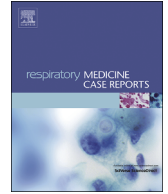




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## Case Report

## Causative antigens of humidifier lung in vapor from a humidifier: A case report

Masafumi Shimoda<sup>a,\*</sup>, Koza Morimoto<sup>a,b,c</sup>, Makiko Hosoya<sup>d</sup>, Asami Osugi<sup>d</sup>, Satoshi Mitarai<sup>d,e</sup>, Yoshiaki Tanaka<sup>a</sup>, Keiji Fujiwara<sup>a,d,e</sup>, Koza Yoshimori<sup>a</sup>, Ken Ohta<sup>a</sup><sup>a</sup> Respiratory Disease Center, Fukuji Hospital, Japan Anti-Tuberculosis Association, Kiyose City, Tokyo, Japan<sup>b</sup> Division of Clinical Research, Fukuji Hospital, Japan Anti-Tuberculosis Association, Kiyose City, Tokyo, Japan<sup>c</sup> Department of Clinical Mycobacteriosis, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan<sup>d</sup> Department of Mycobacterium Reference and Research, The Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Kiyose City, Tokyo, Japan<sup>e</sup> Department of Basic Mycobacteriosis, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

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## ABSTRACT

A 68-year-old man exhibited fever and cough three weeks prior to hospital admission after three months of ultrasonic humidifier usage. Chest computed tomography showed bilateral ground-glass opacities, lymphocyte levels in the bronchoalveolar lavage fluid were elevated (60.8%), and the histological examination of a transbronchial lung biopsy showed lymphocytic alveolitis. He gradually improved without medication after he stopped using the humidifier. Accordingly, humidifier lung was the diagnosis. Humidifier water and vapor collected from the patient's humidifier were investigated. Humidifier vapor was obtained by collecting the condensed moisture. Laboratory examinations exhibited gram-negative rods and a high concentration of endotoxin and (1 → 3)-β-D-glucan in both vapor and water. The serum-precipitating antibodies showed a stronger reaction against humidifier vapor than against humidifier water. 16S rRNA metagenomic analysis revealed a high percentage of sequences of *Spirosoma lacussanctae* and *Sphingomonas* spp. in both the humidifier vapor and water. The percentages of sequence reads were lower in humidifier vapor than in water; conversely, sequences of *Pseudomonas* spp. and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* were more concentrated in the humidifier vapor than in humidifier water. Although the reason for the different bacterial ratios between humidifier vapor and water is uncertain, the bacteria that were more concentrated in humidifier vapor than in humidifier water might have been the causative antigen underlying the humidifier lung diagnosis. This is the first report to indicate the presence of causative antigens in humidifier vapor.

## 1. Introduction

Humidifier lung is a rare phenotype of hypersensitivity pneumonitis (HP) caused by the inhalation of humidifier vapor [1–3], and features of humidifier lung are different from those of other phenotypes of HP [2–4]. Many causative antigens of humidifier lung have been reported, such as fungi, bacteria, amoebae, and endotoxins in humidifier water [1,4]. However, no report has demonstrated

\* Corresponding author. Respiratory Disease Center, Fukuji Hospital, Japan Anti-Tuberculosis Association, 3-1-24 Mastuyama, Kiyose City, Tokyo, 204-8522, Japan.

E-mail address: [shimodam@fukujuji.org](mailto:shimodam@fukujuji.org) (M. Shimoda).

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causative antigens in humidifier vapor. Therefore, we examined antigens in vapor collected from a patient's humidifier by using serum-precipitating antibodies and 16S rRNA metagenomic analysis.

## 2. Case presentation

A 68-year-old man had fever and cough three weeks prior to admission after three months of using an ultrasonic humidifier 24 hours a day in his home for the first time. He had a medical history of esophageal hiatal hernia, duodenal ulcer, gallbladder polyp, and alcoholic liver dysfunction. He had no smoking history and was allergic to cedar, ragweed pollen and house dust. A general physician diagnosed him with pneumonia based on chest radiography, but his symptoms did not improve despite antibiotic treatment (Garenoxacin). Therefore, he visited our hospital.

He showed normal vital signs and inspiratory coarse crackles in both lung bases. Laboratory findings showed a white blood cell count of 25,090 cells/ $\mu\text{L}$  with 93.2% polymorphic nuclear leukocytes, C-reactive protein level of 9.86 mg/dL, and serum Krebs von den Lungen-6 (KL-6) level of 611 U/mL. Test results for serum antibodies against *Trichosporon asahii*, pigeons, budgerigars, and *Aspergillus* were negative. Chest computed tomography (CT) showed bilateral ground-glass opacities (Fig. 1). The bronchoalveolar lavage fluid (BALF) exhibited an elevated cell count ( $8.15 \times 10^6/\text{mL}$ ) comprising lymphocytes (60.8%), neutrophils (6.6%), and eosinophils (5.6%). The ratio of CD4<sup>+</sup> to CD8<sup>+</sup> cells in the BALF was 1.7, which was normal. The histological examination of a trans-bronchial lung biopsy showed lymphocytic alveolitis without granulomas. He gradually improved without medication after he stopped using the humidifier. Accordingly, he was definitively diagnosed with humidifier lung ( $\geq 90\%$  confidence) based on the clinical practice guidelines for HP [5].

Humidifier vapor and water were collected from his humidifier. Humidifier vapor was obtained by collecting the condensed moisture on a sterile spitz without filters. Laboratory examinations exhibited gram-negative rods and a high concentration of endotoxin (more than 25.0 EU/mL in both vapor and water) and (1  $\rightarrow$  3)- $\beta$ -D-glucan (158.0 pg/mL in vapor and 626.3 pg/mL in water). Filamentous fungus was identified in only the humidifier water. Metagenomic analysis revealed a high percentage of sequences of *Spirosoma lacussanctae* and *Sphingomonas* spp. in both the humidifier vapor and water; however, the percentages of sequence reads were lower in humidifier vapor than in water (Fig. 2). On the other hand, sequences of *Pseudomonas* spp. and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* were more concentrated in the humidifier vapor than in humidifier water. Tests for serum-precipitating antibodies against humidifier vapor and water were positive, and the reaction in the humidifier vapor was stronger than that in the humidifier water (Fig. 3).

## 3. Discussion

This is the first report to reveal the presence of causative antigens of humidifier lung in humidifier vapor. Generally, causative agents of humidifier lung are identified through serum-precipitating antibodies against humidifier water and/or specimens that are cultured from humidifier water [1]. However, no study has investigated humidifier vapor. In our study, serum-precipitating antibody results indicated that humidifier vapor also include causative antigens, like humidifier water. Furthermore, the reaction of serum-precipitating antibodies against humidifier vapor was stronger than that in humidifier water, and the content ratio of bacteria that were identified by metagenomic analysis was different between humidifier vapor and water. Specifically, bacteria which were more concentrated in humidifier vapor than in humidifier water might have been the causative antigen underlying humidifier lung.

There are few reports on humidifier vapor. A total of 45% of humidifier vapor samples, which were collected by a microbial air sampler, exhibited the presence of several bacteria and fungi, which were the same as those in humidifier water [6]. Another report demonstrated that the concentration of environmental endotoxin increased in the areas around a running humidifier [7]. However, those experimental studies did not investigate the use of a humidifier by a patient with humidifier lung. Humidifier vapor from the patient's humidifier were collected in our study and revealed the presence of bacteria and endotoxin, similar to in humidifier water.



Fig. 1. Chest computed tomography scans showed bilateral diffuse subpleural nonsegmental ground-glass opacities.

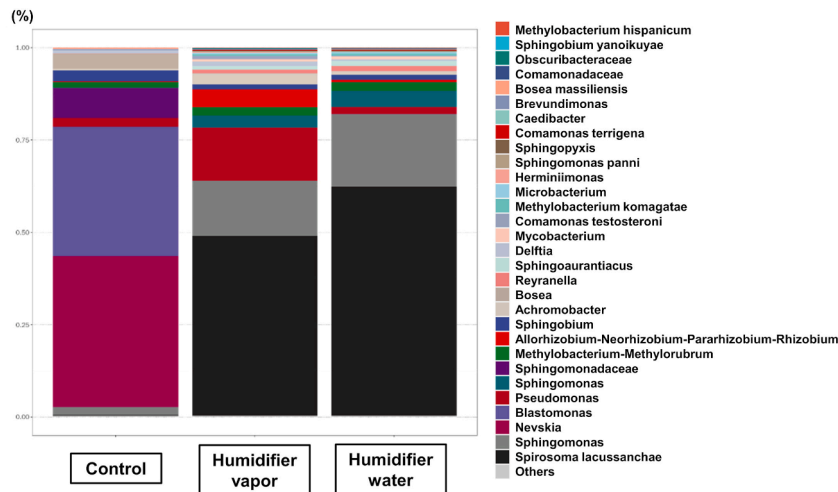


Fig. 2. 16S rRNA gene amplicon sequencing analysis identified the top 30 strains in the V2–V3 hypervariable region. Comparisons of sequence percentages between humidifier vapor, water, and control. *Spirosoma lacussanchae* and *Sphingomonas* spp. made up high percentages of the sequences in both the humidifier vapor and water; however, these sequences made up a lower percentage in the humidifier vapor than the humidifier water. Reads of *Pseudomonas* spp. and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* were concentrated in the humidifier vapor more than in humidifier water. The control was a sample from humidifier water obtained from our hospital. rRNA ribosomal ribonucleic acid.

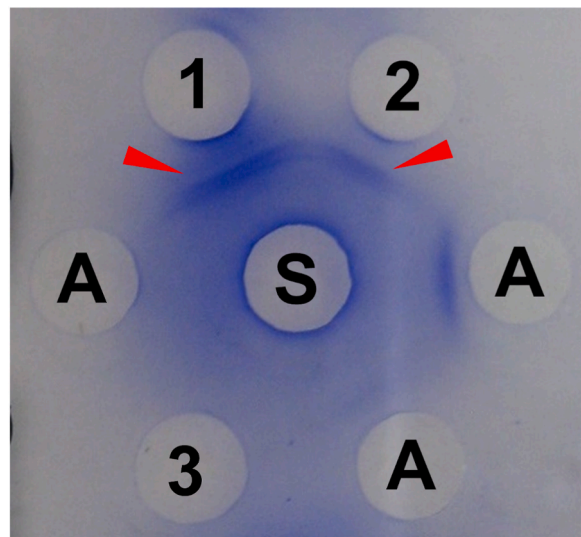


Fig. 3. Results of positivity for serum-precipitating antibodies against humidifier vapor and humidifier water. The red arrows show the precipitation line between the patient's serum and the humidifier water or the humidifier vapor. S: patient serum (5x concentrated), 1: humidifier vapor (10x concentrated), 2: humidifier water (10x concentrated), 3: humidifier water (original solution), A: sample antigens (from other humidifier water that was not analyzed). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Furthermore, our study showed that test results for serum-precipitating antibodies against humidifier vapor were positive. Generally, the appearance of serum-precipitating antibodies is related to type III allergy (immune complex-mediated) [8], which is one of the common mechanisms of other phenotypes of HP [5]. Various fungi and bacteria that have been identified from humidifier water have been reported as causative antigens [1,2,4]; however, the mechanism of humidifier lung might be different from other phenotypes of HP based on differences in the clinical features [2–4]. Furthermore, the reaction of serum-precipitating antibodies against humidifier vapor was stronger, and the content ratio of bacteria in humidifier vapor and water were different based on metagenomic analysis. The reason for the different ratios of bacteria between humidifier vapor and water is uncertain. A previous report suggested that some pathogens are broken down by the vibrations of an ultrasonic humidifier [9], which might help explain the differential bacterial ratio results in our study. However, this study did not statistically analyze this finding or the contribution of individual fungi. Metagenomic analysis can be used to identify bacteria based on genotypic methods more accurately than the use of general phenotypic characteristics [10]; therefore, causative antigens might not be identified by routine culture. In addition, the report demonstrates the presence of concordant antigens in humidifier vapor and water by metagenomic analysis and tests for serum-precipitating antibodies; however, the usefulness of humidifier vapor as a diagnostic tool when compared to humidifier water was uncertain. Further study is needed for accurate identification of causative antigens which might lead to elucidation of the mechanism of humidifier lung.

Another mechanism of humidifier lung is suggested to involve exposure to endotoxin [2,3], which is generated by bacteria in humidifier water broken down by ultrasonic humidifiers [9]. Generally, a humidifier is associated with increased airborne endotoxin levels [7,11], and our study is the first to demonstrate that humidifier vapor also includes high concentrations of endotoxin, similar to humidifier water. Previous reports demonstrated that the different mechanisms of humidifier lung compared to other phenotypes of HP might be related to the different features, such as lower KL-6 levels, fewer centrilobular ground-glass opacities and more common consolidations on CT, and less common granulomas in histopathological findings [2–4]. Although there has been no report that demonstrates this phenomenon, the evidence in our study that humidifier vapor contains high concentrations of endotoxin might be essential for the elucidation of the mechanism.

#### 4. Conclusion

This is the first report to indicate the presence of causative antigens in humidifier vapor, presumably bacteria that were more concentrated in the vapor.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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