Bacterial and Fungal Contamination of Air conditioners filters and Carpets

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Abstract— Background:
Objective*: To study the level of colonization by bacteria, and fungi/molds in air conditioners and carpets used in homes, offices,university classrooms, prayer room and laboratories.*
Methods*: A total of 25 different settled dust samples from air conditioners, and carpet vacuum cleaners were investigated in this study. One gram of each sample was emulsified into 9.0mL sterile normal saline. 10-fold dilutions were made in normal saline and 100 µL of each suspension was inoculated onto 2 plates of* SabouraudDextrose Agar*incubated at 25 °C for 48-72hrs, while 50 µLwas inoculated onto MacConkey and Blood agar plates which were incubated at 37 °C for 48hrs.*
Results*: of the settled dust samples examined, 72 % were found to harbor molds, bacteria and yeasts. Of the total bacteria isolated, Bacillus spp. comprised 60.3%, S.epidermidis (20.7%), S. aureus (17.2%), Gram negative bacilli (1.7%). On the other hand, Aspergillusspp. made up 26.9%, of the total mold isolates, followed byPenicillium spp. (20.9%), Yeast (13.4%), Rhizopus spp. (7.5%), Zygomycetes spp. (3%), Aspergillusniger (3%), Lichtheimiacorymbifera (3%), Microsporum spp. (1.5%) and Unidentified mold in 20.8%. The mean bacterial concentration found was 1.33 x 10^5Cfu/g of dust.*
Conclusion*: The presence of potentially pathogenic bacteria and molds in the dust samples of the air conditioner filters, and home carpets indicated that our environment is full of microorganism that may affect people's health and expose them to different diseases, this mandates regular cleaning and safe removal of dust from A/C filters and carpets.*

Keywords—air conditioner filters, carpets, bacteria, Fungi.

I. INTRODUCTION

Our planet is surrounded by air and dust where variety of microorganisms are found.Aerobiology is the study of the airborne microorganisms and their effect on human health and the environment.As a result of increasing awareness of the variety of health problems caused by airborne microorganisms [1] research in this area has been increasing recently.Airborne contaminants are also known as bioaerosols and include bacteria, fungi, viruses and pollen. These may be present in the air as solid (dust) or as liquid (condensation and water). An aerosol is a two-phase system of gaseous phase (air) and particulate matter (dust, pathogens), thus making an important bacterial vehicle. The micro organisms that can be found in bioaerosolscan be foundattached to dust particles or may survive as freefloating particles surrounded by a coating of dried organic or inorganic material and due to a lack of nutrientstheycannot multiply in bioaerosolsbut they can travel in the air for great distances[2, 3].

The developmental process of using air conditioners and carpets by humans in houses, class rooms, mosques and other placesthough resulted in the help of easing life for humans during times when weather is harsh, however, their use unfortunately brought undesirable effects in that they became a good source and or media for bacterial and fungal/mold contamination[4].The improperly maintained air conditioning systems and exposure to these microbial fragments and metabolites can be a source ofwide variety of illnesses ranging from allergy symptoms to dangerous conditions such as respiratory, dermatological infections, epidemics and food pollution[5].In particular, individuals with poor health or immune compromised patients are at a greater risk to suffer from exposure to these microorganisms that could be present in this environment [6].Recent research findings have shown that certain vacuum cleaners unfortunatelycan make things moreworse, in that they have the ability to spit fine dust back into the air, where they can spread any kind of infections[7] in the surrounding environment, which leads to the believe that microorganisms are linked to poor indoor air quality[8].

It has been estimated in Northern Europe and Canada that 20-40% of homes have mold contamination [9].This number in tropical and subtropical countries isestimated to be much higher [10, 11].Whereas, in United States, as many as 47%of homes have mold contamination [12].Varioushealth effectshave beenrelated to mold exposure[13].A case control study conducted inEurope suggested a relation between increases symptoms of asthmatic patients andincreased mold and moisture problems in homes[14].Jo and Lee (2008), [15] reported that automobile and household air conditioning can discharge up to 2,500 colony forming units per meter

cubed (CFU/m^3) of bacteria, as well as 1,000 CFU/m^3 of fungi at ambient levels during initial startup. Conspicuous growth (up to 10^6CFU/cm^2) of bacteria is reported from air handling cooling coils.

Ali et.al., (2014)[16] have investigated the presence of bacteria, fungi and mold in 50 dust samples from carpets of 50 mosques in Elkhomes city inLibya, and found that12%were positive for *Escherichia coli*, 60% for Staphylococcus spp. and 66%for Klebsiella spp.Similarly, Rahouma et.al., (2010) [17] have collected 57 dust samples from carpets of mosques in Tripoli city in Libya, and reportedthe presence of *E. coli* in 16 samples, *Staphylococcus aureus*in 12 sample, Salmonella spp. in 2 samples and Aeromonas spp. in one sample.

The aim of the present study is to determine the presence of bacteria and fungi in settled dust samples collected from home carpets and air conditioners filters in different places inSakaka city, Aljouf, Saudi Arabia.

II. MATERIALS AND METHODS

Location of study:

Sakaka is located in the northeast of Saudi Arabia.The geographical location is between longitudes 37/42 east and latitudes 29/32 north, and it rises from the sea at 580 feet with an estimated area of about 107.794 km2, which is equivalent to 4.9% of the total area of the Saudi Arabia. Climate fluctuations are evident with the average maximum temperature of 42 °C in the summer, and the average winter temperature of 8.5 °C. The month of July is the hottest months of the year, while temperatures drop in the month of January with an average rainfall of approximately 200 mm. A north-west wind is reported in the area during the month of April, whereas, an eastern-western wind in the rest of other months. The average speed of wind is about 5.7 miles per hour.

Sample collection:

A total of 25 settled dust samples were collected from carpet's vacuum cleaners (*number*=5)and filters of air conditioners(*number*=20).Sampling was performed during the periodDec 2015 to Feb 2016. The carpet dust samples were collected using a 2,000 W and 1400 W household vacuum cleaners; while samples from filters of air conditioners were collected fromWindow type A/C, Split A/C and Tower A/C.Settled dust was carefully removed from the filters and collected onto a clean paper and transferred into sterile petri dishes and transported to the microbiology laboratory at the college of Medicine, Aljouf University,and processed within 1-10 hours of collection.

Bacteriology

After collection, the contents (i.e. dust, settled material, hair, etc.) of each sample was thoroughly mixed together and 1.0 g of each sample was weighed and emulsified into 9.0 ml of sterile normal saline, and vortexedfor 2 minutes to ensure that all the contents were mixed well and to disperse any microorganisms that may have been trapped into the dust. A ten-fold dilution of each sample (10^1 - 10^2) was performed using sterile normal saline, and 100 µL of each dilution was transferred and spread onto two*Sabouraud Dextrose Agar plates(SDA)*(Oxoid LTD., Basingstoke, Hampshire-England) *using a glass spreader. One plate each of* MacConkeyagar(Oxoid LTD., Basingstoke, Hampshire-England) and BloodAgar (Oxoid LTD., Basingstoke, Hampshire-England) were streaked using 50 ml of *each sample.* All plates were incubated at 37°C in air for 24 hours, except forSDA plates which were incubated at 25°C for 48-72 hrs.[18]

After incubation, any visibly distinguishable bacterial or fungal colonies were identified and the number on each plate was counted. The initial observations about the shape, color, size and other visual properties of each isolate werealso recorded.A representative sample colony of each visually differentiable bacteria, fungi or moldwas selected using a sterile inoculating loop, and was transferred by streaking ontoa fresh platesof SDA, Blood agar or MacConkey agar, and incubated as previously described. All bacterial isolates were Gram stained and examined under the microscope using the X1000 lens. [19]. Any suspected yeast's or molds were examined using a drop of lacto phenol cotton blue stain under the X400 lens.

III. IDENTIFICATION OF BACTERIA AND FUNGAI

Biochemical tests:

In order to identify the isolated bacteria some biochemical tests were used including:

Catalase test:To test for the presence of catalase, isolated colonies were selected and transferred via a sterile inoculating plasticloop to a clean slide containing one drop of hydrogen peroxide. If the bacteria contained catalase, hydrogen peroxide was converted to water and oxygen gas, causing bubbles to appear on the slide. Catalasenegative bacteria produce no reaction[20].

Coagulases test: Is an enzyme-like protein and causes plasma to clot by converting fibrinogen to fibrin; usinga plastic pipette aseptically 0.5 ml of 1:3 diluted human plasma was transferred into a test tube, the isolated colonies of the bacteria to be tested were emulsified into the plasma and incubated at 37°C for 3 - 4 hours. Any clot formation was taken as a positive result, however, if no clot was observed by the end of 4 hours, then the test was continued with incubated at 37°C and a final observation was taken at 24 hours[21].

Results:

Of the 25 samples examined in this study, 20 (80%) were made of settled dust collected from different types of A/C filters; while the remaining 20% represented dust from carpet vacuum cleaners (Figure 1). The amount of settled dust collected was greater in the vacuum cleaners then window type air conditioners. However, moderate amounts or very little settled dust was evident on the split or the tower type A/C .

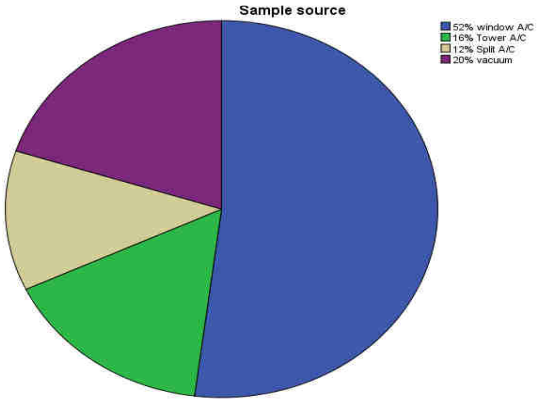

Fig.1: Percentages and sources of the settled dust samples.

As shown in Table 1, of the total 58 bacterial isolates 60% were identified as Bacillus species, 20.7% as*S. epidermidis*; 17% *S. aureus* and only 1.7% was identified as Gram negative bacilli's. On the other hand, a total of 67 isolates were recovered from both the A/C filters and carrept samples. Of the total mold/yeast isolates 26.9% were identified as Asperigillus spp. followed by Penicillium spp. (20.9%), Rhizopus spp. (7.4%) and yeast in 13.4%. While other molds were found in smaller percentages as shown in Table 2. Using the cooton blue technique 20.8% of the total mold isolates could not be fully identified.

Table.1:Bacteria isolated from A/C filters and carpets

Microorganisms	Frequency (%)
Bacillus spp.	35 (60.3%)
S. *epidermidis*	12 (20.7%)
S. *aureus*	10 (17.2%)
Gram negative bacilli	1 (1.7%)
Total isolates	58 (100%)

Table.2: Molds and Yeast isolated from A/C filters and carpets.

Microorganisms	Frequency (%)
Aspergillus spp.	18(26.9%)
Penicillium spp.	14 (20.9%)
Yeast	9 (13.4%)
Rhizopus spp.	5 (7.5%)
Zygomycetes spp.	2 (3%)
Aspergillusniger	2 (3%)
Lichtheimiacorymbifera	2 (3%)
Microsporum spp.	1 (1.5%)
Unidentified mold	14 (20.8%)
Total isolates	67 (100%)

Table.3: Total viable count of the bacterial isolates on McConkey and Blood agar plates.

Total viable count	Number of Samples	Minimum Viable count (cfu/g)	Maximum Viable count (cfu/g)	Mean Viable count (cfu/g)	Standard Deviation
McConkey agar	25	2500	420000	132804	128190
Blood agar	25	11000	520000	133400	129202

The concentration of the viable bacterial count on McConkey agar ranged from 2.5 x 10^3 to 4.2 x 10^5 Cfu/g of settled dust (mean 1.3 x 10^5Cfu/g). While the use of blood agar yielded a growth concentration that ranged from 1.1 x 10^4 to 5.2 x 10^5 Cfu/g (mean 1.33 x 10^5 Cfu/g).

IV. DISCUSSION

Microorganisms such as bacteria, molds and yeast are common contaminants in our environment. The inappropriately maintained air conditioning systems and exposure to metabolites can be a source of wide variety of diseases such as allergy, respiratory, dermatological infections, epidemics and food contamination [4]. In view of the climate changes in the northern part of Saudi Arabia, almost all inhabitants have adopted to the use of A/C at homes, work place, prayer places and in their automobiles. As reported by Abdel Hameed and Farag in 1999[22], most of the people in arid areas tend to spend over 90% of their lives indoors: in houses, offices, and schools where they are exposed to some indoor environmental factors (bioaerosols) that influence their health and physical condition. Fungi reproduce through the production of microscopic spores, many of which are dispersed bywind, rainfall, and physical disturbance. Biological contamination of indoor air is mostly caused by bacteria, molds and yeast.In addition to being

dangerous as pathogenic living cells they can also secrete some substances harmful for health.

As reported in the present study that almost all the dust samples collected from the A/C filters were colonized by bacteria, yeast and molds. This colonization by these microorganisms is partially explained by the fact that condensation of water and increase in relative humidity and temperature in the A/C favors fungal growth. As a common practice the A/C units examined in this study are usually switched on and off during the day, this mandates frequent cleaning to reduce the clogging of the filters by dust and to reduce the likely chance of fungal or bacterial growth [23, 24]. Similarly, home carpets were found to harbor bacteria, yeast and molds, a periodic shampooing and vacuum cleaning is also necessary in order to minimize the likely hood of microbial colonization [25].Although, the common way to clean home carpets is by the use of vacuum cleaners, however, their use has been associated with the release of high concentrations of antigens through the mechanical disturbance of settled dust and release from the vacuum cleaner itself [26]. The results of the present investigation has shown that dust samples collected from carpets contained several types of bacteria including *S. aureus*, *S. epidermidis*, Gram negative bacilli, and other types of molds such as *Asperigillus* and *Penicillum* spp. and others which is in agreement with the findings by other researchers [27]. It has also been reported that aerosolization of carpet dust can be a source to spread other pathogenic bacteria such as Salmonella and *Clostridium botulinum*[28]. What make things worse is that the presence of such microorganisms in dust bags or the chambers of the vacuum cleaners can be extended over long periods of time [29].

The amount of dust present in the A/C and carpets varied considerably. Obviously, vacuum and A/C filters samples exhibited too much amount of dust in comparison with Tower A/C filters, where the least amount of dust was found. As shown in the results section the concentration of the different microorganisms obtained on both McConkey agar and blood agar plates didn't significantly differ as the mean number of viable colonies was comparable on both types of media. Of the bacteria isolated in the different samples of dust, Bacillus spp. Represented 60.3% of the total bacterial isolates followed by *S. epidermidis (*20.7%), *S. aureus* (17.2%) and GNB (1.7%). however, a study conducted in Libya by Ali et.al., (2014) [16] showed the presence of other microorganisms such as *E. coli* and Klebsiella spp.This can be explained by the fact that the source of the dust samples examined in both studies differed. The Libyan samples were collected from carpets at different mosques where large group of worshipers usually gather to perform religious duties. Whereas, in the present study a smaller number of samples from home carpets were used. It also expected that a better cleaning and care is taking at homes which can reduce the amount of dust and therefore bacterial and fungal or mold presence. Other pathogenic bacteria were also isolated from Masjed (mosques) carpet samples. For instance Rahouma et.al., (2010) [17] reported the presence of *E. coli* in 16 samples, *Staphylococcus aureus* in 12 sample, Salmonella spp. in 2 samples and Aeromonas spp. in one sample.Our study has shown the presence of *S. aureus*in a number of samples; however, the predominant bacterial species present were Bacillus spp. Of the 25 samples examined in our study 18/25 (72%) of the samples yielded the growth of Aspergillusspp. which represented 26.9% of the total mold and fungal isolates. Similarly, Penicillium spp. were largely found making up 20.9%, and Yeast 13.4%. We were unable to identify 16.4% of the molds present using the colonial morphology and microscopical examination of these isolates. In a study conducted in Warsaw, Poland by Gołofit-Szymczak&Górny[30],they found almost similar results were Aspergillus spp. and Penicilliumwere the most common isolates they encountered. The presence of certain bacterial species such as *S.aureus*and *S. epidermidis* on carpets is highly suspected, because these bacteria are present in the nose and skin of people as part of their normal flora [31].In view of the sources of the settled dust samplesused in our study and others, it was not possible to make a direct comparison of results.

V. CONCLUSIONS AND RECOMMENDATIONS

The presence of potentially pathogenic bacteria and molds or yeast in the dust samples of the examined air conditioner filters, and home carpets indicated that our environment is full of microorganism that may affect people's health and expose them to risk of different diseases, this mandates the regular cleaning and removal of dust from A/C filters and carpets.

The use of good quality vacuum cleaners isalso recommended to decrease the chance of people being exposed to indoor allergen or microorganisms. It is likewise highly advised to keep cleaning of the air conditioners and vacuums regularly, and changing of the carpet and other furniture.Further studies are recommended to a follow up.

REFERENCES

[1] Bitton, G. (2002). Encyclopedia of environmental microbiology. Wiley InterscienePublication, New York, NY.

[2] Dowd, S.E., Maier, R.M (2000).Aeromicrobiology. In Environmental microbiology. Edited by Maier, R.M.,

Pepper, I.L., and Gerba, C.P. Academic Press, Canada. P.91-122

[3] Douwes, J., Thorne, P., Pearce, N., Heederik, D. (2003).Bioaerosol health effects and exposure assessment: Progress and prospects. Annual occupation hygiene.Vol. 47, No. 3, P. 187-200

[4] Air Conditioning South East (2013).Can air conditioning make you sick, airconditioningsoutheast.com.http://airconditioningsoutheast.com/tips/air-conditioning/can-air-conditioning-make-you-sick.

[5] Aydogdu, H., A. Asan, Otkun, M.T. (2008). Indoor and outdoor airborne bacteria in child day-care centers in Edirne City (Turkey): seasonal distribution and influence of meteorological factors EnvironMonit. Assess. 8:76-89.

[6] Adnan A. (2013). Bacteria is the most useful microorganism in the environment and beneficial for human beings. But it also has some harmful effects on human body,biotecharticles.com.http://www.biotecharticles.com /Biology-Article/Beneficial-and-Harmful-Bacteria-312.html.

[7] Woodford, C. (2016). Vacuum cleaners. http://www.explainthatstuff.com /vacuumcleaner.html.

[8] Fabian, M. P., Miller, S. L., Reponen, T., Hernandez, M. T. (2005). Ambient bioaerosol indices for indoor air quality assessments of flood reclamation. AerosolScience. 36: 763 783.

[9] Brunekreeef B, Dockery DW, Speizer FE, Ware JH, Spengler JD, Ferris BG. (1989). Home dampness and respiratory morbidity in children. Am Rev Respir Dis. 140:1363-1367.

[10] Miller JD. (1990). Contamination of food by Fusarium toxin: Studies from Austral-Asia. Proc Jap AssocMycotox. 32:17–24.

[11] Miller JD.(1992). Fungi as contaminants in indoor air. Atmos Environ. 26A (12):2163–2172.

[12] Mudarri, D., Fisk, W.J.(2007). Public health and economic impact of dampness and mold. Indoor air 17:226–235.

[13] Institute of Medicine (IOM).(2004). Damp indoor spaces and health. Washington D.C: The NationalAcademies Press.

[14] Williamson IJ, Martin CJ, McGill G, Monie RD, Fennerty AG. 1997. Damp housing and asthma: A casecontrol study. Thorax.52:229-234.

[15] Jo, W., Lee, J. (2008). Airborne Fungal and Bacterial Levels Associated Withthe Use of Automobile Air Conditioners or Heaters, Room Air Conditioners, andHumidifiers. Archives of Environmental & Occupational Health, 63(3), 101-107.

[16] *AliM, Alemary F, AlrtailA, RzegM, Albakush A, SifawGhenghesh K. (*2014). High isolation rates of multidrug-resistant bacteria from water and carpets of mosques, Libyan J Med 2014. 9: 25415.

[17] Rahouma A, Elghamoudi A, Nashnoush H, Belhaj K, Tawil K, SifawGhenghesh K. (2010).Isolation of antibiotic-resistant pathogenic and potentially pathogenic bacteria from carpets of mosques in Tripoli, Libya, Libyan J Med 2010. 5: 5536.

[18] Mann D.(2012). Are Vacuum Cleaners Bad for Your Health, WebMD.http://www.webmd.com/allergies/news/20120106/are-vacuum-cleaners-bad-for-health.

[19] Nester, E. W., Andesron D. G., Roberts, Jr., C. E., Nester, M. T. (2007). Microbiology: A Human Perspective (5th ed). Boston: McGraw Hill.

[20] Alexander, S. K., Strete, D.(2001). Microbiology: A Photographic Atlas for the Laboratory. San Fransisco: Benjamin Cummings.

[21] Bascomb, S., M. Manafi. (1998). Use of enzyme tests in characterization and identification of aerobic and facultatively anaerobic gram-positive cocci. ClinMicrobiol Rev. **11:**318–340.

[22] Abdel Hameed A.A., Farag S.A.(1999). An indoor bio-contaminants air quality. InternationalJournal of Environmental Health Research. 9: 313

[23] Yau, Y.H., Chandrasegaran, D., Badarudin, A. (2011). The ventilation of multiple-bed hospital wards in the tropics: a review. Building and Environment 46 (5), 1125–1132

[24] Ruping, M.J.G.T., Gerlach, S., Fischer, G., Lass-Florl, C., Hellmich,M., Vehreschild, J.J., Cornely, O.A. (2011). Environmental and clinical epidemiology of Aspergillusterreus: data from a prospective surveillance study. Journal of Hospital Infection 78 (3), 226–230.

[25] Khan, A.A.H., Karuppayil, S.M.(2011). Practices contributing to biotic pollution in Airconditioned indoor environments. Aerobiologia 27, 85–89

[26] Woodfolk JA, Luczynska CM, de Blay F, Chapman MD andPlatts-Mills TA. (1993). The effect of vacuum cleaners on the concentration and particle size distribution of airborne cat allergen. J. Allergy ClinImmunol. 91:829–837.

[27] Kaarakainen P, Rintala H, Vepsalainen A, Hyvarinen A, Nevalainen A, Meklin T. (2009). Microbial content of house dust samples determined with qPCR. Sci. Total Environ. 407:4673-4680.

[28] Nevas M, Lindstrom M, Virtanen A, Hielm S, Kuusi M, Arnon SS, Vuori E, Korkeala H. (2005). Infant botulism acquired from household dust presenting as sudden infant death syndrome. J ClinMicrobiol. 43: 511–513.

[29] Haysom IW., Sharp K. (2003). The survival and recovery of bacteria in vacuum cleaner dust. J R SocPromotHealth 123:39–45.
[30] M. gołofit-szymczak, R.L. górny.(2010). Bacterial and Fungal Aerosols in Air-Conditioned Office Buildings, Warsaw, Poland, International Journal of Occupational Safety and Ergonomics (JOSE), Vol. 16, No. 4, 465–476
[31] Turnidge J, Rao N, Chang F-Y, Fowler Jr VG, Kellie SM, Arnold S, et al. (2008). Staphylococuus aureus.