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Significant Heterogeneity in Airborne Mold Quantities on the Caribbean Island Of St. Kitts: Health Implications and Impact on Food Preservation

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Abstract

The air we breathe in a given environment can influence the state of health of a person living in that environment. The recent outbreak of fungal meningitis in the USA which led to several deaths, hospitalizations and illnesses due to fungal contamination of a steroid being administered for pain management has underscored the importance of a dire need to have an understanding of the quantities of fungi in the air that surrounds us. Here we report the study results of an analysis of the air for levels of molds from a range of separate locations on the Caribbean island of St. Kitts. The purpose of the study was to establish an initial benchmark for St. Kitts in terms of air quality, with specific attention to airborne fungi, as no such previous analysis has been undertaken and because airborne molds have the potential to negatively impact public health. Our research involved exposure of Sabaroud's dextrose agar plates for fungal growth at predetermined locations around the island followed by determination of the fungal count per hour of exposure. A scoring system proposed here identifies areas with high mold count. The results suggest that areas with abundant vegetation or presence of vegetables also had the highest airborne mold counts. In contrast, areas tested within air-conditioned buildings which were routinely cleaned and well maintained; showed the lowest mold counts. This study should become a simple precursor model for global follow-up studies for monitoring air quality. Ensuring that the air we breathe contains minimal quantities of molds will result in healthier population and help to prevent possible associated allergies and disease, particularly among more susceptible immunocompromised persons either due to AIDS or immunosuppressive therapy.

Keywords: Air pollution; Airborne infection; Air microbiology; Molds (Fungi); St. Kitts; Airborne fungi; Fungi; airborne mold; environmental hazards; air quality; Caribbean; research; Immunocompromised host; Indoor air pollution

Introduction

Since the latter part of the past century, molds (fungi and yeast) have become increasingly recognized for their medical importance [1]. A unique characteristic that separates fungi from other pathogens is its cell membrane containing ergosterol and cell wall containing chitin, glucan and mannose. The mold spores, most important to this research, have thick melanized walls which usually contain complex carbohydrates that are hydrophobic and waxy. The fungal spores are commonly dispersed in the wind, affecting the air we breathe, and potentially invading our bodies via the respiratory system. Airborne fungi have been known to cause allergic reactions and asthma in healthy individuals [2]. However, for an immunocompromised person the same fungi may have consequences that are far more serious. Immunocompromised individuals have lost the ability to fight off the pathogens, and with the main entry way for infectious fungi like *Aspergillus* being inhalation [3], opportunistic infections are readily facilitated, underscoring the medical importance of fungal studies such as this one. Fungal infections are also a concern because there is currently no vaccination or prophylactic treatment available.

In 2012, the US Food & Drug Administration (FDA) and Center for Disease control and Prevention (CDC), released the findings of a multistate investigation into an outbreak of fungal meningitis and other infections among patients who received contaminated steroid injections delivered directly into the spine. Fungal species including *Aspergillus tubingensis, Aspergillus fumigatus, Cladosporium* and *Penicillium* species were found to have contaminated the unopened vials of betamethasone, cardioplegia, and triamcinolone solutions [4,5].

Fungal research on the island of St. Kitts and Nevis is still in its infancy. The only research that has been reported regarding the fungus in St. Kitts was done in a study on the prevalence of Kertinophillic fungi in the soils of St. Kitts and Nevis [6]. In this study investigators gathered 108 soil samples and were able to determine the presence of Geophillic Dermatophyte in the soil in St. Kitts. Other international research has shown that indoor/outdoor spore counts can have considerable variation even between residential locations [7]. Some geographic areas may demonstrate seasonal changes in airborne fungi [8]. Since high quantities of airborne molds found year round maybe present in the air surrounding a tropical Island, which can cause respiratory infections, opportunistic infections, allergies and food spoilage, we engaged in this study.

Materials and Methods

The research project consisted of analyzing the air from a range of separate locations within St. Kitts. Preliminary mold count was obtained as follows. Duplicate Difco Sabaroud's Dextrose agar, SDA (40 g/L dextrose, 10 g/L peptone, 20 g/L agar, pH 5. 6 Petri plates 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 a shorter periods than 1 hour for those that had a too many to count (TMTC) quantities of molds. The mold

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count for those with shorter exposure was then multiplied by a factor equal to the fraction. If there was a 15 min exposure in the second round than 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 were included in the experiment as they have been shown to potentially carry the same risk for mold contamination [9]. In addition, the city of Basseterre is home to a local Caribbean brewery. Research in the Pacific Northwest regions of the United States has found the hop plant, *Humulus lupulus* , to be a source of fungal infection [10]. Upon successful incubation, isolated colonies were confirmed as due to single mold spores.

Each isolate was transferred and cultured on a master plate designated specifically for that location, culture samples were analyzed under light microscopy, and a spore count was determined. Locations where exposed plates yielded too many colonies with resulting overgrowth, a re-sampling was performed, this time exposing SDA plates for a reduced period of time before incubation. Upon a routine one hour incubation period, mold overgrowth was observed at a few locations which were re-exposed for decreased time periods and re-cultured on fresh SDA plates to better isolate and identify the fungal agents involved.

Mold counts for each of the selected locations were obtained by averaging the repeat counts from the duplicate plates that were exposed. The counts obtained were then linked to a map generated from Google maps and shown in Figures 1-5.

Locations yielding mold overgrowth, where there were either too many to count (TMTC) or contained too many fungi, were referred to as TMTC. A scoring system has been developed to assess significant differences. Location with too many to count or over 100 colonies would be designated a score of 5. Those Locations having a colony count, in the range of 50-100 is designated as 4. A count of 20-50 is designated as 3, Counts between10-25, designated as 3. Those between 10-20 as 2 and counts less than 10 designated as 1. Reproducible 2-fold, 3-fold or 4-fold difference in mold count would be considered as a statistically significant difference. Marginal differences would be considered statistically insignificant.

Results

Overall significant heterogeneity of airborne molds was discovered in St. Kitts and summarized in the Table 1. Mapped results of the mold counts obtained are shown in Figures 1-5. Two locations had TMTC or a score of 5. One such area called Mattingly Heights (Figure 6B) is an upscale residential area which lies on the slopes of a dormant volcano and has thick diverse vegetation. The other is a busy coffee shop cum

Location	Score
Mattingly heights, coffee shop in Frigate bay, study area in student residence.	5 (Greater than 100 Mold colonies)
Bridge in Town, farmer's produce market, Outdoor dining area at the University campus	4 (50 or higher , <100)
Peninsula and beach	3 (30-50)
Other outdoor locations along the peninsula	2 (Around 25 ± 5)
Indoor campus areas	1 (Less than 10)

Table 1: Overall significant heterogeneity of airborne molds was discovered in St. Kitts.

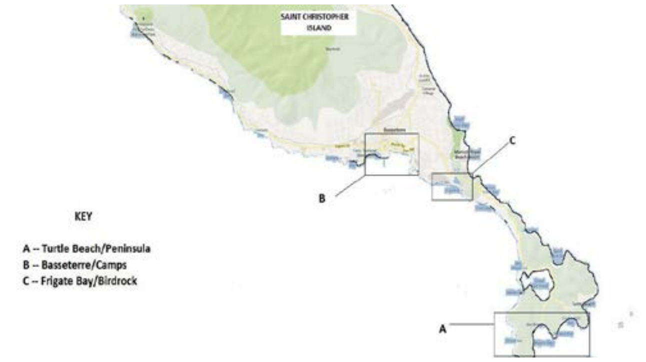

Figure 1: Map of southern St. Kitts and areas which were examined.

Figure 2: The Peninsula and Coastal areas of St. Kitts.

Figure 3: Camps and Basseterre areas of St. Kitts.

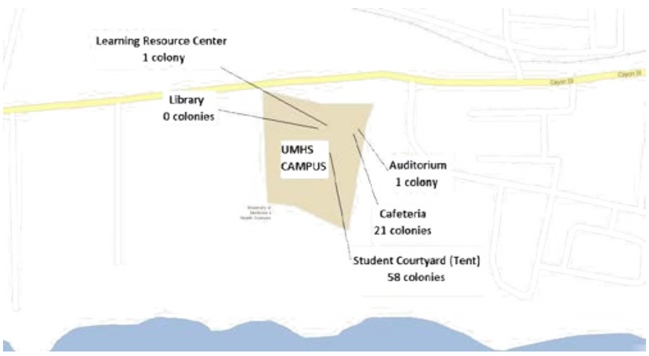

Figure 4: Campus of the University of Medicine and Health Sciences

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Figure 5: Frigate Bay and Bird Rock.

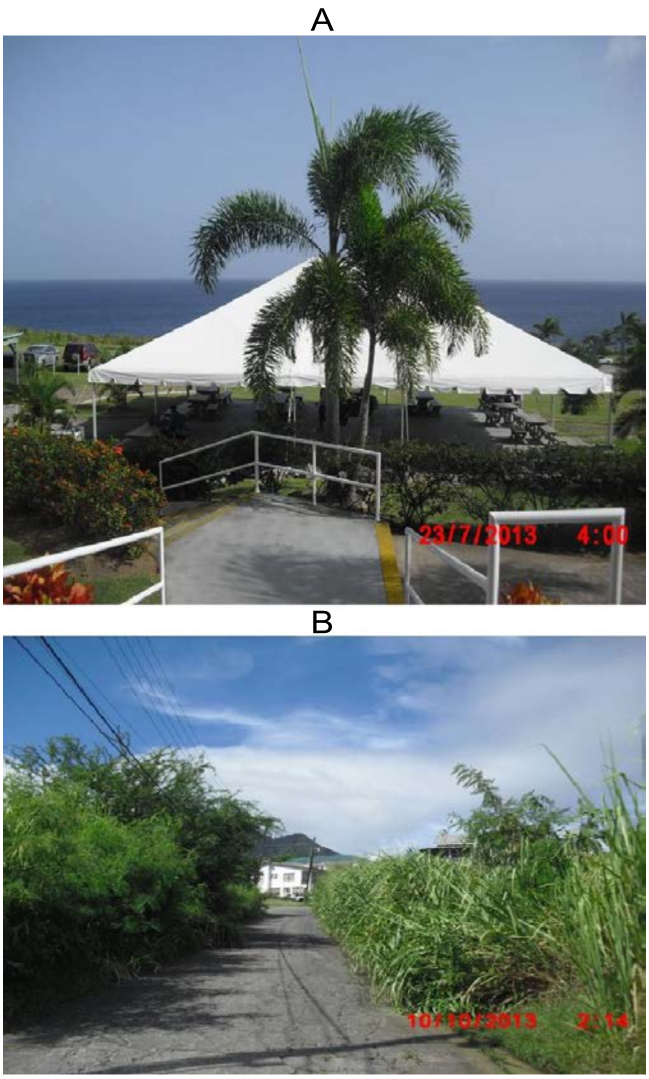

Figure 6: A: University dining area tent (Top). **B:** Mattingly residential area (Bottom).

internet café in town which is frequented by hundreds of customers daily. Locations falling in the second group were ones in which greater than 50 mold colonies were discovered. This group included the

Figure 6: C: The walking bridge over the ghaut in Basseterre (Top). **D:** The sludge heading into the sea (Bottom).

walking bridge in town which is surrounded by vegetation and under which runs a drain or ghaut where water flows from the mountain and empties into the Caribbean Sea (Figures 6C and 6D). The farmer's fruit and vegetable market in town (Figures 6E and 6F) as well as the University campus outdoor dining area (Figure 6A), which is situated in midst of grass and landscaped with tropical trees, scrubs and flowers, complete the second group. The third group was one in which there were approximately 25 colonies. Several locations around the Island were found to fall into this group. The fourth location group included several areas on the UMHS campus which are regularly cleaned; the University lecture halls, auditorium, library and other indoor locations. These areas are air conditioned, with filtered air passing through, and are regularly mopped. The test results from these areas showed minimal presence and levels of molds or fungi. The most surprising result was observed from the butcher's shop in town (Figures 6G and 6H). Despite the presence of an open drain flowing along the entrance to the shop, as well the shop's proximity to the vegetable market, few mold colonies were detected.

Discussion

The rationale for this study was to develop a simple approach to gain a rapid understanding of the diversity of the mold count in the air of a tropical island in the Caribbean region and establish an initial benchmark. The purpose was to then propose this as a model for worldwide monitoring of molds so that instances of possible deadly disasters, resulting in associated disease, such as fungal meningitis, may

Figure 6: E: The butcher's shop (blue building) (Top). **F:** Inside the adjoining fruit and vegetable market (Bottom).

Figure 6: G: Entrance to the butchers' shop (Top). **H:** The meat market (butcher's shop) (Bottom).

be minimized or avoided. If only the air of the manufacturing facility for the steroid formulations for pain relief which was the source of fungal contamination was monitored to determine its mold count it would have become apparent that the formulation also needed to be tested as part of routine quality control prior to the product being shipped to 23 states. Such tests are also important if one has air filtration system; to ensure that the filters are doing the job and the surrounding air from the facility does not contaminate the product being formulated for injection into humans. High mold count areas can be a threat to public health especially for those who are immunocompromised and inhale fungal spores that cause systemic diseases. Those suffering from non-communicable diseases such as diabetes and hypertension can also be adversely affected if they inhale spores of certain fungal species. Patients with HIV/AIDs may suffer a serious reduction in their CD4 count which compromises the immune system and becomes susceptible to systemic fungal infection from molds that would not affect immunocompetent persons. Organ transplant recipients use of immunosuppressant drugs renders them prone to fungal infection, as are persons undergoing prolonged antibiotic therapy. Overall the increased ability of fungi to cause disease among such persons is a public health hazard. Hence, awareness of the existence and location of such high mold areas by persons deemed more susceptible to systemic fungal infections from nonpathogenic airborne molds; would alert them to avoiding exposure and thus lowering the incidence of morbidity and mortality. It is well known that migrating away from high mold count areas (unpublished observation) can offer considerable relief from respiratory discomfort and help prevent progression to more serious health conditions.

Although the findings of this study did not determine the presence of infectious mold spores at critical disease causing levels, a number of the locations tested may still not be suitable environments for the immunocompromised individual. Mattingly Heights (Figure 6B), the café, the ghaut estuary (Figures 6C and 6D), the farmer's fruit and vegetable market (Figures 6E and 6F), as well as the University dining area (Figure 6A), may best be avoided by persons whose immunity is weak or those with hypersensitivity.

The reason for the low mold count in the butchers' shop (Figures 6G and 6H) is not easy to explain even though there is an open drain that flows along the entrance and it is located adjacent to the vegetable market. We had anticipated a higher mold count at this location considering these conditions. Possibly the wind direction at the time of sampling or recent and frequent cleaning affected this result.

The very low mold counts in the University lecture halls, auditorium, library and other air-conditioned indoor facilities on campus, can be attributed to the constant filtering of the air passing through those air conditioners, the regular cleaning of those filters and spaces, along with their minimal exposure to the outside environment. On the other hand, food both ripened fruit like papaya (Figure 7A) and cooked meat (Figure 7B) kept indoors in a refrigerator for no longer than a week is often covered with molds Figure 7A and 7B in tropical places.

Conclusion

The results of the study suggest that areas with high vegetation or presence of vegetables had the highest airborne mold counts. Indoor air-conditioned locations, routinely cleaned, and well maintained buildings, showed the lowest mold counts. Although the findings of this study did not determine the presence of infectious airborne mold spores at critical disease causing levels, a number of locations around St. Kitts yielded spore counts high enough to be determined unsuitable environments for the immunocompromised person. This study should become a precursor model to follow-up studies monitoring air quality for the purpose of ensuring that the air we breathe contains a minimal level and quantity of mold. Such studies would contribute to a healthier population on St. Kitts and elsewhere, and help prevent possible associated disease, particularly among more susceptible persons or those with allergies to high mold count in the air. Additionally, it is important to be aware of mold count to ensure extra precautions for proper food preservation, as even food stuffs kept in refrigerators become moldy if not quickly consumed.

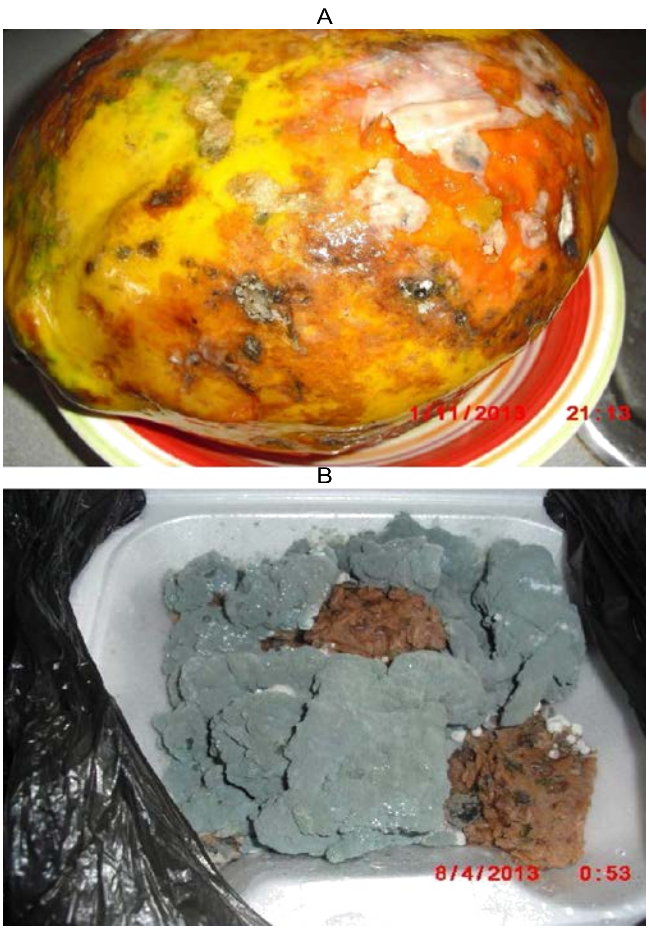

Figure 7: A: Moldy papaya kept in the refrigerator in the Camps residential area (Top). **B:** Moldy cooked meat kept in refrigerator at UMHS (Bottom).

Acknowledgement

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