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Assessment of The Indoor Microbial Air Quality Of A Tertiary Healthcare Institution

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ABSTRACT

This study investigated the presence and concentrations of airborne bacteria and fungi in the indoor environment of a tertiary healthcare institution in South-Eastern Nigeria using the following sampling sites: Accident and emergency ward (A and E), Intensive Care Unit (ICU), Main Operating Theatre (MT), Microbiology Laboratory (ML), Surgical Ward (SW) and Administrative Department (AD) which was the control. The indoor temperatures and relative humidity of the sites were also measured and assessed in relation to the level of indoor microbial air contamination. The results were then compared with indoor microbial air quality (IMAQ) standards for indoor hospital environments. Mean bacterial Total Viable Counts exceeded accepted limits in all the sampled sites both during the morning (A and E: 747CFUs, AD: 388CFUs, ICU: 388CFUs, MT: 546CFUs, ML: 905CFUs, and SW: 603CFUs) and afternoon periods (A and E: 618CFUs, AD: 661CFUs, ICU: 259CFUs, MT: 216CFUs, ML: 661CFUs, and SW: 503CFUs) while Mean fungal TVCs exceeded accepted limits in four of the six sampled locations both during the morning (A and E: 259CFUs, AD: 402CFUs, ML: 445CFUs, and SW: 216CFUs) and afternoon periods (A and E; 172CFUs, AD: 302CFUs, ML: 503CFUs and SW: 273CFUs). This may be as a result of overcrowding, inadequate ventilation, and high temperatures prevalent in those locations throughout the sampling period, the inadequacy of the cleaning agents in terms of their bactericidal or bacteriostatic activity, as well as surgical smoke emitted from surgical energy devices. Thus, the IMAQ levels of studied hospital were not satisfactory. It is therefore recommended that conscious and concerted efforts be made to minimize indoor microbial air contamination and ensure that good IMAQ is maintained within the Hospital environment.

Keywords: Indoor microbial air quality; hospital acquired infections; healthcare institution

INTRODUCTION

Indoor Microbial Air Quality (IMAQ) is the quality of the microbial air within a building (USEPA, 2017) as well as the microbial air quality within and around buildings and structures, especially as it relates to the health and comfort of building occupants (USEPA, 2018).Indoor microbial air contaminants are microorganisms and their biological by-products which may cause disease and allergic responses in building occupants (ASHE, 2016). One of the obligations of a hospital is to establish and maintain a safe and functional environment for its occupants (ASHE, 2016) through putting in place and maintaining mechanisms for a good IMAQ.

Unlike in developed countries, where surveillance systems have been put in place to help reduce the prevalence of hospital acquired infections (HAIs), developing countries like Nigeria undertake limited surveillance (monitoring) activities due to social, economic, and healthcare deficiencies (Ige *et al*., 2011; Nejad *et al.*, 2011). This study will provide surveillance information and other data that will guide interventions against HAIs, especially as it concerns Nigeria and be used to maintain or improve the IMAQ levels of the hospital's facilities such that there is an improvement in patient and staff safety, comfort, and productivity as well as a reduction in the risk of HAIs.

MATERIALSAND METHODS

Research Design

This study investigated the presence and concentrations of airborne bacteria and fungi in the indoor environment of a Federal tertiary healthcare institution in Umuahia using the following sampling sites: Accident and emergency ward (A and E), Intensive Care Unit (ICU), Main Operating Theatre (MT), Microbiology Laboratory (ML), Surgical Ward (SW), and Administrative Department (AD), which served as control.
All six sites were assessed for important indoor microbial air contaminants. Five of the six sites were selected due to the regularity of use by staff and patients as well as the presence of susceptible or vulnerable patients. The sixth(control) site was selected because it is less regularly used by patients. Indoor air sampling was carried out in October 2019 (rainy season) over a two-week period.

A mini thermo-anemometer (Thomas Scientific model) was used to measure indoor temperature and relative humidity according to Asif *et al.*(2018).

Microbial contaminant sampling was carried out using passive method (Pasquarella *et al.*, 2000). Petri dishes were prepared in triplicates for reproducibility and to improve accuracy. The mean values of the TVCs were reported. The number of patients and staff during the period of sampling per site were also noted.

SAMPLING OF AIRBORNE BACTERIA AND FUNGI

Samples of airborne bacteria and fungi were collected twice daily (9am and 3pm) in triplicates for two weeks in each sampling site. Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) media were prepared according to the manufacturer's instructions and autoclaved

at 121ºC for 15 minutes. 30ml of each culture media were poured into 9cm Petri dishes separately and allowed to solidify. Preparation of media was carried out in the Microbiology laboratory of the hospital to be assessed.

The TSA was used to culture the airborne bacteria while the SDA was used for the fungi. Each Petri dish was left open in the sampling site for 1 hour,1m from the floor and 1m away from walls or any relevant physical obstacle (9). Subsequently, the dishes were covered, sealed, and incubated. The TSA plates were incubated for 24 to 48 hours at 37ºC while those containing PDA were incubated for 72 hours at 28.5ºC (Asif *et al.*, 2018).

DETERMINATION OF TOTAL VIABLE COUNTS (TVC)

The colonies present were counted using a colony counter and expressed as colony forming units per cubic metre (cfu/m^3) of air. The total viable counts (TVC) in cfu/m^3 were calculated using Omeliansky's formula:

$$N = 5a \times 10^4/(bt)$$

where **N** is the microbial cfu/m^3 of indoor air, **a** is the number of colonies per Petri dish, **b** is the surface area of dish in square centimetres, and **t** is the exposure time in minutes (Awad and Mawla, 2012). Mean values obtained were compared with WHO and American Conference of Governmental Industrial Hygienists (ACGIH)guidelines for IAQ (biological contaminants) (ACGIH, 1989; WHO, 1990).

RESULTS

ISOLATION AND IDENTIFICATION OF BACTERIA AND FUNGI

Colonies of airborne bacteria obtained using TSA were first identified based on their cultural characteristics after which they were subjected to Gram staining and microscopy for further identification of individual organisms. The bacteria were also identified based on their biochemical properties using biochemical tests such as catalase and oxidase tests (Cheesbrough, 2006).

Colonies of airborne fungi obtained using PDA were characterized based on their morphology. They were also subjected to Lactophenol cotton blue staining and microscopy to identify the morphology of individual fungi and spore formation (Cheesbrough, 2006). Isolation and identification of bacteria and fungi were carried out in the Microbiology laboratory of the hospital was assessed.

STATISTICAL ANALYSIS OF DATA

The data produced from the sampling, isolation and identification of microbial contaminants were analyzed using Microsoft Excel (Microsoft Corporation, USA) and Statistical Package for Social Sciences (SPSS) version 26 (IBM Corporation, USA).One-way ANOVA was used to analyse the statistical differences among different sampling sites in the hospital, as well as between morning and evening periods (Asif *et al.*, 2018).

The ethical clearance to undertake this study was obtained from the Health

Research Ethics Committee (HREC) of the tertiary healthcare institution, while informed consent was sought and obtained from the heads-of-department of all the sampled sites.

Table 1: Description of sampling sites and average number of occupants

Site	Type of Heating, Ventilation and Air Conditioning (HVAC) system	Period	Average number of occupants
A & E	Natural ventilation only.	24 hours	29 (8 staff, 21 patients and caregivers)
AD	Natural ventilation only.	8-12 hours	10 staff
ICU	Natural ventilation with AC	24 hours	5 (4 staff, 1 patient)
MT	Artificial ventilation. (AC & Fan) only during operation. Natural ventilation post-operation.	24 hours	9 during operations (8 staff, 1 patient) 2 staff post-operation
ML	Natural ventilation only.	8-12 hours	10 staff
SW	Natural ventilation only.	24 hours	22 (7 staff, 15 patients and caregivers)

Each of the six sites selected had different HVAC systems, occupational periods and average number of occupants as shown in Table 1. All the locations remain functional with staff and patients present except AD which has no patients therein because it serves mainly hospital administrative purposes while ML is often visited by patients, but the patients are not allowed into the laboratory facility itself. The patients remained in the ML vicinity while they are attended. ML is also the smallest in terms of area covered of the assessed sites.

Mean indoor temperatures ranged from 28.2^{o}C to 32.1^{o}C in the morning and from 25.1^{o}C to 31.8^{o}C in the afternoon (Figure 1). Mean relative humidity ranged from 67% to 79% in the morning and from 65% to 83% in the afternoon. AD (32.1^{o}C) and ML (31.8^{o}C) were the warmest locations in the morning and afternoon respectively while ICU (28.2^{o}C in the morning and 25.1^{o}C in the afternoon) was the coolest of all the sites. ICU was also the most humid in the morning along with SW, with a relative humidity of 79% while A and E was the most humid in the afternoon with a relative humidity of 83%, closely followed by ICU with a relative humidity of 80%.

There were significant differences ($P<0.05$) between the mean temperature and relative humidity in AD (control) and the mean temperature and relative humidity in other locations in the morning. However, there was no significant difference between mean temperature in AD (control) and that in A and E, and SW in the afternoon as well as no significance between mean relative humidity (RH) in the control and mean RH

in ML during the same period. There was significant difference (P<0.05) between mean temperature in the morning and in the afternoon in AD, ICU, ML and SW but not in A and E and MT. Mean RH did not differ significantly between morning and afternoon in AD, ICU and MT but differed significantly in A and E, ML and S.

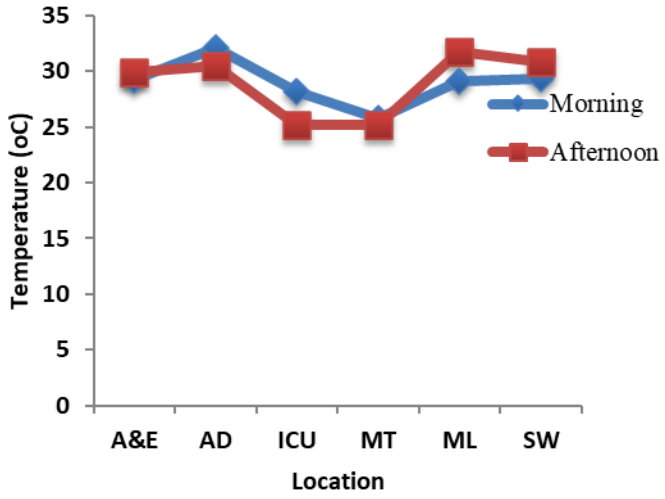

Figure 1: Mean temperature in each of the sites during the morning and afternoon periods.

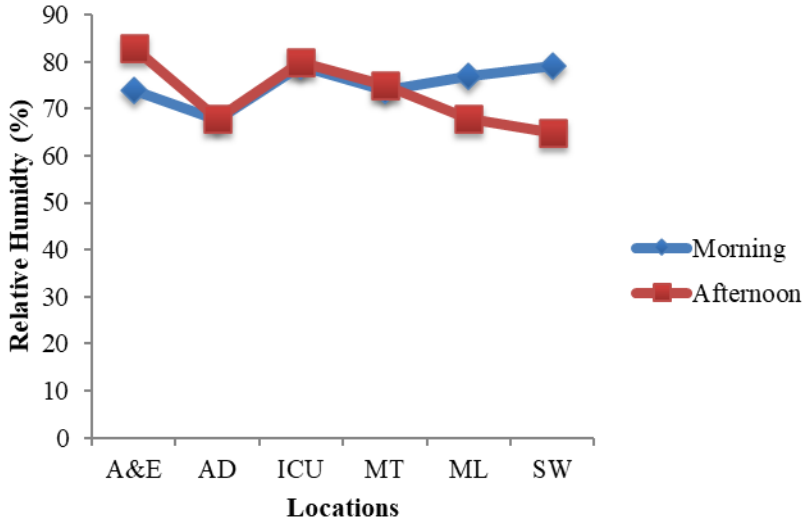

Figure 2: Mean relative humidity in each of the sites during the morning and afternoon periods.

Table 2: Colony Forming Units (CFU) per m^3 of the Sampled Sites during The Morning and Afternoon periods at the tertiary healthcare institution.

Location	Morning	
	Bacteria	Fungi

A and E	747^{b}	259^{c}
AD	388^{e}	402^{b}
ICU	388^{e}	14^{f}
MT	546^{f}	43^{e}
ML	905^{a}	445^{a}
SW	603^{c}	216^{d}
	Afternoon	
Location	**Bacteria**	**Fungi**
A and E	618^{b}	172^{d}
AD	661^{a}	302^{b}
ICU	259^{d}	57^{e}
MT	216^{e}	14^{f}
ML	661^{a}	503^{a}
SW	503^{c}	273^{c}

Values are given in colony forming units (CFUs). Values with different superscript down a column are significantly different from each other ($p<0.05$). Statistical differences among different sampling sites were analysed.

Mean indoor airborne bacterial TVCs ranged from 388CFUs to 905CFUs in the morning and from 216CFUs to 661CFUs in the afternoon (Figures 3 and 4). ML had the highest mean bacterial TVC (905 CFUs) while MT had the lowest TVC(388CFUs) in the morning. In the afternoon, AD and ML had the highest bacterial TVCs (661CFUs each) while MT again had the lowest TVC (216 CFUs). All the bacterial TVCs taken from the sampled locations exceeded acceptable standards for indoor airborne bacteria (WHO 1990).

The bacterial organisms isolated were *Bacillus sp* (26.7%), *Enterobacter sp* (1.3%), *Micrococcus luteus* (10.6%), *Pseudomonas aeruginosa* (3.1%), *Staphylococcus aureus* (44.5%) and *Streptococcus sp* (13.7%) (Table 4). *S. aureus* was the most abundant bacterium found in all the sampled sites while *Enterobacter* was the least prevalent. *Bacillus sp, M. luteus, S. aureus* and *Streptococcus* were the most prevalent as they were isolated from the six sites both during the morning and afternoon periods. *Enterobacter sp* was isolated from A and E and SW only while *P. aeruginosa* was isolated from AD and SW only.

In the morning, there was significant difference ($P<0.05$) between the mean bacterial TVC of AD (control) and those of the other sites except ICU whose bacterial TVC did not vary from that of AD. In the afternoon, AD differed significantly from the other locations except ML where the mean TVC was not different from that of AD.

All the sites sampled varied significantly ($P<0.05$) in mean bacterial TVC between the morning and afternoon periods. However, while all the sites showed relatively high TVCs in the morning and relatively low TVCs in the afternoon, AD

(control) showed low mean TVC in the morning and high TVC in the afternoon.

Table 3: Mean Concentrations of Indoor Bacterial and Fungal Air Contaminants during the Morning and Afternoon Periods

Bacterial Counts		
Locations	**Morning**	**Afternoon**
A and E	747[a]	618[b]
AD	388[b]	661[a]
ICU	388[a]	259[b]
MT	546[a]	216[b]
ML	905[a]	661[b]
SW	603	503[b]
Fungal Counts		
Locations	**Morning**	**Afternoon**
A and E	259[a]	172[b]
AD	402[a]	302[b]
ICU	14[b]	57[a]
MT	43[a]	14[b]
ML	445[b]	503[a]
SW	216[b]	273[a]

Values are given in colony forming units (CFUs). Values with different superscript across a row are significantly different from each other ($p<0.05$). Values are the mean ± standard deviation of three replications for each parameter. Statistical differences between morning and afternoon periods were analysed.

Mean indoor airborne fungal TVC ranged from 14CFUs to 445CFUs in the morning and from 14CFUs to 503CFUs in the afternoon (Table 3). ML had the highest mean TVC in the morning (445CFUs) while ICU had the lowest (14CFUs). In the afternoon, ML again had the highest mean TVC (503CFUs) while MT had the lowest (14CFUs). Of all the sites sampled, only ICU and MT did not exceed accepted standards for indoor airborne fungi both in the morning and in the afternoon.

The fungal organisms isolated included *Aspergillus fumigatus* (36.2% of total mean fungal TVC), *Penicillium sp* (51.1%) and *Cladosporium sp* (12.7%) (Table 4). Both *A. fumigatus* and *Penicillum sp* were isolated from all the sampled sites at one point or the other during the sampling periods while *Cladosporium sp* was isolated from A and E, AD, ML and SW but not from ICU and MT. Thus, *Penicillium sp* was the most prevalent fungus while *Cladosporium* was the least abundant. In the morning, there was significant difference ($P<0.05$) between the mean fungal TVC in AD (control) and those of the other locations (Table 2). This was also the case with the afternoon period where mean fungal TVC in AD differed significantly from those of other sites.

Mean fungal TVC varied significantly (P<0.05) between morning and afternoon periods in the sampled locations (Table 3). However, while A and E, AD and MT showed relatively high TVCs in the morning and relatively low TVCs in the afternoon, ICU, ML and SW showed low mean TVCs in the morning and high TVCs in the afternoon.

Table 4: Mean TVC and Percentage of Specified Airborne Bacteria and Fungi Isolated from the Sampled Sites during the Morning and Afternoon Periods

Characteristics	A & E		AD		ICU		MT		ML		SW		Total
	M	N	M	N	M	N	M	N	M	N	M	N	
Total TVC (B)	**747**	**618**	**388**	**661**	**388**	**259**	**546**	**216**	**905**	**661**	**603**	**503**	**6,495**
													100%
Bacillus	**201**	**244**	**129**	**230**	**14**	**72**	**144**	**72**	**244**	**172**	**86**	**129**	**1,737**
	26.9%	*39.5%*	*33.3%*	*34.8%*	*3.7%*	*27.8%*	*26.3%*	*33.3%*	*27%*	*26.1%*	*14.3%*	*25.7%*	*26.7%*
Enterobacter	**43**	**14**									**29**		**86**
	5.8%	*2.3%*	-	-	-	-	-	-	-	-	*4.8%*	-	*1.3%*
Streptococcus	**158**	**43**	**86**	**43**	**101**	**43**	**86**	**43**	**186**	**72**	**14**	**14**	**889**
	21.2%	*6.9%*	*22.2%*	*6.5%*	*25.9%*	*16.7%*	*15.8%*	*20%*	*20.6%*	*10.9%*	*2.4%*	*2.9%*	*13.7%*
Staphylococcus aureus	**288**	**216**	**115**	**273**	**258**	**130**	**244**	**86**	**359**	**316**	**388**	**216**	**2892**
	38.5%	*34.9%*	*29.6%*	*41.3%*	*66.7%*	*50%*	*44.7%*	*40%*	*39.7%*	*47.8%*	*64.3%*	*42.9%*	*44.5%*
Micrococcus luteus	**57**	**101**	**43**	**72**	**14**	**14**	**72**	**15**	**115**	**101**	**14**	**72**	**690**
	7.7%	*16%*	*11.1%*	*10.9%*	*3.7%*	*5.6%*	*13.2%*	*6.7%*	*12.7%*	*15.2%*	*2.4%*	*14.3%*	*10.6%*
Pseudomonas aeruginosa	-	-	**14**	**43**	-	-	-	-	-	-	**72**	**72**	**201**
			3.7%	*6.5%*							*11.9%*	*14.3%*	*3.1%*
Total TVC (Fungi)	**259**	**172**	**402**	**302**	**14**	**57**	**43**	**14**	**445**	**503**	**216**	**273**	**2,700**
													100%
Aspergillus fumigatus	**130**	**57**	**101**	**172**	-	**14**	**29**	**14**	**144**	**158**	**68**	**91**	**978**
	50%	*33.3%*	*25%*	*57.1%*		*25%*	*66.7%*	*100%*	*32.3%*	*31.4%*	*31.6%*	*33.3%*	*36.2%*
Penicillium	**155**	**86**	**230**	**101**	**14**	**43**	**14**	-	**215**	**273**	**125**	**164**	**380**
	44.4%	*50%*	*57.1%*	*33.3%*	*100%*	*75%*	*33.3%*		*48.4%*	*54.3%*	*57.9%*	*60%*	*51.1%*
Cladosporium	**14**	**29**	**72**	**29**	-	-	-	-	**86**	**72**	**23**	**18**	**342**
	5.6%	*16.7%*	*17.9%*	*9.5%*					*19.4%*	*14.3%*	*10.5%*	*6.7%*	*12.7%*

M: Morning; N: Afternoon (-): Organism not seen

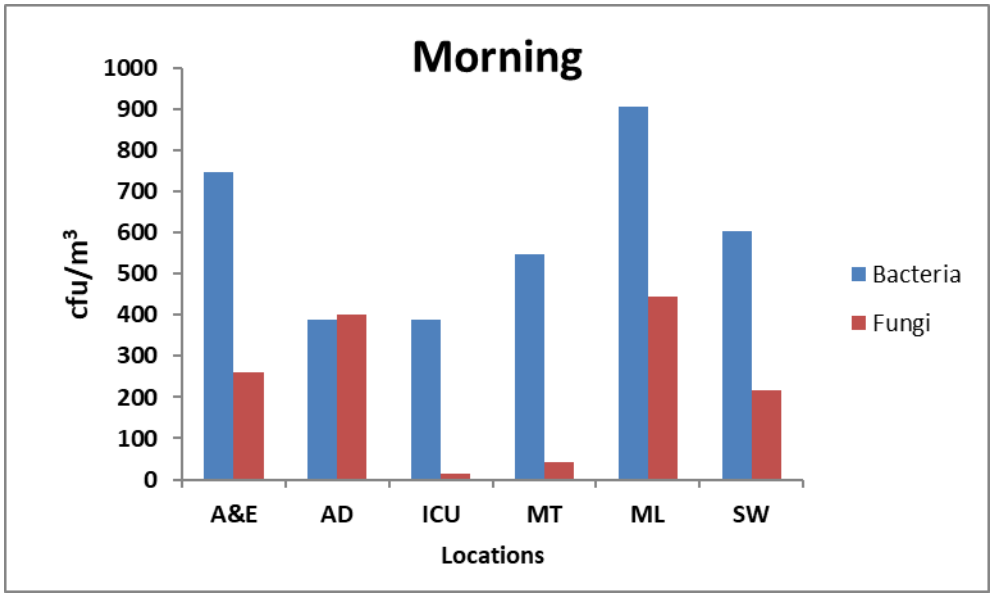

Figure 3: Mean microbial air contaminant TVCs in each of the sampled locations during the morning period.

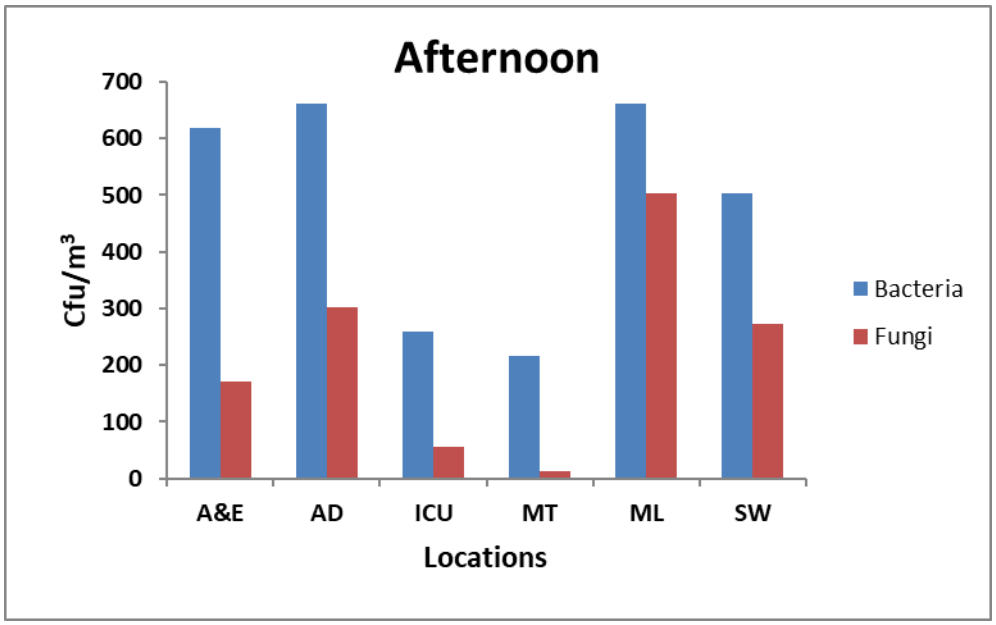

Figure 4: Mean microbial air contaminant TVCs in each of the sampled locations during the afternoon period.

DISCUSSION

This study showed that Tertiary healthcare institution had levels of indoor microbial air contamination which exceeded WHO and ACGIH standards (WHO 1990; WHO, 2010). The IAQ of the hospital was affected by factors such as ventilation, temperature, relative humidity, type and number of occupants and the nature of activities carried out therein.

A and E had the highest average number of occupants (29 persons), followed by SW with 22 persons (Table 1). On the

other hand, ICU had the lowest average number of occupants (5 persons) followed by MT with 9 occupants. Although, ML had neither the highest nor the lowest average occupancy, it exceeded its capacity such that patients were not allowed in. This may have accounted for the high level of indoor microbial air contamination in it.

Mean temperature and relative humidity exceeded accepted guidelines in all the sampled location. This was due to improper building design, inadequate ventilation, and overcrowding (WHO, 2010).High relative humidity has been associated with the triggering and worsening of upper respiratory tract diseases (WHO, 1990). This could also affect occupants of ICU and SW, most of whom are immunocompromised.

Mean bacterial TVCs exceeded accepted limits in all the sampled sites both during the morning and afternoon periods. This may be as a result of inadequate ventilation and high temperatures prevalent in those locations throughout the sampling period (WHO, 1990, Cheesbrough, 2006). This is further compounded by overcrowding in sites such as A and E, AD and ML.

S. aureus was the most prevalent bacterium in all the sites both in morning and the afternoon as a result of being a constituent of normal human microfauna. This is a cause for concern as the organism is renowned as an unselective opportunistic pathogen as well as for possessing antibiotics resistance (Cheesbrough, 2006, Asif *et al.*, 2018).

This study agrees with that of Agbagwa and Onyemaechi (2014) which showed *S. aureus* to be the most prevalent bacterium in hospital environments in Nigeria. That *Staphylococcus*, *Streptococcus* and *Bacillus* were the most prevalent bacteria in this study is in agreement with certain findings (Agbagwa and Onyemaechi, 2014; Emuren and Ordinioha, 2016; Asif *et al.*, 2018 and Pati, 2018) which showed them to be some of the most commonly found bacteria in hospital environments.

Bacillus sp was the second most prevalent bacterium and its presence may be due to its ability to produce spores which thrive in warm temperatures (Cheesbrough, 2006). Also, diathermic surgical procedures carried out in MT produce surgical smoke which may be a source of *Bacillus sp*. and other bacterial pathogens (McCormick, 2008 and Mowbray *et al*., 2013).

Mean fungal TVCs exceeded accepted limits in four of the six sampled locations (A and E, AD, ML and SW). This may have been as a result of their environments being very warm, humid, and overcrowded. Such conditions aid the proliferation of fungal spores (WHO, 1990, Cheesbrough, 2006).

The presence of *Aspergillus*, *Penicillium* and *Cladosporium* in the indoor air of the hospital is consistent with some studies (El-Sharkawy and Noweir, 2014; Asif *et al.*, 2018) which showed these fungi to be some of the most common in hospital environments as well as the commonest fungi found indoors (Bensch, 2018).

The abundance of *Penicillium* may be explained by the ability of its spores to thrive even in unfavourable conditions and the ability of the organism to colonise a wide variety of environments, as longs as there is dampness (Mold Help, 2019 and Ward, 2019). Its presence may also imply that the buildings from which it was isolated are damaged by water, other weather conditions or age (Mold Help, 2019). Consequently, its presence may contribute to the incidence of allergies, asthma, pulmonary inflammation, and other respiratory tract diseases in the hospital especially in persons whose immune system is compromised (Mold Help, 2019 and Ward, 2019).

This microbiological assessment showed bacterial counts to be much higher than fungal counts in all the sampled sites throughout the sampling period (morning and afternoon). This may be as a result of the sites exceeding their capacity or the inadequacy of the cleaning agents in terms of their bacteriocidal or bacteriostatic activities (Guns *et al.*, 2013). This assessment is consistent with studies which showed bacterial counts to be higher than fungal counts in the indoor environments of health care institutions (Agbagwa and Onyemaechi, 2014).

CONCLUSION

The microbiological assessment of the tertiary healthcare institution's indoor air showed high levels of microbial air contamination across all sampled locations both during the morning and afternoon periods. This was due to the prevailing warm and humid atmosphere which supports the growth of microbes as well as high numbers of occupants which serve as carriers of these organisms or their spores.

More efficient and frequent cleaning and disinfection as well as low occupancy ensured that ICU and MT had the lowest microbial load throughout the sampling period such that fungal counts in both sites were well within accepted limits, but bacterial counts still exceeded recommended limits. Other sites had alarmingly high concentrations of airborne bacteria and unacceptable levels of fungal concentrations which may contribute to the incidence of nosocomial infections in the facility. High level of indoor microbial air contamination may also increase the incidence of antibiotic resistance, especially of nosocomial pathogens.

Thus, conscious and concerted efforts (such as adequate sensitisation of Management at all levels and staff on the importance of good IMAQ to their wellbeing and productivity and on ways to maintain it in the hospital; undertaking regular validation of sanitation programmes and Standard Operating Procedures in every department or unit to keep indoor microbial air contamination to the barest minimum; and **i**nstallation of high-efficiency gas scavenging systems or filters in sensitive areas such as operating theatres, ICUs and laboratories to filter out harmful aerosols)must be made to minimize indoor microbial air contamination and ensure that good IAQ is maintained within the environment of Tertiary healthcare institution.

AUTHOR CONTRIBUTIONS

C. Chikwem designed and carried out the research, performed the statistical analysis, wrote the protocol, and prepared the manuscript. C. Nwakanma designed and supervised the research, revised the manuscript, and gave the final approval of the version to be submitted for publication.

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

ABBREVIATIONS

ACGIH: American Conference of Governmental Industrial Hygienists, A & E: Accident and Emergency Ward, AD: Administrative/Ethical Committee Building WHO: World Health Organization, USEPA (EPA): United States Environmental, Protection Agency CFU: Colony Forming Units, TVC: Total Viable Count, SOP: Standard Operating Procedures, MT: Main Operating Theatre, ICU: Intensive Care Unit, ML: Microbiology Laboratory, SW: Surgical Ward, Sp: specie(s).

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