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Assessment of Airborne Fungi in an Indoors Laboratory Toilet

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Abstract: *Fungi are ubiquitous in both indoors and outdoors environment, exposures to them result to allergies, infection and other adverse health conditions. The assessment of indoor fungi prevalent in six toilets (Biotechnology laboratory toilet 1 & 2, Microbiology laboratory toilet 1 & 2 and Applied Biology laboratory toilet 1 & 2) in Presco Campus, Ebonyi State University Abakaliki, was carried out in the month of November, 2015. Potato dextrose Agar was prepared and poured aseptically into Petri dishes, these were exposed in the toilets for five minutes and incubated for seventy two hours. Pure cultures of isolates were produced and identified, assessment of the hygienic conditions of the toilets was also carried out using questionnaires to elicit responses from those taking care of the toilets. The result revealed eight fungi species; Aspergillus, Trichosporonoides, Gibellula, Beauvaria, Stachybotrys, Illosporium, Botrytis and Rhizoctonia. Aspergillus spp were the most predominant in all the toilet with prevalent rate of 75 % , next to Aspergillus were Gibellula and Illosporium. Microbiology lab. toilet had only Aspergillus and also lowest fungi load. The lowest fungi load in Microbiology lab. toilets was related to frequent washing and moderate ventilation.*

Keywords: *Fungi, indoors, toilet, hygiene, detergents*

I. Introduction

The quality of air in an ambient environment especially in an indoors enclosure affects peoples health. Studies have revealed predominant of fungi in both outdoors and indoors environment at any time. Their ubiquitous and persistent presence predispose them as serious threat to public health especially in an indoor environments, where they are in much closer contact with people (Samet and Spengler, 2003; Khan, 2009). The role of indoor fungi in elicitation of allergies has long been recognized, exposure to fungi may result in serious respiratory infections especially in children and immuno compromised individuals (Boyacioglu *et al*., 2007; Varani *et al*., 2009). Studies have shown that majority of fungi which cause allergies belong to sub division Deuteromycotina (Chaturvedi and Chaturvedi, 2013)

Fungi grow almost everywhere, even as lichens inside Antarctic rocks. They grow over a wide temperature range (-5 to 50 ^{0}C and greater), although individual species usually grow within a much narrower range. (Horner *et al*., 2000). One of the most important physical parameters affecting fungal growth is moisture. Their sporulation are facilitated by dampness, reduced ventilation and high humidity (Ezike *et al*., 2016). Inorganic materials like wall coverings or synthetic paints are usually colonized, because they absorb and serve as favourable growth substrates for *Aspergillus fumigatus* , *Aspergillus versicolor* etc. especially in an indoors environment (Smat and Spengler, 2003).

People spend most of their time in indoors than outdoors environment, however it is the outdoors air that are major sources of the fungi spores whenever outside air is introduced into the indoors enclosure (Ebner, *et al*.,2002). Many indoor locations with high humidity favour high sporulation of fungi, especially the bath room, kitchens and toilet. Toilets are more frequently used among members of the family, they are also more frequently used in schools and public places. They harbor microorganisms because of the different microbiomes which are continually being introduced by different users, coupled with resident population growing therein on wet surfaces and organic substances (ISFHH, 2014). Studies have shown that fungi are introduced either by means of passive ventilation or by means of ventilation systems (Yassin and Almouqatar 2010). Also possible sources of biological contamination of indoor air include: people, organic dust, various materials stored in the buildings, and the air inflowing from the ventilation and air conditioning systems (Kalwasinska *et al*., 2012). Fungi could be introduced into the toilet by an infected individual's faeces and subsequent infections could be aided by improper hygiene after using the toilet. The risks of infection becomes greater when ready-to-eat food such as sandwiches are prepared by someone with contaminated hands (ISFHH, 2014).

The latter part of the 20th century has experienced an increase in the prevalence of fungi infections and allergies. Fungal spores have also been known as one of the important environmental bio-particles causing dermatitis, respiratory infections etc (Tosunoglu *et al.*, 2014; D'amato, 2007). Monitoring of fungi load in

laboratory toilet becomes imperative as they are communal areas of the school which are in constant use throughout the day by both students and lecturers.

II. Materials and Methods

Six toilets in Presco campus Ebonyi State University Abakaliki were selected for this research work, the research was carried out in the month of November, 2015. An inspection of each toilet was carried out prior to the research. A questionnaire was developed and given to those who are taking care of each toilet; this was to access how often the toilets were washed, the type of detergent and disinfectant used in washing the toilets. The toilets selected were; Biotechnology lab. toilets 1 & 2, Microbiology lab. toilets 1 & 2 and Applied biology lab. toilets 1 & 2, these toilets were most frequently used by both staff and students and were assumed to be more cleaner than other toilets in the school.

Sample Collection

Potato dextrose agar (PDA) was prepared and aseptically poured into petri dishes, these were exposed in the six laboratory toilets, two petri dishes were exposed in each toilets by placing them at the height of 18 cm above the ground level in various corners for 5 minutes. After 5 minutes exposure, the petri dishes were sealed with masking tape and labeled. They were incubated for 72 hours for a viable growth to occur, cultures were sub cultured to obtain pure cultures.

Microscopy

From the pure cultures the mycelia were picked with a sterile needle onto a slide and a drop of lactose phenol cotton blue was dropped on it covered with cover slip and viewed with a light microscope at x40 magnification and identified.

III. Result

The six studied laboratory toilets showed presence of fungi. Different colony forming units were found in different toilets. The total number of colony forming unit found in plates group 1, exposed in Biotechnology toilets were 34, Microbiology toilets had 8 and Applied biology toilets had 26 (Table 1). The colony forming units in plates group 2, exposed in Biotechnology toilets, Microbiology toilets and Applied biology toilets were 36, 14 and 13 respectively (Table 2).A total number of eight (8) fungi were identified they were; Aspergillus, Trichosporonoides, Gibellula., Beauvaria, Stachybotrys, Illosporium, Rhizoctonia and Botrytis with prevalence rate of 75 %, 2.8 %, 5.6 %, 2.8 %, 2.8 %, 5.6 %, 2.8 % and 2.8 % respectively (Table 3 and Figure1). In biotechnolgy toilet 1, the identified fungi were those of Aspergillus, Trichosporonoides, Gibellula, Beauvaria and Stachybotrys, Biotechnology toilet 2 Aspergillus spp., Gibellula and Beauvaria spp. were found. In Microbiology toilet 1 and toilet 2, the fungi were dominated by Aspergillus spp. In Applied biology toilet one 1, Aspergillus, Rhizoctonia and Botrytis and Gibellula were identified. In Applied biology toilet 2, the identified fungi were those of Aspergillus spp., Gibellula and Botrytis In all, Aspergillus spp. were more abundant. The highest number of fungi species were found in Biotechnology and Applied biology toilets while Microbiology toilets had the least fungi load (Table 4). In assessing the hygienic level of the toilets, the highest number fungi species emerged in Biotechnology and Applied biology toilets which were washed once a week using detergent and disinfectants, ventilation were moderate and the toilets were temporarily wet with dust particles. Microbiology toilets had only 1 fungi and were usually washed twice a week with detergent and disinfectant with moderate ventilation, wetness and dust particles (Table 5).

Table 1: Culture Plate group 1, exposed in Toilets

Locations	Colour of colonies	No of colony Forming Units.
Biotechnology toilet 1	Black Brown Green	9 1 2
Biotechnology toilet 2	Green	2
	Black	15
Microbiology toilet 1	Black	5
Microbiology toilet 2	Black	3
Applied biology toilet 1	Green	4
	Black	7
Applied biology toilet 2	Black	10
	White	5

Table 2: Culture plate group 2, exposed in Toilets

Locations	Colour of colonies	No of colony Forming Units
Biotechnology toilet 1	Black	15
	White	2
Biotechnology toilet 2	Black	13
	Green	6
Microbiology toilet 1	Black	9
Microbiology toilet 2	Black	5
Applied biology toilet 1	Green	4
	White	1
Applied biology toilet 2	Black	6
	Green	2

Table 3: Percentage of fungi in all the toilets

Fungal Spore Types	Colony forming units	Percentage (%)
Aspergillus sp.	27	75
Trichosporonoides sp.	1	2.8
Gibellula sp.	2	5.6
Beauvaria sp.	1	2.8
Stachybotrytis sp.	1	2.8
Illosporium sp.	2	5.6
Rhizoctonia sp.	1	2.8
Botrytis sp.	1	2.8

Table 4: Fungi present in six laboratory toilets

S/N	FUNGAL SPORES	LOCATIONS											
		BT1	BT2	MB 1	MB 2	AP 1	AP2						
1	*Aspergillus*	+	+	+	+	+	+						
2	*Trichosporonoides*	+	_	_	_	_	_						
3	*Gibellula*	+	+	_	_	_	+						
4	Beauvaria	+	+	_	_	_	_						
5	*Stachybotrys*	+	_	_	_	_	_						
6	*Illosporium*	_	_	_	_	_	_						
7	*Rhizoctonia* sp	_	_	_	_	+	_						
8	*Botrytis*	_	_	_	_	+	+						

BT1- Biotechnology lab toilet 1
BT2- Biotechnology lab toilet 2
MB1- Microbiology lab toilet 1
MB2- Microbiology lab toilet 2
AP 1- Applied Biology lab toilet 1
AP 2- Appled Biology lab toilet 2
+ , positive
-, negative

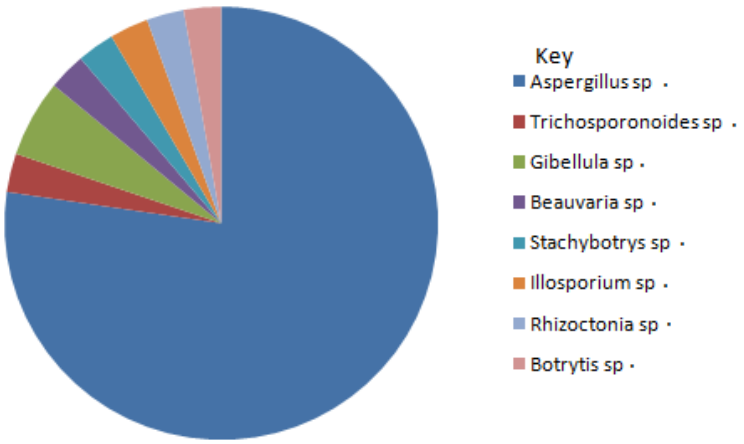

Figure 1: Pie chart showing the percentage of the fungal spores prevalence in all the toilets.

Table 5: Assessment of the Hygienic Level of the Three Laboratory Toilet

Questions	Biotechnology Toilet 1	Biotechnology Toilet 2	Applied Biology Toilet 1	Applied Biology Toilet 2	Microbiology Toilet 1	Microbiology Toilet 2
How often is the toilet wash	Once a Wk ☐ Twice a Wk ☑ Once a Mnth ☐ Twice a Mnth ☐ Everyday ☐ Not at all ☐	Once a Wk ☐ Twice a Wk ☑ Once a Mnth ☐ Twice a Mnth ☐ Everyday ☐ Not at all ☐	Once a wk ☐ Twice a Wk ☑ Once a Mnth ☐ Twice a Mnth ☐ Everyday ☐ Not at all ☐	Once a Wk ☐ Twice a Wk ☑ Once a Mnth ☐ Twice a Mnth ☐ Everyday ☐ Not at all ☐	Once a Wk ☐ Twice a Wk ☑ Once a Wk ☑ Once a Mnth ☐ Everyday ☐ Not at all ☐	Once a Wk ☐ Twice a Wk ☑ Once a Wk ☐ Once a Mnth ☐ Everyday ☐ Not at all ☐
Detergent Used	Klin ☐ Omo ☑ Ariel ☐ Zip ☐ Good mama ☐ Others ☐	Klin ☐ Omo ☑ Ariel ☐ Zip ☐ Good mama ☐ Others ☐	Klin ☑ Omo ☐ Ariel ☐ Zip ☐ Goodmama ☐ Others ☐	Klin ☑ Omo ☐ Ariel ☐ Zip ☐ Goodmama ☐ Others ☐	Klin ☐ Omo ☐ Ariel ☑ Zip ☐ Good mama ☐ Others ☐	Klin ☐ Omo ☐ Ariel ☑ Zip ☐ Good mama ☐ Others ☐
Type of Disinfectant Used	Harpic ☑ Hypo ☐ Dettol ☐ Izal ☐ Others ☐	Harpic ☑ Hypo ☐ Dettol ☐ Izal ☐ Others ☐	Harpic ☐ Hypo ☐ Dettol ☐ Izal ☑ Others ☐	Harpic ☐ Hypo ☑ Dettol ☐ Izal ☐ Others ☐	Harpic ☑ Hypo ☐ Dettol ☐ Izal ☐ Others ☐	Harpic ☑ Hypo ☐ Dettol ☐ Izal ☐ Others ☐
Rate of Ventilation	Well Ventilated ☐ Not Ventilated ☐ Moderate ☑	Well Ventilated ☐ Not Ventilated ☐ Moderate ☑	Well Ventilated ☐ Not Ventilated ☐ Moderate ☑	Well Ventilated ☐ Not Ventilated ☐ Moderate ☑	Well Ventilated ☐ Not Ventilated ☐ Moderate ☑	Well Ventilated ☐ Not Ventilated ☐ Moderate ☑
Wetness	Temporarily Wet ☑ Permanently Wet ☐ Not Wet ☐	Temporarily Wet ☑ Permanently Wet ☐ Not Wet ☐	Temporarily Wet ☑ Permanently Wet ☐ Not Wet ☐	Temporarily Wet ☑ Permanently Wet ☐ Not Wet ☐	Temporarily Wet ☑ Permanently Wet ☐ Not Wet ☐	Temporarily Wet ☑ Permanently Wet ☐ Not Wet ☐
Dusty	Temporarily Dusty ☐ Permanently Dusty ☐ Not Dusty ☐ Moderate ☑	Temporarily Dusty ☐ Permanently Dusty ☐ Not Dusty ☐ Moderate ☑	Temporarily Dusty ☐ Permanently Wet ☐ Not Dusty ☐ Moderate ☑	Temporarily Wet ☐ Permanently Wet ☐ Not Dusty ☐ Moderate ☑	Temporarily Wet ☐ Permanently Wet ☐ Not Dusty ☐ Moderate ☑	Temporarily Wet ☐ Permanently Wet ☐ Not Dusty ☐ Moderate ☑

IV. Discussion

The study affirms that fungi are ubiquitous in toilet and dominated by *Aspergillus*. Some of the fungi found are human pathogens. *Aspergillus* sp., *Botrytis* sp., *Trichosporonoides* sp. and *Stachybotrys* sp. could cause human infections. *Aspergillus* colonize human respiratory tract and also act as a powerful allergen resulting in *Aspergillus* asthma and allergic bronchopulmonary Aspergillosis (Tomee and Vander werf, 2001*). Aspergillus* has been known to cause *Aspergillosis* (chambon – Pautas *et al*., 2001; Larone, 2002; Flavery and Streifel, 2007). *Trichosporonoides* causes low blood pressure (Agranovski, *et al*., and Ristovski *et al*., 2005). Spores and mycelia from *Stachybotrys* sp. have been shown to contain trichothecene mycotoxins (Sorenson *et al*., 1987) and it is reasonable to assume that heavy and prolonged exposure to trichothecene could cause respiratory and dermatological problems. Exposures to *Botrytis* pose a serious health risk to humans and lead to cases of keratomycosis, hay fever and asthma.

In a similar study carried out in residential surfaces, *Cladosporium*, *Cryptococcus*, *Penicillium*, *Candida*, *Malassezia*, *Phoma*, *Exophiala*, *Rhodotorula*, *Wallemia*, and *Fusarium* were identified (Adams *et al*., 2013). The present work found dominance of *Aspergillus* in toilet. *Aspergillus* was also found dominant in indoors concentration in the homes of allergic/asthmatic children in Delhi, India (Sharma *et al*.,2011). Most outdoors sampling seldom reported *Aspergillus* dominant. This could be due to sampling medium used, most studies have reported preponderace of *Aspergillus* in an indoors environment, *Aspergillus* is culturable and grows very prolifically on a PDA media.

Many factors such as moisture level and ventilation system influenced fungi concentration in studied toilet, higher moisture level, favoured prolific growth of fungi. This is in conformity with Hargreaves et al. (2003), who found similar result.. Frequent washing of the toilet with detergent and disinfectant had influence on fungi load. Biotechnology and Applied Biology toilets that were temporarily wet during sampling had the highest number of fungi, water droplets on floors during flushing enhanced the growth of fungi in ambient environment of the toilets. This supports the views of Shaugnessy *et al*. (1999), who stated that microbial growth depends on adequate water supply. All toilet had moderate ventilation , however ventilation was found as one of the key factors that affect particles removal and deposition rate in indoors environment due to air turbulent movement (Jamriska *et al*., 2000; Howard – Reed *et al*., 2003; Wallace *et al*., 2004).

Frequent washing of the toilet with ariel and harpic in Microbiology laboratory toilets seems to be more potent on fungi. Stojanovic *et al.*(2004) found that detergents exhibit significant inhibitory effect on the enzyme activity of some species of fungi. The use of different kinds of detergent; klin, omo, ariel, zip, good mama and disinfectant; harpic, hypo, dettol, izal in the studied toilet would have reduce the flora prevalent in them. Stojanovic *et al*., 2011 also indicated indicated significant changes in bioproduction of amino acids and proteins of *Aspergillus niger* cultivated in the presence of detergent and its component, compared with control experiment. Issa and Ismail (1994) also showed that the number of genera and species of microflora decreased in River Nile water-treated with different doses of detergents.

V. Conclusion

The present research indicated preponderant of *Aspergillus* in laboratory toilets. The abundance of fungi species in Biotechnology and Applied Biology toilets were related to reduced frequency of washing and temporary wetness. Microbiology which had low fungi load were frequently washed with ariel and harpic, other detergents could have also reduced fungi growth, however frequent washing was recognized as a major factor which influence reduction of indoors fungi in the laboratory toilets.

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