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*Original Article*

# Microbiological Indoor and Outdoor Air Quality of Two Major Hospitals in Benin City, Nigeria

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**ABSTRACT**

Air contains large number of microorganisms including bacteria and fungi and their estimation is important as an index of cleanliness for any particular environment. It becomes imperative to undertake a study of the microbiological air quality of the airborne micro-flora in the environments of two major government hospitals, University of Benin Teaching Hospital (UBTH) and Central Hospital, in Benin City metropolis. Both indoor and outdoor air samples were assessed monthly for the three (3) months in the wet season (June – August, 2010) and dry season (November 2010 - January 2011) using the settled plate methods. The study sites were divided into nine (9) units which include accident and emergency ward, laboratory, male ward, female ward, children ward, labour room, treatment room, theatre and outside the hospital gate. The mean airborne bacterial load in the two hospitals ranges from 8.5cfu/min to 172.5cfu/min and 5.5cfu/min to 64.5cfu/min for UBTH and Central hospital in the wet season. While the mean airborne fungal load in UBTH and Central Hospital in dry season ranges from 2.5cfu/min to 9.5cfu/min and 1.5cfu/min to 19.0cfu/min respectively. The female ward, children ward, accident and emergency ward and outside the hospital gate recorded the highest airborne micro-flora. The result revealed the isolation of ten (10) fungal isolates and six (6) bacterial isolates. These include *Aspergillus niger, Aspergillus flavus*, *Botryodiplodia acerina, Rhizopus stolonifer, Nigospora zimm, Mucor* sp., *Monilla infuscans, Penicillium* sp., *Candida* sp. and *Trichoderma viridis* while the six (6) bacterial isolates include *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus* sp., *Serratia marcescens* and *Micrococcus* sp. The result shows the highest fungal population of 26.5cfu/min (outdoor) in UBTH followed by 24.0cfu/min (outdoor) in Central Hospital. The highest bacterial load of 172.5cfu/min (outdoor) was recorded in UBTH. The fungal isolates *Aspergillus niger* (53.0%) and *Monilla infuscans* (43.9%) were showed to be the most frequently isolated airborne fungal organisms while *Staphylococcus aureus* (91.3%) and *Staphylococcus epidermidis* (85.8%) were the most frequently isolated bacterial isolates. The statistical analysis showed no significant difference between the microbial population obtained during the wet and dry seasons in both hospitals studied. Data generated underline the usefulness of monitoring hospital environments.

**Keyword: Aeroflora, Bacteria, Fungi, Hospital, Seasonal variation**

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## INTRODUCTION

Microorganisms such as bacterial and fungal spores are almost always present in the air. The quality of indoor environment, however, is not easily defined or readily controlled, and can potentially place human occupants at risk (Jaffal, *et al.,* 1997a). Airborne transmission is one of the routes of spreading diseases responsible for a number of nosocomial infections (Claudete *et al*., 2006).

Nosocomial infection also known as hospital acquired infection is infection acquired in a hospital environment, which was not present in the patient at the time of admission (Beggs, 2003). Hospitals are potentially conducive for antimicrobial resistant and virulent pathogens to proliferate. Large numbers of microorganisms are found in indoor air and it is of great importance to carry out regular survey as a yardstick of determining

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standard of cleanliness in hospitals (Williams *et al*., 1956). The sources of hospital airborne infection or contamination could be traced to a variety of factors. These include the patient's own normal flora, linens, bed sheets, staff clothes, visitors and the materials such as flowers. Activity of patients (sneezing, coughing, talking, yawning) and the number of patients per room may likewise be sources of hospital infection (Jaffal *et al*., 1997a; Alberti *et al*., 2001; Ekhaise *et al.,* 2008; 2010). It has also been reported that microbiological pollutants such as animal droplets, plants, building materials and air conditioning system have played significant role in the spread of airborne micro-flora. Materials such as files kept close to bedside in surgical wards have been implicated as a viable source (Burge *et al.,* 2000).

Reservoirs of pathogens outside of hospitals are also becoming new area of concern. Generally, outdoor air is the dominant source of indoor fungi (Ponce-Caballero *et al*., 2008). More, interestingly, it has been shown that season can affect the results of an indoor microbial analysis. Reponen and colleagues showed that although the indoor air counts of fungi were significantly lower during winter than other seasons, airborne bacteria did not exhibit clear seasonal pattern (Reponen *et al*., 1992). Rintala *et al.* (2008) reported a clear distinction of the effect of seasons on airborne micro-flora, where the total concentration of culturable microorganisms in indoor air was highest in summer and fall than in winter. This present study was aimed at investigating the types of airborne micro-flora of the two major government hospitals in Benin City, Nigeria and also to determine the effect of seasonal variability on the microbial population as possible consideration in infection control practices for regions with similar climate.

## MATERIALS AND METHODS

### Study Area

This work was carried out at University of Benin Teaching Hospital (UBTH) and Central Hospital, Benin City. The study sites were divided into nine (9) units which includes accident and emergency ward, laboratory, male ward, female ward, children's ward, labour room, treatment room, theatre and outside the hospital gate.

### Sampling Procedure

Air samples were collected once a month for three months in the wet season (June, 2010 – August, 2010) and three months in the dry season (November, 2010 – January, 2011) using the Settle Plate Methods. During the sampling period, the day temperatures in the rainy season ranged from 25$^{o}$C to 33$^{o}$C, while in the dry season, the day temperatures ranged from 26$^{o}$C to 40$^{o}$C. The plates containing nutrient agar (NA) and potato dextrose agar (PDA) were used for the isolation of bacterial and fungal isolates respectively. An antifungal agent (griseofuvin) was incorporated into the nutrient agar medium to inhibit the growth of fungi while antibiotic (chloramphenicol) was incorporated into the potato dextrose agar to inhibit the growth of bacteria. Each plate was exposed at a height of 1m to 1.5m from the floor for a period of 5 to 10minutes (Rahkio and Korkeala, 1997; Bhatia and Vishwakarma, 2010). The bacterial culture plates were incubated at 37$^{0}$C for 24 - 48hrs while the fungal culture plates were incubated at room temperature (20$^{0}$C - 28$^{0}$C) for 3 - 4 days. After incubation, the total number of colony forming units (CFU) for the bacterial and fungal air-flora were enumerated and converted to organism's colony forming unit per cubic meter.

### Identification of Microorganisms

Bacterial colonies were initially characterised by cultural, morphological and microscopic examinations and further identified by biochemical examination of the isolates according to Bergey's Manual of Determinative Bacteriology (Gerhardt *et al.,* 1994). The fungal colonies were identified based on colony appearance and microscopic examination of the spore and hyphae (Barnett and Hunter, 1972). Data obtained were analysed for significant difference using T- test.

## RESULTS

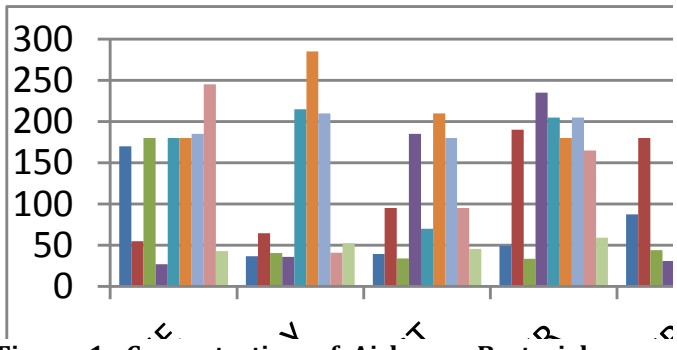


**Figure 1: Concentration of Airborne Bacterial Population (cfu/min) obtained from University of Benin Teaching Hospital between June, 2010 and January, 2011**

***Abbreviation: SS1: Accident and Emergency ward, SS2: Laboratory, SS3: Male ward, SS4: Female ward, SS5: Children's ward, SS6: Labour room, SS7: Treatment room, SS8: Theatre, SS9: Outside the hospital gate***

**Table 1: Distribution of the Aero-flora in the two Hospitals**

| | Wet Season | | Dry Season | |
|---|---|---|---|---|
| **BACTERIAL ISOLATES** | **Microbial Load in UBTH (%)** | **Microbial Load in Central Hospital (%)** | **Microbial Load in UBTH (%)** | **Microbial Load in Central Hospital (%)** |
| ***Staphylococcus aureus*** | 300cfu/mi (100.0) | 300cfu/min (100.0) | 223cfu/min (74.3) | 289cfu/min (96.3) |
| ***Staphylococcus epidermidis*** | 267cfu/min (89.0) | 234cfu/min (78.0) | 256cfu/min (85.3) | 234cfu/min (78.0) |
| ***Bacillus cereus*** | 122.4cfu/min (40.8) | 167.2cfu/min (55.7) | 133.7cfu/min (44.6) | 278cfu/min (92.7) |
| ***Bacillus* sp.** | 167.3cfu/min (55.8) | 200.4cfu/min (66.8) | 122.1cfu/min (40.7) | 189.4cfu/min (63.1) |
| ***Serratia marcescens*** | 44.4.cfu/min (14.8) | 44.4cfu/min (14.8) | 44.4cfu/mi (14.8) | 66.6cfu/min (22.2) |
| ***Micrococcus* sp.** | 189.4cfu/min (63.1) | 223cfu/min (74.3) | 256cfu/min (85.3) | 200.4cfu/min (66.8) |
| **FUNGAL ISOLATES** | | | | |
| ***Aspergillus niger*** | 133.8cfu/min (44.6) | 178.4cfu/min (59.5) | 144.9cfu/min (48.3) | 178.4cfu/min (59.5) |
| ***Aspergillus flavus*** | 122.6cfu/min (40.9) | 55.5cfu/min (18.5) | 66.6cfu/min (22.2) | 111.0cfu/min (37.0) |
| ***Botryodiplodia acerina*** | 100.4cfu/min (33.5) | 134cfu/min (44.7) | 89.3cfu/min (29.8) | 100.4cfu/min (33.5) |
| ***Rhizopus stolonifer*** | 22.2cfu/min (7.40) | 0.00cfu/min (00.0) | 0.00cfu/min (00.0) | 11.1cfu/min (03.7) |
| ***Nigospora zimm*** | 56.0cfu/min (18.7) | 11.1cfu/min (03.7) | 33.3cfu/min (11.1) | 0.00cfu/min (0.00) |
| ***Mucor sp.*** | 122.6cfu/min (40.9) | 111.5cfu/min (37.2) | 88.9cfu/min (29.6) | 144.8cfu/min (48.3) |
| ***Penicillium sp.*** | 44.4cfu/min (25.9) | 77.7cfu/min (25.9) | 134.2cfumin (44.7) | 145.2cfu/min (48.4) |
| ***Monilla infuscans*** | 33.3cfu/min (11.1) | 144.8cfu/min (48.3) | 134.2cfu/min (44.7) | 55.5cfu/min (18.5) |
| ***Candida sp.*** | (77.7cfu/min 25.9) | 122.1cfu/min (40.7) | 89.3cfu/min (29.8) | 100.4cfu/min (33.5) |
| ***Trichoderma viridis*** | 0.00cfu/min (00.0) | 33.3cfu/min (11.1) | 0.00cfu/min (00.0) | 88.8cfu/min (29.6) |

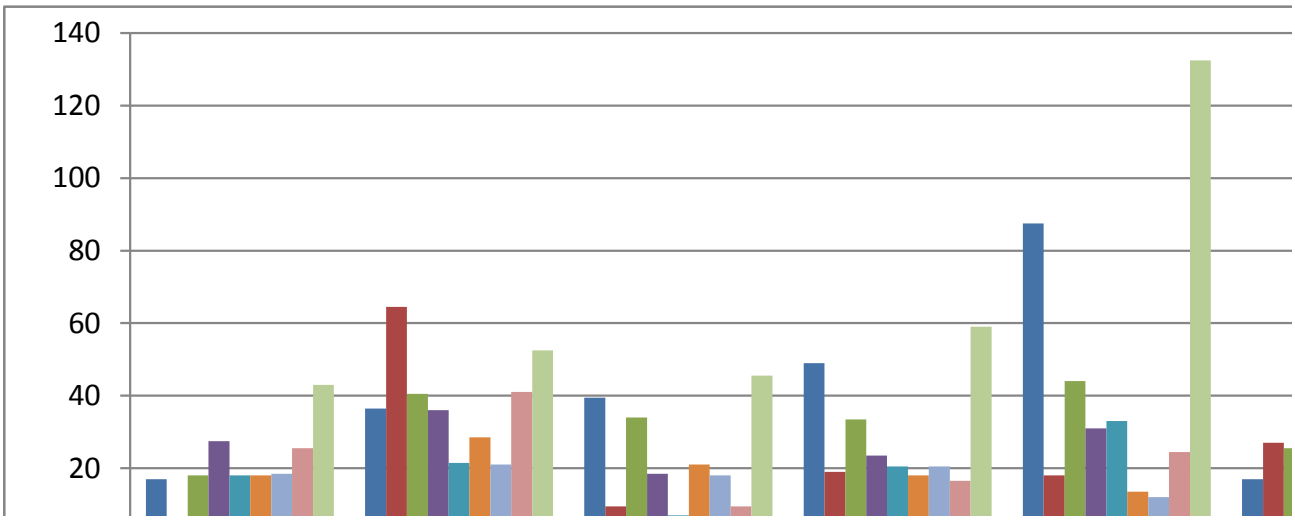


**Figure 2: Concentration of Airborne Bacterial Population (cfu/min) Obtained from Central Hospital Between June, 2010 and January, 2011**

***Abbreviation: SS1: Accident and Emergency ward, SS2: Laboratory, SS3: Male ward, SS4: Female ward, SS5: Children's ward, SS6: Labour room, SS7: Treatment room, SS8: Theatre, SS9: Outside the hospital gate***

The frequency of airborne micro-flora in the different hospital units from the two major hospitals in Benin City are presented in Tables 1. Six (6) airborne bacterial and ten (10) fungal isolates were isolated and characterised from the different units studied. The bacterial organisms include *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus* sp., *Serratia marcescens and Micrococcus* sp. while the airborne fungal isolates include *Aspergillus niger, Aspergillus flavus, Botryodiplodia acerina, Rhizopus stolonifer, Nigospora zimm, Mucor* sp., *Monilla infuscans, Penicillium* sp., *Candida* sp. and *Trichoderma viridis.*

The total airborne microbial load of the nine different units studied in the two hospitals varied from hospital to hospital during the two seasons. The mean total airborne bacterial load ranged from 8.5cfu/min - 172.5cfu/min and 5.5cfu/min 132.5cfu/min in University of Benin Teaching Hospital (UBTH) and Central Hospital respectively (Figures 1 and 2).

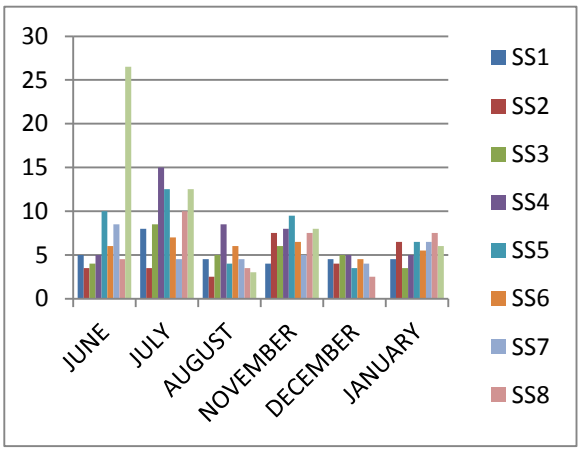


**Figure 3: Concentration of Airborne Fungal Population (cfu/min) Obtained from University of Benin Teaching Hospital Between June, 2010 and January, 2011**

***Abbreviation: SS1: Accident and Emergency ward, SS2: Laboratory, SS3: Male ward, SS4: Female ward, SS5: Children's ward, SS6: Labour room, SS7: Treatment room, SS8: Theatre.***

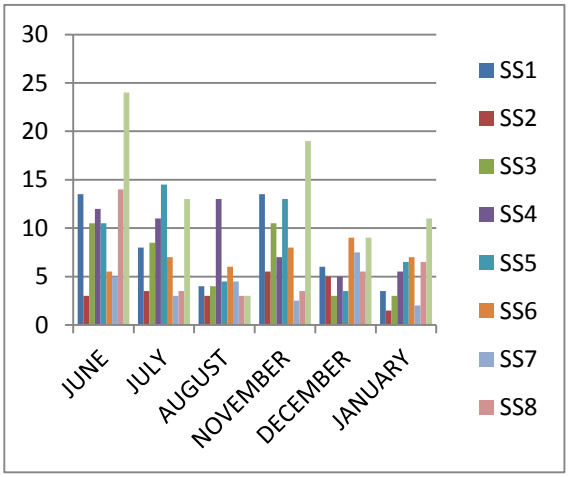


**Figure 4: Concentration of Airborne Fungal Population (cfu/min) Obtained from Central Hospital Between June, 2010 and January, 2011**

***Abbreviation: SS1: Accident and Emergency ward, SS2: Laboratory, SS3: Male ward, SS4: Female ward, SS5: Children's ward, SS6: Labour room, SS7: Treatment room, SS8: Theatre.***

The mean airborne fungal load for UBTH and Central Hospital ranged from 2.5cfu/min - 26.5cfu/min and 1.5cfu/min - 24.0cfu/min (Figures 3 and 4) respectively. Among the different units studied in the hospitals, the accident and emergency ward, laboratory, male ward and outside the hospital gate were observed to record the highest bacterial load in UBTH compared to Central Hospital where the bacterial load was high in the laboratory, male ward, labour room and outside the hospital gate especially in the dry season. The highest fungal load was recorded in the accident and emergency ward, theatre, female ward, children ward and outside the hospital gate for UBTH, while in the Central Hospital, the highest fungal load was recorded in accident and emergency ward, male ward, female ward and outside the hospital gate. The highest fungal load for the two hospitals was recorded between the months of June and August, 2010.

*Staphylococcus aureus, S. epidermidis, Micrococus* sp and *Bacillus* sp. were the most frequently occurring airborne bacterial isolates in the two hospitals both in the wet and dry seasons. The airborne fungal isolates, *Aspergillus niger, A. flavus, Mucor* sp. and *Botryodiplodia acerina* were frequently occurring isolates in the hospitals in the wet and dry seasons. In rainy season, *Staphylococcus aureus* and *Aspergillus niger* was recorded to be the most frequently occurring airborne microbial isolates at 100% and 44.6% occurrence in UBTH, while they occur at 100% and 59.5% occurrence in Central Hospital. While in the dry season, *Staphylococcus aureus* occurs at 96.3% in Central Hospital and *Staphylococcus epidermis* occurs at 85.3% in UBTH. Among the airborne fungal isolates *Aspergillus niger* was also recorded to be the most frequently occurring fungal isolate at 48.3% occurrence in UBTH and 59.5% occurrence in Central Hospital. The presence of these fungal spores in the air poses a great problem for both patient and their visitors as they are capable of causing diseases and infections. The statistical analysis showed no significant difference between the values in the microbial load obtained during the wet and dry seasons of investigation

## DISCUSSION

The study of airborne microorganisms in indoor environments is important to understand the dissemination of airborne microbes particularly the pathogenic ones (Jaffal *et al.,* 1997a). It is believed that the environment where patients are treated has an important influence on the prospect of such patients recovering or acquiring infection that may complicate their conditions (Ekhaise *et al*., 2010). It is therefore, important to evaluate the quality of the air whether indoor or outdoor in the hospital environments. The number and type of airborne microorganisms can be used to determine the degree of cleanliness. In this study, the most frequently isolated bacterial isolates were *Staphylococcus aureus* and *Staphylococcus epidermidis*. These airborne micro-flora obtained

were similar to that obtained by Ekhaise *et al.* (2010), who reported the isolation of bacterial isolates, that includes *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli, Bacillus* spp. *Proteus mirabilis* and *Streptococcus sp,* with *Staphylococcus aureus* being the most prevalent bacterial isolate. Lateef (2003) reported *Staphylococcus aureus* as a cause of infections of the skin, deeper tissue and organs, pneumonia and *Serratia marcescens* causes bacteriuria. These microorganisms are known primary agents of nosocomial infections in hospitals. Similar variety of aero- flora was isolated in a hospital in a desert country (Jaffal *et al*., 1997b).

*Aspergillus niger* and *Aspergillus flavus* were the most frequently isolated fungal isolates in this study. This is similar to that obtained by Jaffal *et al.* (1997a), who isolated seven fungal isolates, from indoor and outdoor environment of houses in the United Arab Emirates. Ekhaise *et al*. (2010) also isolated *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *and Candida* sp., *Verticillium* sp. with *Aspergillus* sp and *Penicillium* from University of Benin Teaching Hospital, one of the study areas. Environmental surveillance of filamentous fungi in three tertiary care hospitals in Greece and indicated that *Aspergillus flavus* and *Aspergillus fumigatus* were the most prevalent species (Panagopoulou *et al.*, 2002). Although, *Aspergillus* may be tolerable for healthy individuals, it may be dangerous for high-risk patients. The spores readily invade the airways and could lead to aspergillosis in immuno-compromised hosts (Gangneux, 2004; Bhatia and Vishwakarma, 2010). Pathogenic actinomycetes usually cause chronic exudative inflammatory infection and also cause acute necrotizing pyogenic infection (Bhatia and Vishwakarma, 2010).

Although, information about the seasonal variation is important for any exposure assessment (Rintala *et al.,* 2008), it is rarely available. Studies on seasonal variation of microbial flora in indoor environments have mostly concentrated on viable counts of fungi (Koch *et al*., 2000; Pitkäranta *et al*. 2008). Nevertheless, Rintala *et al*. (2008) found that seasonal differences between bacterial aeroflora were not statistically significant during the course of their investigation. In Nigeria, two distinct season are noticeable and these are the wet or rainy (April to October) and the dry (November to March) seasons. The wet season is characterised by longer days and warm humid weather, while the dry season is associated with relatively shorter days, and low night temperatures (Owonubi and Yayock, 1981). The results obtained in this study revealed high frequency of airborne bacterial isolates in the rainy season than in the dry season as compared to the frequency of airborne fungal isolates, which predominate during the dry season. It is an established fact that temperature and relative humidity are two important factors for fungal spore generation, release and dispersal; particularly in indoor environments. The dry atmosphere and high temperature in the months of dry season influence the movement of airborne microbial particles and thus support evidences for the concentration of fungal species within the period.

In conclusion, the results generated in this study clearly suggest that regardless of season, indoor environment allows aerosols build up which could potentially lead to infections in the wards. Thus, hospitals should have enhanced practice of good sanitation protocols and infection control measures. Regularly, monitoring of hospital aeroflora is particularly recommended. Thorough hand washing and use of alcohol rubs by medical personnel before and after each patient contact are known effective methods to combating nosocomial infections; these could also limit microbial dispersals within the hospitals.

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