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RESEARCH ARTICLE

ASSESSMENT OF AIRBORNE FUNGAL SPORES IN INDOOR ENVIRONMENT OF LIBRARIES IN NNAMDI AZIKIWE UNIVERSITY

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Abstract

Background: Polluted indoor environments pose health challenges such as allergy, infections, and toxicity. Most indoor air pollution comes from hazardous non-biological and biological agents. Due to the nature of the indoor environment of libraries, it is prone to colonization by fungal species.

Method: Three sampling sites were used for the study and they include; Festus Aghagbo Nwako Library, Main campus Awka, Medical Library, Nnewi Campus and Library Complex, Agulu campus. A total of 100 air samples were analyzed Using the Zefon A6 Single-stage microbial air sampler and Malt Extract Agar supplemented with 0.05mg/ml of chloramphenicol while 16 nasal swabs were collected from the staff present using sterile swab sticks. The mould isolates were identified using the slide culture technique while the yeast isolates were subjected to candida chrom agar and integral yeast plus identification. Antifungal susceptibility was performed using the integral yeast plus system and the agar well diffusion technique.

Results: Out of the 100 air samples, a total of 625 fungal isolates were identified of which *C.lunata*; 201 (32.16%) was the most predominant, while *P. marneffi, P. expansum, A. restrictus, A. infectoria* and *R. rubra*; 1(0.16%) occurred the least. All significant at ($p \leq 0.01$). A total of 7 fungal spores were isolated from the 16 nasal swabs and appeared thus in descending order of frequency: *P. notatum*, 3 (42.85%), *A. niger, C. lunata, C. albicans* and *F. aqueductum*, 1(14.3%). Antifungal Susceptibility of the 28 yeast isolates indicated that *C. famata, C. laurentii* and *C. luteolus*, were all susceptible to commonly used antifungals in the integral yeast plus system with a 100% susceptibility value, while the mould isolates showed relatively moderate susceptibility to selected antifungals.

Conclusion: The organisms isolated are well known to be pathogenic especially to immunocompromised individuals. Their presence in the indoor environment of libraries serves as a risk factor to both the library staff and visitors. Adequate precautionary measures and occasional environmental surveillance need to be inculcated in order to reduce the number of fungi in the indoor environment of these libraries.

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Introduction:-

Air quality of indoor environments is one of the main factors affecting health, wellbeing and productivity of people. Indoor air quality is affected by the presence of microorganisms which include bacteria, moulds and viruses (World Health Organization, 2009). People spend 80%- 90% of their time in indoor environments either working or resting. At rest an adult breathes an average of 14 m^3 of air per day (Brochu et al., 2006).

Fungi are a heterogeneous group of eukaryotic microorganisms acting as saprobes or parasites or less frequently as symbionts that lives in association with other organisms. Some of them possess the ability to cause superficial, cutaneous, subcutaneous, systemic and allergic diseases. They are found in all types of ecosystems carrying out various functions such as breaking down organic substances into simpler molecules easier to assimilate, producing substances toxic to other organisms without causing any harm. However, depending on the degree of infestation and the exposure of the immunological system, they may cause serious illnesses (Ferdinandus et al., 2001). Fungi can exist as yeast or mould or in both form (dimorphic). Yeasts are microscopic fungi consisting single cells that bud, an example is the Candida species. Mould in contrast, occurs in long filaments known as hyphae which grow by apical extension. Example of mould is the Mucor, Penicillium, and Rhizopus. Dimorphic fungi grow as yeast or spherules in vivo as well as in vitro at 37 ^{o}c but as molds at 25^{o}c (Hawksworth et al., 2017).

Libraries are the true backbone of research academy and well-organized collection of information resources in the form of books, periodicals, newspapers, films, recorded music that are made accessible to defined community for reference or borrowing (Kayarkar and Bhajbhuje, 2014). These materials are mostly cellulosic and rarely non-cellulosic in origin, while the binding gum used in books may be organic or synthetic(Verma et al., 2013). The materials allow the growth of mould when they are damaged by flooding or dampness, thereby contaminating the indoor environment, which may be unhygienic affecting the health of library visitors and library staff (Prester, 2011). Majority of fungi need a high relative humidity and temperature to grow and develop, its development is enhanced in microclimatic environments caused by condensation, but some fungal species are able to live at low water activities for that are classified as xerophilic fungi. These fungi are perfectly adapted to indoor environments and thrive in dusty environments, lack of ventilation or water retention by hygroscopic materials (Khan and Karuppayil, 2012). They can be found in the indoor air of archives, libraries and museums where large number of papers is found. Dust is a good source for these fungi to feed and grow; this condition intensifies fungal contamination (Borregoet al., 2010). Fungal degradation and deterioration of documentary materials is a worldwide problem that causes great damage, especially paper documents stored in the archives, libraries and museums (Mesquitaet al., 2009). The ability to do these is due to the production of extracellular enzymes such as cellulase, xylanase, pectinase, e.t.c., which possess hydrolytic ability. Also, they spoil valuable documents mechanically, chemically and aesthetically because they form hyphae, produce and excrete pigments and organic acids (Micheluz. et al., 2015).

The activity of people and equipment within the indoor environments is thought to be the principal factor contributing to the buildup and spread of airborne microbial contamination (Hospodsky et al.,2012). Particular activities like talking, sneezing, coughing and walking can generate airborne biological particulate matter. House plants, flower pots, house dust, textiles, carpets, wood material and furniture stuffing, occasionally release various fungal spores into the air (Kalogerakis et al., 2005). Moreover, environmental factors such as temperature, humidity, air exchange rate, air movement, building structures and location, poor design, ventilation system as well as interior or redesign may also enhance microorganism's growth and multiplication in the indoor atmosphere (Meadow et al., 2014).

A review made by WHO on the number of epidemiological studies showed that, there is sufficient evidence for an association between indoor dampness-related factors and a wide range of effects on respiratory health, including asthma development, asthma exacerbation, current asthma, respiratory infections, upper respiratory tract symptoms, cough, wheeze and dyspnea (World Health Organization, 2009). Thus, microbiological air quality is an important criterion that must be taken into account when indoorworkplaces are designed to provide a safe environment. The high moisture content and moderate temperature in indoor environment of library are conductive for the growth of microbes and accelerate the deterioration process that affect the physical and chemical properties of library collection (Kayarkar and Bhajbhuje, 2014). Fungi frequently isolated from indoor environment of libraries include species belonging to the genera *Aspergillus,Penicilium, Trichoderma, Alternaria, Mucor, curvuleria,*

*cladosporium*and *Rhizopus*. Airborne fungal contaminants are able to provoke infections such as childhood asthma, allergies and mycotoxicity (Aimanianda et al., 2010). Their increased awareness among human population has made the study of fungal airspora essential and hence the aeromycology has acquired a prominent position in various fields of environmental sciences.Exposure to fungi has been reported to cause several types of human health problems, primarily irritations, infections, allergies, and toxic effects, and it has been suggested that toxigenic fungi are the cause of additional adverse health effects (Shelton et al., 2002). However, several investigations have been carried out on indoor environmental microfungal organisms in many different parts of the globe due to their relationship with plants, animals and human. Since diverse fungal species constituting the major components of aeromycoflora, are major causes of respiratory ailment of human beings as well as important agents of degradation of cellulosic and non-cellulosic material in indoor closed environment, there is a great need for understanding, aerobiological studies from indoor environment for the various libraries in Nnamdi Azikiwe University. Presently, prevalence of aeromycoflora has so far not been reported earlier from these places, it was therefore worthwhile to undertake a study of the diversity of aeromycoflora of indoor environment of these various libraries.

Methods:-

The study was designed to determine and identify airborne fungi present in the indoor environment of libraries in various campuses of Nnamdi Azikiwe University.The main libraries within the 3 campuses of Nnamdi Azikiwe University were sampled.

1. Festus Aghagbo Nwako library, Main campus Awka
2. Library Complex, Faculty of Pharmaceutical Sciences, Agulu Campus
3. Medical Library, College of Health Sciences, Okofia Nnewi Campus

As well as the air samples, nasal swabs were also collected from the librarian present in each of the library sampled.

The air samples were collected using Malt extract agar supplemented with chloramphenicol at 0.05mg/ml and Zefon single stage microbial air sampler. The microbial air sampler was operated at an air flow rate of 28.3LPM (litres of air per minute). The sampling time was about 5-10 minutes according to environmental situation of the measurement condition to avoid drying of the agar surface and over loading of the plate. The plate sampler was set up at a high representative of the normal human breathing zone, i.e, 1.5m above floor level. The samples were collected in the morning immediately after cleaning. Hands were cleaned with sanitizer, the springs of the impactor lid unhooked and the inlet cone and the jet classification stage were removed exposing the base. The malt extract agar plate was removed and placed on the base allowing the bottom to rest on the three tines of the base. Next, the lid of the agar plate was removed and placed on a clean surface, sterile side down. The jet classification stage and the inlet cone were immediately placed on to the base making sure the o-rings make a seal at each connection. The device was secured with the three spring clamps and visually checked to be sure of a good seal. The impactor was placed on a level surface and the vacuum pump turned on for the appropriate amount of time. After collection, the pump was turned off and the three clamps unhooked. The agar plate cover was quickly replaced and the agar plate removed from the base. The surface of the agar plate was examined for evidence of impaction. After collection of the air samples, the impaction stage was cleaned with cotton wool soaked in 70% alcohol before the next sample collection. The inoculated plates were sealed with a masking tape to prevent contamination, and incubated at room temperature for 4-14 days. The culture plates were observed daily for yeast growth and from the 4^{th} day for mould growth. After incubation, the colony count was performed on each media plate to obtain the number of colonies.Macroscopically, established mould and yeast colonies were evaluated for the following characteristics

Surface view:

1. Colour
2. Texture
3. Colonial topography

Reverse view:

The plates were checked for

1. Diffusible pigments
2. The margin of the colony was also noted

Slide culture technique and subsequently lacto phenol cotton blue wet mount was performed on the colonies to identify them microscopically. Yeast colonies were further inoculated on 'candida CHROMagar^{TM}' by

DirectStreaking and Incubated for 36-48h at 37°C under aerobic conditions to identify the *Candida* species among them. Carbohydrate Fermentation test was subsequently performed on the yeast isolates using the 'Integral system yeasts plus' in a bid to further type them down.

Yeast isolates were subjected to antifungal sensitivity analysis using the Rapid Yeast plus Test Kit. The tests were evaluated according to growth or inhibition of yeasts in media containing antimycotics; in wells 14-NY to 23-FLU. The antimycotic drugs used include; Nystatin 1.25 µg/mL, Amphotericin B 2 µg/mL, Flucytosine 16 µg/mL, Econazole 2 µg/mL, Ketoconazole 0.5 µg/mL, Clotrimazole 1 µg/mL, Miconazole 2 µg/mL, Itraconazole 2 µg/mL, Voriconazole 2 µg/mL, Fluconazole 64 µg/mL.

Antifungal Susceptibility of mould isolates was carried out using the agar well diffusion method. The antifungal agent's amphotericin B (AMB), Ketoconazole (KTC), Nyastatin (NY) and Griseofulvin (GRI) were used in their commercial presentation to prepare a stock solution adjusted to the concentration of 1.25 mg ml $^{-1}$ (25 µg per well). Each agent was dissolved in its corresponding solvent.

Antifungal drugs	Solvents	Diluents
Nystatin	Dimethylsulphoxide(DMSO)	Sterile Saline solution
Griseofulvin	Dimethylsulphoxide(DMSO)	Sterile Saline solution
Ketoconazole	Dimethylsulphoxide(DMSO)	Sterile Saline solution
Amphotericin B	Dimethylsulphoxide (DMSO)	Sterile Saline solution

The well diffusion test was performed using Potato Dextrose agar. The inoculum used was prepared using moulds from a 4-day culture on Sabouraud dextrose agar. A suspension was made in a sterile saline solution (0.85%). The turbidity of the suspension was adjusted with a spectrophotometer at 530 nm to obtain a final concentration to match that of a 0.5 McFarland standard (0.5–2.5 × 10). 20 ml of Potato Dextrose agar was melted, cooled to 55 °C and then inoculated with 1 ml of the organism suspension. The inoculated agar was poured into a sterile petri dish, and allowed to cool down on a leveled surface. Once the medium had solidified, four wells, each 4 mm in diameter, was cut out of the agar 20µl of the antifungal agent was placed into each well. Then the plates were incubated at 25 °C for about 3 days.

Statistical Analysis:
Data obtained was subjected to Statistical analysis using the Statistical package for the Social Sciences (SPSS) version 21 and statistically significant levelsweredetermined.

Results:-

100 air samples collected from the three libraries in the various campuses of Nnamdi Azikiwe University alongside 16 nasal swab specimens collected from the staff present were analysed to determine the presence of Airborne fungal spores.

The frequency of fungal isolates based on the various libraries is shown in table 1. In medical library, Nnewi campus *C. lunata* 37(28.68%) occurred the most while *P. marneffi, C. laurentii, C. parapsilosis* and *A.terreus*, 1(0.76%) eachoccurred the least. In Festus Aghagbo Nwako library, Awka C. lunata 127(43.20%) occurred the most, while *P. expansum, F. aqueductum* and *M. mucedo* 1(0.34%) eachoccurred the least. In Library Complex, Agulu *A.nidulans* 95(47.03%) had the highest frequency of occurrence while *C. dublinensis, R. rubra, A. infectoria* and *A. restrictus* 1(0.50%) each had the least frequency of occurrence.

Table 2 shows the total frequency and percentage of the fungal isolates from the three libraries. Of all the organisms isolated, *C. lunata* 201(32.16%) occurred most while *P. marneffi, P. expansum, A. restrictus, A. infectoria* and *R. rubra* 1(0.16%) occurred the least.

As Table 3 shows, some of the organisms isolated from nasal swabs of librarian's present correlate with the fungal spores isolated from the indoor environment of these libraries. P. notatum, 2 (28.6%) at Library complex, Agulu and 1(14.3) at Medical library, Nnewi campus occurred the most while A. niger, C. lunata, C. albicans and F. aqueductum, 1(14.3%) occurred the least.

Table 4 shows the temperature and relative humidity of the libraries sampled.It can be observed that the temperature ranged from 26.4◦C to 29.1 ◦C, while the relative humidity ranged from 56.7% to 63%.

The frequency of fungi isolated from libraries that are storey buildings was more than those isolated from the bungalow. it is also be observed that the library that is located directly above the microbiology laboratory has a higher frequency of isolates; 202 (32.32%) than that located close to a refuse dump; 129 (20.64%), however the one located close to the road has the highest frequency of isolates; 294 (47.04%). It is also noticed that the age of the building greatly influences the frequency of fungal pathogens as the libraries with age range of "10 – 15 years ". have more organisms isolated from them than that of "5 – 10 years ". It is also very much evident from the table that the number of students visiting the library contributes to the high number of fungal spores. This is seen in table 5.

Table 6 shows the environmental factors that may have affected the frequency of fungal isolates.Even though all the libraries have the same set of ventilation systems, it can be observed that the maintenance is poor as it is not serviced as often as possible, this may be the reason for the high number of fungal isolates. It is also observed that the higher the number of windows, the more the fungal isolates. Moisture problem also contributes greatly to the presence of fungal spores.

C.krusei is the only yeast specie that doesn't ferment maltose and sucrose, while *C. laurentii* is the only specie that ferments lactose as seen in the result of the carbohydrate fermentation test as shown in table 7.

The susceptibility pattern of yeast isolates is shown in table 8. While some species showed sensitivity to all the antifungal agents, some other species such as *C. parapsilosis* and *C.krusei* showed some atom of resistance to the agents.

Table 9 shows the susceptibility pattern of selected mould species to three different concentrations of four antifungal agents. The zone of inhibition of each concentration of the agent was recorded as an average of the duplicate test carried out.

Table 1:- Frequency of fungal isolates based on Libraries.

Name of Library	Organisms	Frequency	Percentage(%)	X^2	P
Medical Library, College of Health Sciences, Nnewi Campus	*Penicillium notatum*	35	27.13	1419.00	0.00**
	Aspergillus niger	6	4.65		
	Penicillium marneffi	1	0.76		
	Cladosporium cladosporoides	16	12.40		
	Curvularia lunata	37	28.68		
	Mucor mucedo	6	4.65		
	Cryptococcus laurentii	1	0.76		
	Cryptococcus luteolus	3	2.33		
	Fusarium aqueductum	2	1.55		
	Curvularia clavata	20	15.50		
	Candida parapsilosis	1	0.76		
	Aspergillus terreus	1	0.76		
	Curvularia lunata	127	43.20	4410.00	0.00**
Festus Aghagbo Nwako Library, Awka Campus	*Aspergillus flavus*	5	1.70		
	Aspergillus terreus	3	1.02		
	Aspergillus niger	18	6.12		
	Cladosporium cladosporoides	42	14.29		
	Penicillium notatum	29	9.86		
	Penicillium expansum	1	0.34		
	Fusarium chlamydosporum	2	0.68		
	Fusarium aqueductum	1	0.34		
	Candida parapsilosis	6	2.04		
	Candida krusei	2	0.68		
	Cryptococcus laurentii	2	0.68		
	Candida dublinensis	4	1.36		

	Mucor mucedo	1	0.34		
	Candida guillemondi	2	0.68		
	Curvularia clavata	49	16.67		
	Cladosporium cladosporoides	22	10.89	2424.00	0.00**
Library complex,	*Curvularia lunata*	37	18.32		
Agulu Campus	*Penicillium notatum*	28	13.86		
	Aspergillus restrictus	1	0.50		
	Aspergillus nidulans	95	47.03		
	Alternaria infectoria	1	0.50		
	Aspergillus niger	5	2.48		
	Candida famata	3	1.49		
	Mucor mucedo	2	0.99		
	Fusarium aqueductum	4	1.98		
	Candida parapsilosis	2	0.99		
	Candida dublinensis	1	0.50		
	Rodotorula rubra	1	0.50		

** significant($p \leq 0.01$)

Table 2:- Fungal species isolated from the samples collected in the Libraries.

Organisms	Frequency	Percentage(%)	X^2	P
Penicillum notatum	92	14.72	9375.00	0.00**
Aspergillus niger	29	4.64		
Penicillum marneffi	1	0.16		
Cladosporium cladosporoides	80	12.8		
Curvularia lunata	201	32.16		
Mucor mucedo	9	1.44		
Cryptococcus laurentii	3	0.48		
Cryptococcus luteolus	3	0.48		
Fusarium aqueductum	7	1.12		
Curvularia clavata	69	11.04		
Candida parapsilosis	9	1.44		
Aspergillus terreus	4	0.64		
Aspergillus flavus	5	0.80		
Penicillum expansum	1	0.16		
Fusarium chlamydosporum	2	0.32		
Candida Krusei	2	0.32		
Candida dublinensis	5	0.80		
Candida guillemondi	2	0.32		
Aspergillus restrictus	1	0.16		
Aspergillus nidulans	95	15.20		
Alternaria infectoria	1	0.16		
Candida famata	3	0.48		
Rodotorula rubra	1	0.16		

** significant($p \leq 0.01$)

Table 3:- Fungal species isolated from the nasal swabs of library staff on duty.

Library	Organisms	Frequency	Percentage
Medical Library, College of Health Sciences, Nnewi Campus	*Penicillum notatum* *Aspergillus niger*	1 1	14.3 14.3
Festus Aghagbo Nwako	*Curvularia lunata*	1	14.3

Library, Awka Campus	*Candida albicans*	1	14.3
Library Complex, Agulu Campus	*Penicillum notatum* *Fusarium aqueductum*	2 1	28.6 14.3

Table 4:- Temperature and Humidity of Libraries.

Name of Library	Temperature	Humidity
Medical Library, College of Health Sciences, Nnewi Campus	28.3◦C	62%
Festus Aghagbo Nwako Library, Awka Campus	29.1◦C	56.7%
Library Complex, Agulu Campus	26.4◦C	63%

Table 5:- Effect of design and age of building on frequency of Fungal isolates.

Parameters		Frequency of Isolates	Percentage
Type of building	Storey building	496	79.36
	Bungalow	129	20.64
	Others		
Location of the library	Close to the Road	294	47.04
	Close to the hospital		
	Close to a refuse dump	129	20.64
	Others (Directly above the microbiology lab)	202	32.32
Type of Ceiling	Plaster ceiling	294	47.04
	Coffered ceiling		
	Vaulted ceiling	331	52.96
	Suspended tile ceiling		
	Others	202	32.32
Age of the building	1-5 years		
	5-10 years	423	67.68
	10- 15 years		
	>15 years		
		129	20.64
Number of students using the library daily	50	294	47.04
	300	202	32.32
	30		

Table 6:- Environmental factors affecting fungal isolates.

Parameters		Frequency of Isolates	Percentage
Type of ventilation	Air conditioner		
	Natural Ventilation		
	All of the above	625	100
	Others		
Frequency of servicing	Once a year		
	Once in six months		
	Once in two months		
	When faulty	625	100
Number of windows			

	6-8	202	32.32
	8-10	129	20.64
	>10	294	47.04
Type of flooring			
	Cemented		
	Rug		
	Carpeted		
	Tiled	625	100
	Others		
Frequency of sweeping			
	Daily	625	100
	Weekly		
	Monthly		
Renovation within the last two years	Yearly		
	Yes		
Moisture problem	No	625	100
	Damp patches	423	67.68
	Mould or mildew growing on the walls, floor or ceiling	202	32.32
	Leakage of roofs		
	Others		

Table 7:- Carbohydrate Fermentation Test using Integral System Yeast plus.

Organism	Glu	Mal	Suc	Lac	Gal	Mel	Cel	Ino	Xyl	Raf	Tre	Dul
C. famata	+	+	+	-	+	+	+	-	+	+	+	+
C. laurentii	+	+	+	+	+	+	+	+	+	+	+	+
C. guillemondii	+	+	+	-	+	+	+	-	+	+	+	+
R. rubra	+	+	+	-	+	-	+	-	+	+	+	-
C. luteolus	+	+	+	-	+	+	+	+	+	+	+	+
C. albicans	+	+	+	-	+	-	-	-	+	-	+	-
C. krusei	+	-	-	-	-	-	-	-	-	-	-	-
C. dubliniensis	+	+	+	-	+	-	-	-	-	-	-	-
C. parapsilosis	+	+	+	-	+	-	-	-	+	-	-	-

KEY:
+ Positive
- Negative
Glu: Glucose, Mal: Maltose, Suc: Sucrose, Lac: Lactose, Gal: Galactose, Mel: Melibiose, Cel: Cellobiose, Ino: Inositol, Xyl: Xylose, Raf: Raffinose, Tre: Trehalose, Dul : Dulcitol/

Organisms	AFST	NY	AMB	FCY	ECN	KCA	CLO	MIC	ITR	VOR	FLU
C. famata	S	3(100)	3(100)	3(100)	3(100)	3(100)	3(100	3(100)	3(100)	3(100)	3(100)
	I	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0
C. laurentii	S	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)
	I	0	0	0	0	0	0	0	0	0	0
C. guillemondii	R	0	0	0	0	0	0	0	0	0	0
	S	0	0	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
	I	2(100)	2(100)	0	0	0	0	0	0	0	0
R. rubra	R	0	0	0	0	0	0	0	0	0	0
	S	1(100)	1(100)	1(100)	0	1(100)	0	0	0	1(100)	1(100)
	I	0	0	0	1(100)	0	1(100)	0	1(100)	0	0
C. luteolus	R	0	0	0	0	0	0	1(100)	0	0	0
	S	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)
C. albicans	I	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0
	S	1(100)	1(100)	1(100)	1(100)	0	1(100)	1(100)	1(100)	1(100)	1(100)
C. krusei	I	0	0	0	0	1(100)	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0
	S	1(50)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)
C. dublinensis	I	0	0	0	0	0	0	0	0	0	0
	R	1(50)	0	0	0	0	0	0	0	0	0
	S	2(40)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)
C. parapsilosis	I	3(60)	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0
	S	6(66.7)	8(88.9)	8(88.9)	6(66.7)	8(88.9)	7(77.8)	6(66.7)	6(66.7)	8(88.9)	8(88.9)
	I	0	0	1(11.1)	1(11.1)	1(11.1)	1(11.1)	0	2(22.2)	1(11.1)	1(11.1)
	R	3(33.3)	1(11.1)	0	2(22.2)	0	1(11.1)	3(33.3)	1(11.1)	0	0

Table 8:- Susceptibility pattern of Yeast Isolates Using the Integral system yeast plus.

KEY:
AFST: Antifungal Susceptibility Testing, NY: Nystatin, AMB: Amphotericin B, FCY: Flucytosine, ECN: Econazole, KCA: Ketoconazole, CLO: Clotrimoxazole, MIC: Miconazole, ITR: Itraconazole, VOR: Voricanazole, FLU: Fluconazole, S= Sensitive, I= Intermediate, R= Resistant

Table 9:- Susceptibility pattern of selected mould species to antifungal agents using the agar well diffusion technique.

Organis	Zone of Inhibition

ms	Nystatin			Ketoconazole			Amphotericin B			Griseofulvin		
	25ug/ml	50ug/ml	75ug/ml	25ug/ml	50ug/ml	75ug/ml	25ug/ml	50ug/ml	75ug/ml	25ug/ml	50ug/ml	75ug/ml
P.notatum	0.00±0.00	0.00 ±0.00	0.00± 0.00	11.00 ±1.41 (0.058)	13.00 ±1.41 (0.049)*	19.00 ±1.41 (0.033) *	0.00± 0.00	0.00± 0.00	0.00± 0.00	9.00± 1.41 (0.070)	9.00± 1.41 (0.070)	13.00 ±1.41 (0.049) *
C. cladosporoides	5.00±1.41 (0.126)	12.50 ±0.71 (0.025)*	14.50 ±0.71 (0.022)*	0.00± 0.00	1.00± 1.41 (0.500) *	3.00± 1.41 (0.205)	21.00 ±1.41 (0.030) *	26.50 ±0.71 (0.012) *	29.00 ±1.41 (0.022) *	12.50 ±0.71 (0.025) *	15.00 ±1.41 (0.042) *	19.00 ±1.41 (0.033) *
C. lunata	9.00±1.41 (0.070)	21.00 ±1.41 (0.030) *	25.00 ±0.71 (0.012)*	25.00 ±0.71 (0.012)*	30.50 ±0.71 (0.010) *	37.00 ±1.41 (0.017) *	15.00 ±1.41 (0.042) *	19.00 ±1.41 (0.033)*	27.00 ±1.41 (0.024) *	9.50± 0.71 (0.033) *	19.50 ±0.71 (0.016) *	31.00 ±1.41 (0.021) *
A.niger	0.00±0.00	1.50± 0.71 (0.205)	4.50± 0.71 (0.070)	10.50 ±0.71 (0.030) *	14.50 ±0.71 (0.022) *	20.50 ±0.71 (0.016) *	1.50± 0.71 (0.205)	4.50± 2.12 (0.205)	7.50± 3.54 (0.205)	10.50 ±0.71 (0.030) *	13.00 ±1.41 (0.049) *	19.00 ±1.41 (0.033) *
F. aqueductum	0.00±0.00	0.00± 0.00	0.00± 0.00	4.50± 0.71 (0.070)	8.00± 1.41 (0.079)	12.00 ±1.41 (0.053)	1.50± 0.71 (0.205)	4.00± 0.00	5.50± 0.71 (0.058)	6.50± 0.71 (0.049) *	9.00± 1.41 (0.070)	14.50 ±0.00 (0.022) *

*Significant(P≤0.05)

Discussion:-

Microbiological quality assessment of indoor air is one of the most vital investigations to determine the microbial indoor air pollution. The information on the indoor microbial concentrations of airborne fungi is necessary both to estimate the health hazard and to create standards for indoor air quality control. The presence of cellulosic materials in the library serves as a good substrate for fungal species especially when they are damaged by dampness. Due to the high relative humidity and temperature provided by indoor environment of libraries several fungal spores are able to thrive well. Most of the fungal pathogens isolated in this study have been proven to be pathogenic (Aimanianda et al., 2010).

Out of the 625 fungal isolates from the three libraries, a total of 129 were isolated from the indoor environment of Medical Library, Nnewi campus. Of this, *C. lunata* occurred the most with a frequency of 37(28.68%), while *C. parapsilosis, A.terreus, C.laurentii* and *P. marneffi* occurred the least with a frequency of 1 (0.76%) each (Table 1). This is in contrast to a similar research carried out by Suriani et al., (2016) in which *Aspergillus spp*. (27.27%) was the most prevalent. It is also in contrast to a study by Ghiasian et al., (2016) in which *Cladosporium spp* (30.9%) was the most frequently isolated.

In Festus Aghagbo Nwako Library, Awka campus, a total of 294 organisms were isolated, of this *C. lunata*, 127(43.20%) occurred the most while *P. expansum*, *F. aqueductum* and *M. mucedo* occurred the least with frequencies of 1 (0.76%) each(Table 4). This is also in contrast to the study by Ghiasian et al., (2016) in which *Cladosporium spp* (30.9%) was the most frequently isolated.

A total of 202 organisms were isolated from the Library Complex, Agulu campus, out of these *A.nidulans* (47.03%) occurred the most (Table 4); significant at $p \leq 0.01$. This is similar to the result obtained by Rahmawati et al., (2018), in which *Aspergillus* (29.50%) occurred the most, although the particular specie was not stated. This contrasted a study on "Fungal Biodiversity of a Library and Cellulolytic Activity of Some Fungi" by Swapna and Lalchand, (2016) in which *Aspergillus niger* and *Aspergillus fumigatus* were recorded as the most dominant species (no percentage stated).

The total frequency of organisms isolated from all the libraries is 625, of which *Curvularia lunata* (32.16%) occurred the highest (Table 5). This is in contrast with researches by Suriani et al., (2016) in which *Aspergillus spp*. (27.27%) was the most prevalent and Ghiasian et al., (2016) in which *Cladosporium spp* (30.9%) was the most frequently isolated. This difference may have been due to contrasting geographical factors as both studies were conducted in Malaysia and Iran respectively. Both countries have different climatic conditions from Nigeria.

16 Nasal swab samples were collected from the library staff on duty at each library sampled. 7 organisms were isolated, of these *P. notatum* and *A. niger* which were also isolated from the indoor environment of the Medical Library, Nnewi campus were isolated from the nasal swab of the staff present. This suggests that the staff may have inhaled some fungal spores from the indoor environment of the library. In Festus Aghagbo Nwako library, there was a similar case as *Curvularia lunata* was also isolated, however a colony of *Candida albicans* which was not present in the indoor environment was isolated, which may mean that the particular staff might have acquired the infection from another source. Two colonies of *P. notatum* and one colony of *F. aqueductum* was isolated from the nasal swab of the staff present at the library complex Agulu campus. These organisms were as well isolated from the indoor environment of the library suggesting that the staff may have been exposed to these fungal spores.

The results obtained from this study showed a high level of fungal contamination among the three libraries sampled, this might have been affected by the temperature and relative humidity of the libraries. The three temperatures (28.3◦C, 29.1◦C and 26.4◦C) fall within the normal range of room temperature which has been proven to positively affect the growth of fungal spores (Hawksworth et al., 2017). These values agree with that obtained by Rahmawati et al., (2018) who in their research on "The diversity of Indoor airborne moulds growing in the university libraries in Indonesia" showed that the temperatures of the libraries fell within the range of 24◦C - 34◦C which supported the growth of the mould species obtained.

Out of the 625 isolates, 496(79.36%) were isolated from the two libraries which are storey buildings (Festus Aghagbo Nwako Library, main campus Awka and Library complex, Agulu Campus). While 129(20.64%) organisms were isolated from the medical library, Nnewi campus which is a bungalow. Thus, it may be said that the type of building has an effect on the presence of airborne fungal spores. This is however in contrast to an observation by Xueying et al., (2016) which showed that more airborne fungal pathogens were isolated from buildings that are ground floor than in storey buildings. Rousselet al., (2008), also isolated more airborne fungal spores from ground floor apartments than in storey buildings. These contrasts may have been because the two studies mentioned were carried out in homes unlike this study which was carried out in libraries, also the fact that the libraries which are storey buildings are located close to the road may have been another contributing factor. It is also observed that the library located close to the road had the highest frequency of fungal isolates followed by that which is located directly above the microbiology laboratory. The library being close to the road might have made it prone to the presence of airborne fungal spores likewise that located above the microbiology laboratory, due to the fact that some of this organisms are being studied in the laboratory, there is every likelihood that this may have contributed to the large number of fungal spores isolated from the library. Out of the 625 isolates, 294 (Festus Aghagbo Nwako Library) was isolated from the library with plaster ceiling while 331 were isolated from the two libraries with suspended tile ceiling (Medical library Nnewi campus and Library Complex, Agulu Campus). The presence of plaster ceiling might have contributed to the higher frequency of fungal isolates. It is observed that the older the building the more prone it is to fungal contamination which is in agreement with a research by Xueying et al., (2016) which showed that more fungal spores were isolated from the indoor environment of older buildings than that of newer buildings. High frequency of visitors/human activities in these libraries may also have affected the number of fungal isolates, this is in agreement with a similar research by Agnieszka et al., (2012), which showed that increase in human activity in the library contributes to the presence of fungal contaminants. However, it was observed that the medical library, Nnewi campus, though having more visitors than the library complex, Agulu had less number of isolates, this may have been affected by several other factors such as the Library in Agulu Campus being located above the microbiology lab and also close to the road.

The ventilation systems in all the three libraries sampled, were only serviced when faulty. This might have contributed to the high frequency of fungal isolates from all the libraries as emphasized by Ruping et al., (2011). From the study it was also observed that the number of windows, the type of flooring and the frequency of sweeping had no significant effect since all the libraries sampled had the same characteristics based on the three parameters listed above. However, since none of the buildings had been renovated within the last two years, this might have been the reason for the high frequency of fungal contamination. Moisture problem has been proven to contribute to presence of fungal contaminants in indoor environment (WHO, 2009). All the libraries sampled had moisture problems which might have contributed to the high frequency of fungal spores. This is in agreement with a similar research on "Associations of Dwelling Characteristics, Home Dampness, and Lifestyle Behavior with Indoor Airborne Culturable Fungi: On-site inspection in 454 Shanghai Residences" by Xueying et al., (2016) which showed that airborne fungi concentrations were significantly higher in bedrooms with visual indicators of dampness, moldy odor, damp spots on an exterior wall, damp spots and mold spots in interior wall, as well as mold spots on the interior of exterior walls.

The yeast isolates were identified based on their ability to ferment some carbohydrate molecules as seen in Table 7. The yeast isolates were also subjected to antifungal susceptibility testing using the integral system yeast plus. Out of the 28 yeast isolates, all colonies of *C.famata, C. laurentii, C. luteolus*, were susceptible to all the antifungal agents tested. *R.rubra* displayed resistance to Miconazole, one colony of *C.krusei* displayed resistance to nystatin, three colonies of *C.parapsilosis* displayed resistance to Nystatin, one colony to Amphotericin B, two to Econazole, One to Clotrimoxazole and Itraconazole while three were resistant to Miconazole.

Comparing the zones of inhibition obtained for Amphotericin B, which at present is the only antifungal agent used in this study that has a breakpoint value for moulds according to the Clinical and Laboratory Standards Institute (CLSI) (Arikan, 2007) with the breakpoint values; (Susceptible ≥15mm), (Intermediate 14–10mm) and (Resistant ≤9) it is seen that at all concentrations of Amphotericin B, *C. cladosporoides* and *C. lunata* are susceptible while *P. notatum, A. niger* and *F. aqueductum* are all resistant. In a similar research, though on Candida species by Magaldi et al., (2004), it was observed that out of the 108 Candida isolates tested, 106 (98%) were susceptible to Amphotericin B which is similar to the 100% susceptibility observed for *C. cladosporoides* and *C. lunata*, Panizo et al., (2009) however showed that out of 145 isolates of candida, none was susceptible to Amphotericin B. This resistance may have been due to resistance which might be inherent or might have been acquired from other sources. *P. notatum*which from this study displayed total resistance to Nystatin and Amphotericin B may also be resistant to these agents as a result of various factors. At increased concentrations of some of these agents, the organisms which displayed resistance may become susceptible however more research is needed to confirm this.

Conclusion:-

This study has revealed that the indoor environment of libraries in Nnamdi Azikiwe Univrsity is to a considerable extent contaminated with airborne fungal spores. Some factors such as poor maintenance of the ventilation systems, location of the library, environmental factors within and outside the library as well as human activities contribute to this contamination. It was also observed that some staff of the libraries might have been exposed to some of these fungal spores which is of real concern to the health and well-being of other library visitors.

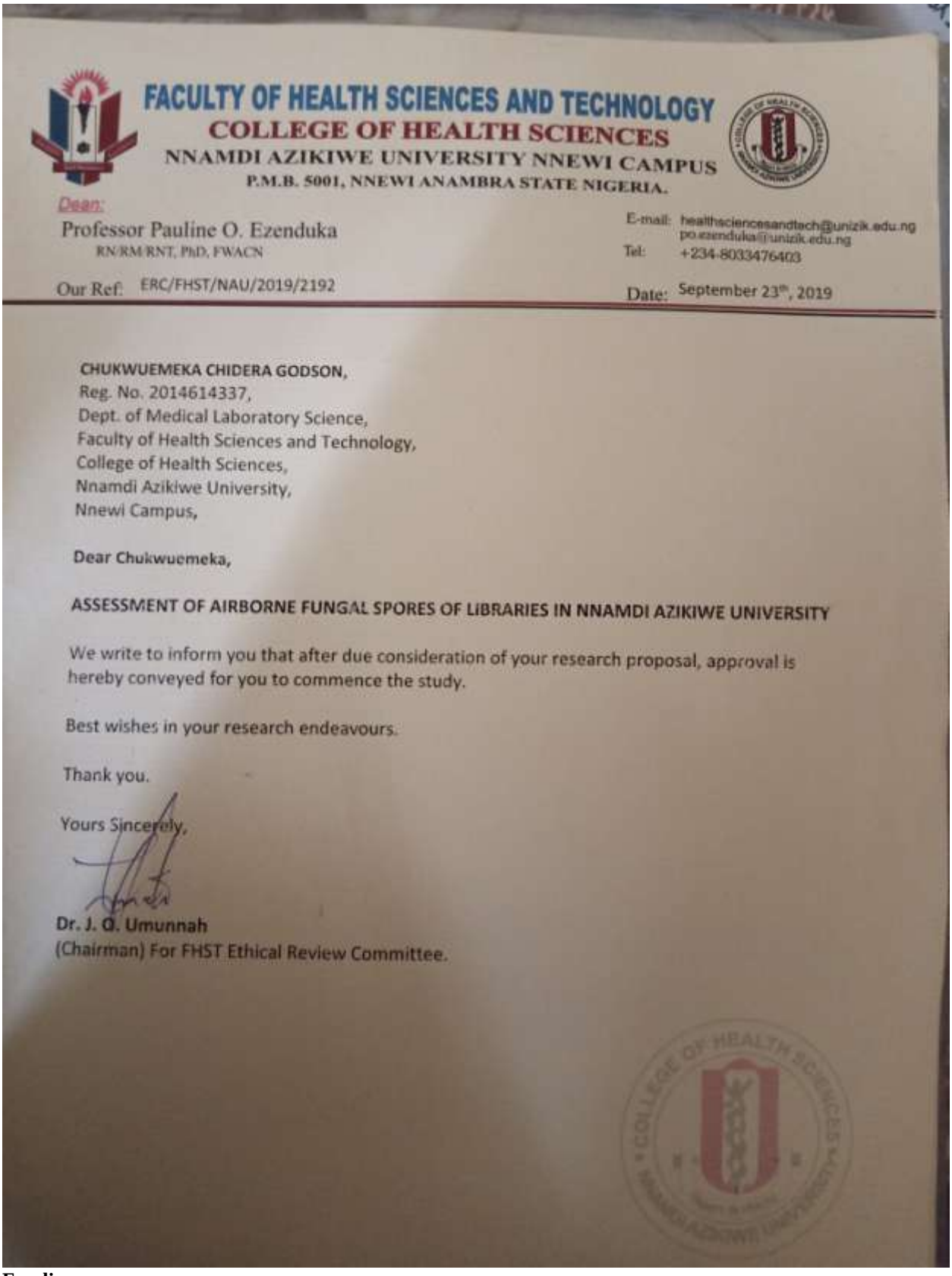
The determination of the antifungal susceptibility pattern showed that some of the organisms were resistant to the antifungal agents used and this might pose a real public health challenge.

Declarations

Ethical Consideration:

The study was approved by the ethical committee of the Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus ERC/FHST/NAU/2019/2192 Date: September 23rd, 2019

All procedure performed in this study, collection of nasal swabs from human participants, were in accordance with the ethical standards of this institution and with the 1964 Helsinki Declaration and its later amendments in 2013.



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Faculty of Health Sciences and Technology,
College of Health Sciences,
Nnamdi Azikiwe University,
Nnewi Campus,

Dear Chukwuemeka,

ASSESSMENT OF AIRBORNE FUNGAL SPORES OF LIBRARIES IN NNAMDI AZIKIWE UNIVERSITY

We write to inform you that after due consideration of your research proposal, approval is hereby conveyed for you to commence the study.

Best wishes in your research endeavours.

Thank you.

Yours Sincerely,

Dr. J. O. Umunnah
(Chairman) For FHST Ethical Review Committee.

Funding:
Funded by researchers

Conflict of Interest:
No conflict of Interest

Consent to Participate:
This was sought and obtained

Availability of data and materials:
Data are available

Code Availability:
Not Applicable

Author's Contribution:
Conceptualization: Chukwuemeka Chidera and Obi Chioma.**Methodology:** Chukwuemeka Chidera and Nwachukwu Chinazo; **Validation:** Chukwuemeka Chidera, Obi Chioma and Ochiabuto Ogochukwu; **Formal Analysis:** Chukwuemeka Chidera and Obi Chioma; **Investigation:** Chukwuemeka Chidera and Obi Chioma: **Resources:** Chukwuemeka Chidera and Nwachukwu Chinazo; **Data Curation:** Chukwemeka Chidera; **Writing Original Draft Preparation:** Chukwuemeka Chidera; **Writing Review and Editing:** Obi Chioma; **Visualisation:** Chukwuemeka Chidera and Ochiabuto Ogochukwu; **Supervision:** Obi Chioma; **Project Administration:** Obi Chioma; **Funding Acquisation:** Chukwuemeka chidera and Nwachukwu Chinazo;

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