Molecular detection of *Blastomyces* in an air sample from an outbreak associated residence

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Abstract

Based on epidemiologic data during a blastomycosis outbreak, exposure within the home was suspected for two case patients that resided together. Soil and air samples were collected from the basement of their residence. Samples were tested for *Blastomyces* by culture and polymerase chain reaction (PCR) to compare with an available clinical isolate. An air sample from the basement of the residence was PCR positive for *Blastomyces*. Sequence data from the air sample and the outbreak clinical isolate were identified as different *Blastomyces* spp. Despite this, our findings suggest that the basement was suitable for the growth of *Blastomyces* and airborne organism was circulating.

Key words: blastomycosis, *Blastomyces*, outbreak, residence, air-sampling.


Blastomycosis, caused by the dimorphic fungal pathogen *Blastomyces* spp., is a potentially fatal disease in humans. *B. dermatitidis* and *B. gilchristii* are endemic in areas of North America, including Wisconsin, USA, where some of the highest annual incidence rates have been reported. In addition, several large outbreaks have occurred in the state. In early 2010, county health officials noticed an increase in blastomycosis cases in Marathon County, Wisconsin, USA. Cases appeared to be geographically clustered within neighborhoods or households with no common outdoor or recreational exposure. Based on epidemiologic data, exposure within the home was highly suspected for two case patients that resided together. Soil and air samples were collected from the basement of the residence of these two outbreak case patients. Samples were tested for *Blastomyces* by culture and polymerase chain reaction (PCR) in order to compare with an available clinical isolate from one of the case patients. We report molecular detection of *Blastomyces* DNA in an air sample collected in the basement of this residence.

The residence was a rental property containing two units, with the basement being provided to tenants for storage. The integrity of the fieldstone walls of the foundation of the basement were compromised, with several holes open to the outside. Evidence of both moisture and small mammal activity, in the form of droppings, were present in the basement.

Environmental samples were collected from the basement of the residence of two outbreak case patients 2 months after blastomycosis symptom onset. Soil samples (*n* = 20) were collected from the earthen floor of the basement into individual sterile 50 ml conical tubes and homogenized in sterile water for culture and DNA extraction. Air samples (*n* = 4) were collected in the basement using a Zefon Bio-Pump® and Via-Cell® sampling cassettes (Zefon International Inc, Ocala, FL, USA) according to the manufacturer’s recommendations. Four separate air samples were obtained, with three collected constantly over a 10 minute period and one sample obtained by intermittent sampling over a 12 hour period. Cassettes were removed from the pump and transported to the laboratory for analysis. The glass slide was retrieved from inside each Via-Cell® cassette and rinsed with sterile water for culture and DNA extraction. Homogenized soil and air samples were plated on yeast extract phosphate agar (Smith’s medium), with one drop of 1 N ammonium hydroxide added to each plate and incubated at 28°C. Cultures were...
examined every other day for *Blastomyces* growth. DNA was extracted from air samples as previously described. Soil samples were extracted using the UltraClean<sup>®</sup> Soil DNA Isolation Kit (MO BIO Laboratories Inc, Carlsbad, CA, USA). Extracted DNA from soil and air samples were amplified with Blasto I and II primers as described previously, with an additional 10 PCR cycles. Extraction and master mix controls were included on each run to ensure purity of the reagents. PCR products were sequenced using the BigDye<sup>®</sup> Terminator v3.1 cycle Sequencing Kit (Applied Biosystems, Warrington, United Kingdom). DNA sequences were aligned using Lasergene 11 software (DNASTAR Inc, Madison, WI, USA). Consensus sequence was nucleotide BLAST searched using the NCBI database. A clinical *Blastomyces* isolate was available for genotyping from one of the case patients. DNA was extracted from the isolate as previously described. The DNA was amplified with Blasto I and II primers and sequenced as described above. In addition, DNA extracted from the cultured *Blastomyces* isolate was able to be further genotyped, due to its purity, using microsatellite typing and its2 sequencing.

All soil samples were PCR and culture negative for *Blastomyces*. Culture plates did grow few fungal and bacterial colonies, but none were morphologically consistent with *Blastomyces* after inspection by a clinical microbiologist. Additionally, all air samples were culture negative. However, the air sample obtained by intermittent sampling over a 12 hour period, was PCR positive for a ∼360 bp product using Blasto I and II primers. Subsequent sequencing of the PCR product and BLAST alignment showed 100% match with GenBank accession FJ427194.1. More recently, we have confirmed the isolate from which sequence FJ427194.1 was derived, as a microsatellite genetic group 2, *B. dermatitidis* isolate. The DNA extraction, amplification, and sequencing of the intermittently collected air sample were repeated and yielded the same results. Amplification of the clinical isolate obtained from the outbreak case patient, using Blasto I and II primers yielded a ∼660 bp product. Subsequent sequencing of the patient PCR product and BLAST alignment showed 100% match with GenBank accession FJ427195.1. Additional genotyping of the clinical isolate confirmed it as a microsatellite genetic Group 1, *B. gilchristii* isolate.

This paper is the result of environmental testing of a residence of two case patients during a blastomycosis outbreak. We were able to detect *Blastomyces* DNA in an air sample obtained from the basement of the residence. The DNA from the air sample was 100% sequence match with a known *B. dermatitidis* isolate, whereas the case patient’s clinical isolate was *B. gilchristii*. However, detection of *Blastomyces* DNA in this residence does suggest that the environment in the basement was suitable for growth of *Blastomyces* and that airborne organism was circulating. Air sampling was conducted within the confines of a basement and therefore we felt our chances of detecting *Blastomyces*, if it was present, were fairly good. Outdoor environmental air sampling and molecular detection has been demonstrated successfully for *Coccidioides*, a closely related species endemic to areas of the southwest United States. The successful use of air sampling for molecular detection of *Blastomyces* provides additional proof of concept for environmental surveillance of dimorphic fungi in endemic areas.

Our attempt to culture *Blastomyces* from air and soil samples was unsuccessful. Successful isolation of *Blastomyces* from the environment is notoriously difficult and despite thousands of attempts, has only been accomplished about 2 dozen times. Most successful isolations have been accomplished via animal inoculation, a resource not available to us. Instead, we selected a culture method specifically developed for contaminated specimens, taking advantage of *Blastomyces* suspected ammonia tolerance. We did observe limited growth of other fungi and bacteria so it is possible that *Blastomyces* was outcompeted by the other organisms present in the soil and air.

Exposure to *Blastomyces* in or near one’s own home has previously been considered controversial. During the epidemiologic outbreak investigation, described by Roy et al., cases were geographically clustered within neighborhoods or households and no common outdoor or recreational activity could be associated with the cases. In addition, outbreak case-patients were not more likely to engage in outdoor activities, which are typical risk factors and significantly more outbreak case-patients had a household member who was also diagnosed with blastomycosis as compared to historic cases. This, in combination with molecular detection of *Blastomyces* in the home of two case patients, indicates that residence (both condition and neighborhood) was a risk factor in this outbreak.

One limitation of this study was that the collection of environmental samples was 2 months after symptom onset in the outbreak case patients, which affected our ability to detect the etiologic strain of the outbreak case patient. Despite this, we report the first successful use of air sampling for molecular detection of *Blastomyces*.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

References


17. Proctor RA. Blastomycosis is a serious disease, but let’s not raise fears without conclusive data. *Wis Med J.* 2001; 100: 8–9.