Adverse Health Effects of Indoor fungi during a Rainy season in Northern part of Anambra State Nigeria

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Abstract – Excessive exposure to mould contaminated materials in indoor environment can cause adverse health effects in susceptible persons, regardless of the type of mould or the extent of contamination. Aspergillus and other fungi can cause allergic and life-threatening illnesses in immunocompromised patients. The study was carried out to determine the prevalent fungal species in homes, offices and hospitals and to determine the health-related experiences of occupants of these environments. The investigation was carried out using health base pre-tested questionnaires and A6 Single Stage microbial air sampler with malt extract agar. Five hundred and forty-nine (549) air samples and 226 nasal swabs of occupants were examined. A total of 55 species of fungi were isolated out of which 12 species were allergenic fungi. The predominant airborne fungi in homes, offices and hospitals were Aspergillusniger (76%), A.niger and Penicilliumnotatum (61%) and P.notatum (63.8%) respectively. Consequently A.niger and P.notatum were the dominant fungi in the nasal swabs of occupants of these environments. There was positive correlation between airborne isolates and fungal nasal carriage of occupants. Total fungal counts were found to be consistently higher in homes where different health problems were reported, reflecting the public health implication of these indoor airborne fungi. Considering the role of airborne fungi in respiratory diseases, appropriate measures should be taken in our indoor environment to prevent mould growth and subsequent dissemination.

Keywords: (1) Health effects, (2)Indoor fungi, (3)Rainy season,(4) Anambra State.

INTRODUCTION

Fungi are ubiquitous in the environment and spores of these fungi especially mould are a common component of household, hospital and workplace dust. Humans spend 90% of their time in indoor environment. Excessive exposure to mould contaminated materials in the indoor and outdoor environment can cause adverse health effects in susceptible persons regardless of the type of mould. Sufficient evidence exist to conclude that exposure to mould in indoor environment is associated with asthma and asthma like symptoms, upper respiratory tract symptoms, wheeze,cough and hypersensitivity pneumonitis in susceptible individual (Mendell, 2011).These have become more prevalent in developing countries such as Nigeria, where proper sanitation and the standard of housing are deplorable.

Studies have shown that people who are atopic or suffer from allergies or have compromised immune system and occupy damp or mouldy indoor environment are at elevated risk of these health problems (Mendell 2011).

The ability of fungi present in indoor spaces to cause different health problems depends to a large extent on the growth environment and secondly on the type of substrate. The allergic symptoms mostly caused by fungi in indoor environment are theIgE dependent type 1 hypersensitivity reactions. These reactions includes sinusitis, rhinitis, asthma, eye irritations, skin irritations, and urticaria.

The antigen that induces this type of reaction is commonly mannan found in the cell walls of genus *Aspergillus, Candida, Cladosporium, Alternaria, Penicilluim*and *Trichoderma.*

MATERIALS AND METHODS

Significance for public health

The high prevalence of allergies, and airborne fungal infections in developed and developing countries, andthe airborne invasive fungal infections in various risk groups of people suffering from immunodeficiency,are an increasing problem for modern medicine. This leads to the increased public concern about indoor airquality in homes, offices and most importantly hospitals resulting in an increase in demand for assessmentsof buildings for evidence of indoor fungal growth.Studies has shown that as many as 10 -15% of deaths among kidney, liver, bone marrow and lung transplantrecipients all of whom have weakened immune systems are of fungal infection caused by inhalation offungal spores (Mayaud and Cadranel, 2000).Most people spend over 90%of their lives indoors where they are exposed to some indoor environmentalfactors that influence their health and physical condition (Ayanbimpe et al., 2010).

The study was carried out in Northern part Anambra State Nigeria. It covered the rainy season period from May to June 2017. The investigation was carried out using health based pre-tested questionnaires structured out of extensive literature review on indoor air contamination by fungi, and A6 single stage microbial air sampler with malt extract agar supplemented with chloramphenicol 0.05mg/ml. The focus of the questionnaire was on those variables in the homes, offices, and hospitals as well as activities and behaviors of the occupants that can influence the presence, growth, and concentration of airborne fungi in indoor environment.

Using stratified random sampling technique air samples were collected from 84 homes, 28 offices, 7 hospitals, giving a total of 549 air samples. The temperature and relative humidity of indoor environments were taken. Two hundred and twenty six (226) nasal swabs were also collected from occupants of homes, personnel in offices and care givers in hospitals sampled. The microbial air sampler is operated at an air flow rate of 28.3 LPM (litres per minute). The sampling time was 5-10minutes according to the environmental situation of the measurement condition to avoid drying of the agar surface and overloading of the plate. The sampler was set up at a height representative of the normal human breathing zone i.e 1.5m above floor level (Obard and Fang 2003). The samples were collected in the morning hours immediately after morning cleaning. The inoculated plates were sealed with masking tape to prevent contamination and incubated at room temperature for 4-14 days and observed daily for yeast and mould growth (Cheesbrough 2006). The nasal swabs were streaked on Sabouraud dextrose Agar, supplemented with chloramphenicol at 0.05mg/ml and incubated at room temperature for 2-7 days.

When growths were adequate, the mature fungal growth wereexamined macroscopically and microscopically. The procedures used were those employed in most mycological examinations involving a pathogenic and non-pathogenic fungi (Ochei and Kolhatker 2000).

Identification of fungal isolated was based on gross colonial morphology, microscopic image observed which were matched against those contained in colour atlases of pathogenic fungi by Fery*et al.,* 1979 and de Hoog, et al. (1995).

RESULT

A total of 54 species of fungi were isolated from homes, 46 species from offices and 52 species from hospitals. Out of these, 12 species were allergenic fungi. The predominant fungi in these environments were homes, *A. niger*(76.2%) and *P.notatum*(75.4%), offices, *C.famata*(64%), and *A.niger* (61.46%)and *P.notatum*(61.4%) and Hospitals, *P.notatum*(63.8%), *A. niger*(58.6%). Nasal swabs collected from occupants of these environments showed that*A.niger* 66(23%) was the most frequently isolated fungi from occupants of homes followedby P.notatum 46(16%) and C.famata 33(12%).The least isolated fungi were C.guillermondii, C.cruzei and P.citrinium (1%).The most prevalent fungal isolate from nasal swabs of office occupants were *A.niger* and *P.notatum* 19(23%), followed by *A.fumigatus* 10(12%) and *C.famata* 10(12%) while the least isolates were*C.dublinensis*, *C.tropicalis*, *P.piceum, P.citrinium, P.gerundense and* R.*glutinis* 1(1%). *Penicilliumnotatum* 18(31%) was the predominant isolate from nasal swabs of care givers in hospitals, followed by *A.niger* 14(24%) and *C.famata* 6(10%), while the least isolated fungus was *C.dublinensis* 1(2%).Table 2 refers to the relationship between airborne fungal isolates and it's nasal carriage by occupants of same environment in the Northern part of Anambra State during the rainy season. In

Anambra East and Anambra West, significantrelationship was observed between airborne and nasal carriage of C.dublinensis (p =0.0017) and C.famata (p = 0.0143). In Ayamelum, significant relationship was observed between airborne isolate and nasal carriage of A.flavus(p = 0.001). In Onitsha North, there were significant association between airborne isolates of A.fumigatus (p = 0.0173), A.niger (p = 0.0263), C.famata (p = 0.0414) and P.notatum (p = 0.0115) and their nasal carriages, no significant associations were observed between the presence of airborne and nasal carriage of other isolates present in this LGA. The correlation of other isolates are as shown in Table 2. These findings suggest that the nasal presence of these isolateswere influenced by the airborne concentration of the fungi in the Local Government Areas concerned.

Table 3concerns the different clinical symptoms studied. To obtain the table, we first isolated all the allegernic funginamely; *A.fumigatus, A.niger, A.clavatus, A.penicillioides, A.candidus, C.herbanum, Alternariaalternata, Bipolaris, Fusariumsolani, F.oxysporium, Trichodermaharzianium, Penicilliumnotatum and Candida albicans*.For a given health problem, two datasets were obtained consisting of wherethe given health problem is present and where it is absent. N refers to the total number of sample site covered. Also, n0 is the number of sample sites where a given health problemis absent and n1 is the number of sample sites where the health problem is present. Similarly, $\bar{x}0$ and $\bar{x}1$ are respectively the mean fungal colony counts where the health problem is absent and where it is present. Thep-value is obtained from the Wilcoxon rank sum test between total colony counts of fungal isolates fromthe sample sites where given health condition is absent and where it is present. The choice of the Wilcoxonrank sum test for independent cases is because our datasets (total colony counts of fungi where the givenhealth problem is present and total colony counts of fungi where the given health problem is absent) failedthe normality assumption using the Shapiro test for normality. From the table it is observed significantly higher colony count in 50% of the different health problems studied. The health problems include asthma, eye irritation, itchy skin, nasal congestion and sore throat, at p-values of 0.001637, 0.003972, 0.001146, 0.03227 and 0.001895respectively. We rejected in each case, the null hypothesis that the colony counts where a given health problem isabsent is the same with the colony count where it is present.

Table 1: Prevalence of Fungal isolates from Homes, Offices and Hospitals Located in Northern part of Anambra StateDuring a Rainy Season.

S/Nos	Isolates	Homes(%)	Offices(%)	Hospitals(%)
1	*Aspergillusfumigatus*	51.41	34.29	37.61
2	*A.tamari-kita*	38.09	22.86	16.30
3	*A.terreus*	29.46	14.29	16.83
4	*A.versicolor*	0.96	0.00	2.03
5	*A.niger*	76.17	61.43	58.64
6	*A.flavus*	43.30	11.43	18.71
7	*A.clavatus*	24.27	2.86	7.86
8	*A.alliaceus*	24.73	20.00	17.14
9	*A. candidus*	12.61	7.14	11.21
10	*A.aculeatus*	37.13	24.29	34.03
11	*A.penicillioides*	14.76	1.43	17.96
12	*A. ochraceus*	34.73	24.29	17.19
13	*Curvuleriaclavata*	68.07	48.57	39.87
14	*C.geniculata*	52.81	37.14	38.26
15	*Phialophorarepens*	26.40	0.00	16.29
16	*P.reptans*	24.73	2.86	8.41
17	*P.europoea*	0.00	0.00	7.86
18	*Phialomoniumobovatum*	12.61	18.57	14.19
19	*Phialomoniumcurvatum*	34.23	28.57	15.07
20	*Paecilomycesliliacinus*	14.50	7.14	8.06

21	*Paecilomycesvariotti*	4.76	7.14	4.89
22	*Fusariumincanatum*	36.17	4.29	10.94
23	*Fusariumsolani*	41.43	25.71	23.54
24	*F.subglutinans*	3.33	10.00	10.00
25	*F.chlamydosporoides*	4.76	1.43	5.60
26	*F.oxysporum*	11.66	8.57	4.64
27	*Penicilliumnonatum*	75.37	61.43	63.80
28	*Penicilliumpiceum*	33.26	14.29	23.46
29	*Penicilliumcitrinium*	19.04	12.86	12.70
30	*Penicilliumgerundense*	1.19	0.00	0.00
31	*Penicilliumgriseofulvin*	3.10	0.00	0.00
32	*Talaromycesmarneffei*	13.10	0.00	11.63
33	*Alternariaalternata*	25.21	11.43	15.97
34	*Exophialasalmonis*	30.44	14.29	22.21
35	*Cladosporiumcladosporoides*	71.79	52.86	46.43
36	*Cladosporiumherbanum*	47.31	21.43	29.03

Table1 Cont: Prevalence of fungal isolates from Homes, Offices and Hospitals in Northern part of Anambra State During a Rainy Season.

s/no	*Isolates*	Homes	Offices	Hospitals
37	*Bipolarisspecifera*	29.74	25.71	26.57
38	*Beauveriebassiana*	11.17	4.29	2.86
39	*Scopulariopsisfusea*	15.23	8.57	14.89
40	*Scadosporiuminflatum*	2.29	0.00	7.03
41	*Trichosporonpullulans*	17.84	1.43	5.36
42	*Tritirachiumoryzae*	16.64	5.71	7.34
43	*Trichodermaharzianium*	18.80	1.43	4.59
44	*Candida tropicalis*	61.77	44.29	41.53
45	*Candida cruzei*	44.01	27.14	29.20
46	*C. dublinensis*	59.21	44.29	46.07
47	*C.glabrata*	23.31	20.00	10.46
48	*C famata*	64.71	64.29	46.49
49	*C.parapsilopsis*	27.37	22.86	26.57
50	*Candida guillermondii*	7.84	4.29	12.14
51	*Candida lusitaniae*	8.31	7.14	9.39
52	*Candida albicans*	11.41	1.43	18.94
53	*Candida haemolinus*	2.86	0.00	4.29
54	*Rhizopusoryzae*	5.23	0.00	0.00
55	*Rhodotorulaglutinis*	26.64	14.29	21.50

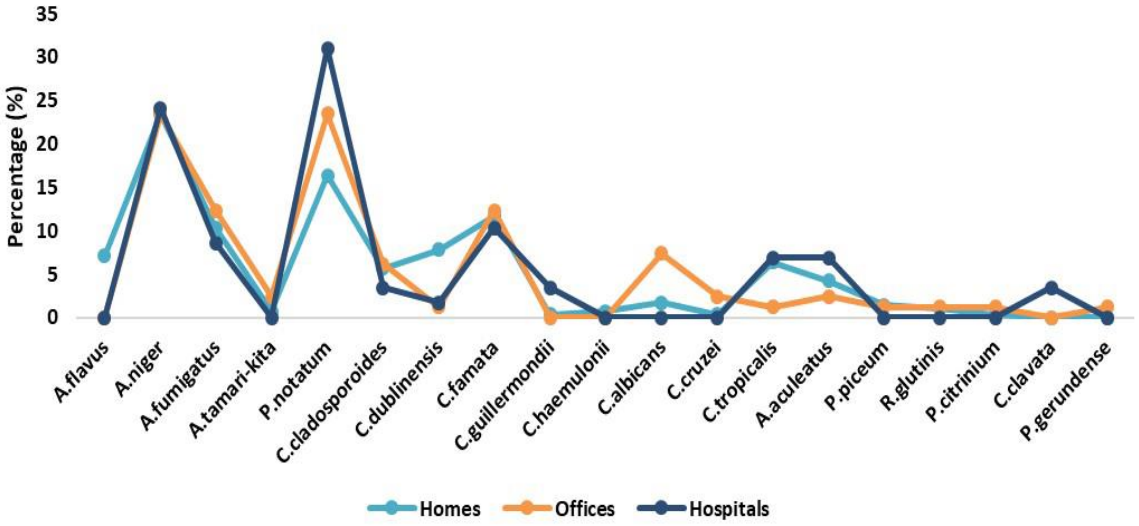

Figure 1: Frequency of fungal isolates from the nasal swabs of sampled individuals of homes, offices and hospitals inNorthern part of Anambra State during rainy season.

Table 2: Correlation between airborne fungal isolates and fungal Nasal carriagefromoccupants of homes, offices and hospitals in Northern part of Anambra State during rainy season.

Air/Narsal Isolates	Anambra East Ø (p-value)	Anambra West Ø (p-value)	Ayamelum Ø (p-value)	Ogbaru Ø(p-value)	Onitsha North Ø(p-value)	Onitsha South Ø(p-value)	Oyi Ø(p-value)
A.aculeatus	-	-	-	0.6042(0.0406)*	-	0.1491(1.0000)	-
A.flavus	-	-	0.8051(0.001)*	0.201(1.0000)	0.175(0.6030)	-	0.1485(1.0000)
A.fumigatus	-	0.1581(1.0000)	0.0769(1.0000)	-	0.6364(0.0114)*	-	0.2236(1.0000)
A.niger	0.2485(0.4737)	0.3162(0.2800)	0.4564(0.2105)	0.1217(1.0000)	0.5774(0.0263)*	0.6708(0.0260)*	0.2236(1.0000)
A.tamari	-	-	-	-	0.2132(1.0000)	-	0.1409(1.0000)
C.albicans	-	-	0.1409(1.0000)	-	-	0.4629(0.0909)	-
C.cladosporoides	-	-	-	-	0.05(1.0000)	0.1491(1.0000)	0.7921(0.0175)*
C.clavata	0.3218(0.2982)	-	-	-	-	-	-
C.cruzei	-	-	0.18(1.0000)	-	-	-	-
C.dublinensis	0.7738(0.0017)*	0.1574(1.0000)	0.0808(1.0000)	0.0548(1.0000)	0.3311(0.2714)	0.1614(0.4805)	0.0556(1.0000)
C.famata	0.201(1.0000)	0.7947(0.0143)*	0.0808(1.0000)	0.1021(1.0000)	0.6111(0.0414)*	0.7327(0.0065)*	0.2435(0.3860)
C.tropicalis	-	0.3443(0.1842)	0.1771(1.0000)	0.1021(1.0000)	-	-	-
P.citrinium	-	-	-	0.4824(0.0709)	-	-	0.7921(0.0175)*

P.nonatum	0.1021(1.0000)	0.0725(1.0000)	0.1217(1.0000)	0.233(1.0000)	0.6912(0.0115)*	0.6421(0.0093)*	0.2485(0.4737)
P.piceum	-	-	0.1021(1.0000)	-	-	0.0541(1.0000)	-
R.glutinis	-	-	-	-	-	0.0896(1.0000)	-

Table 3: Clinical symptoms reported by occupants of homes, offices and hospitals in Northern parts of Anambra State during the Rainy Season

Problem	N	N0	N1	X0	X1	P-Value
Asthma	140	134	6	37.04	95.67	0.001637
Cough	140	88	52	36.1	45.38	0.05941
Eye Irritation	140	84	56	32.41	50.14	0.003972
Head Ache	140	31	109	34.77	40.91	0.4514
Hoarseness	140	114	26	36.83	51.46	0.1651
Itchy Skin	140	106	34	34.91	54.03	0.001146
Nasal Congestion	140	82	58	34.88	46.16	0.03227
Rhimitis	140	135	5	38.26	94.4	0.223
Sinusitis	140	139	1	39.09	103	0.1311
Sore throat	140	110	30	34.65	57.53	0.001895

Key N= Total Number of sample sites

No = Number of sample sites where a given health problem is

N_1= Number of sample sites where a given health problem is present.

X_0= mean fungal colony conts were the health problem is absent

X_1= Mean fungal colony conts were the health problem is absent.

DISCUSSION

The fungi isolated in this study are known allergenic, pathogenic and toxigenic fungi that induces allergic reactions in susceptible people. The predominant fungi isolated from this study isin consistent with the report of a study by Shelton*et al.,* 2002, Ayanbinpe*et al.,* 2010. *A.niger, A.fumigatus, P.notatum*, *C. Cladosporoides*and *C.clavata* are knownallergenic and pathogenic fungi that causes rhinitis, sinusitis, aspergillosis and hypersensitivity pneumonitis in immune compromised individuals. The high prevalence of these fungal isolates in indoor environments were due to high population, type of indoor activities, temperature and relative humidity of indoor environment, moisture problems, and area of study. It was observed that the indoor environments were contaminated through open doors and windows because 90% of homes, offices and hospitals sampled used natural ventilation. The dominance of *Aspergillus* and *Penicillium* species is probably due to theirxerophilic property, longer viability and speed and small sizes of their spores. These features make them air borne for longer period. This is similar with observation by Sussman and Ainsworth (2013).

The genera of yeast flora recorded in our study and reported as opportunistic pathogens by Rippon*et al.*, (1982) and McGinnis (2012) were *Candida, Cryptococcus, Rhodotorula, and Trichosporon. Candidaalbicans* isolated in this study, though at low prevalence is one of the *Candida*species most frequently associated with nosocomial and allergenic infections. Candida guilliermondii, C.tropicalis and C.crusei arespecies of Candida that were incriminated as etiologic agents in the different types of candidiasis, namelyvulvovaginal candidiasis, bronchial and pulmonary candidiasis, oropharyngeal candidiasis, esophagealcandidiasis, paronychia and onychomycosis as reported by Habibipour (2016). Rhodotorulaglutinis is acommon airborne contaminant that is reported in several acute infections of the lung, urinary tract, eye andmeninges (Menon et al., 2014).Regarding the comparison of cultured yeast propagules among different environments, the result of thisstudy showed that homes yielded higher count of

yeast isolates than offices and hospitals. This result is similar with the study of Pasanen et al. (1992), who reported that yeastswere more common and significantly higher in damp residences and old rural houses than other residence .This may be due to higher human activities and habits in homes like washing, sweeping, bed making andcooking that generates heat which increases the indoor relative humidity favoring the growth of yeast.

The dominant fungal species from nasal swabs of occupants of various environments were consistently the dominant airborne fungiin the corresponding indoor environment. This result is similar to the findings of astudy by Buzina et al. (2003), who isolated species of *Penicillium, Aspergillus, Cladosporium, Alternaria*and*Aureobasidium* as the most prevalent isolates from nasal mucosa of patients suffering from chronicrhinosinusitis and from healthy control persons. Study of Eidi*et al.,* (2016) also demonstrated similar fungifrom nasal cavity of healthy people. One of the nasal isolates *A.fumigatus* is the main agent of aspergillosis andallergic bronchopulmonaryaspergillosis in immunocompromised patients and a lot of other diseases (Hohl andFeldmesser, 2007). *Cladosporiumcladosporoides*is a strong allergenic fungi that severely affect asthmaticsand people with respiratory diseases. It also causes infections of the skin and toe nails, as well as sinuses andlungs.

The result of the correlation analysis is similar with the findings of Ponce-Caballero *et al*., (2013). This is reflected by the respiratoryhealth problems reported by some occupants of these indoor environments as shown in Table 3. The health effects observed are similar with report of Mendell*et al*., 2011 who stated that evidence from epidemiologic studies and meta-analyses showed indoor dampness and mould to be associated consistently with increased asthma development and exacerbation, dyspnea, wheeze, cough, respiratory infections, bronchitis, allergic rhinitis, eczema and upper respiratory tract. Summarily, the isolation of these airborne fungi from nasal of occupants of the various environments (Figure 1); the positive correlation between some airborne isolate and their nasal carriages, and the health problems reported by occupants of these indoor environments, offer supportto the role of these airborne fungi in respiratory health effects.

CONCLUSION

Considering the role of airborne fungi in respiratory diseases appropriate measures like environmental monitoring, housing improvement and establishment of awareness programme should be established to prevent growth and subsequent dissemination.

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