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## Air Conditioners as a Source of Bacterial Contamination in an Indoor Environment

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### Abstract

The objectives of this study were to assess the bacterial content of air conditioning and indoor air of some hospitals in the south of Libya. This study was conducted in a 2<sup>nd</sup> of March hospital and Brack general hospital in southern Libya. Samples were collected from indoor air, and from filters, cooling coils, and water drainage basins of air conditioning units in those hospitals. Bacterial colonies were counted and identified characteristics of the bacteria on blood agar and MacConkey agar, also identified bacterial species. Bacterial numbers in the samples taken from 2<sup>nd</sup> March hospital formed 56.57% of the total indoor air samples, 79.9% of the total filters samples, 72.07% of the total coils samples, and 54.63% of the total water samples. The bacterial numbers in samples taken from a Brack general hospital contained 43.43% of the total indoor air samples, 20.1% of the total filter samples, 27.93% of the total coils samples, and 45.37% of the total water samples. Hemolytic bacteria were present in smaller numbers on blood agar than non-hemolytic bacteria, and were presenting more non-fermented at a higher rate than fermented bacteria on MacConkey. The bacterial species that were identified included some species of pathogenic bacteria as *S.aureus*, which existed in the various samples, *K. pneumoniae*, Bacillus and Pseudomonas, those species involved in nosocomial infections.

**Conclusion:** The occurrence of high bacterial numbers on parts of air-conditioning followed by its spread to the internal environment which constitutes a health risk to people exposed to it especially if this environment are the hospitals where patients with various injuries.

**Key Words:** air conditioning, hospital, indoor air, pathogenic bacteria.

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## **Introduction**

A number of studies by a group of health and environment scientists have shown that indoor air pollutants are a major health hazard in a number of cities, and the world health organization has estimated that 30% of recently built or recently renovated buildings contain a contaminated air [1]. Furthermore, the American society of heating, cooling and air conditioning has indicated that more than 20% of the residents of these buildings suffer from health problems caused by contaminated indoor environment [1]. The health hazard related to residential buildings, or work places such as offices and schools became extremely important factor for community health. The severity and frequency of these problems has increased in 1980's altogether with increased reports about infections described to be connected with insufficient air circulation and caused by air borne contaminants inside these buildings which contain air conditioning units. A number of studies have shown that there is a relationship between contaminated air conditioning units and a health problem known as a building syndrome [2, 3]. In addition in a number of studies to investigate the relationship between the type of air condition unit and the symptoms related to work places that are common between office workers it has been shown that symptoms such as headache, weakness, upper respiratory tract and mucosal infections are always associated with air conditioned places [4]. In one study in Hong Kong for the quality of indoor air in some air conditioned offices has shown that the mechanical air conditioned spaces has a great effect on the microorganisms load i.e. bacteria and fungi in air [5], and that these machines are a habitat for microbial communities and a

source for their distribution in the surrounding environment [6]. Air conditioning units as described by *Muyshoundt* and colleagues are a safe place for microbes and subsequently spreading it in to the environment, the same observation was reported by Harrison and co-workers, as they have found that the bacterial count ranged between 2-960 colony forming unit/ml in a 15 English office contain different air conditioning units and ventilation systems [7, 8]. Other studies have reported that the accumulation of dust on the air conditioning filters provided a good environment for microbial growth [9], this was further confirmed by studies on the prevalence of microbes on different components of air conditioning units [10, 11], as these studies have proved that the contaminated air conditioning units provided suitable environment for the microbial growth on the different components of these units such as filters, water tanks etc., as these microbes tend to grow on the biofilm on the surface of the units components [12]. A Japanese study has examined the prevalence of bacteria and fungi in the internal parts of the air conditions units before and after cleaning and in the inside air, they concluded that the contaminated internal tubes is a source of spreading of microbes in the internal environment [13]. Pathogenic bacteria such as *Legionella* species which is one of the most common bacteria isolated from an indoor environments [14]. Another Gram positive and Gram negative bacteria were also isolated from air conditioning units [11].

## **Material and Methods**

This study was conducted in Brack general Hospital and 2<sup>nd</sup> of March hospital. Samples

from these institutions were collected from an indoor air according to the standard method [1]. Samples from air condition parts (filters, cooling coils) were collected by sterile cotton swabs, and samples of water were taken from the drainage basins. Samples were inoculated onto culture media known Trypticsoy Agar as primary cultures. Plates were incubated at 28°C for 48 hours, after the incubation, and the total viable count of bacterial colonies were performed using standard methods, and selected colony of each type of bacteria as a representative of this type was inoculated on the culture media MacConkey agar and Blood Agar. Bacterial isolates were identified by using the standard methods used in the microbiology laboratories of this department.

### **Results**

Bacterial numbers in samples taken from the 2<sup>nd</sup> of arch hospital ranged between 2-32 cfu/m<sup>2</sup>/h in air samples average 15.16, which is 56.57% of the total indoor air samples in both hospitals). Bacterial counts on filters ranged between 40-3000 cfu/cm<sup>2</sup>, average 528.71 (79.9% of the total filter samples in both hospitals). Counts on coils ranged from 10 to 2710 cfu/cm<sup>2</sup> average 466.48 (72.07% of the total coils sample in both hospitals). Water tanks contained between 10 to 11.000 cfu/ml average 3060 (54.63% of the total samples of water in both hospitals). Isolated bacteria were classified on the basis of Gram stain, morphology and culture characteristic, on MacConkey Agar and Blood Agar. Table 1.

Bacterial counts on samples taken from Brack general hospital ranged between 2-31cfu/m<sup>2</sup>/h in air samples, average 11.64 (43.43% from the total internal samples in both hospitals). Filters from air conditioning units in the same hospital contained an average of 133cfu/cm<sup>2</sup> (20.1% from the total filter samples of both hospitals). Bacterial counts on coils ranged between 0-1710, average 180.8cfu/cm<sup>2</sup> (27.93% from the total coil samples from both hospitals), and averaged between 20-12070 cfu/ml, average 2541.4 in drainage water tanks 45.37% from total samples taken from the drainage water from both hospitals, as shown in table 2 which also shows the average number of bacterial groups classified on the basis of Gram reaction, morphology and culture characteristics on blood and MacConkey agar media.

In certain experiments an attempt was made to identify the isolated bacteria, and as shown in table 3, some isolates were identified as *Staphylococcus aureus* and *Micrococci* species; these comprised the largest proportion of the total isolated bacteria from air samples from Brack hospital. Coagulase negative *Staphylococci* species were also isolated from both hospitals. Certain *Bacillus* species were present and isolated from coil samples taken air conditioning units in the 2<sup>nd</sup> of March hospital. Gram negative bacilli species were also present, these included *Klebsiella pneumonia* and *Flavobacterium*. Water from the drainage tanks contained certain *Psuedomonas* species.

Table 1: Total number (percentage) of colonies forming units in samples of 2<sup>nd</sup> of March

		Water	Cooling coils	Filters	Indoor air
<b>Morphologic group</b>	Negative bacilli	(55.11) 132.25	(28.35) 132.25	(63.88) 337.74	(11.87) 1.8
	Positive bacilli	1686.43	(33.54) 156.48	(15.74) 83.23	(33.84) 2.13
	Positive cocci	(2.7) 82.5 (42.19) 1291.07	(38.1) 177.74	107.74 (20.38)	(84.29) 8.23
<b>Total</b>		(100) 3060	(100) 466.48	(100) 528.71	(100) 15.16
<b>Blood agar</b>	Hemolytic	(2.73) 83.57	(32.09) 149	(17.39) 91.94	(45.71) 6.93
	Non- hemolytic	(97.29) 2976.42	(67.91) 316.77	(82.61) 436.77	(54.29) 8.23
<b>Total</b>		(100) 3060	(100) 466.48	(100) 528.71	(100) 15.16
<b>MacConkey agar</b>	Lactose fermenting	(17.03) 521.07 (48.65)	(1.04) 4.84 (98.96) 461.64	(21.96) 116.13 320.32	(28.5) 4.32 (10.84)
	Non- Lactose fermenting	1488.57 (34.32)	(0) 0	(60.59) (17.45) 92.26	(0)0
	No growth	1050.35			
	<b>Total</b>	<b>(100) 3060</b>	<b>(100) 466.48</b>	<b>(100) 528.71</b>	<b>(100) 15.16</b>

Table 2: Total number (percentage) of colonies forming units in samples of Brack general hospital

		Water	Cooling coils	Filters	indoor air
<b>Morphologic group</b>	Negative bacilli	(51.75) 1315.24	(17.04) 30.8	(31.13) 41.4	(3.26) 0.38
	Positive bacilli	(20.94) 532.14	(16.7) 30.2	(28.12) 37.4	(13.4) 1.56
	Positive cocci	(27.31) 694.05	(66.26) 119.8	(40.75) 54.2	(83.34) 9.7
<b>Total</b>		(100) 2541.4	(100) 180.8	(100) 133	(100) 11.64
<b>Blood agar</b>	Hemolytic	(0.8) 20.24	(28.43) 51.5	(36.69) 48.8	(36.6) 4.26
	Non- hemolytic	(99.2) 2521.19	(71.57) 129.4	(63.31) 84.2	(63.4) 7.38
<b>Total</b>		(100) 2541.4	(100) 180.8	(100) 133	(100) 11.64
<b>MacConkey agar</b>	Lactose fermenting	(27.43) 697.14	(22.68) 41	(31.73) 42.2	(40.38) 4.7
	Non- Lactose fermenting	(61.3) 1557.86	(77.32) 139.8	(50.07) 66.6	(57.39) 6.68
	No growth	(11.27) 286.43	(0)0	(18.2) 24.2	(2.23) 0.26
	<b>Total</b>	<b>(100) 2541.4</b>	<b>(100) 180.8</b>	<b>(100) 133</b>	<b>(100) 11.64</b>

Table 3: Number (percentage) of bacterial species in 2<sup>nd</sup> of March and Brack general hospitals

	Water		Cooling Coils		Filters		Indoor air	
	Brack general hospitals	2 <sup>nd</sup> of March	Brack general hospitals	2 <sup>nd</sup> of March	Brack general hospitals	2 <sup>nd</sup> of March	Brack general hospitals	2 <sup>nd</sup> of March
CONS	(1.44) 36.47	(25.83) 790.36	(5.86) 10.6	(39.28) 183.23	(20.15) 26.8	(1.89) 10	(46.39) 5.4	(22.56) 3.42
<i>S. aureus</i>	(0.02) 0.59	(0) 0	(1.81) 3.27	(0.83) 3.85	(15.49) 20.6	(0) 0	(32.13) 3.74	(21.9) 3.32
<i>Micrococcus spp</i>	(27.54) 700	(0.02) 0.71	(15.38) 27.8	(0.14) 0.65	(21.8) 29	(17.57) 92.9	(2.41) 0.28	(10.62) 1.61
<i>Flavobacterium spp</i>	(29.53) 750	(1.34) 41.07	(12.72) 23	(6.98) 32.58	(0.6) 0.8	(5.55) 29.35	(0) 0	(0) 0
<i>Klebsiella Pneumonia</i>	(3.22) 81.76	(0) 0	(0) 0	(0) 0	(0.45) 0.6	(0) 0	(0) 0	(0) 0
<i>Bacillus spp</i>	(2.08) 52.94	(2.7) 82.5	(15.04) 27.2	(30.77) 143.58	(21.95) 29.2	(74.5) 393.87	(3.78) 44	(7.85) 1.19
Non-spore forming bacillus	(29.49) 749.41	(0) 0	(1.18) 2.14	(1.45) 6.77	(11.13) 14.8	(0.12) 0.65	(7.9) 92	(24.47) 3.71
<i>Pseudomonas spp</i>	(5.51) 140	(1.35) 41.18	(0) 0	(0) 0	(0) 0	(0) 0	(0) 0	(0) 0
Unidentified bacteria	(1.17) 29.73	(68.76) 2104.18	(48) 86.79	(20.55) 95.82	(8.42) 11.2	(0.37) 1.94	(7.39) 0.86	(12.6) 1.91
<b>Total</b>	<b>(100) 2541.4</b>	<b>(100) 3060</b>	<b>(100) 180.8</b>	<b>(100) 466.48</b>	<b>(100) 133</b>	<b>(100) 528.71</b>	<b>(100) 11.64</b>	<b>(100) 5.16</b>

CONS: Coagulase-negative Staphylococcus

## **Discussion**

In this study the bacterial contents in an internal air samples and parts of air conditioning units installed at different locations in the 2<sup>nd</sup> of March hospital in Sebha and Brack general hospital. Results obtained were variable; however, bacteria were isolated from both hospital and from almost all samples tested. Numbers and percentages of bacteria isolated were lower than numbers reported in similar study carried out in Brazil [11], and comparable to a study in Jordan [12]. Regarding the type of bacteria isolated in this study Gram positive cocci bacteria were the predominant in both hospitals, and was different than those recorded in a hospital in Brazil were Gram positive bacilli was predominant [11]. Three hospitals in India have reported similar finding to this study [13].

Filters and coils contained the highest number of bacteria and reached 3000 cfu/cm<sup>2</sup> in some samples from the 2<sup>nd</sup> of March hospitals and 1150 cfu/cm<sup>2</sup> in samples from Brack hospital, when compared to numbers recorded in 2005 we find that it quite high [14]. This finding suggest that it is a contaminated surface, and probably will act as a source of contamination in the internal air, especially as the air passes through these surfaces during operation hours, and as shown by certain studies these contaminated filters will spread bacteria in the internal environment [15]. The isolation of Gram negative bacilli in higher proportions from the 2<sup>nd</sup> of March hospital (63.88%) in agreement with study reported in 2009 [14]. Cooling coils are considered as a suitable place for the growth of microbes, as it forms what is known as the biofilm [16, 17]. Furthermore, it is also shown that microbes growing on the biofilm are more resistance to harsh conditions

and certain antimicrobial agents [18-20]. The results of this study has indicated that the number of bacterial isolates in the samples collected from the internal air and from air conditioning units installed in the 2<sup>nd</sup> of March hospital were more than those isolated from Brack hospital (72.07% and 27.93% of the total samples from both hospitals respectively) , this was probably expected as the Brack hospital is smaller, more recent and deals with fewer patients than hospital Sebha, these factors will help to reduce the contamination as the small size and fewer patients and staff will make it easier to clean and easier to manage and this will subsequently reduce the bacterial contamination.

Drainage water from the drainage tanks contained the higher bacterial numbers as compared to other parts as it reached in some samples to 11000 cfu/ml in the drainage tanks of Sebha hospital and 12070 cfu/ml in samples taken from Brack hospital. These numbers are indicative of contamination as the stagnant of water in the drainage tanks provide suitable condition for the growth of many microbes [17]. The prevalence of large numbers of bacteria in the internal environments may allow for the spread of certain bacterial components which can be inhaled and become a source of infection in the respiratory tract and sensitivity [22, 23].

The presence of pathogenic bacteria in the internal environments especially in hospitals or students dormitories is a great risk to those inside these places, as people attending hospitals are in greater risk of the nosocomial infections. *S aureus* isolated from 12.9% from internal air samples collected from Sebha hospital and as it is known that this bacterium is capable of causing disease of variable

severity from simple skin and wound infection to more complicated serious infection [23]. Furthermore, a recent report by the center of disease prevention and control has shown that 18650 persons in the united states of America died by methicilline resistant *S. aureus* [24]. Bacillus species were isolated from both hospitals, Bacillus is capable of causing disease as it may be transferred into the body by dust and cause the disease known as building related illness [17]. In addition to the isolation of

certain *Pseudomonas* species from the drainage tanks and it is known that this bacteria is resistant to a variety of antibacterial agents and it is a major causative agent in nosocomial infections.

The results of this study have proved the use of air conditioners units in our buildings is a source of bacterial contamination and subsequently source of infection. To avoid the risk of dangerous infections a great care should be used to reduce this risk.

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