MICROBIOLOGICAL ASSESSMENT OF AIR-CONDITIONERS USED IN CALABAR, NIGERIA

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ABSTRACT

Background: There is a rising heat level in Nigeria that has been associated with climate change which has a global reach, exposing millions of people to the risk of several health problems. Usually air conditioners are deployed in human dwellings to eradicate heat conditions but may serve as potential reservoirs of infection if not appropriately used and maintained.

Aim: This study was aimed to evaluate in-use air conditioners in Calabar, Nigeria for their role in the transmission of microbial infections between April and August, 2021.

Methodology: A cross sectional study was conducted to examine 100 air conditioners' filters and 50 filtered air samples of the same air conditioners in use for potential bacterial and fungal pathogens. Each filter was wiped with sterile cotton wool swab moistened with sterile peptone water, while the filtered air samples were collected by placing the appropriate culture media plates against the air conditioner air stream in use. The culture plates were incubated at 37^0C for 24-48hours and one Sabouraud Dextrose agar plate at room temperature for 2-7 days. Identification of the isolates was done using standard bacteriological and mycological methods.

Results: Comparatively, the filters were more significantly contaminated with bacteria 100(100.0%) and fungi 73(73.0%), respectively (x^2=62.567, P=0.00001) than the filtered air samples 24(48.0%) and 17(34.0%), respectively (x^2=21.13, P=0.00001). All (100%) of the 100 air conditioner filter samples studied carried bacteria compared to 73(73.0%) which carried fungi (x^2=31.12, P=0.0001). Also, air conditioner's air stream samples carried insignificantly more bacteria 24(48.0%) compared to fungi 17(34.0%) (x^2=2.0258, P=0.1546). The most frequently encountered bacteria and fungi in the study were *Bacillus* species (50.8%) and *Aspergillus niger* (60.0%), respectively. Others were *Aspergillus flavus* (40.0%), *Pseudomonas aeruginosa* (20.2%), *Staphylococcus aureus* (13.7%), *Klebsiella pneumoniae* (9.7%), and *Escherichia coli* (5.6%).

Conclusion: This study concludes that air conditioners in-use in Calabar can play role in the transmission and spread of air borne infections. Measures should be taken to ensure implementation of the recommended healthy, periodic cleaning and maintenance as well as from microbiological screening of in-use air conditioners.

Key words: Air conditioner, Infection, Transmission, Pathogens

INTRODUCTION

Variations in weather condition and climate change are increasingly issues of public health importance (Guirguis *et al.,* 2018). The high level of heat exposure as a result of global climate change in cities and villages is a factor exposing millions of people to the risk of several health problems. People living in the urban areas are at higher risk due to higher population density. Heat is the top weather-related killer in the world. Air conditioners have however been used to reduce the prevalence of weather-related challenges in the world.

Many buildings in the city especially in public places have installed air conditioners to reduce heat exposure and improve indoor conditions (Lundgren *et al.,* 2013). Air conditioners are indoor devices that provide an atmosphere with controlled temperature, humidity and purity at all times regardless of the weather conditions (Anas *et al.,* 2016). It cools air by reducing the humidity following condensation of the water vapors (Lundgren *et al.,* 2013; Vidya *et al.,* 2014). The air conditioners have been discovered to cause some form of physiological discomforts and allergic symptoms which is also known as sick building syndrome that were noticed when occupants spend considerable time indoor in a room that is equipped with active air conditioners (Anas *et al.,* 2016). The mucous membrane irritation, breathing difficulties, irritated skin, headache and light headedness, fatigue, feverish conditions, chest pain, persistent cold, sore throat, catarrh, cough, watery eye, prolonged muscle cramps and joint pain were common ailments that have been discovered to be suffered by individuals who have spent considerable time in air-conditioned rooms (Rohizan *et al.,* 2013).

Researchers have proven that air conditioners can be contaminated with organic pollutants, bacteria, fungi and particulate matter from mice, insects and the contaminants can grow and survive in areas that meet their environmental requirements (Maus *et al.,* 2001). Currently, most air conditioners use internal filters that extracts microorganisms and dust but sometimes they do not completely remove these microorganisms and they may remain viable and can be returned to the surrounding atmosphere during inefficient operation, period of maintenance and due to temporary malfunction (Maus *et al.,* 2001). Particle pollutants are very dangerous to the human body; most of the particle pollutants in air are very small which enables them to easily enter into the respiratory canal deeply together with inhaled air (Morawska *et al.,* 2004; Tham *et al.,* 2005). These pollutants are circulated in a close environment with the air from the air conditioning device. It is very possible for the surface of these particle pollutants to adsorb harmful or potential pathogens or microbes which increases their harmfulness to human body (Morawska *et al.,* 2004).

Microbial contamination of air conditioners has been attributed to the operation of most air conditioners outside their designed parameters due to inappropriate filters (Anas *et al.,* 2016), neglect or over use, poor maintenance and hygiene practices which results in dust accumulation (Nordell, 2000). The dust accumulation coupled with humidity, especially at the downstream section of the cooling equipment leads to the proliferation of potential harmful microorganisms capable of surviving the prevailing conditions (Chang *et al.,* 1996; Nordell, 2000). Organisms like *Legionella pneumophilia, Streptococcus pneumoniae, Staphylococcus aureus, Klebsiella pneumoniae* and *Bacillus species* have been isolated in air conditioner filters (Kemp *et al.,* 1999; Nordell, 2000). Species of *Penicillium, Pneumocystis carinii* and *Aspergillus, Rhizopus, Fusarium* and *Alternaria species* have been isolated too (Tabien*et al.,* 1996; Prescott *et al.,* 2000; Roponen *et al.,* 2001). The aim of this study was to evaluate in-use air conditioners as a possible source of microbial infection transmission.

MATERIALS AND METHODS

Study Area

This study was carried out in Calabar Metropolis. Calabar is the capital of Cross River State in the southern Nigeria. Calabar is often described as the tourism capital of Nigeria, especially due to several initiatives implemented during the administration of Donald Duke (1999-2007), which made the city the cleanest and environmentally friendliest city in Nigeria. Administratively, the city is divided into Calabar Municipal and Calabar South Local Government Areas. It has an area of 406square kilometers (157sq mi) and a population of 371,022 as at 2006 census (Ottong *et al*., 2010).

Calabar features a tropical monsoon climate with a lengthy wet season spanning ten months and a short dry season covering the remaining two months. The harmattan which significantly influences weather in West Africa is noticeably less pronounced in the city. It has a temperature ranging from 25-28^{0}C. Calabar is a large urban city with several hotels, good road network, hospitals, schools and many other establishments. The main occupation of the residents of Calabar is farming and trading, many are civil servants but combine work with either farming or trading. The annual Calabar carnival attracts thousands within and beyond Nigeria into the city.

Study design

A cross sectional study design was employed in the study. Hotels, offices and hospitals equipped with air conditioners in Calabar Metropolis were enrolled for this study.

Administration of questionnaires

The evaluation of air conditioners was done by giving questionnaires to those that were willing to participate in the study. Structured questionnaires were given to the management of the enrolled hotels, offices and hospitals who were asked to supply answers to reflect the duration/frequency of use, frequency of cleaning/maintenance, functional state, etc.

Sampling technique and sample collection

Convenient sampling technique was employed in the study. Air conditioners used in this study were those reported to have been in constant use; samples were grouped according to the site of collection, which were the air conditioner filters and the filtered air coming out of the air conditioner's air vent.

Two samples were collected from each of 150 air conditioners, one from each of the 100 air conditioners' filters and the other from the filtered air coming out from 50 of the same air conditioners while being used. A sterile cotton wool swab moistened with sterile peptone water was used to swab the filters. Air samples were collected unto a blood agar, cysteine lactose deficient agar and two Sabouraud dextrose agar by exposing the content of the media plates directly to the air coming out of the air conditioners for three to five minutes before the plates were closed and appropriately labeled. The swab sticks and the air sampled plates were taken to the laboratory within an hour of collection for processing (Cheesbrough, 2006).

Laboratory processing of the samples

The swab stick containing the swabbed sample were rolled gently on a clean sterile glass slide making a thin smear and allowed to air dry. The smear was stained by Gram's method and examined microscopically for its Gram reactions (Cheesbrough, 2006).

Cultural and isolation procedures

Each swab was inoculated into a sterile container containing 5mls of peptone water (This formed the sample stock solution) and allowed for organisms' recovery for 3 hours in the incubator at 37°C. Each recovered sample was cultured on one blood agar, one cysteine lactose deficient agar and two Sabouraud dextrose agar. These culture plates were incubated aerobically at 37°C for 24 to 48hours and one Sabouraud dextrose agar plate incubated at room temperature for 2 to 7 days for fungi growth (Cheesbrough, 2006).

Identification of bacterial isolates

At the end of incubation period, bacterial colonies were identified macroscopically based on their colonial morphology. They were also identified microscopically using Gram staining method and motility test. The bacterial organisms were further identified using standard biochemical tests for Catalase, coagulase, indole, urease, oxidase, carbohydrates fermentation and citrate utilization (Cheesbrough, 2006).

Identification of fungal isolates

This was done based on colonial and microscopic morphology of the isolates. Fungal colonies on Sabouraud dextrose agar plates were observed macroscopically for consistency, color, size and shape.

Slide smears of the colonies, stained with lactophenol cotton blue were observed microscopically for typical hyphae and conidia (Baker *et al.*, 2001; Sangeetha and Hangadurai, 2013).

RESULTS

Figure1 displays the occurrence of bacteria and fungi in air conditioner filters and air samples. Overall, air conditioners' filters were more contaminated with bacteria and fungi than their air stream samples 100/100(100.0%) and 73/100(73.0%), respectively versus 24/50(48.0%) and 17/50(34.0%), respectively. These differences were statistically significant (x^2 = 62.57, P = 0.00001) and (x^2 = 21.13, P = 0.00001). All (100.0%) of the 100 air conditioner filter samples studied carried bacterial contaminants compared to 73(73.0%) which tested positive for fungi. This difference was statistically significant (x^2 = 31.106, P = 0.00001). Also, 24(48.0%) of the 50 air conditioner's air stream samples carried bacteria compared to 17(34.0%) with fungi, although this difference was statistically insignificant (x^2 = 2.0256, P = 0.154665).

Table 1 shows the distribution of bacteria and fungi in air conditioner filters and air based on location. All, 100 (100%) of the air conditioner filter samples studied, irrespective of location carried bacterial contaminants. Air samples from office air conditioners had the highest occurrence of bacterial contaminants 9(52.9%) followed by the hotels 8(50.0%) and hospitals 7(41.2%). These differences were statistically insignificant (X^2 = 0.509, P = 0.775285). Air conditioner's filter samples from hotels had the highest number of fungal contaminants 20(83.3%) followed by the offices 25(73.5%) and the hospitals had the least number 28(66.7%). These differences were statistically insignificant (x^2 = 2.1597, P = 0.339638). The air conditioner's air samples from the offices had the highest number of fungal contaminants 9(52.9%) while the hotels and hospitals had similar rates of occurrence 4(25.0%) and 4(23.5%), respectively. These differences were statistically insignificant (x^2 = 4.1261, P = 0.12707).

Figure 2a is on the frequency of bacteria and fungi species isolated in the study. *Bacillus* species was the most frequently encountered bacteria (50.8%) followed by *Pseudomonas aeruginosa* (20.2%), *Staphylococcus aureus* (13.7%), *Klebsiella Pneumoniae* (9.7%) and *Escherichia coli* (5.6%).

Figure 2b is on the frequency of fungi species isolated in the study. *Aspergillus niger* (60.0%) occurred more frequently than *Aspergillus flavus* (40.0%) among the fungal contaminants.

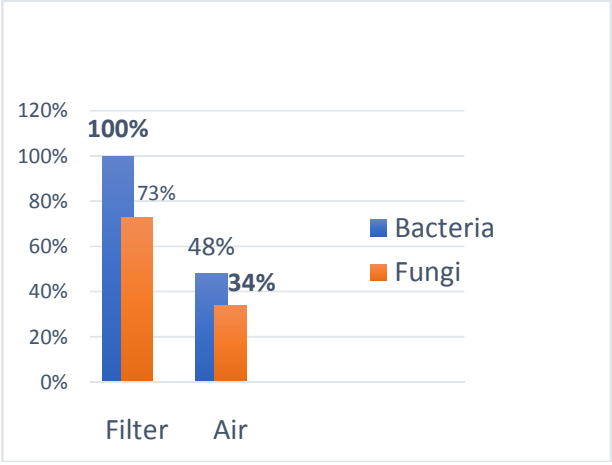

Fig.1: Occurrence of bacteria and fungi in air conditioners' filters and air samples

Table 1: Distribution of bacteria and fungi in air conditioners' filters and air based on location

Air conditioners' Location	No. Examined		No. (%) with Bacteria		No. (%) with Fungi	
	Filter	Air	Filter	Air	Filter	Air
Hotels	24	16	24(100.0)	8(50.0)	20(83.3)	4(25.0)
Offices	34	17	34(100.0)	9(52.9)	25(73.5)	9(52.9)
Hospitals	42	17	42(100.0)	7(41.2)	28(66.7)	4(23.5)
Total	100	50	100(100.0)	24(48.0)	73(73.0)	17(34.0)

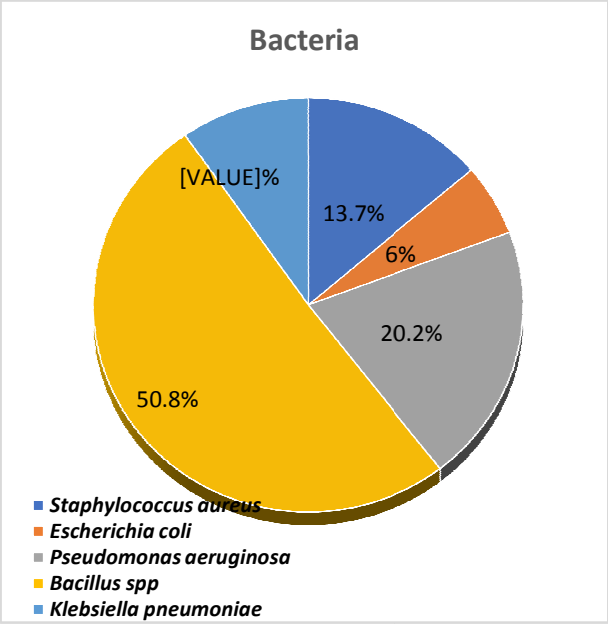

Fig.2a: Frequency of bacteria species isolated in the study

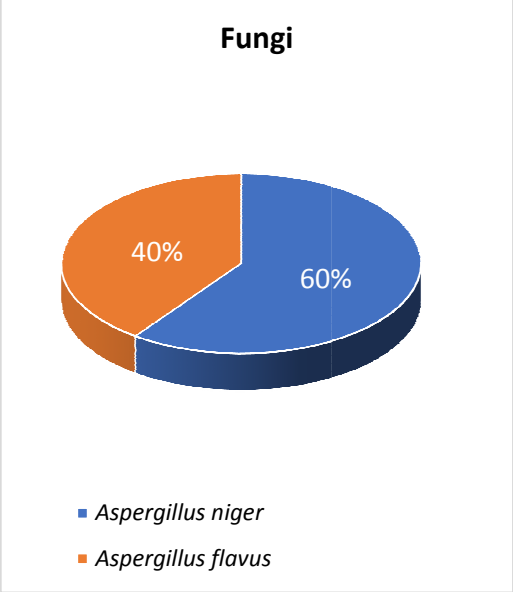

Fig.2b: Frequency of fungi species isolated in the study

DISCUSSION

The results available from this study show the contamination of air conditioners by bacteria such as *Staphylococcus aureus* (13.7%), *Escherichia coli* (6.0%), *Pseudomonas aeruginosa*(20.2%), *Klebsiella pneumoniae* (9.7%) and *Bacillus* spp(50.8%) and also by fungi such as *Aspergillus niger* (60.0%) and *Aspergillus flavus* (40.0%). This compares with the work of Almoffarreh *et al.*, 2016 in Saudi Arabia they also isolated different species of bacteria and fungi, including *Bacillus* spp. (60.3%), *S. aureus* (17.2%), Gram negative bacilli (1.7%), *Aspergillus niger* (3.0%).

All the air conditioners filter samples studied carried bacterial contaminants (100.0%) compared to 73.0% which tested positive for fungi. Overall, air conditioner filters harbored more bacteria (100.0%) and fungi (73.0%) contaminants, respectively than the filtered air samples (48.0%) and (34.0%), respectively from the same air conditioners. These differences were statistically significant (x^2 = 62.5667, p = 0.00001) and (x^2 = 21.13, p = 0.00001) respectively. This significant difference observed in the distribution of microbes on air conditioner filters and filtered air is in line with work done by Nordell (2000) in Sokoto, Nigeria and Al-Abkari (2014) in eastern region of Saudi From this study, it was observed that the air conditioners in the hotels had the highest occurrence of microbial contaminants on their filters (bacteria (100.0%) and fungi (83.3%).

The office air conditioners had the highest number of bacterial and fungal contaminants (52% versus 52.9%) followed by the hotels (50.0% versus 25.0%) and hospitals (41.2%versus 25.0%), respectively. Although the frequency of bacteria and fungi colonization of these air conditioners tend to differ according to the location, no association was established between this microbial colonization and the air conditioners' locations(X^2 = 0.509, P = 0.775285) versus (x^2 = 4.1261, P = 0.12707), respectively.

Bacillus, the most encountered bacterial species in this study, are known to cause pneumonia and other respiratory tract infections while a few species of *Aspergillus* are capable of causing both benign and fatal human infections called aspergillosis, when their spores are inhaled from the environment in dust, and in outdoor and indoor air (Grbić *et al*., 2008). Some are also known to produce carcinogens and cause secondary infections, especially in immunocompromised persons, producing symptoms such as persistent cold, watery eye, prolonged muscle cramps and joint pain (Alexoupolous and Mims, 1990).

The huge number of bacteria and fungi species isolated in this study could be justified by the observation during this study that most of the air conditioner users lacked the culture of subjecting their air-conditioning systems to regular maintenance.

CONCLUSION

This study has recorded a high rate of occurrence of various potential bacterial and fungal pathogens in in-use air conditioners in Calabar with *Bacillus* spp and *Aspergillus niger* being the most frequently isolated. The various species of potential pathogens isolated in this present study implies that the air conditioners could be contaminated with microorganisms which pose the risks of hypersensitivity reactions and infections to the users and the general population. Measures should be put in place to ensure the implementation of recommended healthy, periodic cleaning and maintenance and microbiological screening of in-use air conditioners.

Conflict of interest

The authors declare none.

REFERENCES

Al-Abkari, H.A. (2014). Studies on microbial contamination in air conditioning systems in the Eastern region of Saudi Arabia Kingdom and their control [M.Sc. thesis in Microbiology]. Dammam, Saudi Arabia: University of Dammam.

Alexoupolous, C. J. & Mims, C. W. (1990). Introductory Mycology 3rd edition. Edinburg, New York. Pp. 112.

Almoffarreh, H. K., Alsaleh, F. M., Alruwaili, M. S. (2016). Bacterial and Fungal Contamination of Air conditioners filters and Carpets. *International Journal of Environment, Agriculture and Biotechnology*, 1(3): 399-404.

Anas, G., Aligbe, D.S., Suleiman, G. & Warodi, F.A. (2016). Studies on microorganisms associated with air-conditioned environments. *IOSR J Environ Sci Toxicol Food Technol. 10*(7):16-18. doi:10.9790/2402-1007011618

Baker, F.J, Silverton, R.E. & Pallister, C.J. (2001). Baker and Silverton's Introduction to Medical Laboratory Technology 7th Ed PP 309-319.

Chang, J.C.S, Foarde, K.K., & Vanosdell, D.W (1996). ASTM STP Characterizing Sources of Indoor Air Pollution and Related Sink Effects. Pp 87-97

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press United Kingdom (7): PP 45-70.

Grbić, M. L., Vukojević J., & Stupar, M. (2008). Fungal colonization of air-conditioning systems. *Arch. Biol. Sci., Belgrade*, 60 (2), 201-206. DOI:10.2298/ABS0802201L

Guirguis, K., Basu, R., Al-Delaimy, W.K., Benmarhnia, T. & Clemesha, R.E.S, (2018). Heat, disparities, and health outcomes in San Diego County's diverse climate zones. *Geography Health. 2:*212-23. doi:10.1029/2017GH000127

Kemp, P. C., Neumeister-Kemp, H. G., Lysek, G., & Murray, F. (1999). Survival and growth of microorganisms on air filtration media during initial loading. *Atmospheric Environment*, 35, 4739-4749.

Lundgren, K. &Kjellstrom, T. (2013). Sustainability challenges from climate change and air conditioning use in urban areas. *Sustainability. 5*:3116-28. doi:10.3390/su5073116

Maus, R, Goppelsröder, A. &Umhauer, H. (2001). Survival of bacterial and mold spores in air filter media. *Atmospheric Environment. 35*:105-113

Morawska, L., Thomas, S., Hofmann, W., Ristovski, Z. & Jamriska, M. (2004). Exploratory cross-sectional investigations on ambient sub-micrometer particles in Salzburg, Austria. *Atmos Environ. 38*(21):3529-33.

Nordell, E.A. (2000). *American Review of Respiratory Diseases*. 162 (2): 501-503. Available from:https://www.researchgate.net/publication/332304828_Studies_on_Microorganisms_Associated_with_Air-Conditioned_Environments [accessed Dec 18 2021].

Ottong, Ering & Akpan (2010). The population situation in Cross River State Nigeria & its implications for socio- economic development; Observations from the 1991 &2006 censuses. Journal of Emerging Trends in Educational Research and Policy Studies. 1(1): 36 - 42

Prescott, M.L., Haley, J.P. & Klein, D.A. (2000). Microbiology, 4th edition. Pp. 312-343.

Rohizan, N.A, & Abidin, E.Z. (2013). Assessment on physical factors of thermal comfort, sick building syndrome symptoms and perception of comfort among occupants in a public research university laboratory building. *Int J Public Health Clinical Sci.* 2(3):59-70.

Roponen, T., Letitonen, M. &Taunema, T. (2001). *Journal of Aerosol science*, 32 (5): 663-666. Available from: https://www.researchgate.net/publication/332304828_Studies_on_Microorganisms_Associated_with_Air-Conditioned_Environments [accessed Dec 18 2021].

Sangeetha, J. & Thangadurai, D. (2013). Staining Techniques and Biochemical Methods for the Identification of Fungi. In V. K. Gupta, M. G. Tuohy, M. Ayyachamy, K. M. Turner, A. O'Donovan (Eds.), *Laboratory Protocols in Fungal Biology: Current Methods in Fungal Biology*. DOI 10.1007/978-1- 4614-2356-0_19:Springer Science + Business Media, LLC.

Tabian, O.C., Anderson, L.J., Arden, N.H. & Berman, R.F. (1996). www.portableairshop.com:Available from:https://www.researchgate.net/publication/332304828_Studies_on_Microorganisms_Associated_with_Air-Conditioned_Environments [accessed Dec 18 2021].

Tham, K.W. & Zuraimi, M.S. (2005). Size relationship between airborne viable bacteria and particles in a controlled indoor environment study. *Indoor Air. 15*:48-57. doi:10.1111/j.1600-0668.2005.00303.x

Vidya, G. Kumar, B.A., Kalpana, M. & Chand K. (2014). Pulmonary function tests in air conditioner users. *Int J Med Biomed Res*. 2014; 3(2):75-80. doi:10.14194/ijmbr.3.2.3