A Simple, Cost Effective and Rapid Air Borne Mold-Monitoring Model Developed in St. Kitts for Ensuring Global Public Health Safety and Food Security

Elise Landa², Harish C Gugnani², Atandra Burman, Kristen Duman, Zachary Ciocchetto, Harleen Saini², Irshad Prasla, James Bassford, Torib Uchel, Samuel Park, Alyssa Mahon, Nalliene Chavez and Girish J. Kotwal*¹

University of Medicine and Health Sciences, St. Kitts, West Indies and Saint James School of Medicine, Bonaire, West Indies
*Contributed equally to the final publication

Abstract
The morbidity and mortality caused by pathogenic and opportunistic airborne molds (ABM) has been on the rise. ABM are not confined by national boundaries but can travel distances over time and can cause respiratory dysfunction, crop damage, food spoilage and serious meningitis outbreaks due to contamination of pharmaceuticals. Presence of mold spores in abnormally high numbers can present hazardous risks to human population world-wide causing mycosis, hypersensitivity reactions or poisoning by mycotoxins. Tropical places and places receiving heavy rains in the monsoon season can end up with a massively higher mold count and the aftermath of hurricanes and floods can result in dangerously high levels of mold counts in the environment. ABM does not uniformly affect the population but is more dependent on individual hypersensitivity, age, nutritional status, vegetation, ventilation etc. While the world has been acutely aware of particulate and minute organic and inorganic pollutants from automobile emissions and industrial release of gases in the air, the presence of molds in the environment has received little attention until the recent outbreak of Meningitis, which resulted in over 60+ deaths and 100s of hospitalizations in the USA. There is an urgency now to develop the tools that can be universally adopted to monitor the mold count in the air around the landmass of the world and then to characterize the type of mold to be followed by ways of cleaning the environment of harmful molds. Here we propose a simple, cost effective and rapid model for monitoring molds that does not require sophisticated infrastructure to gain initial insight of what is in the air in terms of molds we breathe locally and assess the impact it could have. Developed countries could certainly go steps ahead in terms of sophistication in identifying the molds such as by use of PCR but for now to establish a baseline of what is the mold count and what are the general types of molds in surrounding air, a simple low cost model could be a start. Our results from assessment of molds on an island nation have revealed that mold count varies within certain regions and settings. The diversity of the molds is proportional to the mold count. Most molds found can cause little direct harm to individuals but can contribute to crop damage and food spoilage. Overall the benefit to the inhabitants in getting to know the types of molds in the surrounding air they breathe is going to be of considerable benefit.

Introduction
Molds are found both indoors and outdoors. Estimates of species of airborne molds range from tens of thousands to perhaps three hundred thousand. Molds grow best in warm, damp, and humid conditions, and spread and reproduce by spores [1,2]. Mold spores can survive harsh environmental conditions, such as dry conditions, that do not support normal mold growth. Some of the more common air molds include Cladosporium, Penicillium, Alternaria, and Aspergillus. Several studies have suggested a potential link of early mold exposure to development of asthma in some children, particularly among children who may be genetically susceptible to asthma development [1,2]. There is enhanced awareness of the importance of mold contamination in residential environments. Individual immune responses to these fungi may range from no symptoms to severe, depending on each person's susceptibility to the allergen [3]. Several investigators have reported on the prevalence of outdoor and indoor air molds in different countries [4-7].

A wide variety of microorganisms including fungi are dispersed into the Caribbean region by the Sahara dust aerosols during the summer months. These microorganisms can cause diseases in plants and animals, and might be responsible for an increase in incidence of asthma and respiratory diseases in this region [8,9]. Information on the prevalence of air molds in the Caribbean region has been very much lacking. In a study from Cuba, the principal airborne molds detected were Cladosporium, Aspergillus, and yeasts, with many other genera being encountered occasionally. In another study in Cuba, Aspergillus, Cladosporium, and Penicillium spores were detected abundantly at different times of the day [10,11]. A Dominican study identified six genera of air born fungi including Aspergillus, Penicillium, Cladosporium, Fusarium, Curvularia, and Nigrospora [12]. The rationale for the present 2-part study is to propose a simple, cost effective and rapid model to monitor airborne molds around the world by first obtaining a mold count within the air of one of the smallest independent nations in the world, using Google maps to catalog the mold counts by location, and further characterizing these mold species [13]. We hope this 4-step model will provide the know-how to identify regions of high mold count, followed by characterization of the diversity of both pathogenic and non-pathogenic molds distributed world-wide. Here, we report the latter part and together, the model could be adopted to cover the air over the landmass of the rest of the world. This information would be vital for ensuring the public health safety and food security.

Materials and Methods
The methodology to trap the molds is essentially the same as...
described earlier [13]. Duplicate Petri plates of Difco Sabaroud's Dextrose agar (SDA (40 g/L dextrose, 10 g/L peptone, 20 g/L agar, pH 5.6)) were exposed for one hour at various pre-determined locations followed by a 48 hour incubation period at room temperature inside a bio-safety hood. Final mold count was obtained by repeating the entire process once again in duplicate with an exposure for periods shorter than 1 hour for those that had too many to count (TMTC) quantities of molds. The mold counts for those with shorter exposure were then multiplied by a factor equal to the fraction. If there was a 15-minute exposure in the second round then the mold count was corrected for 1 hour by multiplying by a factor of 4. The average count/hour of exposure was obtained by averaging the mold count from the 2 separate exposures for the same location. Exposure locations included apartments and places of business because even these locations are susceptible to pathogenic fungal colonization. Places using electrical appliances to artificially modify the environment are susceptible to pathogenic fungal colonization. Places using electrical appliances to artificially modify the environment were included in the experiment as they have been shown to potentially carry the same risk for mold contamination [14]. Identification of the molds was attempted by a detailed study of colonial morphology and microscopic features as observed in lactophenol cotton blue mounts, and comparing the observed characteristics with descriptions given in standard books and manuals [15-17].

The distribution of molds identified was then, using Google maps, organized into pockets of air sampled providing a map of the location with the corresponding molds in the air of that location (Google map 1 and 2).

**Results**

Twelve different locations on the island of St. Kitts were examined for fungi present in the air. More than 20 different species of fungi and yeast were identified. *Cladosporium cladosporioides, Mucor sp* and *Aspergillus sp.* were all found in three or more locations throughout the island. Other infrequently recovered molds included *Curvularia, Alternaria, Fusarium solani, Metarrizum, Stachybotry, Trichosporon, Geotrichum,* and *Sporobolomyces.* The brief detail of the isolations of molds from different locations are mentioned below.

**Mattingly Heights-Residential Housing**

A kitchen area in a house in Mattingly Heights was examined. This was one of the sites that showed mold overgrowth and the plates of SDA were re-exposed to air in this location for a shorter duration of 15 minutes and incubated until mold growth was manageable enough to isolate colonies. Based on microscopic analysis of fungal growth in Mattingly Heights, it was noted that predominant fungi prevalent in the air in the Camps Area were *Mucor sp* and *Cladosporium cladosporioides* (Figure 1).

**Sea View Gardens- Neville’s restaurant**

A restaurant by the main road in greater Camps area called Neville’s was examined. This site also showed mold overgrowth and the plates of Sabouraud dextrose agar were re-exposed to air in this location, and incubated until appreciable mold growth occurred to recover cultures. *Cladosporium cladosporioides* was the predominant fungal agent in the air; a few colonies of *Geotrichum candidum* were also observed (Figure 2).

**HalfMoon Bay-Horizon’s villa**

Residential housing area in the Half Moon Bay was examined. This site showed significant growth of *Aspergillus fumigatus* and *Mucor sp,*
as evidenced by a large number of colonies of both species appearing on the plates (Figure 3).

**Frigate bay**

Rituals Coffee House, a restaurant and coffee house in Frigate Bay area was examined. This location showed growth of several colonies of fungi, mainly *Cladosporium sp*, *Alternaria* (*A. alternata*) and the yeast, and *Trichosporon sp*. (Figure 4).

Rituals Sushi Restaurant in the Frigate Bay area was examined. Location showed multiple colonies of *Cladosporium sp* and *Candida sp* (Figure 5).

**Camps**

A residential housing in the Camps area was examined. Significant overgrowth was seen on test plates as well as master plate. Growth included several colonies of species of *Aspergillus* (mainly *A. niger*, *A. fumigatus*, and *A. flavus*), *Mucor*, *Cladosporium sp*, and *Trichosporon sp*. (Figure 6).

The Best Buy Grocery Store located in Camps was examined. Large amount of mold growth was seen, including that of *Rhodotorula sp* and *Cladosporium sp* (Figure 9).

**UMHS campus**

The lunch tent on the UMHS campus where most students eat meals while on campus was examined. Significant growth was seen and fungal species included *Mucor sp*, *Aspergillus sp* (Figure 7).
The Student Lounge on the East side of the Administration building was examined. Large colonies of growth were seen, including fungi and yeasts. The predominant species was *Aspergillus fumigatus*, followed by *Curvularia* species. A few colonies of *Bipolaris* sp were also observed (Figure 8).

Discussion

The present investigation constitutes the first study of its kind dealing with the types of airborne molds in an island nation of St. Kitts. It is noteworthy that a variety of species were recovered in the agar plates exposed to air in different types of locations. The predominant fungi were *Cladosporium* (*C. cladosporioides*) and *Aspergillus* (mainly *A. fumigatus*, *A. Niger* and *A. flavus*). The presence of *Aspergillus fumigatus* in quantity in air has important health implications for humans and animals [18]. *A. fumigatus* is quite common in house dust, indoor and outdoor, in different types of soil, on decaying plant material, compost, and also on hay and crops. It is also an important etiological agent of systemic mycosis in domestic animals and in humans, especially the immunosuppressed and immunocompromised.
Location | Mold Species
---|---
Residential Housing- Mattingly Heights | Cladosporium cladosporioides (Mucor sp. )
S. chartarum | Aspergillus niger (Aspergillus fumigatus)
Neville’s Restaurant- Sea View Gardens | Cladosporium cladosporioides (Geotrichum candidum)
Horizon’s Villa- Half Moon Bay | Mucor sp. (Aspergillus fumigatus)
Ritu’s Coffee House- Frigate Bay | Cladosporium sp. (Trichosporon sp. Alternaria alternata)
Ritu’s Sushi Restaurant- Frigate Bay | Cladosporium sp. Candida sp. 
Residential Housing- Camps | Aspergillus niger (Aspergillus fumigatus)
S. chartarum | Cladosporium sp. (Mucor sp. Trichosporon sp. Alternaria alternata)
UMHS Lunch Tent | Aspergillus fumigatus
UMHS Campus- Camps | H. capsulatum
Camp- Best Buy Grocery Store | R. stolonifer
Species infrequently found on St. Kitts | M. arthureum, F. solani, S. chartarum

Table 2: Location of mold species.

Mold II Sheet 1.

Coccidioides immitis has been sufficiently studied in the past making this one of the earliest models present in the Ohio valley in USA, Coccidioides immitis present in the Southwest region of USA etc. The diversity of fungi that could directly or indirectly cause harm has not been sufficiently studied in the past making this one of the earliest studies and attempts to obtain a handle at what else is in the air surrounding human inhabitants, plant and animal life. Capturing the molds on culture plates, followed by their morphological identification and comparison with molds from the immediate vicinity can serve as a simplified and cost-effective model for recognizing and monitoring airborne molds in much of the atmosphere. We envision that by ultimately expanding our model of organizing the information gathered and emphasizing the unique molds that have been identified, studies similar to ours can be conducted in other geographical areas.

[19,20]. A. Niger and A. flavus have also been reported to cause allergies, asthma, and rhinitis in children and adults [20]. C. cladosporioides, a dematiaceous fungus is frequently encountered in indoor and outdoor environment occasionally linked to health problems [14]. Fusarium solani, a species infrequently recovered in this study is known to cause mycotic keratitis and skin infections in humans [20, 21]. A. alternate, another dematiaceous mold occasionally encountered in this study has been recognized as risk factor for asthma [22]. Stachybotrys, occasionally encountered in our study is known to cause human disease through direct irritation, type I hypersensitivity, or production of toxins. A variety of respiratory, dermatological, eye, and constitutional symptoms have been associated with heavy and prolonged exposure to S chartarum [23]. The design and methodology detailed in this novel study can be adopted globally to generate a Google map of the total count and types of air borne molds prevalent worldwide. This information can also be used to ensure public health by using appropriate masks, and ensure food security by its proper handling and preservation. Currently such a map exists for a few pathogenic molds like Histoplasma capsulatum present in the Ohio valley in USA, Coccidioides immitis present in the Southwest region of USA etc. The diversity of fungi that could directly or indirectly cause harm has not been sufficiently studied in the past making this one of the earliest studies and attempts to obtain a handle at what else is in the air surrounding human inhabitants, plant and animal life. Capturing the molds on culture plates, followed by their morphological identification and comparison with molds from the immediate vicinity can serve as a simplified and cost-effective model for recognizing and monitoring airborne molds in much of the atmosphere. We envision that by ultimately expanding our model of organizing the information gathered and emphasizing the unique molds that have been identified, studies similar to ours can be conducted in other geographical areas.
Acknowledgement

The authors gratefully acknowledge the Research Committee for evaluating the suitability of the project for medical students and the office of the Dean Camacho for assisting in allowing the use of the facilities for performing the experiments.

References

17. Ellis D, Davis S, Alexiou H, Handke R, Bartley R (2007) Descriptions of Medical Fungi, Mycology Unit, Women’s and Children’s Hospital, and School of Molecular and Biomedical Science, University of Adelaide, University of Adelaide:Adelaide: 198.