

Comparative Analysis of Airborne Microbial Concentrations in the Indoor Environment of Two Selected Clinical Laboratories

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Abstract: The airborne microbial concentrations of two clinical Laboratories located in the main campus of Ahmadu Bello University (LBU) and Sarkin-pawa Street (LBS) in Samaru-Zaria town were investigated within a period in the dry season (January-march) and a period in wet season (July-September) 2007. Bacterial counts in the dry season ranged from 1.8×10^3 cfu/ml to 0.03×10^3 cfu/ml with the highest count in LBS in the third week of sampling while those of wet season ranged from 8.0 to 0.01×10^3 cfu/ml with the highest in LBS. The fungal count ranged from 3.6 to 0.08×10^3 cfu/ml in the dry season and 0.54 to 0.04×10^3 cfu/ml in the wet season. The highest fungal count occurred in LBS and LBU in dry and wet seasons respectively. There was no statistical difference between the bacterial concentrations of the laboratories using paired sample t-test. In fungal counts, there was also no significant difference between the laboratories. The correlation between the sum total of bacterial and fungal concentrations at 0.05 level of significance (2-tailed) using Kendall's tau *b*, and Spearman's rho was significant. The predominant bacteria and fungi isolated from investigated air samples included *Staphylococcus* spp, *Proteus* spp, *Streptococcus* spp, *Micrococcus* spp, *Aspergillus* spp, *Rhizopus* spp, *E. coli*, *Bacillus* spp. This comparative information could be useful for the medical and public health practitioners on common airborne bacteria and fungi, and their roles in the indoor air quality of clinical laboratories.

Keywords: Airborne, bacteria, fungi, laboratory, season

I. Introduction

Microorganisms suspended in the air of both occupational and residential indoor environments are now appropriately recognized and that exposures to them are associated with a wide range of adverse health effects with major public health impact. The quality of indoor air is one of the most significant factors affecting the health and well-being of people who inhale at least 10m³ of the air every day, and spend between 80-95% of their lives indoors (Dacarro *et al.*, 2003). The air inhaled by people is abundantly populated with microorganisms which form so-called bioaerosols (Wojtatowicz *et al.*, 2008). Bioaerosol is a colloidal suspension, formed by liquid droplets and particles of solid matter in the air, whose components contain or have attached to them viruses, fungal spores and conidia, bacterial endospores, plant pollen and fragments of plant tissues (Karwowska, 2005). They account for 5-34% of indoor air pollution.

In many environments such as hospitals, animal sheds, clean-rooms, pharmaceutical facilities, train and spacecraft, the presence of bioaerosols can compromise normal activities, making efficient monitoring crucial (Venkateswaran *et al.*, 2003; Gorny, 2004; Stetzenbach, 2007; Okafor and Opuene, 2007). Infectious aerosols tend to be extremely small (< 5µm) and can therefore remain suspended and viable in the air stream over long periods of time, resulting in extremely high risk of airborne infection in confined places. Nosocomial infection is a serious and widespread problem with many of the infections associated with person-to-person contact and increasingly airborne route transmission. This may account for as much as 10-20% of all endemic nosocomial infections (Brachman, 1970).

Microbial damage in indoor and outdoor areas is caused most frequently by molds and bacteria. This constitutes a common problem all over the world. In

Finland, 70 % of day care centers have signs of moisture problems (Ruotsalainen *et al.*, 1993), as well as 55 % of homes (Nevalainen *et al.*, 1998) and 53 % of schools (Kurnitski *et al.*, 1996). The microbial spores may become airborne, entering indoor areas either by means of passive ventilation or ventilation systems. Microbes are launched into the air via humans, animals, vegetation and could be deposited on surfaces. Aerosolization of microorganisms and their survival in the air is affected by certain climatic conditions including desiccation, humidity, temperature, and radiation (Pepper, 2009). It is known that excessive humidity and or a high water content of building materials could determine the extent of microbial growth and all these factors might lead to adverse health effects, unpleasant odors, and destruction of materials (Yassin and Almouqatea, 2010). However, some microbes can resist the factors by forming endospores, pigments and others including Gram positive bacteria could be tolerant.

The findings of epidemiological research indicate that exposure to high concentrations of microbes in the air frequently lead to allergies, asthma, hay fever (Björnsson *et al.*, 1995; Newson *et al.*, 2000), pneumonia (Siersted and Gravesen, 1993) and many other infections (Renn *et al.*, 2001. Allsopp *et al.*, 2004). Long term exposure in poor indoor air quality including total bacterial counts, total fungal counts, has been shown to have potential threat to human health (Fabian *et al.*, 2005; Huboyo *et al.*, 2011). Biological factors such as fungal spores and mites are involved in sick building syndrome (SBS), a complex situation in which occupants experience a variety of symptoms and become generally unwell, recovering only when they cease to frequent the building (Allsopp *et al.* 2004; Ross *et al.*, 2000).

Sources of indoor air pollutants may be brought about by contaminated outdoor air, air conditioning or office equipment, human activity, building components and furnishings, and other accidental events (Lai *et al.*, 2010; McNamara *et al.*, 2011). Other sources include building maintenance, cleanliness, relative humidity, temperature, type of furniture, and carpeting (Dharmage *et al.*, 1999; Smedge and Norback, 2001).

Exposure to bioaerosols unlike exposure to chemicals do not have threshold limits to assess health impact/toxic effects, due to the complexity in their entity, variations in human response to their exposure and difficulties in recovering microorganisms that can pose hazard during routine sampling. Their role in various industrial settings has been well studied (Douwes *et al.*, 2003). Increasing incidences of nosocomial and occupational diseases due to bioaerosol exposure (Eickhoff, 1994; Beggs, 2003) indicate the need for a thorough knowledge in this respect. Bioaerosol monitoring in hospitals provides information for epidemiological investigation of nosocomial infectious diseases, research into airborne microorganism spread and control, monitoring biohazardous procedures and use as a quality control measure to determine the quality of indoor air (Stetzenbach, 2005).

II. Materials And Methods

Study area: The study was carried out in two selected hospital microbiology laboratories namely Jama'a (LBJ), a private-owned and sick bay, Ahmadu Bello University main campus clinic (LBA). Both are located in Samaru-Zaria. LBJ is located in Sarkin-pawa Street, Samaru- Zaria. Kaduna State, Nigeria. It is close to the north gate of Ahmadu Bello University (ABU), Zaria. The clinical laboratory measuring approximately 7.3m², is located on the ground floor of the hospital. There are no potential sources of microorganisms except an open dustbin. The only entrance which is from the reception room is always opened and it has a large burglary-proof window. LBA is located on the main campus in Samaru. It measures about 13.75m², air-conditioned, uncarpeted with tiled workbench surface. There is direct ventilation link with the external environment via a window and door.

Air sampling and examination: This was conducted using impaction method. Single-stage MAS-100 (Merck Eurolab, Switzerland) air sampler with flow rate of about 25L/minute was used based on the principle of the Andersen air sampler corresponding to its 5th stage (Pascual *et al.*, 2003; Karbowski *et al.*, 2011) which guarantees that all particles >1µm were collected. There is however no internationally accepted recommendations on sampling flow-rate and the media used for sampling but reports suggested that high-containment laboratory and hospitals require air samplers with flow-rates ≥ 25 L/minute for monitoring (Agnieszka *et al.*, 2012). Air samples were collected for 1min within the average temperature range of 32-38⁰C and 28-34⁰C in dry and wet seasons respectively. During the sampling, the device was placed at a height of 1.0-1.5m above the floor for indoor measurements and at the ground level for outdoor measurements. This is to simulate aspiration from the human breathing zone. Petri dish filled with nutrient agar (NA) was used as sampling surface for bacteria while potato dextrose agar (PDA) supplemented with the antibiotic chloramphenicol to inhibit the growth of bacteria was used for sampling fungi. The NA plates were incubated at 37⁰ C for 24 hours while the PDA plates were incubated at room temperature for at least 3 days after which colonies were counted and expressed in colony forming units (cfu/ml). Each colony was isolated for identification including the fungal growths.



Fig 1: Air sampling using MAS sampler

Each bacteria isolate was gram stained to ascertain whether it is positive or negative. Further identification processes were carried out by subjecting the isolates to various biochemical tests including coagulase, oxidase, triple sugar iron, and others.

The fungal colonies were identified based on the manual of Barnett and Hunter, 1972 and Beneke, 1980. They were observed by morphological and microscopical examination of the spores and hypha using lactophenol cotton blue reagent.

III. Results

The microbial concentrations at temperature range of 28-34⁰C and 32-41⁰C in wet and dry season respectively, obtained in this investigation are presented in a table and charts. The highest total microbial mean count (bacteria): 23.1cfu/ml during the period of study occurred in the air of LBJ in wet season, followed by bacteria: 9.88cfu/ml in the same laboratory but in dry season as shown in Fig.1. The fungal concentrations were moderately low in both laboratories. The highest bacterial concentration: 8.0cfu/ml in LBJ occurred in 7th week of sampling in the wet season while that of LBA: 2.6cfu/ml occurred in the 3rd week of wet season. On the other hand, the highest fungal count: 3.6cfu/ml in LBJ was obtained in dry season at 1st week of sampling while the highest count: 1.0cfu/ml in LBA occurred in same dry season but at the 2nd week as shown in Figures 2, 3 and 4. The two laboratory environments frequently were dominated by members of the bacterial genera *Micrococcus*, *Bacillus* and *Staphylococcus* all of which contain several species representatives. *Pseudomonas*, *Proteus*, *Escherichia coli*,

Table 1: Indoor Airborne Microbial Concentration of the Laboratories (cfu/ml)

LBJ:Jama'a lab; **LBA:** ABU lab; **DAB:** air bacteria in dry season; **WAB:** air bacteria in wet season; **DAF:** air fungi in dry season; **WAF:** air fungi in wet season; **MC:** mean count.

WK	1	2	3	4	5	6	7	8	MC
LBJ									
DAB	1.10	1.60	1.80	1.10	1.40	1.60	0.66	0.62	9.88
WAB	0.10	2.90	1.30	1.10	0.90	1.30	8.00	7.50	23.1
DAF	3.60	0.40	0.46	0.08	0.08	0.56	0.74	0.42	6.34
WAF	0.04	0.06	0.26	0.28	0.14	0.52	0.28	0.26	1.84
LBA									
DAB	1.40	1.50	0.85	0.03	0.07	0.68	0.82	1.10	6.45
WAB	1.40	0.48	2.60	2.00	0.31	0.92	0.54	0.50	8.75
DAF		0.34	1.00	0.14	0.51	0.14	0.17	0.10	0.61 3.01
WAF	0.54	0.27	0.27	0.17	0.14	0.84	0.20	0.25	2.68

Klebsiella, and *Streptococcus* were present in LBJ but fewer. *Klebsiella* and *Proteus* were not found in LBA. The outdoor microbial concentrations of each of the laboratories were to a considerable extent higher than the indoors and predominated by *Bacilli* and *Corynebacteria*. The isolated indoor fungi in LBJ included *Aspergillus*, *Fusarium*, *Penicillium*, *Cladosporium* and *Candida* but *Penicillium* was not found in LBA.

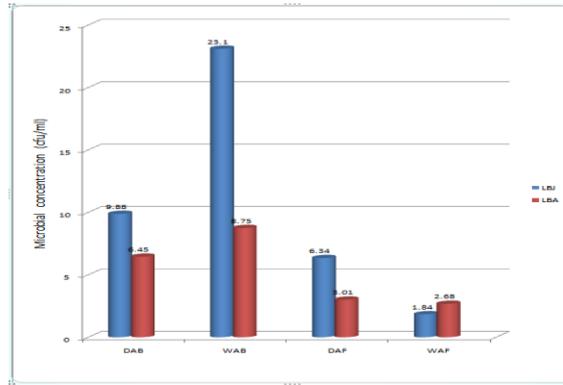
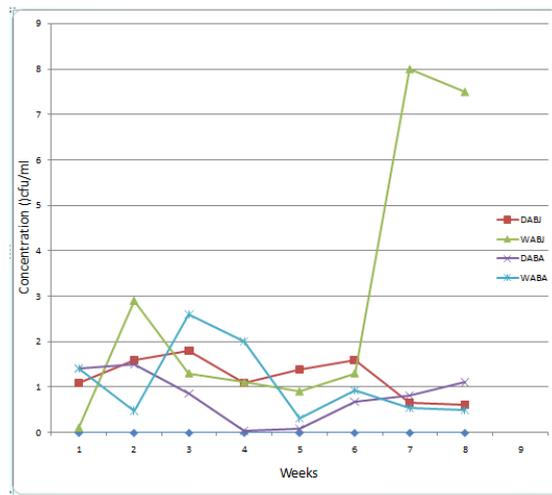
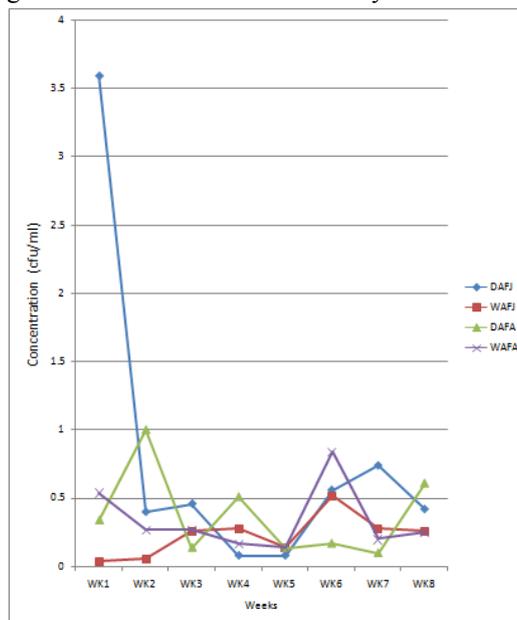


Fig 2: Mean concentration of microbial counts per season (cfu/ml)



DABJ: dry season air bacteria in Jama'a lab. **WABJ:** wet season air bacteria in Jama'a lab. **DABA:** dry season air bacteria in ABU lab. **WABA:** wet season air bacteria in ABU lab.

Fig 3: Bacterial concentration for dry and wet season



DAFJ: dry season fungi in Jama'a lab. **WAFJ:** wet season air fungi in Jama'a lab. **DAFA:** dry season air fungi in ABU lab. **WAFJ:** wet season air fungi in ABU lab.

Fig 4: Fungal concentration in dry and wet season

IV. Discussion

Dispersion of pathogenic microorganisms in the laboratory indoor environment occurs during analyses of clinical specimen and these agents may not induce any immediate effects in humans even after fatal exposures. In the absence of a reliable surveillance system, there would most likely be unawareness of the incidence until the exposed individuals seek medical assistance up to several days later (Marius *et al.*, 2012). Regular air inflow into interiors is the main process resulting in biological contamination of the indoor environment. This implies that some of the isolated organisms including *Cladosporium*, *Corynebacterium*, *Aspergillus*, might have originated from outdoor environments of the laboratories. In this study, *Aspergillus* and *Penicillium* were significantly higher in the indoor environments of the laboratories. This was in line with the results of Yassin and Almouqatea, 2010.

Cladosporium spp, *Alternaria* spp, *Aspergillus* spp, and *Fusarium* spp are amongst the most common allergenic genera, and their metabolites are believed to irritate the respiratory system (Kalogerakis *et al.*, 2005). The frequent and high level of *Aspergillus* spp encountered in both indoor and outdoor environments of the laboratories during dry season could lead to pulmonary aspergillosis when the spores are inhaled. They are thought to enter hospital buildings through ventilation ducts with inadequate filtration. The immunocompromised individuals are particularly vulnerable to infection from *Aspergillus* spp and mortality rates are significant.

Micrococcus and *Bacillus* species are regularly found on human skin (Kloos and Musselwhite, 1975). The more frequent observation of *Bacillus* spp in outdoor air than the indoor air suggested local source differences or specific inactivation of non-spore forming bacteria in the outdoor atmosphere. It is generally acknowledged that spore-forming bacteria such as *Bacillus* spp are present mainly as spores in the environment (Stenfers *et al.*, 2008). This is supported by the high amount of *Bacillus* spp derived from this study although they exist as both spores and vegetative cells in the airborne environment.

Staphylococcus aureus, most especially the methicillin resistant strain (MRSA) is a problem in hospitals worldwide. Though, its infection is mostly associated with contact route, the airborne route is thought to play an important role in intensive care and burns units. *Pseudomonas aeruginosa* is an opportunistic and as well common nosocomial pathogen associated with air and surfaces. It is known to be notorious for its resistance to antibiotics making it dangerous and dreadful and might account for nosocomial pneumonia and UTI. This could be the reason why it was found in LBA despite the high level of sanitation practice and tiled work surface.

Outdoor microbial concentrations vary according to the season and time of day, and these variations are also reflected in this study. For instance, the highest concentrations of bacteria were detected in wet season and fungi in dry season usually characterized by wind, cold, dryness and to a certain extent high humidity (Dharmage *et al.*, 2002; Lee and Jo, 2006).

Humidifiers are an important source for bacterial exposure that may lead to allergic disease. Improper ventilation causes condensation and hence moisture buildup inside buildings. *Actinomycetes* that originate from environmental sources rather than from humans are regarded as moisture indicator in indoor environment (Bhatia, 2011).

The relatively high bacterial concentration recorded in dry season in LBJ was due to the use of ceiling fan as mechanical ventilation at the time of sampling. This facilitated the aggravation of the aerosols into the air. The concentration was still on a higher side at the following week of sampling and could be as a result of warm humid weather condition, agreeing with the result of Tseng *et al.*, 2011 in their study on indoor bacteria and fungi in Taiwan. Bacterial concentrations were generally higher than fungi similar to published reports of some researchers including Yassin and Almouqatea, 2010, and Shiaka *et al.* 2011.

Data resulting from this study showed that the concentrations of both viable bacterial and fungal aerosols in both laboratories did not exceed 10^3 cfu/ml suggesting low level of contamination probably due to proper hygiene maintained most especially in LBA. This is similar to the report of Maigorzata, 2011 in his investigation on bacterial and fungal aerosols in air conditioned office buildings in Warsaw, Poland.

V. Recommendations

The IAQ of hospital laboratories should be greatly improved since pathogenic organisms or contaminated materials are usually handled. These might include application of appropriate disinfectant-detergent solution for cleaning of surfaces (floor, workbench, equipments, and workers hand), proper discarding and disposal of wastes, removal of contaminants from building by passive or active fresh air ventilation, frequent fumigation and use of air sanitizer to reduce aero microflora. All parts of the humidification and dehumidification systems must be kept clean and dry to prevent bacterial and fungal growth.

VI. Conclusion

The consequences of indoor-air and indoor environment problems indicate that much is to be done in identifying and managing indoor-air deficiencies. Multidisciplinary approach is required to handle the complexity of bioaerosols.

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