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Fungal spore distribution in two hospitals in Kabale district, Uganda

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ABSTRACT

Fungi infections are a danger to the value of human life as they cause health issues such as depression, lack of self-confidence and seclusion in infected individuals. To date, the prevalence and distribution of airborne fungi spores are still not well documented in Uganda. Airborne fungi spores in Kabale Referral and Rugarama hospitals were monitored for two months. This study also examined the proportion of fungal infection cases reported in the two hospitals regularly. The fungal colonies were collected for two months using the open plate sedimentation method with Petri dishes of Potato Dextrose Agar (PDA) media, cultured and identified morphologically. The obtained results were analyzed using analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMTR) with a level of significance at $P < 0.05$. A total of 398 fungal colonies were obtained from the two locations for the period of study. *Aspergillus flavus* (15.3%) followed by *Aspergillus fumigatus* (10.6%) recorded the highest number of fungal colonies while *Nigrospora* (1%), *Fusarium spp.* (2.3%) and *Trichoderma spp.* (1.3%) had the least number of species in all the locations sampled. A number of the fungi isolated are opportunistic and are allergens that cause various diseases, irritations and allergic reactions in humans. Therefore, this study recommends routine aerobiological monitoring in both outdoor and indoor hospital environments for better management of fungal infections.

KEYWORDS: fungi, health, airborne, hospital.

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INTRODUCTION

Fungal spores are easily dispersed in the air because of their size and are aerosolized in the atmosphere throughout the world. The dispersal of airborne fungal spores is connected with health problems and affects the quality of life of affected individuals (Adekunle, 2000; Asan, 2004). They are more abundant during the wet season and less in the dry season (Makut *et al.*, 2014; Odebode, 2017). Fungi are everywhere and can sometimes be life-threatening to the health of people in indoor environments (Samet & Spengler, 2003; Khan, *et al.*, 2009). The major route of exposure is by inhalation or ingestion of these spores. Products of fungi growth such as Microbial volatile organic compounds (MVOC) may be part of the cause and contribute to symptoms of illness or discomfort alone on exposure to fungal biomass (Beezhold *et al.*, 2008). Fungi can grow on almost all organic and man-made materials, most especially if they are hygroscopic or damp. Non-living objects get commonly inhabited because they absorb dust which assists the growth of *Aspergillus species* as they serve as substrates for quick growth. The ubiquitous nature of fungi makes them one of the common isolates in healthcare facilities. Several studies have emphasized the fact that hospital infections are triggered by diverse species of fungi, like *Candida albicans*, *Aspergillus*, *Cladosporium*, and

Penicillium (Faure *et al.*, 2002). Data on fungal infections are not readily available in some parts of the world. The problem is more prevalent in developing countries and also varies across countries, for instance, the occurrence of dermatophytoses in Tunisia is 30.3%, in Brazil is 38.4% and in Iran 21.1% (Hashemi *et al.*, 2009). Fungi reside everywhere including hospitals we envisaged would be germ-free places. Instead, hospitals harbor pathogenic organisms such as fungi. There are an estimated 1 million fungal infections per year in Uganda and the overall fungal disease burden in Africa is not well described (Wordria *et al.*, 2012). Also, in Uganda, not much research has been done in the study of outdoor airborne microorganisms, especially in hospital environments. This study, therefore, investigates the composition of airborne fungi for two months in two hospitals in Kabale, South West, Uganda.

MATERIALS AND METHODS

The study was carried out in two Hospitals, Kabale Regional Referral Hospital and Rugarama Hospital. The bed capacity of Kabale Hospital is quoted at 280 while that of Rugarama hospital is 150 in-patient capacity. They are both within the Kabale district which is located in the Southwestern region of Uganda.

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Fungal Collection

Air samples were collected during June and July 2019, using the open plate method. Sampling locations were chosen to cover the major wards in each hospital. Plastic plates (9 mm) containing Potato dextrose agar with lids open were placed in sampling sites at human height above the floor for 10 min and thereafter covered. The plates were then taken to the laboratory for further work. Samples were collected in triplicates and immediately taken to the Microbiology Laboratory of the Kabale Referral hospital for further observation and studies

Isolation and Identification Of Airborne Fungi

The plates were incubated at 28°C for 2 weeks for proper morphological identification of fungi. Plates were examined daily for any visible fungal growth. Fungal isolates were identified at the genus level according to their microscopic and macroscopic morphological features.

Patient Data Review

Data on the patients were obtained from each hospital. Hospital record books, reports and manuals that contain information related to the topic of the study were gathered.

Data Analysis

Data obtained from all locations and hospital records were analyzed using multiple analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMTR) with the level of significance at $P < 0.05$ (95% confidence interval). Histograms and line graphs were also used for graphical representations

Meteorological data were also obtained from Kabale Meteorological Agency, Uganda to estimate the relationship between weather parameters and the distribution of fungi spores in the environment.

RESULTS

Means with different superscripts are significantly different. Mean separation done with Duncan Multiple Range Test at $P < 0.05$.

Means with different letters across the column are significantly ($p < 0.05$) different from one another for each parameter (One-way Anova).

Results showed that *Aspergillus flavus* (15.5) was the highest fungal spore collected in Kabale Referral hospital compared to Rugarama hospital (15.0). *Aspergillus fumigatus* was also abundant in both hospitals while *Nigrospora spp* was found to be the least observed fungal specie in both hospitals (Table 1).

Means with different superscripts are significantly different. Mean separation done with Duncan Multiple Range Test at $P < 0.05$.

In June, *Aspergillus flavus* (15.0) and *Aspergillus fumigatus* (10.50) had the highest occurrence while *Nigrospora spp* (1.00) and *Alternaria spp* (1.00) had the least occurrence in both hospitals. In July, *Aspergillus flavus* (15.50) had the highest occurrence followed by *Aspergillus fumigatus* and *Yeast* (10.50). *Nigrospora spp* and *P. marneffeii* had the least occurrences (1.0). The research findings indicate that July shows a higher distribution of fungi species than June (Table 2).

A total of 189 colonies were obtained during the study in June. Fungal species isolated include *Aspergillus flavus* (17.9%), *Aspergillus fumigatus* (12.3%), *Aspergillus oryzae* (3.8%), *Penicillium notatum* (10.4%), *Penicillium citrinum* (8.5%), *Rhizopus stolonifer* (6.6%), *Fusarium spp* (6.6%), *Neurospora spp* (2.8%), *Trichoderma spp* (7.5%), *Aspergillus niger* (2%), *Aspergillus sydowii* (6.6%), *Alternaria sp* (2.8%), *Nigrospora* (1%), *Yeast* (1%), *Unidentified species* (12.3%). For Kabale hospital, fungal species isolated include; *Aspergillus flavus* (13.3%), *Aspergillus fumigatus* (9.6%), *Penicillium notatum* (10.8%), *Penicillium citrinum* (10.8%), *Rhizopus*

Table 1: shows the frequencies and species of airborne fungi present in Rugarama and Kabale Referral hospital

Organisms	Rugarama Hospital	Kabale Hospital
<i>Aspergillus flavus</i>	15.00a	15.50a
<i>Aspergillus fumigatus</i>	10.00ab	10.50ab
<i>Aspergillus oryzae</i>	2.00b-e	1.00d
<i>Penicillium notatum</i>	10.00ab	9.00a-d
<i>Penicillium citrinum</i>	9.00abc	9.50ab
<i>Rhizopus stolonifer</i>	5.50b-e	7.00bcd
<i>Fusarium spp</i>	2.50b-e	2.00cd
<i>Neurospora spp</i>	4.50b-e	3.50bcd
<i>Trichoderma spp</i>	1.50cde	1.50cd
<i>Aspergillus niger</i>	6.00b-e	7.00bcd
<i>Aspergillus sydowii</i>	5.00b-e	5.00bcd
<i>Alternaria sp</i>	3.50b-e	3.50bcd
<i>Nigrospora spp</i>	1.00de	1.00d
<i>Yeast</i>	4.50b-e	10.50ab
<i>Neurospora crassa</i>	2.00b-e	3.50bcd
<i>P. marneffeii</i>	0.00e	1.00d
<i>Unidentified colonies</i>	6.50b-e	7.00bcd

Table 2: Monthly distribution of airborne fungi species in Rugarama and Kabale hospitals

Organism	June	July
<i>Aspergillus flavus</i>	15.00a	15.50a
<i>Aspergillus fumigatus</i>	10.50ab	10.50ab
<i>Aspergillus oryzae</i>	2.00b-e	1.00d
<i>Penicillium notatum</i>	10.00abc	9.00a-d
<i>Penicillium citrinum</i>	9.00a-d	9.50abc
<i>Rhizopus stolonifer</i>	5.50b-e	7.00bcd
<i>Fusarium spp</i>	2.50b-e	2.00cd
<i>Neurospora spp</i>	4.50b-e	3.50bcd
<i>Trichoderma spp</i>	1.50cde	1.50cd
<i>Aspergillus niger</i>	6.00b-e	7.00bcd
<i>Aspergillus sydowii</i>	5.00b-e	5.00bcd
<i>Alternaria sp</i>	3.50b-e	3.50bcd
<i>Nigrospora spp</i>	1.00cd	1.00d
<i>Yeast</i>	4.50b-e	10.50ab
<i>Neurospora crassa</i>	2.00b-e	3.50bcd
<i>P. marneffeii</i>	0.00d	1.00d
<i>Unidentified species</i>	4.50b-e	5.00bcd

stronifer (4.8%), *Fusarium spp* (2.4%), *Neurospora spp* (1.2%), *Trichoderma spp* (1.2%), *Aspergillus niger* (6%), *Aspergillus sydowii* (6%), *Alternaria spp* (4.8%), *Nigrospora* (1.2%) Yeast (9.6%), *Neurospora crassa* (4.8%), unidentified species (10.8%) (Figure 1).

A total of 209 colonies was obtained from the study in the two hospitals throughout July. These include- *Aspergillus flavus* (18%), *Aspergillus fumigatus* (10.4%), *Aspergillus oryzae* (2%), *Penicillium notatum* (8.5%), *Penicillium citrinum* (7.5%), *Rhizopus stolonifer* (7.5%), *Fusarium spp* (2%), *Neurospora spp* (5.7%), *Trichoderma spp* (2%), *Aspergillus niger* (6.6%), *Aspergillus sydowii* (3.8%), *Alternaria sp* (2.8%), Yeast (11.3%), *Un identified species* (13.2%). In Kabale hospital, fungi species

obtained include; *Aspergillus flavus* (12.6%), *Aspergillus fumigatus* (9.7%), *Penicillium notatum* (8.5%), *Penicillium citrinum* (10.7%), *Rhizopus stonifer* (5.8%), *Fusarium spp* (2%), *Neurospora spp* (1%), *Trichoderma spp* (1%), *Aspergillus niger* (6.8%), *Aspergillus sydowii* (5.8%), *Alternaria sp* (3.9%), *Nigrospora* (2%) Yeast (8.7%), *Neurospora crassa* (5.8%), *P. mamefei* (2%), unidentified species (9.7%) (Figure 2).

From the figure 3, in June, at Kabale Referral hospital- the OPD department (28.4) had the highest number of colonies, followed by the Paediatrics Ward (26.6), Maternity ward (24.8) and Emergency department (20.2) while in Rugarama Hospital, the OPD department (27.2) had the highest number of colonies, followed by the Paediatrics ward (26.3), Maternity ward (25.4)

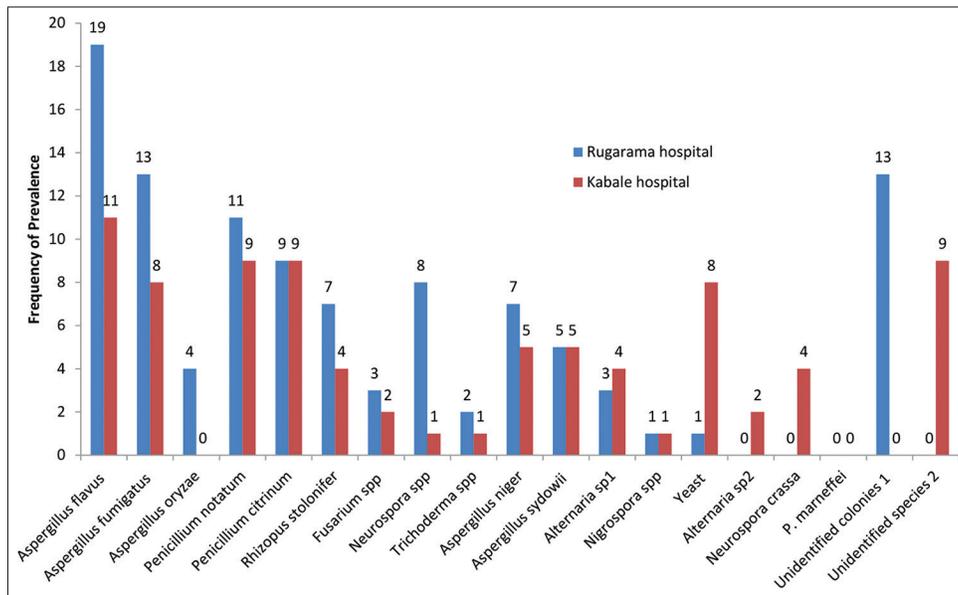


Figure 1: Distribution of fungi species in Kabale Referral hospital and Rugarama hospital in the month of June

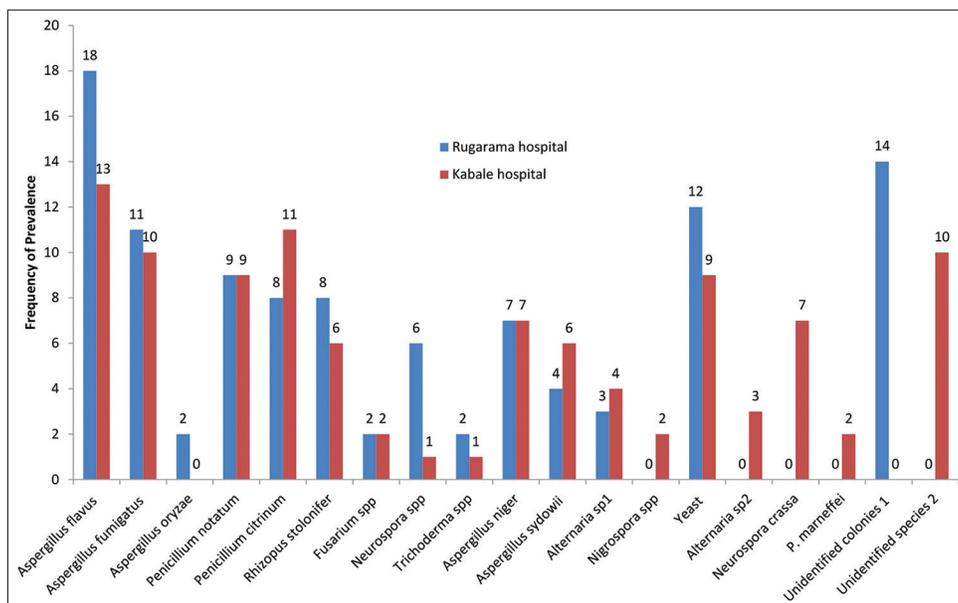


Figure 2: Distribution of fungi species in Kabale Referral and Rugarama hospitals in the month of July

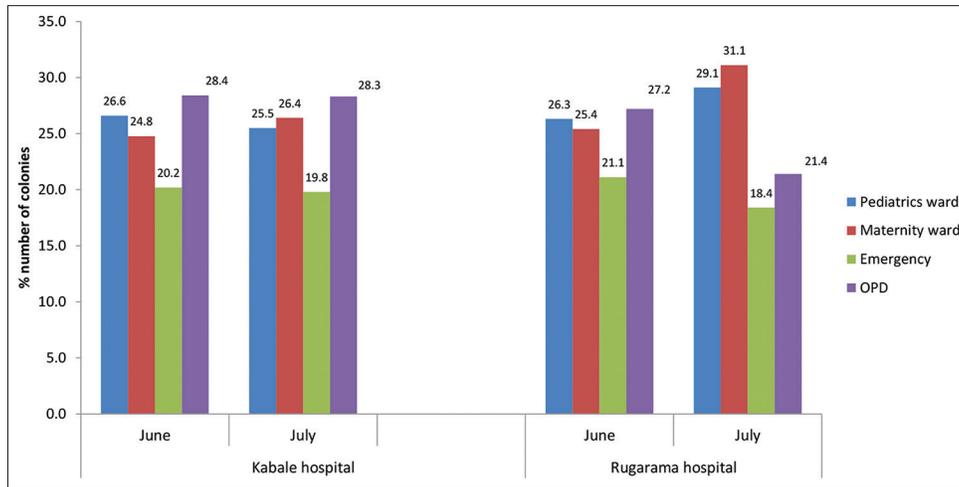


Figure 3: Percentage number of fungi spores recorded in each hospital

and Emergency department (21.1) with the least number of colonies. In July, Kabale Referral hospital OPD department (28.3) had the highest number of colonies, followed by the Maternity ward (26.4), Pediatrics ward (25.5) and Emergency department (21.1) with the least number of colonies. While at Rugarama hospital, the maternity ward (31.1) had the highest number of colonies, followed by the paediatrics ward (29.1), OPD department (21.4) and Emergency department (18.4) had the least number of colonies.

Figure 4 shows that a high number of female patients (23) had asthma cases and the least cases observed in male patients (14) at Kabale Referral hospital in June, and these were distributed as follows; 25 of the patients were aged between 6 to 59, 08 patients were aged 60 years above and the least cases were in those aged below 5 years. While there were no registered fungal infection cases in females and a few cases in males (02) in June, and these were mostly in patients aged between 6 to 59 years (07) and least in patients aged 5 and below (03). In Rugarama hospital, there were many registered fungal cases (10) in females compared to asthma (01) while fungal and asthma cases (03) in males were equal in June, and these were common in the patients aged 6 to 59 years (11) and a few in patients aged 5 and below (02).

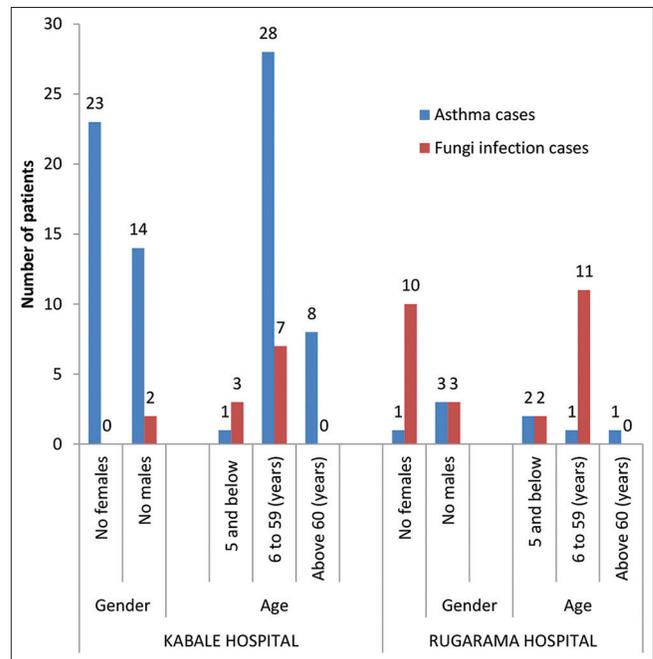


Figure 4: Number of patients with fungal related cases in Kabale and Rugarama hospitals in the month of June

From Figure 5, there were many registered asthma cases in females (23) and a few in males (14) at Kabale Referral hospital, and these were many in patients between 6 to 59 years (28), 5 years and below (03) and above 60 years (02). While there were fewer asthma cases both in males (01) and females (03), these were high in patients aged between 6 to 59 years. In Rugarama hospital in July, there were 08 asthma cases in females and 01 case in males, and there were 04 cases in patients aged 5 and below, 03 cases in patients aged between 6 to 59 years and 02 in patients aged 60 and above, while there were 09 fungal cases in females and 08 fungal cases in males.

The meteorological conditions for the months of June and July 2019 at Kabale as given by the Kabale Meteorological Agency (Figure 6 & 7) indicate that the Dry bulb temperature for these months was 23.5 in June and 23.6 in July. The wind speed was

20.4 in June and 10.9 in July. The Relative Humidity was 98 in June and 97 in July. The rainfall was 62.8mm in June and 107.5mm in July.

DISCUSSION

Fungi cause three major harmful effects on human health, which include inflammatory, allergic and toxin effects. Inflammatory and allergic effects as a result of fungal spores most commonly happen through the airborne channel to humans (Balloy & Chignard, 2009). The major challenge in the hospital environment concerning the spread and influence of fungal infections is that patients are continuously exposed to fungal spores in high quantities in hospital wards (Enoch *et al.*, 2006). It is therefore pertinent to always assess the

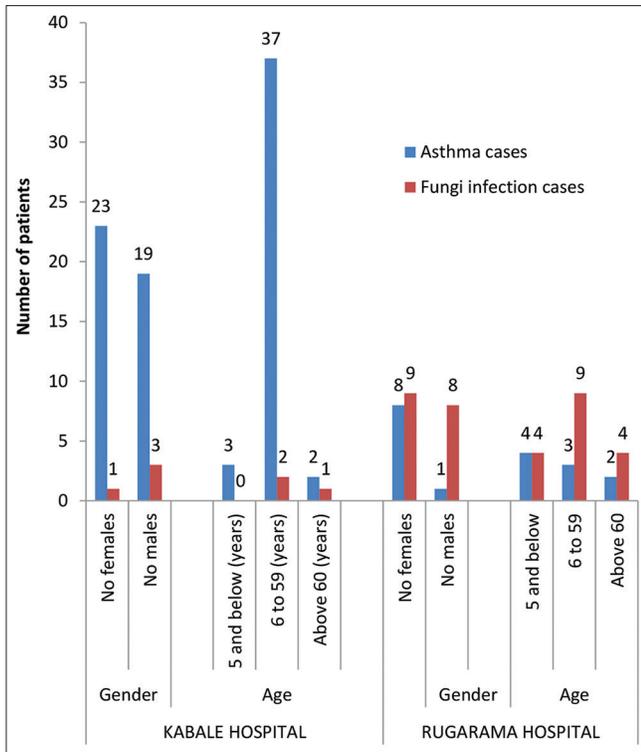


Figure 5: Number of patients with fungal related infections at Kabale and Rugarama hospitals in the month of July

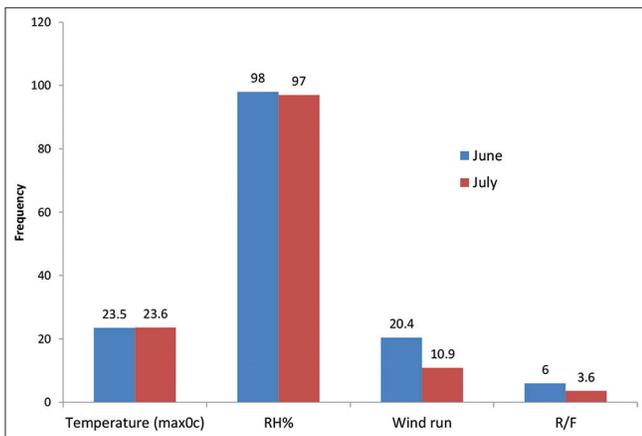


Figure 6: Meteorological Mean values from Kabale Meteorological Agency for the months of June and July

distribution of airborne fungi in hospitals. There is also a need to monitor fungal spores suspended in the air so it can further help in the control of hospital infections. More attention should be focused on these public health issues, including infectious diseases that can occur from inhalation of fungal spores (Chang *et al.*, 2008).

This research work provides information on the distribution of fungi from two different hospitals in Kabale municipality, Southwest Uganda. Earlier research has investigated the composition of airborne fungi spores in indoor and outdoor environments but few studies have been done in Uganda. To the best of our knowledge, this is the first study of fungal

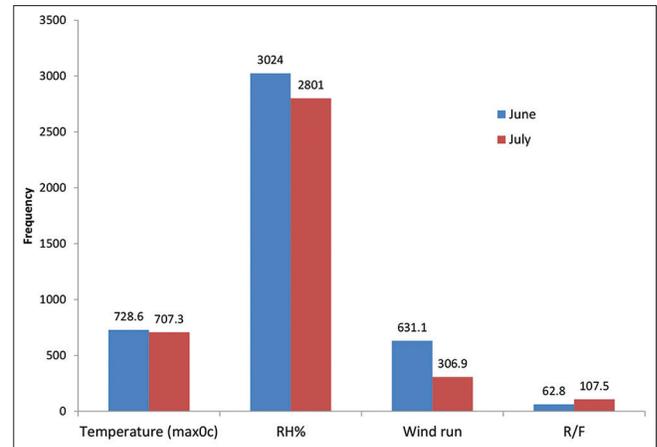


Figure 7: Meteorological Total values Recorded at Kabale weather station

distribution in the hospital environment in the Kabale Municipality atmospheric environment.

In this study, results showed that a total of 398 fungal spores were obtained from the selected locations in the hospital throughout sampling. For both hospitals, during the period of investigation *Aspergillus flavus* had the highest occurrence in all sampled locations. *Nigrospora*, and *Trichoderma spp*, had the least occurrences in all sampled locations. *Aspergillus* and *Penicillium species* had the most occurrences in all the sampled locations which are in agreement with the works of Boyacioglu *et al.* (2007) and Odebode (2017) who also observed a higher number of *Aspergillus* fungal spores during the rainy season. Exposure to spores of fungi from *Aspergillus* and *Fusarium spp*. may result in respiratory infections especially in immunocompromised individuals as opined by Hedayati *et al.* (2007), Jain *et al.* (2010), and Uztan *et al.* (2010) in their findings. Patients who have impaired immune systems and who spend most of their time indoors contaminated by fungal spores may risk fungal infections (Marcoux *et al.*, 2009; Wang *et al.*, 2010). There were more fungi species in Kabale Referral hospital than in Rugarama hospital. This could be attributed to the location and size of the hospital. Kabale Referral hospital has more human personnel working in the place who can be carriers of these fungal spores. This may also be due to the high numbers of patients and visitors received daily in the hospital. This corroborates the works of Lemaire *et al.* (2018) who observed that the principal source of microorganisms in ambient air in hospitals is the patient. Naruka and Gaur (2012) in their work opined that the various activities going on in the hospital (professionals and visitors), and materials that are brought in by people are also sources of fungi in the environment.

The fungal infections present in the two hospitals include asthma and other allergic infection which is common in people aged 6 to 59 years who are normally immune-compromised. Asthma cases were more reported in Kabale Referral hospital than in Rugarama hospital. Also, asthma cases were more in females than males. The higher number of fungal spores recorded in July compared to June may imply that the month of

July was characterized by favourable weather conditions suitable for fungi growth compared to June. Also, the number of fungal infections reported in the hospital was almost equal for both male and female patients, this implies that both males and females at Kabale Referral hospital were affected by the fungal infections especially those aged 6 to 59 years. It implies that these people are more exposed to the risk of a fungi infection and may be mostly immune-compromised.

The outpatient department had the highest number of fungal spores isolated than all other wards which were closely followed by the Maternity ward at Kabale Referral hospital while the emergency department had the least number of fungal spores isolated. This can be attributed to the high number of activities going on at these places every day. For Rugarama hospital, the Maternity ward had the highest number of fungal spores which was followed by the paediatrics section and the emergency ward also had the least number of spores collected.

Information gathered from Kabale Meteorological Agency, showed there was a positive correlation between months with a high number of fungi colonies and months with moderate rainfall and Relative humidity. According to Gravesen (1985), temperature is also a significant factor because airborne fungi especially allergenic types are mainly mesophilic. The abundance of fungi spores in the air during the period of study shows that temperature was favorable for the growth of fungi.

CONCLUSION

Monitoring of airborne fungi is important so as to take steps in reducing fungi load and to further prevent fungi infections. Since fungi infections are a serious threat to public health, environmental monitoring of airborne fungi is therefore required to reduce fungal concentrations in the hospital environment and to avert nosocomial infections. The result of this research work will guide policy experts which can form the basis for action aimed at improving the air and surface quality of the wards sampled. Periodic inspection and constant cleaning alongside restriction of visitors into the various wards may be an effective measure to reduce the load of fungal spores in the hospital environment. Also, good sanitation practices and fumigation should be encouraged in various health facilities and other indoor environments. Factors that are favourable to the growth of fungi should also be abated. Air conditioning systems should be well taken care of and leakages should also be repaired on time so as to prevent fungi growth. There should also be an elimination of the habit of carrying food into and eating from the wards. In summary, Public health practitioners have a huge role to play in educating the general public and creating awareness on the dangers of these microorganisms because they are a part of the air we breathe in and we have no choice but to breathe in air.

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