Bacillus Contaminated Air- conditions (A/Cs) in Homes at areas of Umul- Hamam, Saudi Arabia

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This study evaluated the bacterial contamination of air conditions in houses of Umul-Alhamaam in Saudi Arabia; by determining the bacteria and associated antibiotic resistance of the isolates. Cultures from 10 surface air-conditions rooms were obtained. The newly filtered air-conditioned rooms were found to have significantly smaller number of bacteria 10^{3cfu}. But it was found a significantly greater number of Bacillus in unfiltered air-condition 106 cfu. This study was conducted in Riyadh, Saudi Arabia during 2011-2013. People living in these houses were exposed indirectly to additional bacterial reservoirs through breathing of contaminated air in their rooms. The newly filtered airconditioned rooms were found to have significantly smaller numberof Bacillus colonies and Staphylococcus colonies while it was found significantly greater number of Bacillus colonies in unfiltered air-conditions without Staphylococcus colonies. Antibiotic sensitivity test were prepared against bacterial isolates and the results were interpreted. A preliminary health status survey revealed that the occupants of air-conditioned homes without filter had more complaints of eye irritation, sneezing, nasal congestion, and cough. To address of this issues of bacterial related illness, there should be an urgent need for studies using objective markers of illness, relevant animal models, proper epidemiologic techniques, and examination of confounding factors. Here I report of severe bacterial pathogenic as a result of indoor bacterial contamination.

Key words: Air-condition filter, cough, Pathogenic bacteria, Contamination, Bacillus and Antibiotics Resistance

Air-conditions (ACS) contamination is a major complication following living in hot parts of the world. Microbial contamination of unclean ACS is precursor for infection. For most homes of these countries, the source of pathogens is the indoor air coming from the ACS without filter or with old ones. However, it is generally accepted that the main factor causing indoor air contamination after clean operations of home ACS are microorganisms, predominantly caused by bacteria and mold shaded from filter or surface ACS. Correlation between airborne bacteria contamination levels and the incidence of postoperative wound infections was reported and the use of ultraclean operating areas with laminar airflow ventilation has been recommended in orthopedic implant surgery, to reduce postoperative surgical site infection¹. Manual small incision cataract surgery (SICS) and phacoemulsification (Phaco) bacterial contamination of the anterior chamber (AC) as well as conjunctive flora bacterial contamination of AC between well-controlled diabetics and nondiabetics was reported². The majority of postoperative endophthalmitis are presumed to be due to introduction of microorganisms during surgery being the major sources of intraocular contamination the conjunctiva and lid margin flora³.

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A recent report by the US National Academy of Sciences stated that there was inadequate or insufficient evidence to determine whether or not an association exists between fungal exposure and the development of asthma. However, two recent reviews of the effects of home dampness and fungi have shown several positive associations between fungal exposure and increased risk of adverse respiratory symptoms in children⁴. Fungal species which are pathogen can be identified using morphological and molecular methodologies based on PCR and ribosomal DNA of the Internal Transcribed Spacer region⁵. A prospective study of Endotracheal aspirate samples of 369 patients under mechanical ventilation for >48hrs who were suspected of having ventilator associated pneumonia (VAP) were conducted. Out of the 369 patients studied, quantitative culture was positive in 166(44.99%), 143 cases (38.75%) showed no growth and in 60 cases (16.26%) growth was <10(5)both of which were included under No Ventilator associated pneumonia (NO-VAP) group.Out of 166 culture isolates, Pseudomonas species 56(33.73%) was the most common, followed by Klebsiella. pneumoniae 28 (16.87%), Acinetobacterspecies20 (12.05%), Escherichia. Coli 16(9.64%), Penicillin resistant Staphylococcus aurous 9(5.42%) and Citrobacter. freundii 7(4.21%) (6). Bacterial colonization and infection is not limited only A/Cs at homes and cars and hospitals. for example; Legionella can colonize water treatment plants, such as refrigeration towers, potable water pipes and can cause infections in humans, when infected aerosols are inhaled despite efforts to keep water systems free of Legionella, this pathogen is still causing infection throughout the world(7). The efficacy of a mobile laminar airflow (LAF) unit in reducing bacterial contamination at the surgical area in an operating theatre supplied with turbulent air ventilation was evaluated⁸. Air-conditioners (AC) produce much dew and wet conditions inside their apparatus, when in operation. Fungal contamination in AC was studied and found that the average fungal contamination of AC filters was about 5-fold greater than that of a carpet, and Cladosporium and Penicillium were predominant in AC filters9. To describe the benefits and optimum use of prophylactic antibiotics in users of therapeutic contact lenses it is necessary to carry out the test of resistance to antibiotics of bacteria isolates¹⁰. Alternatives to antibiotics are urgently needed in animal agriculture. The form these alternatives should take presents a complex problem due to the various uses of antibiotics in animal agriculture, including disease treatment, disease prevention, and growth promotion, and to the relative contribution of these uses to the antibiotic resistance problem¹¹. Information about the prevalence of Staphylococcus aureus resistance to antimicrobial drugs has mainly been obtained from invasive strains, although the commensal micro biota is thought to be an important reservoir of resistance¹². Components of fixed orthodontic appliances as received from the manufacturers were tested for free of contamination. After growth on blood-rich media, cultured bacteria were identified by 16S rDNA polymerase chain reaction amplification and sequence phylogeny. Results were as reported the findings of most commonly bacterial species of Staphylococcus epidermidis, followed by Kocuria, Moraxella, and Micrococcus species. And it was concluded that new materials "as received" from the manufacturers and those exposed to the clinical environment are not free from bacterial contamination before use in patients, but this contamination is low considering the potential for aerosol and operator contamination and could be considered insignificant¹³. Computer terminals in schools were sampled for the investigation of S. aureus and methicillin-resistant staphylococci. The investigators reported that the overall prevalence Methicillin-resistant Staphylococcus aureus of on computer keyboards was low: 0.68% for a postsecondary institution and 2% and 0% for two secondary institutes. Although the prevalence was low, the presence of MRSA combined with the high volume of traffic on these student computer terminals demonstrates the potential for publicaccess computer terminals and computer rooms at educational institutes to act as reservoirs¹⁴.

MATERIALSAND METHODS

Cultures from ten air-conditions (AC)in homes of Umul-Alhamam were obtained. In total, 238 bacterial colonies were obtained from 10 surfaces sampled 47.058823% of the colonies grew on nutrient agar plate were from air-condition with new filters while 52.941176% were from aircondition with old filters. This study was conducted in Riyadh, in the mid of June 2011-2013. People living in these houses were found to be exposed indirectly to additional bacterial reservoirs through breathing of contaminated air in their homes. Gram and simple staining was performed. Disks of Bacitracin (BA), Chloramphenicol (C), Cotrimoxazole (TS), Gentamicin (GM), Neomycin (NE), Penicillin G (PG), Polymyxin B (PB) and Tetracycline (T)were tested against isolates.Because of convenience, efficiency and cost, the disk diffusion method was used for determining antimicrobial resistance in in this study. A growth medium, Mueller-Hinton agar was first seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration (approximately 1 to 2 x 10⁻⁷. Antibiotic discs, each of which is impregnated with a standard concentration of a particular antibiotic are then placed and lightly pressed onto the agar surface. After an overnight incubation, the bacterial growth around each disc is observed. The zones formed with each disc weremeasured in (mm) by using ruler.

RESULTS

A culture from 10 surfaces air-conditions rooms see fig (1)were obtained and bacterial colonies were obtainedsee fig (2).Colony morphology and Gram staining of the isolates indicated that the isolates were bacillus strains added to this the isolates were also catalase positive both of new and old ACs isolates see fig (2 &3). Diluted colonyfrom ACs with old filters was found to have 106 cfu. While diluted colonyfrom ACs with new filter had $_{10}^{3}$ cfu. Out of 238 colonies 112 colonies were from ACs with new filter and 126 colonies were from ACs with old filter. The percent of the isolated colonies from old ventilators was 52.941176 % while from new ventilator airconditions was only 47.058823% indicating families living in houses with air-conditions without filter in higher risk of bacterial contamination than those living with old ventilators see fig(4). Antibiotic sensitivity test of *Bacillusspp* against different antibiotics was prepared see Table (1). People living in these houses were exposed indirectly to additional bacterial reservoirs through breathing of contaminated air in their homes. The newly filtered air-conditioned rooms were found to have significantly smaller number of bacteria104 ^{cfu}; but it was found a significantly greater number of bacteria in unfiltered air-condition106 CFU.Disks of Bacitracin (BA), Chloramphenicol (C), Cotrimoxazole (TS), Gentamicin (GM), Neomycin (NE), Penicillin G (PG), Polymyxin B(PB) and Tetracycline(T) were placed on the media after smearing the bacteria on the media and pressed onto the surface of Mueller-Hinton agar. After an overnight incubation, the bacterial growth around each disc was observed see fig (5, 6, &7).A preliminary health status survey revealed occupants of air-conditioned homes without filter had more complaints of eye irritation, sneezing, nasal congestion, and cough ..

Zone inhibition (mm) Name of Name of **Bacillus**spp Antibiotics Antibiotics B1 B2 B3 BA - R -R -R Bacitracin С Chloramphenicol -R 16mm (I) -R TS Cotrimoxazole 27mm(I) 30mm(S) 30mm(S) 25mm(S) GM Gentamicin 24mm(S) 24mm(S) NE Neomycin 21mm(I) 20mm(I) 21mm(I) PG Penicillin G 10mm(R) - R - R PB Polymyxin B 7(R) 10 (I) 9(R) Т Tetracycline 16mm(S) 19mm(S) 20mm(s)

Table 1. Antibiotic sensitivity test of Bacillus spp against different antibiotics



Fig. 1. A type of aircondition in room of Umul hamaam



Fig. 2. Gram strain of isolated *Bacillus* from air condition without filter in rooms of Umul-hamaam



Fig. 3. *Bacillus* contamination prevalence of old and new air-conditions



Fig. 4. *Bacillus* colonies isolated from Air-condition in rooms of Uml-Alhamaam



Fig. 5. Antibiotic disks of Bacitracin (BA), Chloramphenicol (C), Cotrimoxazole (TS), Gentamicin (GM), Neomycin (NE), Penicillin G (PG), Polymyxin B (PB) and Tetracycline(T) against *Bacillus* isolates



Fig. 6. Antibiotic disks of Bacitracin (BA), Chloramphenicol (C), Cotrimoxazole (TS), Gentamicin (GM), Neomycin (NE), Penicillin G (PG), PolymyxinB(PB) and Tetracycline(T) against *Bacillus* isolate



Fig. 7. Antibiotic disks of Bacitracin (BA), Chloramphenicol (C), Cotrimoxazole (TS), Gentamicin (GM), Neomycin (NE), Penicillin G (PG), Polymyxin B (PB) and Tetracycline(T) against *Bacillus* isolates

DISCUSSION

Various air-conditioning systems have been installed in buildings to control environmental conditions and to prevent the development of biological agents. Nevertheless, it has been found that very often these systems present a high risk of proliferation of fungi and bacteria and spread contaminated aerosol that damages objects as well as human health. In particular, the humidifier components of these systems are favorable to microbial growth. Spores in a dormant state are commonly present in the air and on the surfaces of objects. However, it is the moisture content of materials that allows microbial growth, because it determines the water available for the germination of spores. In AC filters with severe bacterial and fungal contamination, these were suggested to actually grow on the filter rather than simply alight and accumulate there. More bacterial contamination was found in AC used with higher frequency and in lower parts of the AC filter, which absorbed more dew with dust. These results support the idea that dew, produced by airconditioning, promotes bacterial and fungal contamination of the AC filter and thus the validator. Humidity around AC as well as internally produced moisture thus appears to affect the levels of bacterial contamination. A higher floor level and sunshine appear to dry the AC, and suppress fungal contamination. Houses on a slope are more humid than those on flat land, and fungal contamination of AC in such households was alsohigher (Hamada, 2002).

It is necessary to pay attention to environmental circumstances of use to control fungal contamination. Inhouseholds; airconditioners (AC) also producemuch dew and humid conditions within the unit duringoperation. Fungal contamination of the air-conditionerfilter, which the air current passes through, appears todistribute fungal spores and bacteria throughout the atmosphere of the room. Sometimes an unpleasant smell or cough is experienced when the AC is switched on, which may berelated to fungal contamination inside the AC.Studies on thebacterial and fungal contamination in AC and clarifying the cause promoting them are essential forcontrolling the air quality in rooms with AC and protecting AC users from mold allergy and pathogenic bacteria. The presentstudy examined the*Bacillus*contamination in new and old filters ofhouse in Umul-Alhamaam area; in Saudi Arabia during 2012-2013. I found that old filters were contaminated with *Bacillus* only while the new filters were contaminated with *Bacillus* and *Staphylococcus*; may be due to human manual and thus of contamination of *Staphylococcus* saprophyte. The isolates of *Bacillus* colonies were tested for their sensitivity against eight types of antibiotics namely Bacitracin, Chloramphenicol, Cotrimoxazole

Gentamicin, Neomycin, Penicillin G, Polymyxin B and Tetracycline and the Bacilluscolonies (type B1, B2and B3) see Fig 5,6, & 7) was sensitive to Gentamicin, Cotrimoxazole and Tetracycline. And they were resistant to Bacitracin, Chloramphenicol and Penicillin G for the rest two antibiotics they were intermediate. The area of the inhibition zone was measured with ruler in millimeter (mm). According to Antibiotic disc HiMedia Mumbai India; Resistance (R), Intermediate (I) and sensitive (S) was determined see table 1 & Fig 3. A preliminary health status survey revealed occupants of air-conditioned homes without filter had more complaints of eye irritation, sneezing, nasal congestion, and cough. I conclude that families living in houses with airconditions without filter higher bacterial and contamination were found. I suggest that the usage of air conditions with filter and keep it clean in all season. The higher densities of airborne bacteria were obtained from air condition without filter that has been used for long seasons. This also suggests correlation between the periods used the air condition and bacterial contamination. House dust containing spore allergens and bacterial lipopolysaccharides increases the incidence of asthma in children. I propose that the houses in which bacterial and fungal contamination should be controlled yearly and all air conditions be cleaned. Anything or system that is useful tool for the detection of increased bacterial and fungal proliferation, which cause adverse effects on human health, should be emphasized.

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