



Microbial Air Quality in Medical Laboratory Rooms at Benghazi Center of Infectious Diseases and Immunity

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Abstract

The control of indoor air quality plays an important role in the prevention of cross infection in hospitals to protect both hospital staff and patients. This study was carried out to determine the variation in microbiota of medical laboratory at Benghazi center infectious diseases and immunity. Samples were collected by using the settled plate techniques for the enumeration of bacterial and fungal isolates. The air specimens were collected through four seasons in the morning between the hours of 9 am and 11 am from air indoor medical lab and swab samples collected from the filters of the air conditioning units in autumn, spring and summer seasons. Indoor air gram positive bacteria, accounting (99.72%) was significantly higher than that of gram negative bacteria (0.28%). The most common bacteria genus found in all seasons were *Staphylococcus* (46.62%) *Micrococcus* (27.31%), followed by *Kocuria* (13.42%). *Micrococcus luteus* and *Kocuria rosea* were found frequently occurring airborne bacterial isolates in the four seasons. Gram negative bacteria were only found in autumn and summer seasons only. Rest room had the highest bacterial count (43.47 CFU/m³) while the microbiology room had the lowest (3.66 CFU/m³). The susceptibility pattern of all isolates revealed sensitivity to all tested antibiotics. *Aspergillus* spp., *Penicillium* spp. and *Alternaria* spp. were frequently dominant air borne fungal isolates. Bacterial concentration (0.26 CFU/m³ air - 38.49 CFU/m³ air) in investigated filters of air conditioning unites while fungal concentration was (0.26 CFU/m³ - 51.59 CFU/m³). The most abundant isolated bacteria in the three seasons was *Bacillus circulans* while *Penicillium* sp. was the most abundant fungi in air conditioning isolates. In indoor air samples of medical laboratory rooms showed contamination with bacteria and fungi under the acceptable levels, when compared with cited in this study. Indoor airborne bacteria and fungi concentration were depended on place of isolation and seasons.

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Introduction

Most biological contamination of indoor air is caused by bacteria, moulds and yeast (Aboul-Nasr *et al.*, 2011).

Indoor air problems have been associated with a decrease in employees' comfort, work efficiency and may also be the cause of some work-related symptoms and diseases (Reijula and Sundman-Digert, 2004; Mahbob, 2011).

In indoor environments, the main source for microbes is mainly originated from outdoor sources (Shelton *et al.*, 2002; WCB Publication, 2005). In addition to outdoor sources, indoors microbes can originate from indoor sources. These can be the occupants themselves and their activities (Ferro *et al.*, 2004; Kopperud *et al.*, 2004). Other factors influencing the microbial population include building maintenance, cleanliness, indoor temperature and relative humidity, type of furniture, and carpeting (Ross *et al.*, 2004; Mandal and Brandl, 2011; Park *et al.*, 2013).

The control of indoor air quality (IAQ) plays an important role in the prevention of cross infection in hospitals to protect both hospital staff and patients. Poor hospital IAQ may cause outbreaks of sick hospital syndrome (SHS), causing headaches, fatigue, sneezing, eye and skin irritations, and other symptoms (Leung and Chan, 2006; WCB Publication, 2005).

Previous studies showed that the microbial flora of the indoor air depends on several factors including the number and hygiene of people who occupy the building (Ekhaise *et al.*, 2008; WCB Publication, 2005), The physical layout of the building, the quality of the hospital system and mechanical movement within the enclosed space (Ekhaise *et al.*, 2010, WCB Publication, 2005).

The counting of microbes in air is not an easy. There are different methods of choice for sampling airborne microbial loads (Pasquarella *et al.*, 2000; Jaffal *et al.*, 1997). Each method has strong and weak points, and in general more than one is necessary to accurately assess most situations (Jaffal *et al.*, 1997). However, Culturing is the conventional method for microbial characterization, although it underestimates total microbial concentrations (Amann *et al.*, 1995).

The present study was aimed to gain knowledge regarding the air quality of medical laboratory indoor air and, to determine the types of airborne micro-flora in the medical laboratory at Benghazi center infectious diseases and immunity (BCIDI). The data can be used to set standards for levels of acceptable microbial population.

Materials and Methods

Cross sectional study conduct to measure indoor air microbial quality of medical laboratory rooms of BCID from October to July 2013-2014. The Samples were collected in four seasons of the year. About 38 samples

each season from indoor air and 11 samples from air conditioners collected by using Settle Plate Method (Passive Air Sampling) on blood agar for bacteria culture (Genet *et al.*, 2011; Kelkar and Kulkarni, 2011) and Sabouraud's Dextrose Agar (SDA) plate plus 50µg/ml of gentamicin for fungi culture (Kelkar and Kulkarni, 2011).

Each plate was leaving open to the air for a 30 minutes (Kelkar and Kulkarni, 2011), a meter to 1.5m above the floor and a meter from the wall (Ekhaise and Ogboghodo, 2011). The air samples collected (at 9-11 AM) within a given day. The blood agars settle plate then incubate at 35- 37° C for 24-48 hours (Ekhaise and Ogboghodo, 2011) while the fungal culture plates are incubate at room temperature (22°C – 26 C) for 3 –7 days (Ekhaise *et al.*, 2010; Garcia-Cruz *et al.*, 2012; Kelkar and Kulkarni, 2011; Bhaita and Viskwakarma, 2010).

The number of microorganisms expressed as colony forming unit (CFU/m³) was estimated according to the equation (Stryjakowska-Sekulska *et al.*, 2007; Bhaita and Viskwakarma, 2010):

$$CFU/m^3 = a \cdot 10000/p \cdot t \cdot 0.2$$

Where:

- a – the number of colonies on the Petri plate
- p – The surface of the Petri plate
- t – The time of Petri plate exposure

Isolation of Bacteria and fungi was performed by sub streaked on the same medium to obtain pure colonies. Bacterial colonies were identified by using gram stain, API 20E (bioMerieux, France) and/or Phoenix™ Automated Microbiology System (Becton Dickinson, USA) (BD) of the isolates. The fungal colonies were identified based on colony appearance and microscopic examination of the spore and hyphae (Barnett and Hunter, 1998; Koneman and Roberts, 1992; Chaturvedi and Ren, 2007; Ellis *et al.*, 2007).

The Antibiotic susceptibility test was done on Mueller-Hinton agar (MHA) (BD) by using Kirby-Bauer disk diffusion method according to the British society for antimicrobial chemotherapy (BSAC) guidelines (BSAC, 2011). *Staphylococcus aureus* ATCC25923 and *Escherichia coli* ATCC 25922 strains were used as control organisms. The antibiotics were: Ampicillin

(10µg), Amikacin (30µg), amoxicillin-clavulanic acid (30µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), Clindamycin (2 µg), Erythromycin (15µg), Gentamicin (10µg), imipenem (10 µg), aztreonam (30 µg), cefoxitin (30 µg), trimethoprim-sulfamethoxazole (25µg), ciprofloxacin (5 µg), Tetracycline (30µg), Vancomycin (5µg) (Oxoid Ltd., Cambridge, UK). The results were recorded as susceptible, intermediate and resistant according to the British society for antimicrobial chemotherapy (BSAC, 2011) recommendations.

Results and Discussion

Air samples from each the sampled medical lab rooms studied were taken and used for enumeration and isolation of airborne bacteria on BA plates, and for the enumeration and isolation of airborne fungi on SDA plates. Table 1 shows microorganisms isolated from the medical lab rooms studied in four seasons.

The percentage of gram positive bacteria (99.72%), were significantly higher than that of gram negative bacteria (0.28%) in the air in medical lab of BCID. With regard to the bacterial groups, a total of 1054 bacterial isolates distributed across 17 genera and 33 species of culture able airborne bacteria were identified from different sites (Table 1). As a whole, the most common bacteria genus found in all seasons were *Staphylococcus* (46.62%) *Micrococcus* (27.31%), followed by *Kocuria* (13.42%), and they totally accounting for about 87.35% of culturable airborne bacteria in medical lab. The most common bacterial species identified in the air was *Staphylococcus capitis* sub *capitis*, *Micrococcus luteus*, followed by *Kocuria rosea*, contributing to 31.50%, 26.94%, and 11.39% of the total airborne bacteria, respectively. *Micrococcus luteus* and *Kocuria rosea* were found frequently occurring airborne bacterial isolates in the four seasons. All other bacteria were detected in small numbers or not detected in all seasons. Namely; *Aerococcus*, *Bacillus*, *Dermacoccus*, *Globicatella*, *Leifsonia*, *Macroccoccus*, *Kytococcus*, *Paenibacillus*, *Pantoea* and *Pediococcus* (Table 1).

The total airborne bacterial load of the different sites studied in the medical lab during the four seasons is presented in figure 1, which showed that the rest room had the highest bacterial count (43.47 CFU/m³) then storage (24.35CFU/m³) and coffee (24.09CFU/m³) rooms while the microbiology room had the lowest (3.66

CFU/m³). The bacterial concentration CFU/m³ air in this medical lab was highest in autumn (105.27 CFU/m³), followed by spring (62.33 CFU/m³), and, winter (58.93 CFU/m³) and lowest bacterial concentration was observed in summer (49.49 CFU/m³) (Figure 2). The susceptibility patterns of all isolates revealed sensitivity to all the antibiotics tested.

Gram positive bacteria appeared higher percent in autumn (38.15%) and spring (22.64%) than winter (21.41%) and summer (17.79%). Gram negative bacteria were isolated only in autumn and summer seasons (0.28%) included *Moraxella* species and *Acinetobacter lwoffii/ haemolyticus* respectively, Table 1.

149 fungal isolated demonstrate to 10 genus were isolated and characterised from the different studied rooms. *Aspergillus* spp., *Penicillium* sp., *Alternaria* spp. were the dominant and the frequently in the four investigated seasons while *Cladosporium*, *Candida* spp., *Fusarium*; *Gliocladium*, *Rhizopus*, *Epicoccum* and *Diplodia* were the least frequent fungi (table 1). The highest fungal load was recorded in coffee room and storage room (5.76 CFU/m³, 6.02 CFU/m³), respectively while the lowest fungal load was recorded in the microbiology room (0.26CFU/m³) (Figure 1).

The degree of contamination by was calculated in percentage terms and CFU/m³ (Table 1). Overall, the least frequently genus encountered was *Rhizopus* and *Diplodia* (0.67%). The fungal counts ranged from 0.26 CFU/m³ in microbiology room to 6.02 CFU/m³ from coffee room. The result showed the fungal CFU/m³ of the autumn was higher than that of the spring and other seasons (Table 1).

33 swab Samples were collected in three seasons (autumn, spring and summer) of year from the filters of the air conditioning units (ACU). Their culturing yielded 10 genera of bacteria and 8 genera of fungi, table 2. Bacterial isolation rate was higher in reception ACU (19.37%) than other air conditioning units (Figure 3). Fungal isolation rate was higher in biochemistry 1 ACU (26.18%) and microbiology (18.33 CFU/m³) than the other air conditioning units, figure 3. Average number of bacteria and fungi present in ACU of different rooms in three seasons were compared in figure 3.

Table.1 Concentration levels of the airborne bacteria and fungi isolated during four seasons

Type and No. of bacteria and fungi isolated	Seasons								All total	%
	Autumn		winter		spring		Summer			
	Total	CFU/m ³	Total	CFU/m ³	Total	CFU/m ³	Total	CFU/m ³		
Gram Positive Bacteria										
Micrococcus									287	27.31
<i>Micrococcus luteus</i>	104	27.24	86	22.52	45	11.79	49	12.83	284	26.94
<i>Micrococcus lylae</i>	1	0.26					2	0.52	3	0.28
Kocuria									141	13.42
<i>Kocuria rosea</i>	9	1.83	89	23.31	2	0.52	20	5.24	120	11.39
<i>Kocuria varians</i>	21	5.49							21	1.99
Staphylococcus									490	46.62
<i>Staphylococcus capitis sub capitis</i>	223	58.40			89	23.31	20	5.24	332	31.50
<i>Staphylococcus kloosii</i>	14	3.67			4	1.05	2	0.52	20	1.89
<i>Staphylococcus hominis</i>	22	5.76			43	11.26			65	6.17
<i>Staphylococcus haemolyticus</i>			25	6.55			4	1.05	29	2.75
<i>Staphylococcus lentus</i>					1	0.26	2	0.52	3	0.28
<i>Staphylococcus epidermidis</i>							22	5.76	22	2.09
<i>Staphylococcus capitis sub urolyticus</i>							10	2.62	10	0.95
<i>Staphylococcus saprophyticus</i>							4	1.05	4	0.38
<i>Staphylococcus lugdunensis</i>							4	1.05	4	0.38
<i>Staphylococcus aureus</i>							1	0.26	1	0.09
Bacillus									22	2.09
<i>Bacillus cereus</i>			4	1.05					4	0.38
<i>Bacillus thuringiensis</i>			9	2.36					9	0.85
<i>Bacillus circulans</i>							2	0.52	2	0.19
<i>Bacillus megaterium</i>							3	0.79	3	0.28
<i>Bacillus coagulans</i>							4	1.05	4	0.38
Corynebacterium									6	0.57
<i>Corynebacterium amycolatum/ minutissimum</i>							4	1.05	4	0.38
<i>Corynebacterium matruchotii</i>							2	0.52	2	0.19
Others										
<i>Aerococcus viridans</i>	7	1.83			23	6.02	1	0.26	31	2.94
<i>Macrococcus caseolyticus</i>			1	0.26	2	0.52	6	1.57	9	0.85
<i>Pediococcus pentosaceus</i>			6	1.57	8	2.09	1	0.26	15	1.42
<i>Leifsonia aquatica</i>			1	0.26			7	1.83	8	0.76
<i>Pantoea agglomerans</i>			1	0.26			3	0.79	4	0.38
<i>Paenibacillus alvei</i>			3	0.79					3	0.28
<i>Dermacoccus nishinomiyaensis</i>					4	1.05	12	3.14	16	1.52
<i>Globicatella sanguinis</i>					11	2.88			11	1.04
<i>Kytococcus sedentarius</i>					6	1.57			6	0.57
<i>Arcanobacterium pyogenes</i>							2	0.52	2	0.19
Total	401		225		238		187		1051	99.72
Gram Negative Bacteria										
<i>Acinetobacter lwoffii/ haemolyticus</i>							2	0.52	2	0.09
<i>Moraxella species</i>	1	0.26							1	0.19
Total	1		0		0		2		3	0.28
Fungi										
<i>Aspergillus spp.</i>	45	11.79	9	2.36	3	0.79	9	2.36	66	44.30
<i>Penicillium sp.</i>	9	2.36	13	3.40	4	1.05	6	1.57	32	21.48
<i>Alternaria spp.</i>	1	0.26	5	1.30	8	2.09	3	0.79	17	11.41
<i>Candida spp.</i>			1	0.26	5	1.30	10	2.62	16	10.74
<i>Cladosporium sp.</i>	1	0.26			3	0.79			4	2.68
<i>Gliocladium sp.</i>	3	0.79			1	0.26			4	2.68
<i>Fusarium sp.</i>	1	0.26	4	1.05					5	3.36
<i>Epicoccum sp.</i>					2	0.52	1	0.26	3	2.01
<i>Diplodia sp.</i>	1	0.26							1	0.67
<i>Rhizopus sp.</i>			1	0.26					1	0.67

Table.2 Airborne microorganisms isolated from the filters of the air conditioning units

Type and No. of bacteria and fungi isolated	seasons									For three seasons		
	Autumn			spring			summer			All total	%	CFU/m ³
	Total	%	CFU/m ³	Total	%	CFU/m ³	Total	%	CFU/m ³			
Bacteria												
<i>S. capitis sub capitis</i>	18	15.79	4.71	5	4.54	1.30	11	5.53	2.88	34	8.04	8.90
<i>Staphylococcus klossii</i>	30	26.32	7.86				4	2.01	1.05	34	8.04	8.90
<i>Staphylococcus homonis</i>							23	11.56	6.02	23	5.44	6.02
<i>Staphylococcus warneri</i>							8	4.02	2.09	8	1.83	2.09
<i>Kocuria rosea</i>							6	3.02	1.57	6	1.42	1.57
<i>Kocuria varians</i>	12	10.53	3.14							12	2.84	3.14
<i>M. luteus</i>	36	31.58	9.43							36	8.51	9.43
<i>Rhizodium radiobacter</i>	9	7.89	2.36							9	2.13	2.36
<i>Bacillus circulans</i>	9	7.89	2.36	46	41.81	12.05	92	46.23	24.09	147	34.75	38.49
<i>Bacillus cereus</i>				34	30.90	8.90				34	8.04	8.90
<i>Bacillus coagulans</i>				7	6.36	1.83				7	1.65	1.83
<i>Bacillus megaterium</i>				3	2.72	0.79				3	0.71	0.79
<i>Aerococcus viridans</i>				15	13.63	3.93				15	3.55	3.93
<i>Arcanobacterium pyogenes</i>							1	0.50	0.26	1	0.24	0.26
<i>Corynebacterium urealyticum</i>							2	1.01	0.52	2	0.47	0.52
<i>Dermacoccus nishinomiyaensis</i>							51	25.63	13.36	51	12.06	13.36
<i>Enterococcus faecium</i>							1	0.50	0.26	1	0.24	0.26
Total	114			110			199			423		110.77
Fungi												
<i>Penicillium sp.</i>	9	30	2.36	92	53.17	24.09	96	63.16	25.14	197	55.49	51.59
<i>Aspergillus spp.</i>	19	63.33		75	43.35	19.64	45	29.61	11.78	139	39.15	36.40
<i>Alternaria spp.</i>							2	1.32	0.52	2	0.56	0.52
<i>Cladosporium sp.</i>	1	3.33	0.26				3	1.97	0.79	4	1.13	1.05
<i>Glaciocladium</i>							2	1.32	0.52	2	0.56	0.52
<i>Fusarium sp.</i>							1	0.66	0.26	1	0.28	0.26
<i>Candida spp.</i>							3	1.97	0.79	3	0.85	0.79
<i>Rhizopus sp.</i>	1	3.33	0.26	6	3.46	1.57				7	1.97	1.83
Total	30			173			152			355		92.97

Fig.1 Concentration airborne microorganisms isolated from indoor air during different sites of medical room lab

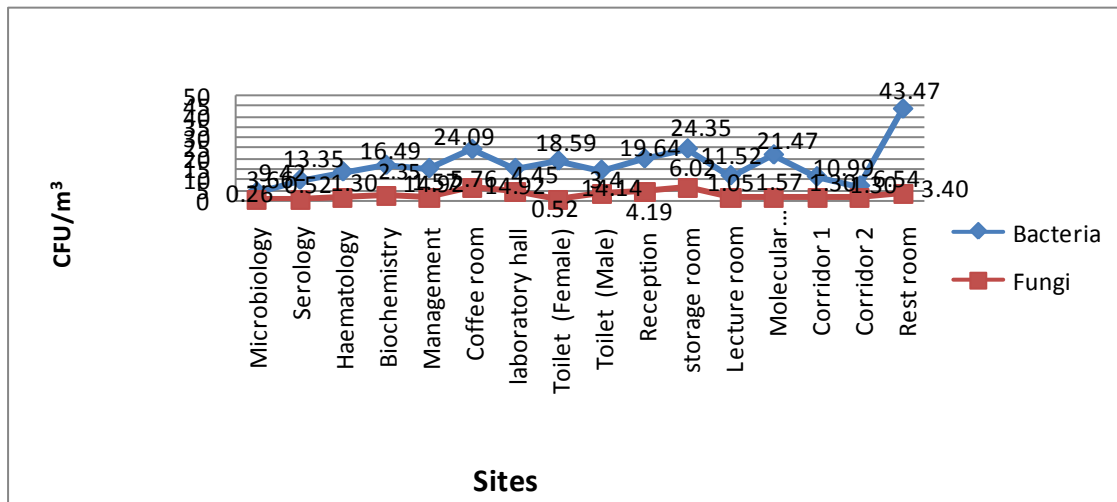


Fig.2 Concentration airborne microorganisms (CFU/m³) isolated from indoor air during four seasons

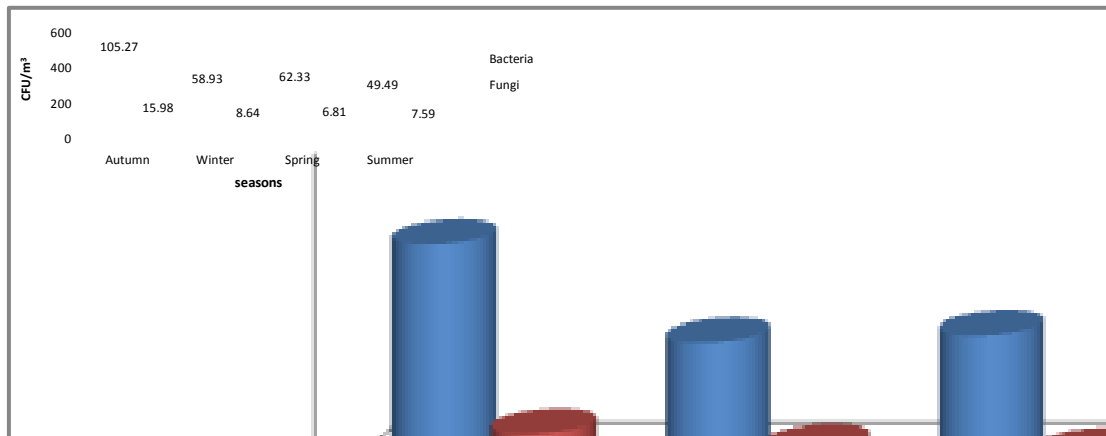


Fig.3 Concentration airborne microorganisms isolated from ACU during different sites of medical lab

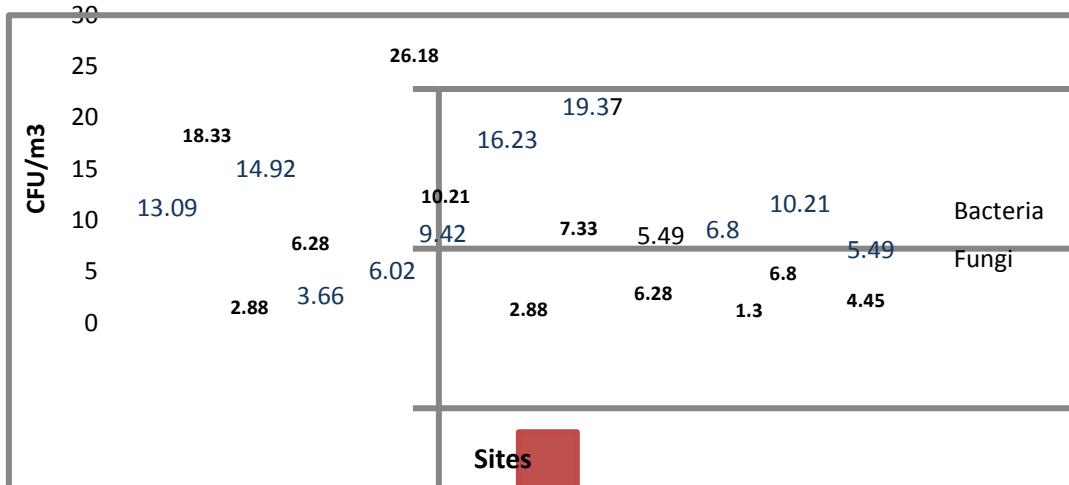
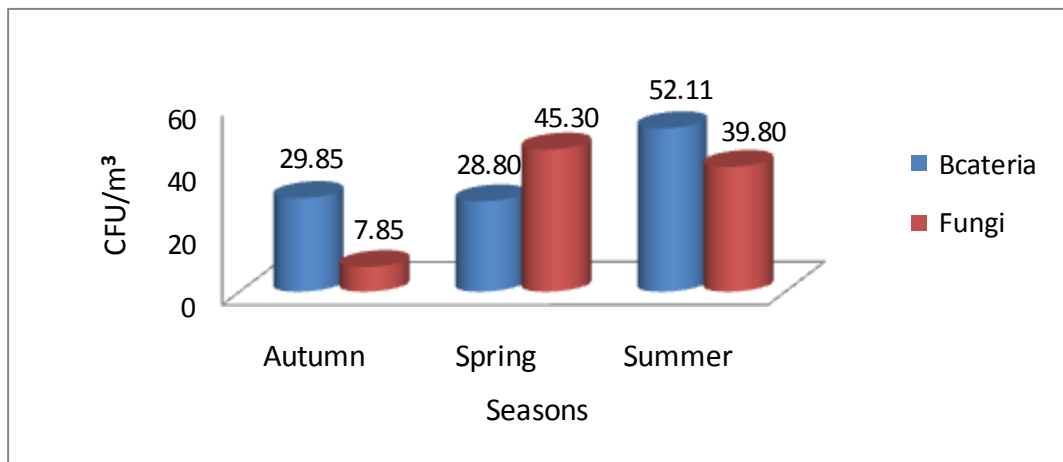


Fig.4 Concentration airborne microorganisms isolated from ACU during three seasons



Concentrations of isolates vary depend on season and the day time. In summer season a concentration of bacteria (52.11 CFU/m³) was higher than autumn and spring respectively (29.85 CFU/m³ and 28.80 CFU/m³), while the concentration of fungi was in spring higher (45.30 CFU/m³) than autumn and summer respectively (7.85 CFU/m³ and 39.80 CFU/m³). Variations in concentration of bacteria and fungi in ACU filters were observed in figure 4. The bacterial counts (CFU/m³) ranged from 0.26 CFU/m³ to 38.49 CFU/m³. The fungal counts (CFU/m³) ranged from 0.26 CFU/m³ to 51.59 CFU/m³ (Table 2).

The types of microorganisms isolated from the air of the different times are shown in table 2. The most abundant of isolated bacteria in the three seasons was *Bacillus circulans* (38.49 CFU/m³) followed by *Dermaococcus nishinomiyaensis* (13.36 CFU/m³), and *M. luteus* (9.43 CFU/m³) and for fungi *Penicillium* (51.59 CFU/m³) then *Aspergillus* spp. (36.40 CFU/m³) were the most abundant in ACU. While other genes from bacteria and fungi were last frequently encountered table 2.

Air quality in medical lab is important for staff, worker health and environment. The main factor affecting the indoor bacterial concentration is the density and activities of occupants (Fox *et al.*, 2005). The concentration of airborne microflora in air medical lab environment of BCID, showed that, rest, storage and coffee rooms (Figure 1) recorded the highest airborne bacterial and fungal population, while the least airborne bacterial and fungal population was recorded in the microbiology and serology rooms (Figure 1).

The degree of bacterial contamination in a tested area of current study was in permitted levels, according to Toth (1992) who suggested that the counting of human normal flora bacteria above 200 CFU/m³ air be considered high. Bacterial concentrations in homes and hospitals have varied between 10 CFU/m³ to 104 CFU/m³ in different studies (Awad and Farag, 1999; Lee and Jo, 2006; Obbard and Fang, 2003). For hospital environments, the maximum number of bacteria CFU allowed by the WHO (1988) is 100 CFU/m³.

The concentrations of gram-positive bacteria are generally higher than those of gram-negative bacteria in the indoor air (Fox and Rosario, 1994) this was in agreement with current study. Among the microbial isolate, *Staphylococcus* spp. was reported to be the most prevalent bacterial isolate followed by *Micrococcus* and *Kocuria*, also they were found in four seasons, while gram negative bacteria found in some of them. These

airborne micro-flora obtained were similar to that obtained by Ekhaise *et al.*, (2010).

The diversity of bacterial species present is wide and in agreement with that found by (Jaffal *et al.*, 1997; Kim and Kim, 2007; Naddafi *et al.*, 2011).

Indoor air samples in this study showed contamination with fungi under the acceptable levels. For the hospital environments, WHO, 1988 recommends shouldn't exceed 50 CFU/m³ of fungi, the National Institute of Occupational Safety and Healthy (NIOSHI-USA) (Jensen and Schafer, 1998) up to 1000 CFU/m³, and the Brazilian Health Ministry (ANVISA, 2000) 750 CFU/m³. However, Jensen and Schafer (1998), reinforces the fact that a low concentration of microorganisms in indoor air environments does not mean they are healthy, but needs to identify the microorganisms to evaluate the conditions of the air-conditioning systems. In this study, nevertheless, the fungal contamination levels were from 1 to 66 CFU/m³ for indoor samples.

Dominant indoor air fungi in this study were *Aspergillus* sp. (44.30%) and *Penicillium* sp. (21.48%) then *Alternaria* sp. (11.41%) and *Candida* sp. (10.74%). Other fungal isolates include *Fusarium* (3.36%), *Cladosporium* (2.68%), *Gliocladium* (2.68%), *Epicoccum* (2.01%), *Rhizopus* and *Diplodia* (0.67%). These results are comparable to those from previous study (Omolgberale *et al.*, 2014; Qudiesat *et al.*, 2009; Herbarth *et al.*, 2003 and Jaffa, 1997) who also isolated similar fungi in indoor environments. Ekhaise *et al.*, (2010) also isolated *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., and *Candida* sp., *Verticillium* sp. from the study areas. Higher isolation rates were made at storage room and the coffee room probably due to poor ventilation, poor cleaner, and overcrowding in this study area.

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