Microbiological Quality Assessment of Indoor Environment of Major Departments at a Medical Centre in Abia State, Nigeria

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Abstract: As people spend most of their time in enclosed surroundings, concerns about microbiological contaminants such as bacteria, fungus, and viruses in the air are critical. This study assessed the microbiological quality of indoor environment of major Departments ata Medical center in Umuahia, Abia State. Settled plate method was adopted, using three (3) 8.5 cm diameter Petri dishes containing different culture media for bacteria and fungi. Samplings plates of Nutrient agar, Blood agar (BA) and Sabouraud dextrose agar (NA, BA, and SDA) were exposed at about 3 meters apart. The result showed that the bacterial load in the Intensive Care Department (ICD), Postnatal Department (PN), and Emergency Department (ED) was higher in the morning hours than in the afternoon hours, with mean values of 2.40 $\times 10^4$ CFU/m^3, 2.85$\times$ 10^3 CFU/m^3, and 2.85$\times$ 10^3 CFU/m^3, respectively. The control sample had the lowest load in the morning (1.45 CFU/m^3) and the highest load in the afternoon (2.36 CFU/m^3).Fungi load observed in ICD and ED was higher in the morning and lesser in the afternoon, with a mean value of 6.85$\times$ 10^3CFU/m^3 and 5.89 $\times$ 10^3CFU/m^3, respectively. In PN, fungi load was higher in the afternoon, and lower in the morning with a mean value of 2.19 $\times$ 10^3 CFU/m^3. The control sample had the lowest load at 2.19 CFU/m^3 in the morning and 2.56 CFU/m^3 in the afternoon. Public health requires constant investigation of the aero-microbiological contamination of indoor air. It is recommended that natural ventilation through proper windows and doors be upheld.

Keywords: Bacteria, Fungi, Indoor microbiological quality, Medical Center

INTRODUCTION

Microbial pollution involves hundreds of viruses, bacteria and fungi that grow indoors when sufficient moisture is available. The presence of many biological agents in the indoor environment is due to dampness and inadequate ventilation. Excess moisture may result in increased chemical emissions from building materials and floor covers (Obumneme*et al*., 2019)**.** Indoor environments contain a complex mixture of live and dead microorganisms, fragments, toxins, allergens, volatile microbial organic compounds, and other chemicals (Asif*et al*., 2019; Ayodele *et al*., 2019)**.**As people spend most of their time in closed environments, concern about the concentrations of contaminants in the air in such places is justifiable. Bacteria, fungi, and viruses, may form biological air contaminants, and their distribution may vary according to the environment, the area within the environment, and the location within a given area, which may add them to the environment(Shittu *et al*., 2019). Indoor air quality is one of the most important factors that influence our general life quality. We breathe 10 m^3 air every day, and we spend 80–95% of our lives indoors. Indoor air pollution can result to health problems and even increased human mortality (Awad *et al*., 2018)**.**Heating, ventilation, and air conditioning systems may also be microbial sources that affect indoor air quality (IAQ) (Kakumanu *et al*., 2020).

There is growing evidence that exposure to biological agents in the indoor environment can have adverse health effects. The World Health Organization's report on indoor air quality, dampness, and mould (WHO, 2009) provided sufficient epidemiological evidence that residents of damp or mouldy buildings, both homes and public buildings, are at an increased risk of respiratory symptoms, infections, and asthma exacerbations (Udochukwu *et al*., 2016).Several factors can affect the indoor air quality, including the building's physical layout, the building's heating, ventilation, and air condition. The microbiological quality of the indoor environment is influenced by the outdoor climate,

the people who work in these facilities, and contaminants within and outside the structures (Brgoszewska *et al*., 2018).

Several health effects have been associated with fungal and bacterial species in the indoor environment. These health effects include rhinitis, upper respiratory symptoms, asthma, and other effects such as allergic skin reactions, tiredness, and headaches (Bjelić *et al*., 2020). Temperature and relative humidity appear to be significant factors influencing fungi and bacteria levels in the indoor environment. Mold and dampness can be reduced by improved ventilation that may reduce any potential health effects of fungi and bacteria indoors (Al-Taweil*et al*., 2020). Therefore,this study was aimed at assessing the microbiological quality of indoors environment of major Departments at a medical center in Umuahia, Abia State.

MATERIALS AND METHODS

The Study Area

This study was carried out in a medical center in Abia State. The State is located on Latitude 5.4309°N and Longitude 7.5247°E. Abia State occupies about 5,243.7sq.km with a population density of 450/km. It is bounded in the north and north-east by Ebonyi State. It is bounded in the West by Imo State, to the east and South-east are Cross-River, and Akwa Ibom State, and to the South, is Rivers State. It is bounded in the west by Imo state, to the East and Southeast by Cross River and Akwa Ibom State, and to the South is Rivers State. By the National Bureau of Statistics' projection, based on the 2006 census and the National Population Commission Allocation 2006, Abia State is about 2,833,999 in the population (NPC, 2006).

Microbiological air sampling

Ethical clearance was first obtained from the Heads of the selected Departments in the Medical center to access the sample collection wards. The settle plate method described by (Pasquarella *et al*., 2000) was adopted. Bacteria were collected on Nutrient agar (NA) and Blood agar (BA) to which an antifungal agent (Griseofulvin) was incorporated to inhibit the growth of fungi; In contrast, fungi were collected on Sabouraud dextrose agar (SDA) plates to which an antibacterial agent (Chloramphenicol) was incorporated to inhibit the growth of bacteria.

The media plates were placed on a table 2m above the floorwhile samplingwas carried out at two different periods of the day: Morning (7.00 - 9.00 am) and Afternoon (3.00- 5.00 pm). A set of three plates (NA, BA, and SDA) wereexposed with their lids open, about 2 meters apart, and allowed to stay for 30 minutes. Afterward, the plates were covered, and transported to the Microbiology Laboratory and incubated at 37°C for 24-48 hours for bacteria and at 25°C for 5-7 days for fungi as described by Naruka and Gaur(2013).Temperature and Humidity were measured twice daily (morning and afternoon) using Thomas traceable Temperature and relative humidity meter (TH 338).

Determination of Microbial Density

Following incubation of culture plates, bacterial and fungal colony forming units (CFU) were enumerated using colonies counter. After the microbial density of the resultant colonies on each plate that was determined, the colonial morphology of the different colonies formed was noted and identical colonies were sub-cultured into Nutrient Agar (NA) or Sabouraud's Dextrose Agar (SDA) plates, incubated appropriately, and stored for further identification and characterization. Humidity and temperature at each ward and department were determined using a digital thermo-hygrometer with an indoor temperature and relative humidity. Data analysis was conducted using IBM SPSS Statistics program Ver 26.A regression test was used to examine the relationship between fungi, bacteria and Humidity, temperature.

The statistical t-test and One-way ANOVA were used to compare independent variables for the continuous variables as count of colonies at a significant level (P> 0.05).

RESULTS

Table 1 shows the bacteria load at different sampling sites. The result showed that the bacterial load in the Intensive Care Department (ICD), Postnatal Department (PND), and Emergency Department (ED) was higher in the morning hours than in the afternoon hours, with mean values of 2.40 × 104 CFU/m3, 2.85 × 103 CFU/m3, and 2.85 × 103 CFU/m3, respectively. The control sample had the lowest load in the morning (1.45 CFU/m3) and the highest load in the afternoon (2.36 CFU/m3).

Table 1. Bacteria load, associated with different sampling locations

Location	Time	Bacterial load (CFU/m^3)
ICD	Afternoon	1.57×10^4
	Morning	3.24×10^4
	Total	2.40×10^4
ED	Afternoon	8.44×10^4
	Morning	2.16×10^4
	Total	1.50×10^4
PN	Afternoon	4.75×10^4
	Morning	8.44×10^4
	Total	6.59×10^4
Control	Afternoon	1.45×10^4
	Morning	2.36×10^4
	Total	1.91×10^4

ICD: Intensive Care Department, ED: Emergency Department, PN: Postnatal Department

Table 2 shows the fungi load at different sampling sites and time. From the result, the Fungi load observed in ICD and ED was higher in the morning and lesser in the afternoon, with a mean value of 6.85 × 103 CFU/m3 and 5.89 × 103 CFU/m3, respectively. In PND, fungi load was higher in the afternoon, and lower in the morning with a mean value of 2.19 × 103 CFU/m3. The control sample had the lowest load at 2.19 CFU/m3 in the morning and 2.56 CFU/m3 in the afternoon.

Table 2. Fungi load, associated with different sampling locations

Location	Time	Fungi (CFU/m^3)
ICD	Afternoon	6.45×10^3
	Morning	6.85×10^3
	Total	6.65×10^3
ED	Afternoon	1.29×10^4
	Morning	5.89×10^3
	Total	9.38×10^3
PN	Afternoon	4.88×10^3
	Morning	2.19×10^3
	Total	3.53×10^3
Control	Afternoon	2.56×10^3
	Morning	2.19×10^3
	Total	2.38×10^3

ICD: Intensive Care Department, ED: Emergency Department, PN: Postnatal Department

Table 3 shows the Temperature (^{o} C) and Humidity (%) levels at different sampling sites and expressed time. The humidity (%) observed in ICD was lower in the afternoon and higher in the morning, with an average of 72.0 %. In ED, Humidity was lower in the afternoon and higher in the morning with an average mean of 70.0 In PN, Humidity was lower in the afternoon and higher in the morning, with an average mean of 70.5 %. The means of ICD, ED, PN, and Control did not differ significantly from each other (P>0.05). The control sample had its humidity levels at 64.0 CFU/m^3 in the afternoon and 75.0 CFU/m^3 in the morning.

Table 3. Evaluation of Temperature (^{o} C) and Humidity (%) at Sampling Locations

Location	Time	Temperature (°C)	Humidity (%)
ICD	Afternoon	31.3 ± 0.4	68.0 ±1.0
	Morning	30.5 ± 0.7	76.0 ±7.0
	Total	30.9^{c}	72.0b^{c}
ED	Afternoon	27.7 ± 0.1	64.3 ±3.5
	Morning	29.2 ± 0.1	75.7 ±2.1
	Total	28.5^{ab}	70.0^{b}
PN	Afternoon	28.4 ± 0.7	62.0 ±5.3
	Morning	28.2 ± 0.3	79.0 ±1.0
	Total	28.3^{a}	70.5^{bc}
Control	Afternoon	28.7 ± 0.4	64.0 ±2.4
	Morning	29.2 ± 0.3	75.0 ± 0.7
	Total	28.95^{a}	69.5^{a}

ICD: Intensive Care Department, ED: Emergency Department, PN: Postnatal Department

Table 4 shows a Regression summary of Bacteria load with humidity and temperature at different sampling sites and expressed time. From the result, the dependent relationship between the percentages Humidity, Temperature with bacteria load was significantly (P≤0.05).

Table 4. Regression Summary of Bacteria load with Humidity and Temperature

	B	Std. Error	Beta	t	Sig.
(Constant)	-33695.5	40023.54		-0.842	
Humidity	-917.74	291.302	-0.568	-3.15	0.016*
Temperature	2854.852	1047.964	0.489	2.724	0.03*
R	0.884a				
R Square	0.781				
Sig. F Change	0.01***				

Table 5 shows a Regression summary of Fungi load with humidity and temperature at different sampling sites and expressed time. The dependent relationship between humidity and temperature with bacteria load was not significantly (P>0.05).

Table 5. Regression Summary of Fungi load with Humidity and Temperature

	B	Std. Error	Beta	t	Sig.
(Constant)	9827.507	24688.74		0.398	0.702
Humidity	-317.03	179.691	-0.519	-1.764	0.121
Temperature	590.141	646.442	0.267	0.913	0.392
R	0.646a				
R Square	0.418				
Sig. F Change	0.258				

DISCUSSION

Although there was no pattern observed in the variety of fungi and bacterial load in the morning and afternoon hours, a seemingly increase observed in the morning in most of the sites may suggest that some of the observed load in the environment may have been a result of human activities and may not be harmful as it is introduced to the air through human activities. Bacteria from outdoor air and those originating from people areharmless; bacteria growing actively or accumulating in the indoor environment, however, may affect health, but this has not been studied extensively (Abbasi *et al*., 2019).

Bacteria and fungi coexisted in the air in all of the hospital units, indicating that the spores delivered by air are those of organisms that can coexist. Bacteria and fungi both flourish in the same environments;this is in tandem with Almoffarreh *et al*. (2016) findings.Omolola *et al*. (2018) state that Common levels of cultivable microorganisms from air vary from 10 to 10^4CFU/m^3;this study indicates the existence of a significant level of microbial contamination in the air which is, nevertheless, below or within the normal range of pollution found in similar studies performed in other cities (Adeleye *et al*., 2018; Larrey *et al*., 2020; Okpalanozie *et al*., 2018). In all the sites, a higher bacteria load was observed over the fungi load; this agrees with the report of Enitan *et al*. (2017), which suggests that people may be more endangered to hazards from bacteria load over that of fungi. However, this does not rule possible hazards known to come from fungi. WHO (2009) reported that many fungal species produce type I allergens, and immunoglobulin (Ig) E sensitization to the most familiar outdoor and indoor fungal species, like *Alternaria, penicillium, Aspergillus,* and *Cladosporium* spp., is strongly associated with allergic respiratory disease, especially asthma. They are found in spores, hyphae, and fungal fragments but are released in more significant amounts during germination and mycelial growth, which may occur inside the airways (Umana*et al*., 2019).

As observed in this study, the higher bacterial population in air disagrees with the report of Asif*et al*. (2019).The findings showed that viable fungal counts frequently exceeded airborne bacterial concentrations, implying that fungal spores are well suited to survive in the absence of available water and nutrients in the environment, according to him. As observed in this study across the various sites, the microbial counts varied below most standards limits. According to Naruka and Gaur (2013), the air is considered contaminated in 1000 viable colony-forming units in a cubic meter of air. The levels of fungal spores not to exceed 500 CFU/m^3 in residential buildings. The Swedish and Singaporean standards set the limit for bacteria to be not more than 500 CFU/m^3 and 300 CFU/m^3 for fungi. Keeping these limits in mind,it is evident that this study's observed microbial levels were lower as it disagrees with the observation by Brągoszewska*et al*. (2018).

The regression analysis observed that the dependent relationship between humidity and temperature with bacteria load was significantly ($P \leq 0.05$)but it was not significant ($P > 0.05$) in relation to fungal load. Similar findings were made by Sidra *et al.* (2015) in assessing airborne microflora in the indoor micro-environments of residential houses of Lahore, Pakistan. Factors that may affect the rate of release of spores or fungal fragments include air velocity, time, colony structure, desiccation stress, moisture condition, and vibration. These factors may affect the rate of aerosolization of spores and fungal fragments differently (Obumneme *et al*., 2019).Fungal spores are ubiquitous in outdoor air, ranging from less than 100 to more than 105 spores/m^3. The indoor levels are usually lower than those outdoors but may be increased by unintended fungal growth in damp buildings (Larrey *et al*., 2020). Studies in damp indoor environments have shown a wide diversity of fungal species and generally due to differences in climate, indoor temperature,humidity, building materials, and differences in sample collection and subsequent culture. Fungi are often found on wet window frames and damp walls of bedrooms, living rooms, and kitchens (Udochukwu*et al.,* 2016).

The medical center's humidity levels were generally high, which may have increased the survivability of fungi spores in the air. Fungal growth can occur only in the presence of moisture, and many fungi grow readily on any surface that becomes wet or moistened; that is, virtually all fungi readily germinate and grow on substrates in equilibrium with relative humidity below saturation (i.e., below 100%) (WHO, 2009). The observed correlation between humidity to bacteria availability superposes the fact; bacteria require higher water activities than most fungi. Temperature and nutrient demands are generally met in most indoor environments. Surprisingly, few studies have been conducted on bacterial growth in damp houses, suggesting that bacteria grow in the same areas as fungi. In particular, *Streptomycetes* (Gram-positive spore-forming bacteria that are not normal indoor flora in urban environments) may grow on damp or wet building materials (Ayodele *et al*., 2019). Their presence in indoor air may, therefore, indicate that a building has a moisture problem. Although no clear association with dampness has been found, it has been suggested that endotoxins from Gram-negative bacteria occur at increased levels in damp buildings (Wemedo and Beke, 2020).

CONCLUSION

Public health requires constant investigation of the aero-microbiological contamination of the air. It should be highlighted that, considering this study was able to establish that air remains an effective vehicle for the movement of viable microbial spores. The microbial load did not take a pattern; hence, no sample units of the hospital maybe said to be more endangered. The study also confirms that humidity and temperature are significantly causal for bacteria load ($p \leq 0.05$). There was no discernible pattern in the variations of load, regardless of the time of data collection (morning or afternoon); however, they differed with time, implying that the difference in time affected the load of microorganisms, which was further confirmed by the significant effect ($p \leq 0.05$) of time as a factor on microbial load as seen in this study.

This study recommends that natural ventilation through proper windows and doors openings should be upheld. Educational programs to increase awareness about indoor air pollution in hospitals should be promoted.

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