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Studies on Microorganisms Associated with Air-Conditioned Environments

Gide Anas[1] David Sunday Aligbe[2] Gide Suleiman[3] and Fatima Adam Warodi[3]
[1]*Nigerian Institute for Trypanasomiasis Research Sokoto State.*
[2]*Ahmadu Bello University Zaria.*
[3]*Desert Research Monitoring and Control Centre, Yobe State University Damaturu P.M.B 1144.*

Abstract: *Air conditioners are temperature cooling equipment used in modifying air temperature of homes, offices, and industries for various reasons. Nevertheless, such artificial environments may be favorable to fungi, bacteria, protozoan and mites growth, which may bring health risks to users, either by hypersensitivity or infections. The microbial contamination of these air conditioner filters was examine in Tertiary Institution in Sokoto north western part of Nigeria using culture techniques. Four Bacterial species of Bacillus lentus, two species of Bacillus alvei, Providencia spp and three fungal species belonging to three genera Aspergillus niger, Asprgillus flavus and Aspergillus versicolor were isolated. Aspergillus niger was the most frequently encountered fungi associated with air conditioner filters, while Bacillus lentus was the most frequently encountered bacteria associated with air conditioner filter.*

***Keywords*:** *Air-conditioning filters, bacteria, fungi, microbial contamination, cooling equipment*

I. Introduction

Air conditioning system consists of centralized equipment that provides an atmosphere with controlled temperature, humidity and purity at all times regardless of the weather conditions. Many of the air conditioning units consist of merely a blower equipped with refrigerating unit that provides only the flow of cool filtered air. Air conditioners are also temperature cooling equipments used in modifying indoor air temperature of homes, offices, cars and industries for various reasons hence they influence human health (Nester *et al.,* 1996).

In most tropical countries, air temperatures are always on the high side except during the harmatan period (Brown *et al.,* 2000). The air conditioners are therefore used to modify the indoor air quality and are installed in homes, offices, vehicles and industries for various reasons including good operational output of equipment and comfort (Han *et al.,* 1998 Brown and wake, 2000). Miller Laiden *et al.,* (1997) reported that most air conditioners in used are operating outside their designed parameters due to inappropriate filters. This assertion had been collaborating by Decosemo *et al.,* (2000). These authors attributed the situation to neglect or over used, which resulted in dust accumulations. The dust accumulation coupled with humidity especially at the downstream section of the cooling equipment lead to the proliferation of selected group of microorganisms capable of surviving the prevailing conditions (Nordell 2000; Chang *et al.,* 1996). This situation has often resulted in what is referred to as " sick house syndrome" noticed only when users of such buildings spends considerable time in it and develop some form of allergic symptoms (Reponen *et al.,* 2001; Rothwell 1998; Leonard 2000). The sick house syndrome symptoms include headache, watery eyes, skin disorders and weakness.

Organisms like *Legionella pneumophilla, Streptococcus pneumonia* and *Bacillus spp* have been *reported* in air conditioner filters (Nordell 2000; kemp *et al.,* 1999). Chang *et al.,* (1996) and Hyarinem *et al.,* 1998 observe species of *Penicillium pneumocystis, Carinii* and species of *Aspergillus, Rhizopus, Fusarium* and *Alternaria species* have been reported too (Prescott *et al.,* 2000; Reponen *et al.,* 2001; Tabien *et al.,* 1996). *Legionella pneumophila* is part of the natural microbial community of soil and aquatic ecosystem (Prescott 2000). Most of the information available deals with the European and American countries, but not much has been reported for tropical countries such as Nigeria. The aim of this study was to assess the level of bacterial and fungal contamination in air-conditioned at Departmental buildings in Usman Dan Fodio University Sokoto north western Nigeria.

II. Materials And Methods

Experimental design

The present research work had the following experimental design; four Departments equipped with air conditioners (AC) were selected at Faculty of Science thus; Chemistry, Biology, Biochemistry and Microbiology Department respectively.

Sampling site

Air conditioners (AC) used in the research work are reported to have been in used for three to five years; the samples were grouped according to the part of the buildings, which are upstairs and downstairs respectively.

Sample Collection

The air conditioners (AC) were opened to expose the filters, settled dust samples were taken from the inner surfaces of the ventilation duct using sterile cotton swabs. Scrubbing was done at both diagonals of each rectangular filters as described by Kemp *et al.,* 1999 and Chang *et al.,* (1998). The sampling was done in duplicates and was taken to the Microbiology laboratory.

Isolation and morphological study of bacteria

Tryptic Soy agar plus Benlate (casein peptone 17 g, soy peptone 3 g, dextrose 2.5 g, sodium chloride 5 g, dibasic potassium phosphate 2.5 g, agar 15 g, Benlate 20 mg; dH_2O 1000 mL, pH= 6.2) was used. After samples inoculation, the Petri dishes were incubated for three days at 28°C. The number of bacteria colonies were assessed in the first and third days of incubation, and the number of CFU/g of filter was determined.

For the study of the morphological groups, the strains was stained by Gram method and examined by microscopic and morphological characteristics of the bacterial cells was evaluated, ordering them in positive and negative Gram rods and coccus.

Isolation and taxonomic study of fungi

The culture medium was semi-solid malt extract agar (malt extract 20 g, glucose 20 g, peptone 2 g, agar 7 g, dH_2O 1000 mL, pH 5.5), the dishes were incubated for 5 days at 28°C. The number of fungi colonies present was assessed in the third and fifth days of incubation, and the number of colony forming units was determined per gram of filter (CFU/g filter). After that, the colonies considered morphologically distinct were isolated and again compared with each other, so that only one isolated strain representative of each morphological group found was selected. The taxonomic identification of fungi was performed considering the morphological characteristics of the vegetative mycelium and the reproductive structures (Barnett and Hunter, 1972; Larone, 1995; Ainsworth *et al*., 1995; Alexopoulos *et al*., 1996; Lacaz *et al.*, 1998).

III. Results

Table 1 presents the location and positions of the air-conditioning (AC) systems at various departmental buildings, most of the AC where placed at down stairs of the buildings.

Table shows the number of bacterial counts on filter dust layer of the AC of the various departments, microbiology laboratory with the highest number of colonies per gram of dust sample.

Table 3 present the number of fungal counts on filter dust samples of the various departmental air-conditioning systems .

Table 4 shows all the isolated bacterial and fungal strain from the inner surfaces of the AC filter duct, four bacterial species and three fungal species were identified.

Table 1. Characteristics of sampling departmental air-conditioning system (AC)

Investigated Dept.	**Microbiology**	**Biochemistry**	**Chemistry**	**Biology**
Characteristics	Old	Old	New	Old
Location	Lab	Lab	Auditorium	Lab

Table2. The count of bacteria on filter layers of the AC.

Samples	**Microbiology**	**Biochemistry**	**Chemistry**	**Biology**
	cfu/g filter	cfu/g filter	cfu/g filter	cfu/g filter
Upper stairs	320	210	133	230
Down stairs	510	360	200	450

cfu/g filter – Colony forming units per gram filter.

Table3: The count of fungi on filter layers of the AC.

Samples	**Microbiology**	**Biochemistry**	**Chemistry**	**Biology**
	cfu/g filter	cfu/g filter	cfu/g filter	cfu/g filter
Upper stairs	267	400	133	222
Down stairs	480	620	530	500

cf u/g filters – Colony forming units per gram filter.

Table 4: Microorganisms identified in settle dust sample from the filters of air-conditioning system.

Bacteria		**Fungi**	
Genus	Species	Genus	Species
Bacillus	*Bacillus lentus*	*Aspergillus*	*Aspergillus niger*
	Bacillus alvei		*Aspergillus versicolor*
Providencia	*Providencia spp*		*Aspegillus flavus*

Note: Species from the genus *Aspergillus* were isolated in over 54 % of the experiments.

IV. Discussion

In our study, the pathogenic fungi identified are *Aspergillus niger, Aspergillus flavus,* and *Aspergillus versicolor*. They are known to cause invasive aspergillosis and produce carcinogens. Most infections commonest being aspergillosis can occur in immunocompromised host or as a secondary infection following inhalation of fungal spores or the toxins produce by them. Symptoms include persistent cold, watery eye, prolong muscle cramps and joint pain (Alexoupolous *et al,* 1990).

Aspergillus flavus and *Aspergillus fumigates* can cause nosocomial infections, allergic bronchopulmonary aspergillosis (ABPA) and sinusitis. Chronic asthmatic may progress to have their bronchial passages colonized by *Aspergillus fumigates.* Constant allergic response maintains the fungal colonization and the first line therapy with steroids brings down the level of contamination of the colonizing organism (Douwes *et al,* 2003).

The bacteria identified are *Bacillus alvei, Providencia stuartii and Bacillus lentus.* More organisms were reported during the dry season, this could be attributed to the changes in weather condition with dry season having a lot of dust particle that could have contained microbial spores as they are blown into the air by the wind. Prescott *et al,* 2000 have implicated *Bacillus species* in pneumonia and other respiratory tract infections. Only particles in the air can contaminate the filters, this is because all spore forming organisms were most prevalent in the dry season. However, non spore formers could have contaminates the filters during repair by touch, human aerosols from cough, sneezing, talking and other similar mechanisms (Alexis *et al;* 2001).

The gram positive isolates confirmed are *Bacillus lentus* and two species of *Bacillus alvei.* Decosemo *et al,* (2000) had also reported the presence of *bacillus lentus* on cooler filters. This species are known to cause pneumonia and other respiratory tract infections. *Providencia stuarti*is the gram negative bacteria isolated, known to cause sick house building syndrome and also causes illness due to endotoxins. Endotoxins are the lipo-polysaccharides of gram negative bacterial cell wall. These are potent pyogens capable of causing fever in very low concentrations. High exposure to endotoxins is often associated with nausea and diarrhea (Nordell *et al,* 2000).

V. Conclusion

Dust and liquid drops are the important medium for microorganisms to spread. It is necessary to termly clean the components that are easy to be infected in air-conditioning systems (e.g., filter, heat exchanger and muffler) and to replace them in time in order to avoid the aggradations of pollutants. Moreover, the condensing water should be eliminated in time in air-conditioning systems to prevent bacteria from propagating.

Filtration is a quite economical and efficient method of improving air-conditioning system, the air filtration systems represent a good solution for the improvement of Indoor Air Quality (IAQ), and the Anti-microbial treatments of filters may be a solution to these problems. It is possible to prevent the accumulation and dispersion of microorganisms by adding anti-microbial agents on the surfaces of filter, which contributes to the improvement of air quality

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