment in sealed plastic bags as recommended by some manufacturers contained no detectable animal allergen when placed in homes with cats and dogs, further study is needed to determine whether this is an appropriate recommendation. For example, it is unknown whether similar storage techniques could prevent contamination with cockroach allergen and whether it could potentially increase exposure to other allergens, such as mold. This study was limited by our inability to directly make comparisons between the allergen levels found in nebulizer equipment and the specific patient's environmental and clinical data. Future studies linking environmental and clinical data with nebulizer data are warranted.

In conclusion, nebulizer equipment can become contaminated with common indoor environmental allergens, which may result in serious consequences in certain sensitive individuals. Preliminary results suggest that contamination of nebulizer equipment may be avoided by keeping the nebulizer sets, including the reservoir, mask, mouthpiece, and tubing, in sealed plastic bags; however, further study is needed to determine optimal storage techniques to avoid allergen exposure.

REFERENCES

1. Butz AM, Eggleston P, Huss K, Kolodner K, Rand C. Nebulizer use in inner-city children with asthma. *Arch Pediatr Adolesc Med.* 2000;154:984–990.
2. Bollinger ME, Wolf B, Schwindt C, Hamilton RG. Contamination of nebulizer equipment with cockroach allergen: there's a bug in the system. *Ann Allergy Asthma Immunol.* 2004;92:475–477.
3. Ball BA, Folinsbee LJ, Peden DB, Kehrl HR. Allergen bronchoprovocation of patients with mild asthma after ozone exposure. *J Allergy Clin Immunol.* 1996;98:563–572.
4. Kelly EAB, Busse WW, Jarjour NN. A comparison of the airway response to segmental antigen bronchoprovocation in atopic asthma and allergic rhinitis. *J Allergy Clin Immunol.* 2003;111:79–86.
5. Uy CG, Yound RC Jr, Chehreh MN, Scott RB. Bronchial challenge studies with cockroach antigen in asthmatic children. *Ann Allergy.* 1973;31:407–412.
6. Lluch M, Sastre J, Fernandes-Nieto E, Quirce S. Eosinophilic and neutrophilic sputum response to bronchial challenge with cockroach. *J Allergy Clin Immunol.* 2003;112:802–803.
7. Liu L-U, Swenson CA, Kelly EA, Kita H, Jarjour NN, Busse W. Comparison of the effects of repetitive low-dose and single-dose antigen challenge in airway inflammation. *J Allergy Clin Immunol.* 2003;111:818–825.
8. Ihre E, Zetterstrom O. Increase in non-specific bronchial responsiveness after repeated inhalation of low dose allergen. *Clin Exp Allergy.* 1993;23:298–305.

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9. Litonjua AA, Carey VJ, Burge HA, Weiss ST, Gold DR. Exposure to cockroach allergen in the home is associated with incident doctor-diagnosed asthma and recurrent wheezing. *J Allergy Clin Immunol.* 2001;107:41–47.
 10. Rosenstreich DL, Eggleston P, Kattan M, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N Engl J Med.* 1997;336:1356–1363.
 11. Sears MR, Rea HH, Fenwick J, et al. 75 deaths in asthmatics prescribed home nebulisers. *BMJ.* 1987;294:477–480.

Requests for reprints should be addressed to:
Mary E. Bollinger, DO
Division of Pediatric Pulmonology/Allergy
University of Maryland School of Medicine
737 W Lombard St, Suite 314
Baltimore, MD 21201
E-mail: mbolling@peds.umaryland.edu