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Microbial Assessment of Indoor Air Quality of Ventilation Systems

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Authors' contributions

This work was carried out in collaboration among all authors. Author HOS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BO and CJU managed the analyses of the study. Author CJU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the study was to compare the indoor levels of airborne bacteria and fungi of air conditioned (AC) buildings and naturally ventilated (NV) buildings using a Supermarket, a Laboratory and an Eatery as a case study. The predominant bacterial isolates were *Staphylococcus*, *Micrococcus* spp., *Escherichia coli*, *Bacillus* spp., *Streptococcus* spp., *Enterococcus* spp., *Klebsiella* spp. The fungal isolates belong to the genera *Penicillium*, *Aspergillus*, *Mucor*, *Trichophyton*, *Fusarium*, *Candida* and *Chaetomium*. The levels of airborne bacteria and fungi were determined using settle plate method. In AC buildings the average air levels of bacteria (supermarket: 24.2 CFU m^{-3}; laboratory: 29.2 CFU m^{-3}; eatery: 51.0 CFU m^{-3} air) were higher than in NV (respectively: 54.3 CFU m^{-3}; 100.7 CFU m^{-3}; 134.3 CFU m^{-3} air). The average air levels for fungal isolates were higher in the eatery due to presence of poorly maintained AC system (supermarket: 7.8 CFU m^{-3}; laboratory: 11.5 CFU m^{-3}; eatery: 56.7 CFU m^{-3} air) than in NV (28.6 CFU m^{-3}; 19.6 CFU m^{-3}; 13.5 CFU m^{-3} air respectively). Findings from this study showed that AC buildings had lower levels of bacterial and fungal contamination compared to buildings with natural ventilation.

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1. INTRODUCTION

Indoor Air Quality (IAQ) refers to the air quality within and around built environments especially as it relates to the health and comfort of the occupants [1]. Indoor air is influenced by the outdoor air such that regular outside air inflow into interiors is recognized as the main source of biological contamination of the indoor environment [2], though human-associated microorganisms (e.g., *Staphylococcus aureus* and *Streptococcus* spp.) contribute to indoor pollution. Improving IAQ in buildings can greatly improve the wellbeing of occupants.

The purpose of most ventilation systems is to provide thermal comfort and an acceptable IAQ for occupants. With the improvement of standard of living, occupants require more and more comfortable and healthful indoor environment. We spend about 90% of our time indoors and on an average, inhales $14m^3$ air per day [3], a consequence of this life style is an increased exposure of individuals to indoor air microorganisms with a concomitant increase in the incidence of respiratory diseases.

The factors affecting indoor environment mainly include temperature, humidity, exchange rate, air movement, ventilation, particle pollutants, biological pollutants, and gaseous pollutants [4]. Pollutants in a building's air can cause dizziness, headaches, aggravate allergies and asthma, cancer, heart disease, Chronic Obstructive Pulmonary Disease (COPD), as well of infections such as Legionnaire's disease, tuberculosis, flu, pneumonia, rhinitis, bronchitis, pharyngitis, pneumonia, keratitis, conjunctivitis and severe acute respiratory syndrome (SARS) [5-9]. All of these are indications for indoor environment problems related to poorly ventilated systems.

Under air-conditioning systems, the combination of low ventilation rates and the presence of numerous synthetic chemicals and wood furnishing, as well as human activities results in elevated concentrations of indoor particle pollutants and volatile organic compounds (VOCs) (e.g benzene, toluene, and formaldehyde). This is deemed to be a major contributing factor to compound hypersensitiveness in this environment [10]. According to the Commission of the European Communities [11] mechanical ventilations (heating, ventilation and air conditioning) systems have been shown to cause adverse effects on residents. Seppanen and Fisk [12] reported that there has been an increase in prevalence of sick building syndrome (SBS) between 30% and 60% in the buildings with air-conditioning systems when compared with natural ventilation systems. The aim of air-conditioning systems is to provide occupants with a more comfortable controlled environment. However, such artificial environments may be favourable to microbial pathogens.

It is fair to say that indoor environment problems still exist in many mechanically ventilated buildings, even though some comfortable and healthy air-conditioning systems have been proposed and standards set. In this study, focus was on microbiological pollutants of air-conditioning and natural ventilation systems in selected public places in Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1 Experimental Design

In this study, three sites: medical laboratory, eatery, shopping centre, that use either natural ventilation or air conditioned system were chosen. These include two supermarkets, two laboratories, and two eateries with different ventilation system. One of each pair of sample sites had air condition and the other used natural ventilation. Samples were collected in triplicates by exposing three plates of each media used for analysis by passive deposition method in the sites.

2.2 Sampling Procedure

The assessment was carried out by exposing Petri dishes containing the appropriate culture media at a convenient place in each of the three study sites, and at approximately one meter above the floor to simulate the breathing zone. Nutrient Agar plates were used for the bacteria, Potato Dextrose Agar plates were used for fungi. The Petri dishes were exposed for 30 minutes to allow time for the microorganisms in the ambient air to settle into the plates which was kept at the centre of each sample collection buildings. Thereafter, the plates were covered immediately wrapped in aluminium foil and transported to the laboratory for incubation. The plates for assessment of bacteria were incubated at 37^0C for 24 hours, while the plates for the assessment

of fungi were incubated at room temperature (25^oC) for 5 days.

Microbial assessments were carried out for 3 days, at the time between 12noon when the cooling system must have circulated around the buildings. Number of isolates were enumerated and expressed as Colony Forming Units per cubic meter (CFU m^{-3}) using the equation Omeliansky described in Awad and Mawla [13].

$$N= 5ax10^4 (bt)^{-1}$$

Where:

N= microbial CFU/m^3 of indoor air,
a= number of colonies per Petri dish
b= dish surface cm^2
t= exposure time in minutes

2.3 Identification of Bacterial Isolates

Identification of the isolates was achieved by the observation of colonial characteristics such as size, colour, shape, elevation, consistency and margin. Gram Staining as well as various biochemical tests were used to identify the isolates. The isolates were identified with reference to Bergey and Holt [14] and Cheesbrough [15].

2.4 Identification of Fungal Isolates

A drop of lacto- phenol blue was placed on a clean grease free slide with using a sterile wire loop. Fungi isolates were each teased in the lacto-phenol blue and then covered with a cover slip. Slides were observed under the light microscope with an objective lens of ×40 and the morphological characteristics of the fungi were recorded. The colonial morphologies were noted with reference to Cheesbrough [15].

2.5 Evaluation of Air Quality

Evaluation of air quality was done according to the sanitary standards for non-industrial premises as shown in Table 1 [11].

2.6 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) statistics 20 software was used to determine the statistical significant differences between the concentrations of bacteria and fungi isolated at different sampling sites.

3. RESULTS

The fungal and bacterial contaminants of the various indoor airs were studied by obtaining 18 plate samples from the three sample locations: two supermarkets, two laboratories, and two eateries with different ventilation system. The bacteria isolated from the sample locations are presented in Table 2. The predominant bacteria isolated from the study sites were, *Staphylococcus*, *Micrococcus* spp.*, Escherichia coli*, *Bacillus* spp., *Streptococcus* spp., *Enterococcus* spp., *Klebsiella* spp. *Staphylococcus* spp. *Micrococcus* spp. and *Escherichia coli* were present in all samples from the air conditioned and naturally ventilated buildings. *Streptococcus* spp., *Enterococcus* spp., *Klebsiella* spp. were not present in the Eatery samples. For all sample sites, *Staphylococcus* spp. was the dominant isolate and *Escherichia coli* the least present.

The average bacteria count varied significantly in the various sample sites as shown in Table 3. The highest count was recorded in the naturally ventilated samples which are in increasing bacteria population per sample site: Supermarket (54.3 CFU m^{-3} air), Laboratory (95.7 CFU m^{-3} air), Eatery (134.3 CFU m^{-3} air). While the air conditioned sample had lower bacterial population in the order: Supermarket (24.0 CFU m^{-3} air), Laboratory (29.2 CFU m^{-3} air) Eatery (51.0 CFU m^{-3} air).

Table 4 shows the percentage occurrence of fungal contaminants in the various sites. The relative frequency of fungi varied according to sample locations. The genera that occurred most in the supermarket were in decreasing order, in the AC samples, *Aspergillus* (62.5%), *Penicillium* (25%) and *Trichophyton* (12.5%). As for the NV samples; *Aspergillus* (48.3%), *Penicillium* (31.1%), *Trichophyton* (10.3%) and *Fusarium*(10.3%). In the laboratory the genera in the AC samples were *Aspergillus* (58.3%), *Trychophyton* (25%) and *Penicillium* (16.7%). While in the NV samples the genera were: *Aspergillus* (45%), *Penicillium* (35%), *Trichophyton* (15%) and *Fusarium* (5%). In the eatery, the most frequent fungi genera in the AC were *Penicillium* (71.9%), *Mucor* (19.3%), *Aspergillus* (7%), and *Chaetomium* (1.8%) and in the NV were *Penicillium* (42.9%), *Aspergillus* (28.6%), *Mucor* (14.3%) and *Chaetomium* (14.2%) as shown in Table 4.

Table 1. Indoor air quality criteria

Group of microbes	Range of values (CFU/m^3)	Pollution degree
Bacteria	< 50	Very small
	50-100	Small
	100-500	Medium
	500-2000	High
	>2000	Very High
Fungi	<25	Very small
	25-100	Small
	100-500	Medium
	500-2000	High
	>2000	Very High

Table 2. Percentage occurrence of bacterial isolates from different sample locations

Type of organism	Supermarket		Laboratory		Eatery	
	AC	**NV**	**AC**	**NV**	**AC**	**NV**
Bacillus spp.	-	12.2	6.2	4.9	8.5	11.6
Staphylococcus spp.	57	49.8	53.6	48.6	62.4	69.3
Micrococcus spp.	27.1	11.6	15.3	20.4	23.7	16.5
Klebsiella spp.	-	-	6.6	13.1	-	-
Streptococcus spp.	11.8	19.4	9.2	10.8	-	-
Escherichia coli	4.1	3.2	4.4	2.2	5.4	2.6
Enterococcus spp.	-	3.9	4.7	-	-	-
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**

AC = Air conditioned buildings; NV = Naturally ventilated building

Table 3. Average bacterial count from different locations

Samples	Supermarket CFU m^{-3} air	SE	Laboratory CFU m^{-3} air	SE	Eatery CFU m^{-3} air	SE
AC	24.0	0.8	29.2	0.8	50.1	4.9
NV	54.3	7.9	95.7	5.5	134.3	35.0

AC = Air conditioned buildings; NV = Naturally ventilated building; SE=Standard error

Table 4. Percentage Occurrence of fungal isolates from different sample locations

Genera	Supermarket		Laboratory		Eatery	
	AC	**NV**	**AC**	**NV**	**AC**	**NV**
Penicillium spp.	25	31.1	25	35	71.9	42.9
Aspergillus spp.	62.5	48.3	58.3	45	7	28.6
Mucor spp.	-	-	-	-	19.3	14.3
Trichophyton spp.	12.5	10.3	-	15	-	-
Fusarium spp.	-	10.3	-	5	-	-
Candida spp.	-	-	16.7	-	-	-
Chaetomium spp.	-	-	-	-	1.8	14.2
Total (%)	**100**	**100**	**100**	**100**	**100**	**100**

AC = Air conditioned buildings; NV = Naturally ventilated building

The average fungal count in the samples of air conditioned building was significantly higher in the Eatery with average CFU of (56.7 CFU m^{-3} air) than in the Laboratory (11.5 CFU m^{-3} air) and the Supermarket (7.8 CFU m^{-3} air). On the other hand, for the building with natural ventilation, the average fungal count s were (13.5 CFU m^{-3} air), (19.6 CFU m^{-3} air) and (28.6 CFU m^{-3} air) for Eatery, Laboratory, and Supermarket respectively. It was observed that in all the sites the natural ventilation building indoor air had a higher number of fungal isolates when compared with the air conditioned indoor air except in the Eatery (Table 5).

Table 5. Average fungal count from different locations

Samples	Supermarket CFU m^{-3} air	SE	Laboratory CFU m^{-3} air	SE	Eatery CFU m^{-3} air	SE
AC	7.8	1.2	11.5	1.3	56.7	4.0
NV	28	1.1	19.6	2.9	13.5	1.8

AC = Air conditioned buildings; NV = Naturally ventilated building; SE=Standard error

4. DISCUSSION

Microbiological assessment of indoor air in the study sites revealed varying levels of bacterial and fungal contaminants. Fungi and bacteria aerosols in the indoor environment of the naturally ventilated buildings (NV) had more bacterial load than the air conditioned buildings (AC). The air in NV had higher loads of microbial pollutants probably because it relies solely on the natural process of air flow through a building, which is usually not efficient. The Eatery had the highest bacterial population probably due to the high amount of human activities during sample collection. This is agreement with findings of Bomala et al. [16] which suggested that areas characterized by a large circulation of people usually have the highest level of air contamination.

According to the sanitary standards for non-industrial premises (CEC, 1993), the level of bacterial pollution in the air conditioned buildings (AC) was very small (<50 CFU m^{-3} air) for Supermarket and Laboratory, while that of the Eatery was small (50-100 CFU m^{-3} air). For the naturally ventilated buildings (NV), the degree of pollution was small (50-100 CFU m^{-3} air) for Supermarket and Laboratory, while the Eatery is considered moderately contaminated (100-150 CFU m^{-3} air). This shows that the buildings with air conditioning systems were less contaminated with bacteria compared to those relying on naturally ventilated systems, which is in congruence with the findings of Lugauskas et al. [17] and Gorny [18]. Said and Salihu [19] reported higher bacterial load (94 – 461 CFU m^{-3}) in selected buildings at Modibbo Adama University of Technology, in Nigeria. The difference in result may be due to the number of person and the activities taking place in the different locations.

Following the same classification, the degree of pollution with respect to fungal contamination for air conditioned buildings (AC) was very small (<25 CFU m^{-3} air) for the Supermarket and Laboratory, and small (50-100 CFU m^{-3} air) for the Eatery. For the natural ventilated buildings (NV), the degree of population was general very small. The results revealed that the fungal count in the Eatery was higher in the air conditioned building than in the naturally ventilated buildings. The reason was probably due to the high contamination from the air conditioning system in the eatery which was very old and poorly maintained and in condition which provided high humidity which were favourable for fungi and mould growth. Again, the air conditioning system in the eatery was found to be of old design with dirty filter coil that trapped microbial contaminants like fungal spores which are then circulated within the indoor air environment. Said and Salihu [19] reported a higher fungal load (83 -418 CFU m^{-3}) in their study. The buildings in their study rely on natural ventilation and are usually overcrowded (particularly the classrooms and laboratories), which are factors that might have contributed to the high level of fungal contamination. The bacterial and fungal counts for all the buildings were within recommended values (< 400 CFUm^{-3}) according to the guidelines of National Ambient Air Quality Standard [20].

It is true that air coming into air conditioned environment are filtered, holding most air pathogens in the filter. But when the filter is not cleaned at specific intervals, it becomes source of biological indoor air pollution. This research demonstrated that *Staphylococcus* spp. and *Micrococcus* spp., were the most commonly found bacteria in indoor air, in agreement with Gorny and Dutkiewicz [21]. As averred by Cox and Waters [22] *Staphylococcus* spp. and *Micrococcus* spp. are significant contributors to emissions in environment with people and animals. The predominant fungal isolates include *Aspergillus* spp . and *Penicillium* spp. In the study by Said and Salihu [19] *Staphylococcus* spp. and *Micrococcus* spp. were reported as the predominant bacterial pollutants, while *Aspergillus* spp., *Penicillium* spp. and *Cladosporium* spp were the predominant fungal species. *Aspergillus* spp. and *Penicillium* spp. are recognized as opportunistic pathogens for humans and often associated with clinical manifestations of allergy, rhinitis, asthma and

conjunctivitis, and are considered potential candidates involved in the establishment of sick building syndromes [23].

5. CONCLUSION

The air quality of the buildings was within the recommended values, as they were either small or moderately contaminated. Some of the isolates in this study are known to be involved in the establishment of sick building syndromes.

ETHICAL CONSIDERATION

The approval to undertake the study was sought and obtained from my supervisor through the consent of the Head of microbiology department in the University of Port Harcourt. The department also communicated to the personnel in the various establishment used as study site.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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