Effect of time on the occurrence of fungi species isolated from a University female hostel, eastern Nigeria.

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ABSTRACT

The Fungal air quality of rooms in female hostels in a University setting in eastern Nigeria was investigated in this study. The aim of the study was to assess fungi in the air and number of fungi spores present in the different levels of the hostels. A total of 36 rooms in 4 levels were investigated in this study and samples were collected twice in a week Exposure was done for 15minutes and 30minutes in duplicates and counts were taken after 3-7 days for fungi estimation. Fungi count had a range of 3-17CFU/m^3 for 15 minutes and 6-34 CFU/m^3 for 30 minutes. Fungi isolated were *Aspergillus flavus* (3, 8.3%), *Trichophyton sp* (27,75.0%) *Candida albicans* (20,55.5%), *Aspergillus niger* (14,38.8%), *Auerobasidum pullulans* (13,36.1%), *Mucor sp* (18,50%), *Penicillium sp* (18,50%), *Microsporium audonii* (6,16.6%), *curvularia sp* (11,30.5%), *Paecilomyces sp* (5,13.8%), *Aspergillus versicolor* (6,16.8%), *Epidermophytum sp* (6,16.6%), *Aspergillus ochraceus* (5,13.8%), *Rhizopus sp* (8,22.2%), *Trichoderma sp* (3,8.3%) and *Yeasts* (2,5.5%). *Trichopyton sp*, *Candida albicans*, *Mucor sp*, *Penicillium sp* and *Aspergillus niger* were most frequently recorded in rooms at all levels. This study has showed the presence of medically important fungi; therefore, awareness of proper hygienic practices and maintenance of these hostels should be done regularly.

INTRODUCTION

Indoor air quality is becoming an increasingly important issue for occupational and public health (Dudzinska, 2011). Indoor air quality can be said to be the quality of air in a closed environment such as a room. The quality of indoor air is one of the most significant factors affecting the health and well-being of people who inhale at least 10m of the air every day and spend between 80-95% of their lives indoors (Daccaro *et al*., 2003). The quantity of Fungi in a particular area depends on the presence of water and other nutrient sources in that particular environment where they develop extensively. Usually, Fungi enter into buildings through the doors, windows, air conditioners and also by people entering from outside. The type of species and amount of organisms present depends on viscosity, temperature, lightening and food available in that particular environment (Daccaro *et al*., 2003).

Female hostels has been implicated with some unhygienic practices that influences the proliferation of fungi and these include, eating and spilling crumbs, combing hair within the rooms, hanging of wet wears inside these rooms to avoid theft if outside, etc. This has led to this study.

A Fungus is a heterotrophic and filamentous organism that depends on external sources of organic carbon and its cells are parasitic where they absorb nutrients through cell membranes. The air quality of hostel can be affected by particles and microbial contaminants such as fungi. The presence of this contaminants in the air, specifically fungi and its spores are not a serious issue until it is inhaled by the body. The Fungus that entered the body through the respiratory system could lead to mild health problems or also make certain conditions in immuno-compromised patients much worse. Symptoms such as runny nose, nasal congestion, eye irritation, cough, asthma aggravations, fatigue, headaches and difficulty in concentrating are common when exposed to the fungus. Headaches, pressured on the head and throbbing, feeling of tiredness are the most common signs. However, in some cases, people who inhale these fungal spores are without symptoms.

The main factors helping in growth of and multiplication of both pathogenic and non-pathogenic fungi are temperature, humidity and the unhygienic conditions present in the different areas of

the building (Bornehag *et al.*, 2001). Fungi flora can be hazardous for health, particularly in rooms with heating, ventilation and air conditioning (HVAC) systems (Gutatowska *et al*., 2002 ; Stryjakowska-sekulska *et al.*, 2007) and can breed allergies (La-serna *et al.*, 2002; Stryjakowska-sekulska *et al.*, 2007) SBS symptoms (Sick building syndrome) causing irritation of mucous membrane, bad physical condition, vertigo, decrease of concentration, memory and intellectual work ability. Air quality refers to the condition of the air within our surroundings. Good air quality pertains to the degree to which the air is clean, clear and free from pollutants and bioaerosols, in this context, fungi. Air quality is determined by assessing a variety of pollution indicators. Much time is being spent in numerous different indoor environments by people (Dike *et al*., 2020; Mostafa *et al*. ,2012) reported that people spend their lifetimes up to 80% in workplace or rooms. The targeted objectives were

- To quantify the fungi spores, present in the rooms assessed
- To characterize and identify fungi species present in the rooms assessed
- To determine the spread of fungi according to time of exposure

Fungi are long known to affect the health of humans in many ways. Fungi spores are spread through the air and therefore can be classified as an airborne in such cases. These spores if pathogenic can pose a great threat when inhaled in immune compromised individuals. However, non -pathogenic spores can become opportunistic in healthy individuals. Indoor air quality of hostels in tertiary institutions is of paramount importance as these rooms are usually damp with little sunlight and are also compacted with reduced ventilation. This makes the Fungi Indoor air quality of rooms a very important research, to ascertain the quality of air inside these rooms to avert impending health issues arising from the presence of fungi spores.

METHODOLOGY

SAMPLING AREA: The sampling area included rooms selected randomly from the 4 different levels of the hostels in the university. There are 48 rooms in University Female hostel divided into 4 levels, each level having 12rooms each. Each room has not more than 5 occupants who spend their morning and evenings in these rooms daily.

STERILIZATION

The bench surface was sterilized with 70% ethanol and cotton wool, while glass wares were sterilized by washing with detergent and rinsing thoroughly in water. This was then dried in a hot air oven at 160^{o}C for 2 hours (Cappuccino & Sherman, 2014).

MEDIA PREPARATION

The media used for this study was Sabouraud dextrose agar (SDA). It was weighed according to the manufacturer's instruction. All samples were cultured on freshly prepared Sabouraud dextrose agar medium seeded with chloramphenicol. This seeding was done to prevent bacteria growth. (Cappuccino & Sherman, 2014).

AUTOCLAVING

The media was covered and autoclaved at 121^{o}C for 15 minutes and allowed the molten medium to cool for 45-50^{o}C at a pressure of 15 PSI. (Cappuccino & Sherman, 2014).

STERILITY TEST

Freshly prepared plates were incubated at 27^{o}C for 24hours and observed for growth. Plates that showed no observable growth were marked sterile and used for the analysis. (Cappuccino & Sherman, 2014).

2.6 SAMPLE COLLECTION

Fungi indoor air quality was investigated in the selected female rooms specified. The methods of Pasquarella *et al.,(*2002) was adopted with slight modifications. The culture medium was positioned at the middle of the room, 0.5m above the floor. Plates were exposed in the evenings and mornings for 15mins and 30 mins respectively per room. Samples were collected twice in a week.

INCUBATION

After exposure and collection, the plates were taken to the laboratory and incubated at 27^{o}C for 3-5days.

FUNGAL COUNT

After incubation, the total number of yeast and moulds collected from different sites were determined. The total number of colony forming unit (CFU) was done by direct counting of growth formed per plate.

ISOLATION OF PURE CULTURES

Sub-culture was done to isolate individual fungal in their pure form on freshly prepared SDA. These were incubated for 3-5 days (Larone *et al.*, 2016).

FUNGI CHARACTERISATION IDENTIFICATION

The fungi were identified based on their microscopic and macroscopic characteristics as described by Larone *et al*., (2016). Macroscopy was done based on their morphological features on the SDA plates while Microscopy was done based on direct slide preparations stained with lactophenol cotton blue.

STATISTICAL ANALYSIS

This was done using Microsoft excel 2010 package. Results obtained were analysed using descriptive statistics and expressed as tables.

RESULTS:

Results showed that fungi counts where higher when exposed for longer periods. Hence, when plates were exposed for 30mins, fungi present were higher than when plates were exposed for 15minutes (Table 1). The morphological characteristics of the fungi isolated based on their macroscopic and microscopic features showed that the fungi present were *Aspergillus flavus, Trichophyton sp, Candida albicans, Aspergillus niger, Auerobasidum pullulans, Mucor sp, Candida spp, Penicillium notatum, Penicillium chrysogenum, Microsporium audonii, curvularia spp, Paecilomyces spp, Aspergillus versicolor, Epidermophytum sp, Aspergillus ochraceus, Rhizopus sp, Trichoderma sp and Yeasts. (*Table 2). Percentage occurrence showed that *Trichophyton sp* had the highest occurrence of (27, 75.0%), this was followed by *Candida albicans* (20, 55.5%) while the least was *Yeasts* (2, 5.5%) from various levels in the female hostel. (Table 3). The distribution of the isolates according to their occurrence level was also investigated. In level 1, *Trichophyton (*10, 83.3%), had the highest occurrence, this was followed

by *Mucor species* (9,75%), while the least were *Yeasts* and *Trichoderma species* (1,8.3%). In level 2, *Trichophyton* (10,83.3%) had the highest occurrence, this was followed by *Candida albicans* (6,50%) and *penicillium notatum* (6,50%), *A. flavus* and *Yeasts* were however not present. In the level 3, *Trichophyton* (5, 83.3%) had the highest occurrence, this was followed by *Candida albicans* (4,66.6%), *Yeasts* was not present in this floor. However, in level 4, *Trichophyton* (4,66.6%) had the highest occurrence, Followed by *Candida albicans* (3, 50%) and *Aspergillus niger* (3,50%), *A. ochraceus* and *Trichoderma* did not occur in this floor.(Table 4).

TABLE 1: FUNGI COUNT IN CFU (COLONY FORMING UNITS) FOR THE ROOMS SAMPLED

LEVELS	ROOMS SAMPLED	EXPOSURE AT 15MINS (CFU/Mins/plate)	EXPOSURE AT 30MINS (CFU/Mins/plate)
Level 1	201	9	12
	202	10	17
	203	14	15
	204	5	11
	205	6	8
	206	8	11
	207	7	11
	208	5	12
	209	7	9
	210	5	11
	211	4	6
	212	4	11
Level 2	301	3	12
	302	10	10
	303	11	13
	304	14	30
	305	7	34
	306	15	16
	307	12	20
	308	13	24
	309	14	17
	310	17	18
	311	11	23
	312	5	13
Level 3	401	8	15
	402	14	19
	403	8	15
	404	9	17

	405	11	21
	406	12	30
Level 4	501	17	21
	502	15	19
	503	10	21
	504	14	23
	505	12	10
	506	13	16

Source: data represents the different levels in the hostel, the rooms per level and the number of colonies present per exposure

Key: CFU- Colony forming units
MINS- minutes

TABLE 2: CHARACTERIZATION AND IDENTIFICATION OF THE FUNGI ISOLATED

ISOLATE	MACROSCOPY	MICROSCOPY	SUSPECTED ORGANISM
A	Powdery radial dark brown with white edges growth. Reverse is colourless.	Non septate hyphae, condiospores scattered round, spores colour is brown	*Aspergillus niger*
B	Cottony, fluffy white growth. Reverse is white	Nonseptate hyphae, Sporangiospores, spore colour is brown	*Mucor sp*
C	Suede likeradial light green with white edges. Reverse is yellow.	Septate hyphae, condiospores shooting upwards out. Spore colour is bluish green.	*Aspergillus versicolor*
D	Powdery Concentric to radial blush with white edges. Reverse is white to cream.	Septate hyphae with condiospores arranged in rows of 8 on phalides.Spore colour is light green to colourless	*Penicillium chrysogenum*
E	Velvety flat yellow to dull brown growth. Reverse is colourless	Septate hyphae with condiospores pointing upwards. Spore colour is bluish.	*Aspergillus ochraceus*
F	Wooly, fluffy white then grey later growth. Reverse is pale brown.	Non septate hyphae, unbranched sporangiospores, stolons. Spore colour is blue.	*Rhizopus sp*
G	Pasty, smoothy bulging cream coloured growth. Reverse is reddish brown.	Septate hyphae with spherical cells	*Candida sp*
H	Pasty smooth bulging cream coloured growth. Reverse is white.	Septate hyphae with spherical short pseudophyphae not conscripted.	*Candida albicans*
I	Lumpy folded at the centre, olive grey to	Septate hyphae with macroconidia containi9ng 2 to 6	*Epidermophyton sp*

	khaki growth. Reverse side is brownish with thin yellow border.	cells.	
J	Velvety flat orange green growth. Reverse side is pinkish yellow to pale brown.	Septate hyphae with longer phalides, condiophores are oblong.	*Paecilomyces* sp
K	Velvety concentric olive gray growth. Reverse is black.	Sepatate hyphae with oval conidia. Spore colour is colourless.	*Curvularia* sp
L	Suede concentric dark green growth. Reverse is off white.	Septate hyphae with condiospores arranged in rows on phalides. Spore colour is light green.	*Penicillium notatum*
M	White colony, occasionally pale pink. Brown or black growth appears when chlamydoconidia develops with a white fringe. Reverse is dark.	Budding cells yeast like cells, presence of blastoconidia that are hyaline and oval.	*Aureobasidium pullulans*
N	White fluffy, compact and wolly growth. Reverse is colourless or light orangery tan to yellow.	Septate hyphae. Condiospores are short and often b ranched at wide angles.	*Trichoderma sp*
O	White colonies, waxy or slightly downy; heaped or folded. Reverse is colourless or pale yellowish orange to tan.	Septate hyphae. Chlamydoconidia are numerous. Microconidia and macroconidia are present.	*Trichophyton sp*

Source: microscopic and microscopic identification of the fungi species in the microbiology laboratory. Key: Sp- Specie; SPP- species

TABLE 3: PERCENTAGE OCCURRENCE OF THE FUNGI ISOLATES

ISOLATES	OCCURRENCE	%
Candida albicans	20	55.5
Aspergillus flavus	3	8.3
Trichophyton sp	27	75.0
Aspergillus niger	14	38.8
Auerobasidium pullalans	13	36.1
Mucor	18	50
Penicillium sp	18	50
Microsporium audonii	6	16.6
Curvularia sp	11	30.5
Paecilomyces sp	5	13.8
Microsporium	6	16.6
Aspergillus versicolor	6	16.6
Epidermophytum sp	6	16.6
Aspergillus ochraceus	5	13.8
Rhizopus sp	8	22.2
Yeast sp	2	5.5
Trichoderma sp	3	8.3

Source: the percentage of occurrence = number of occurrence per isolate / total number of occurrences x 100.
Key: sp- species
%- percentage

TABLE 4: DISTRIBUTION OF THE INDIVIDUAL ISOLATES IN THE DIFFERENT HOSTEL LEVELS

ISOLATE	LEVEL1 (n-12)	LEVEL 2 (n=12)	LEVEL 3 (n=6)	LEVEL 4 (n=6)
Candida albicans	7(58.3)	6(50)	4(66.6)	3(50)
Aspergillus flavus	2(16.6)	-	-	1(16.6)
Trichophyton sp	10(83.3)	8(66.6)	5(83.3)	4(66.6)
Aspergillus niger	4(33.3)	4(33.3)	3(50)	3(50)
Auerobasidium pullulans	6(50)	4(33.3)	2(33.3)	1(16.6)
Mucor	9(75)	5(41.6)	3(50)	1(16.6)
Penicillium	7(58.3)	6(50)	3(50)	2(33.3)
Microsporium audnii	3(25)	1(8.3)	1(16.6)	1(16.6)
Curvilaria spp	5(41.6)	3(25)	2(33.3)	1(16.6)
Paecilomyces sp	2(16.6)	1(8.3)	1(16.6)	1(16.6)
Aspergillus versicolor	2(16.6)	2(16.6)	1(16.6)	1(16.6)
Epidermophyton spp	3(25)	1(8.3)	-	2(33.3)
Aspergillus ochraceus	3(25)	1(8.3)	1(16.6)	-
Rhizopus sp	2(16.6)	2(16.6)	2(33.3)	2(33.3)
Yeast spp	1(8.3)	-	-	1(16.6)
Trichoderma sp	1(8.3)	1(8.3)	1(16.6)	-

Source: Number of occurrence per specie/N x100
Key: n = total no of rooms per floor. Numbers in parenthesis represent percentage of occurrence per level.
sp: species
%: percentage

DISCUSSION

The Fungal Indoor air quality of rooms in University hostels is one of the most vital investigations. The information on the indoor concentration of fungi is necessary both to estimate health hazard and to create standards for indoor air quality control. The quality of indoor air is one of the most significant factors affecting the health and well-being of people who inhale at least 10m of the air every day, (Dacarro *et al*., 2003).

The concentration of fungi in the indoor air quality of University female hostels, estimated with the use of settle plate method, ranged between 3-17CFU/m^3 for 15 minutes and 6-34 for 30 minutes (Table 3). According to current Swedish requirements the number of 500 colony-forming units (cfu) of bacteria and 300 cfu of fungal spores in 1 m3 can be accepted in an indoor environment (Abel *et al*., 2002). Results shown in The Netherlands Research Methods in Biological Indoor Air Pollution in 1989 described the amount of fungi over 104 cfu/m3 or the amount of particular species of mould over 500 cfu/m3 as dangerous for health. In 2001 the American Industrial hygiene Association (AIhA) published a proposition of guidelines for the amount of fungal spores in different indoor environments, for example residential and commercial buildings. Guideline for residential buildings are less than 500 cfu/m3 and for commercial buildings are less than 250 cfu/m^3 . other countries' requirements are similar. In Brazil total amount of airborne microorganisms (especially fungi) in enclosed space shouldn't exceed 750 cfu/m^3,(De aquino neto & De Góes siqueira, 2000). Universally applicable standards defining an acceptable level of indoor air contamination with microorganisms have not yet been established.(Samuel &Abayneh, 2014).These above values has shown that our findings were within the acceptable range mentioned.

Fungi counts done showed that counts where higher when exposed for longer periods. Hence, 30mins of plate exposure had higher fungi counts than when plates were exposed for 15minutes, this could imply that the longer individuals are exposed in these rooms, there is a tendency of inhaling these spores which may or may not be detrimental to health especially in the immunocompromised.

The morphological characteristics of the fungi isolated were done based on their macroscopic and microscopic features. The fungi isolated from these rooms were *Aspergillus flavus,*

Trichophyton sp, Candida albicans, Aspergillus niger, Auerobasidum pullulans, Mucor sp, Penicillium notatum, Penicillium chrysogenum, Microsporium audonii, curvularia spp, Paecilomycesspp, Aspergillus versicolor, Epidermophytum, Aspergillus ochraceus, Rhizopus sp, Trichoderma sp and Yeasts. These outcomes are in full agreement with Enitan *et al*., (2017) except for *Alternaria sp.*

Presence of *Aspergillus species* and *penicillium species* were reported by other researchers (Mostafa *et al*., 2012; Shelton, 2002). *Aspergillus species* can cause invasive Aspergillosis and produce mycotoxins which are known to be carcinogens (Augustowaka *et al*., 2006). *Aspergillus* species are moulds found in organic matter transmissible via inhalation (Alwakeel ,2007). Large amounts of *Aspergillus* were found in the indoor air because that fungal genius is abiquitous (Gniadek and Macaura, 2007). It can cause a broad spectrum of disease in humans, ranging from hypersensitivity reactions to direct angioinvasion (Alwakeel, 2007). Fungi in these and other genera affect humans in complex ways and are capable of causing a variety of diseases such as infection, allergy and irritation and toxicosis.

A high occurrence of *Candida species* was found. *Candida species* are yeasts that are widely distributed in the environment and are members of the normal microbial flora in the skin, mouth, vaginal tract and gastrointestinal tract of the human body. (Larone *et al*., 2016) Therefore, it is often present in stools without significance. Infection with the yeast *Candida* is the most frequent cause of fungal disease. *Candida species* is the most common cause of Candidiasis which is an acute or chronic infection involving any part of the body. This agrees with Enitan *et al*.,(2019) that notwithstanding the session of the day, the indoor environment seems to allow bioaerosol to build up and this could serve as possible risk factors for the quick spread of infections among female students. It also agrees with Andualem *et al*., (2019) that attention should be given to controlling any physical factor which will favour growth and multiplying of fungi within the indoor environment of the rooms with the aim of safeguarding the health of students. (Dike & Wekhe, 2020).

Penicillium species are known to cause corneal, cutaneous, external ear, respiratory and urinary tract infections as well as endocarditis after insertion of valve prostheses. Many strains of *Penicillium* produce toxins. (Larone *et al*., 2016).

Epidermophyton species is a dermatitis that produces infection in skin and nails. Trichophyton is a dermatitis that causes flavus, a severe, chronic, scarring scalp infection that results in permanent hair loss, sometimes infects the skin and nails (Dike & Wekhe, 2020).

Rhizopus species are the most common ethiologic agents of zygomycosis (Larone *et al*., 2016). *Paecilomyces species* are increasingly associated with disease especially sinusitis and eye infections. They have been reported to occasionally cause endocarditis, nephritis, nail, cutaneous and subcutaneous infection (Larone *et al*., 2016). *Aureobasidium pullulans* cause corneal, peritoneal, cutaneous, pulmonary and systematic infections (Larone *et al*., 2016). *Trichophyton* had the highest occurrence of 27(75.0%) in the fungal indoor air quality of rooms in University Female hostels (Table 3). This could be because it a dermatophyte and could be transmitted through sharing of towels, students sitting on other student's bed, making use of the same pillow and bed sheet, body to body contact. It causes eczema and this is common among females. This was followed by *Candida albicans* 20(55.5%) which is a normal microbial flora in the skin, mouth, vaginal tract and gastrointestinal tract and also common among youths. The least that occurred was *Yeasts* 2(5.5%).

The presence of many biological agents in indoor environments is attributable to dampness and inadequate ventilation. Excess moisture on indoor materials can lead to growth of fungi too which subsequently emit spores into the indoor air (NYCDHM,2008).

CONCLUSION

According to the results shown, the presence of medically important fungi in the University female hostel was observed. The main fungi pathogens isolated from air samples were *Trichophyton sp, Candida sp, Penicillium chrysogenum, Mucor* and *Aspergillus niger*. Therefore further studies will be needed to help check the various routes of entry that increase fungi growth among University female hostels.

ETHICAL DECLARATION

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article"

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