International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Variability in Airborne Bacterial and Fungal Population in Educational Dental Hospital, Umm Al-Qura University

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Received: 16/01/2017 *Revised: 25/01/2017* *Accepted: 30/01/2017*

ABSTRACT

Background: Airborne particles are produced from the oral cavity during dental treatment using high speed hand pieces, ultrasonic and air – water syringing or exposure to the microorganisms present in blood, saliva and suspended in the air. These microorganisms settle some time later on the surfaces prone for transmission of various diseases among the patients, hospital workers and visitors.
Objectives and Methods: The purpose of this research was to determine the quantity, quality and identify the viable (bacterial and fungal) and nonviable airborne particles in sixty four dental clinics (DCs) during manned (during treatment) and non-manned (No patient or staff was present in the DC at these times) conditions into Educational Dental Hospital, Umm Al-Qura University, Makkah, Saudi Arabia. The colony forming units (CFUs) were determined in the air by using the settle plate and air sampling methods and on the surface by using contact plate method. The bacterial and fungal isolates were identified by using standard microbiological procedures. The particle count, temperature and relative humidity for each DC unit was measured.
Results and Conclusion: In the present research the viable and nonviable air and surface count of the DCs was significantly higher in manned than non-manned conditions ($p < 0.001$), with their quantity and quality presented in the accepted grade C and D limits according to the standard level guide. The isolated species from DCs was commensal and no risk found to healthcare staff and patients that due to present a good and strict air and sterilization system applied in the dental college hospital.

Keywords: Airborne particles, Air quality, settle plate, air sampling, contact surface plate, particles count.

INTRODUCTION

Dental hospital infection is considered a dangerous risk for DCs in the whole world. The risk of cross-infections may be produced from microbial aerosols or particle generation during dental treatment using handpieces, ultrasonic scalers, turbine burs, bicarbonate polishers, polishing cups and other aerosol forming instruments. It may also be produced during treatment from

the air- water sprays inside the oral cavity or produced from the patient own microflora or may be obtained either from healthcare staff or the related environment with a high risk of infection to healthcare workers and patients. [1,2] Several pathogens are able to survive for long periods on DC surfaces and microbial aerosols thus become reservoirs of infection and considered a good indicator of the quality of air and surface contamination. [3]

The viable and nonviable airborne particles are the important indicators of the standard of air contamination and considered the source of the diseases which are transferred from an infected to a susceptible person. [4, 5] Microorganisms can cause respiratory diseases such as allergies, asthma, rhinitis, pneumonia, tuberculosis and nosocomial infections. [6-10]

The indoor environment is affected by the number of personnel, relative humidity, temperatures, air flow and air pressure, which due to increase the airborne microorganism count and cause acute diseases and infections such as *Streptococcus pneumoniae* and *Staphylococcus aureus* (MRSA), *Acinetobacter* sp., *Pseudomonas* sp., fungal spores and viruses which present on dust particles. Also, in recent years the transmission of the respiratory diseases through airborne was increased due to circulating air systems for temperature control in many new buildings. [10, 11]

The viable airborne particles depend on the particle size and their source to cause the diseases. Particles less than 10 µm in diameter are able to cause lower respiratory tract diseases. [12] The smaller particles (<5 µm) which are generated during dental treatment, can remain in the air and reach the respiratory system, while larger particles settle onto environmental surfaces and act as reservoirs of microorganisms transferred to instruments and other environmental surfaces by cross-infection or to the eyes, mouth or nose of patients and healthcare workers. [13]

Controlling of the airborne pathogens is important for: a. reducing the exposure to infection risk and safety control procedures for the patients, healthcare staff and the hospital visitors, b. reducing the amount of indoor airborne particles in DCs, c. prevent the transmission of the microbes from one area to another or cross infection, especially when an epidemic case appears, d. identify the viable and nonviable airborne and collection of pathogenic microorganisms which is very important for the quantification of contamination. [14-16]

The available information about viable and nonviable particles in DCs and their dangerous effect is still not clear, and for that reason this study was initiated.

MATERIALS AND METHODS

Sampling sites:

This research was done in the Educational Dental Hospital, Umm Al-Qura University, Makkah, Saudi Arabia. The samples were collected from sixty four DCs, which are shown in DCs layout Figure 1. The settle plate and air sampling plates were done during manned and non-manned conditions, while the contact plate was collected after treatment and sterilization of the DCs during non-manned condition. The particle count, temperature and relative humidity were measured. All samples were distributed among the DCs, according to the standard method as shown in Figure 2 and the results were recorded during all experiments.

Evaluation of viable air contamination:

a- Settle plate methods:

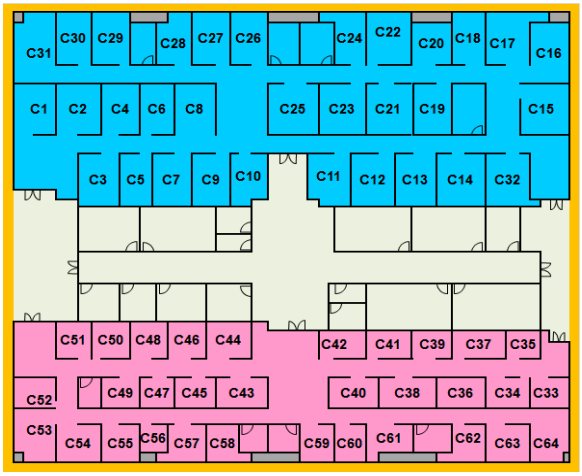
The settle plate method was carried out according to Pasquarella C *et al.*, 2000 [17] by using 90 mm, tryptic soy agar (TSA) disposable petri plates in four locations into the DCs. The petri plates were exposed to air for one hour and set up at a height one meter above floor level. The samples were immediately brought to the laboratory and incubated at 37ºC for 24-48 hours for bacterial growth and 3-5 days at 25°C for fungal growth.

b- Air sampling methods: [18]

The air samples were collected from three locations in the DCs by using air sampler model Microbio MB2 International, United Kingdom. The air sampler was run at an air flow-rate of 100 L/min and aspirated onto 90 mm, TSA disposable plate. The sampling time was one minute after delayed sample 30 sec to avoid overloading of the collection plate and drying of the agar surface. The air sampler was set up at a height one meter above floor level. The air sampler head was autoclave sterilized at 121ºC, 1.5 pas for 20 min and cleaned by swabbing with alcohol 70% between each sample. The samples were immediately brought to the laboratory and incubated at 37ºC for 24-48 hours for bacterial growth and 3-5 days at 25°C for fungal growth.

c- Contact surface plate method: [19]

The contact surface plate was collected from five locations in the DC (patient chair, doctor's chair, dental unit tray, table and dental unit switch) by using 55 mm, TSA disposable petri plates medium and sterilized by swabbing with alcohol 70% after samples taken. The samples were immediately brought to the laboratory and incubated at 37ºC for 24-48 hours for bacterial growth and 3-5 days at 25°C for fungal growth. The CFU/cm^2 derived from the microbial count on 55 mm diameter plates.



C: clinic number.

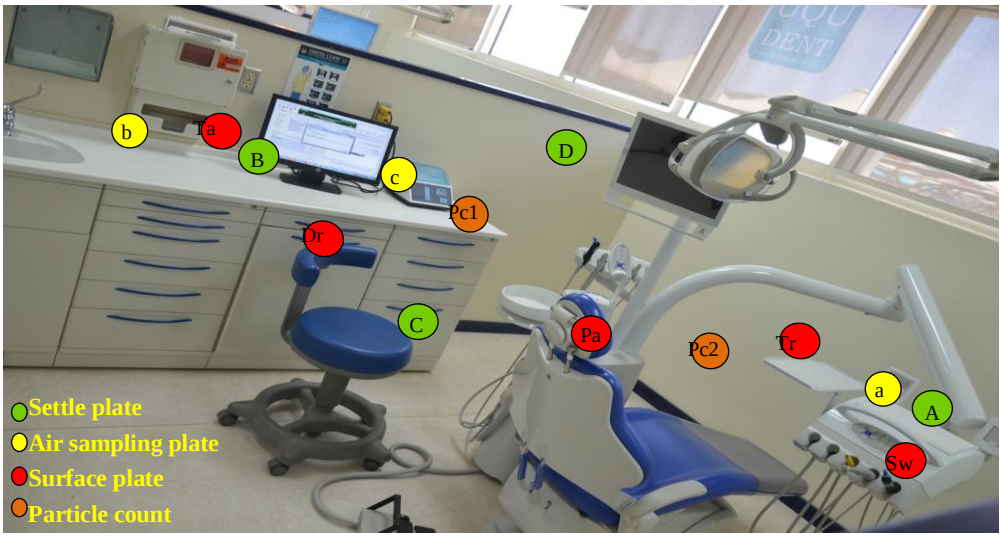
Figure 1: The layout of the DCs.

Evaluation of nonviable air contamination: [20]

The particles count (0.5, 0.7, 1.0, 3.0, 5.0 and 10.0 µm) were collected from two samples in each DC by using portable particle counters model APC 9510-02 AeroTrak International, USA. The portable particle count was set up at a height one meter above the floor level. A zero check should be performed before conducting the first sample by zero filter assembly. The portable particle count sample was measured three times and the mean and standard deviation (SD) were calculated, the temperature and relative humidity were recorded for each sample.

Guide and reference for microbial air contamination in DC:

In order to determine a guide for index air microbial contamination (IAM) during manned condition, the exposing disposable petri plates (90 mm) were used in the DC for one hour and set up at a height one meter above floor level, the mean value was used in the normal results at the target level and the 75th percentile as the alert level. [17] The Swiss Hospital Association's (2007) recommended that the mean value of settle plate in DC should be around 15 – 25 CFU/h during the manned condition which was similar to the European Commission Guide to Good Manufacturing Practice recommended in grade C and D for pharmaceutical industries, the air sampling was 100, 200 CFU/m^3 and settle plate 12.5, 25 CFU/h and contact plates (diameter 55 mm) 25, 50 CFU/plate respectively. The maximum permitted airborne 0.5 and 5.0 µm particle concentration for grade C during non-manned condition was 352000 and 2900 respectively, while during manned condition was 3520000 and 29000 respectively. The grade D during non-manned condition was 3520000 and 29000 respectively, while during manned condition was not defined. [21-22]



Settle plate site: A,B,C,D; Air sampling plate site: a,b,c; Surface plate site: Doctor's chair (Dr), Patient chair (Pa), Dental unit tray (Tr), Dental switch unit (Sw), Table (Ta); Particle count:

Figure 2: Distribute the settle, air sampling, contact surface plates and particle count sites into the DC.

Culture media and microbial identification:

a- Isolation and identification of bacteria:

Bacterial isolates were characterized and identified by using standard microbiological procedures (morphology, microscopic appearance, physiological and biochemical tests) according to Bergey's Manual of Systematic Bacteriology. [23] The bacterial identification was confirmed by using vitek 2 analyzer (bioMerieux, UK). All data were analyzed automatically and the identification was collected.

b. Isolation and identification of fungi: [24]

A wet fungal colony was examined microscopically by using Lactophenol-cotton-blue solution and identified by morphology, spore and hyphal characteristics and microscopic appearance.

Statistical analysis:

The mean values, SD, percentiles and 95% confidence interval of CFU were calculated. Due to the normal distribution of data, their mean used a statistical descriptor. The paired *t*-test was used for analyzing the difference between microbial counts during manned and non-manned conditions. Statistical significance was assumed for *p* values lower than 0.001. Statistical analysis was carried out using portable SPSS statistics version 19.

RESULTS AND DISCUSSION

The airborne particles in the DC were generated from the dental treatment processes or from outside sources such as air and dust, which were considered the micro-organisms sources for the dental team worker, patients and visitors. The newness of our research was to determine the quantity, and the quality of viable and nonviable airborne particle by using standardized techniques (settle, air sampling plates and nonviable particles by the portable particle counter), and study the microbial surface monitoring by using the contact surface plate during non-manned conditions in sixty four DCs.

In our study, the air sampling plates and settle plates mean values, SD, percentiles and 95% confidence interval (CI) of CFU were collected in Table 1. In our research, the viable count means were higher during manned than non-manned conditions in DCs, which affected by the air conditions, relative humidity, personnel activity and number of DCs.

Messano GA *et al.,* (2013) was emphasizing that viable airborne could come from patients and staff worker, which increase the levels of airborne bacterial contamination inside the dental units which was higher during manned than non-manned conditions and cause diseases transmission.

[25] Cellini L *et al.*, (2001) have noted that the number of airborne bacteria in DC was approximately two fold greater than that present before such activity. He also mentioned that the increase in the number of microbes in manned conditions should be observed when compared with non-manned condition and that more activity was generated around the plated during the treatment. [26]

The mean or target values were measured in male and female DCs during the manned condition of air microbial contamination 24.47, 14.91 CFU/h and 23.69, 17.84 CFU/m^3 respectively. The 75th percentiles or alert values were measured in male and female DCs during the manned condition of air microbial contamination 25.9, 16 CFU/h and 25, 19 CFU/m^3 respectively.

Pasquarella C *et al.*, (2012) determined the IAM as median value in ten DCs in Italy giving 27.5 CFU/m^3 during the activity and the alert values as 75th percentiles was 43.7. [19] Cellini L *et al.*, (2001) determined the microorganisms level in the area of the dental office was present in acceptable value which gave the mean 4-18 CFU/h. [26] Kedjarune U *et al.*, (2000) measured the level of air contamination by using a slit to agar sampler (Bio air checker Bac1, Nikken, Japan) and plates were incubated for 48 hours at 37 °C in aerobically at 5% CO_2 to give 232.49 CFU/m^3. [27]

In our studies found a significant correlation between the settle plate and air sampling methods in DCs during manned and non-manned conditions revealed in male 0.0006 and 0.0003, while in female 0.0005 and 0.0009 respectively.

Most published studies of the DCs found a significant correlation between the settle plate and air sampling methods, [28-32] while Petti S *et al.*, (2003) found a significant correlation between settle plate and air sampling methods during the high contamination level, but not found during the low contamination level. [33]

Table 1: Microbial air contamination values measured by settle and air sampling plates during manned and non-manned conditions in DCs.

Dental clinic	Sampling Technique	Sampling condition	Mean	95% CI range	S.D	Minimum	Maximum	25th Percentiles	75th Percentiles	*P* value
C1-C32	SP CFU/CFU/h	Non-manned	3.91	3.59 - 4.31	+ 0.99	2	6	3.14	4.68	0.0006
		Manned	24.47*	23.16 - 25.16	+ 2.53	20	30	22.56	25.9**	
	ASP CFU/CFU/m^3	Non-manned	5.72	5.38 - 5.88	+ 0.73	4	8	5.14	6.35	0.0003
		Manned	23.69*	23.19 - 24.5	+ 2.0	20	29	22.23	25.0**	
C33-C64	SP CFU/CFU/h	Non-manned	2.84	2.59 - 3.09	+ 0.95	1	4	2	4	0.0005
		Manned	14.91*	14.44 - 15.47	+ 1.57	12	18	14	16**	
	ASP CFU/m^3	Non-manned	4.97	4.688 - 5.25	+ 0.86	3	7	4	5.7	0.0009
		Manned	17.84*	17.50 - 18.65	+ 1.82	14	22	16	19**	

* IAM mean value was proposed standard or target value, ** The alert value was the 75th percentiles, N=32, CFU/m^3: colony forming unit per cubic meter, CFU/h: colony forming unit per hour, ± SD: standard deviation, CI: confidence interval, SP: settle plate, and ASP: air sampling plate.

In our study, the mean value, SD, percentiles and 95% CI of CFU were estimated for five critical sides include tray, table, Dr chair, patient chair and unit switch in male and female DCs by using the surface contact plates after finishing from sterilization after the end of the clinic day, all data were collected in Table 2.

The (25-75th) percentiles values in tray, table, Dr chair, patient chair and unit switch in the male DC revealed 3.17 – 7.67, 3.43 – 7.0, 4.63 – 8.77, 6.26 – 10.33 and 3.31 – 5.89 CFU/m^2, while in the female were 2.27 – 5.36, 2.5 – 5.36, 3.1 – 4.90, 2.19 – 3.71 and 1.73 – 4.1 CFU/m^2 respectively.

In previous studies of Castiglia P *et al.*, 2008 had observed that some surfaces were already contaminated at the end of the day and before the beginning of clinical

activity due to the usage of inappropriate disinfection, which lead to settling the airborne particles during the night on the surfaces and the absence of a proper air extraction system [34] and Decraene V *et al.*, 2008 referred to the importance of preventing cross-infection and hence transmission the antibiotic resistant between DC surfaces. [35] Schel AJ *et al.*, (2006) reached to the same conclusion in DCs have insisted on the importance of using a strict system, appropriate and frequent disinfection procedures to reduce the infection risk in DC aerosols. [36] Guida M *et al.*, (2012) reported that the surface contamination was increased at the end of the activity and recommended microbiological monitoring and improving the disinfection procedures and air treatment systems in the dental environment to detect the presence of risk factors and to adopt control measures. [37]

Table 2: Microbial surface contamination values measured by surface contact plate during non-manned condition in DCs.

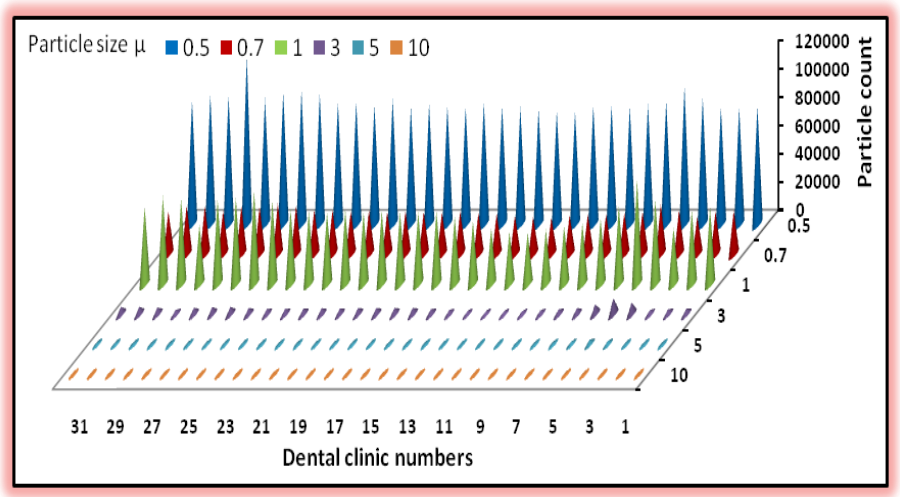
Dental clinic	C1-C32					C33-C64				
Surface plate site (CFU/m^2)	Tray	Table	Dr. chair	Patient chair	Switch unit	Tray	Table	Dr. chair	Patient chair	Switch unit
Mean*	5.56	5.40	7.06	8.31	4.87	3.75	3.94	4.06	2.94	2.97
95% CI lower	4.16	4.19	6.34	7.33	4.28	3.28	3.59	3.56	2.53	2.47
95% CI upper	7.18	6.53	8.03	9.81	5.75	4.34	4.40	4.56	3.44	3.48
S.D	3.14	2.53	3.42	3.51	2.23	1.76	1.68	1.50	0.94	1.55
Minimum	0	1	1	2	2	1	1	1	1	0
Maximum	12	11	15	17	11	7	7	8	5	6
25th Percentiles	3.17	3.43	4.63	6.26	3.31	2.27	2.5	3.1	2.19	1.73
75th Percentiles **	7.67	7.0	8.77	10.33	5.89	5.36	5.36	4.90	3.71	4.10

* IAM mean value was proposed standard or target value, ** The alert value was the 75th percentiles, CFU/m^2: colony forming unit per square meter, ± SD: standard deviation, CI: confidence interval, Temp. was 18-20°C and Rh. was 60-62 % in the male while the female was Temp. 16-18°C and 55-53 %.

In our study, the mean of particles count size 0.5, 0.7, 1, 3, 5 and 10µ in male DCs during non-manned conditions were 80439–119556, 26130–36174, 36795–74475, 2121–11502, 341–3361 and 37–363, While in female DCs were 61763–79646, 19588–31629, 24258–53800, 1499–5268, 316–1085 and 52–385 respectively. All data were shown in Figures 3 and 4. The particles count size 0.5, 0.7, 1, 3, 5 and 10µ were increased during the manned condition in male DCs to give 223522–165750, 50977–33294, 62634–38773, 3685–1783, 670-304, 386–108, While in female DCs were 196083–131920, 62179–41401, 51470–82903, 8745–3034, 2376–512 and 727–57 respectively. All data were shown in Figures 5 and 6.

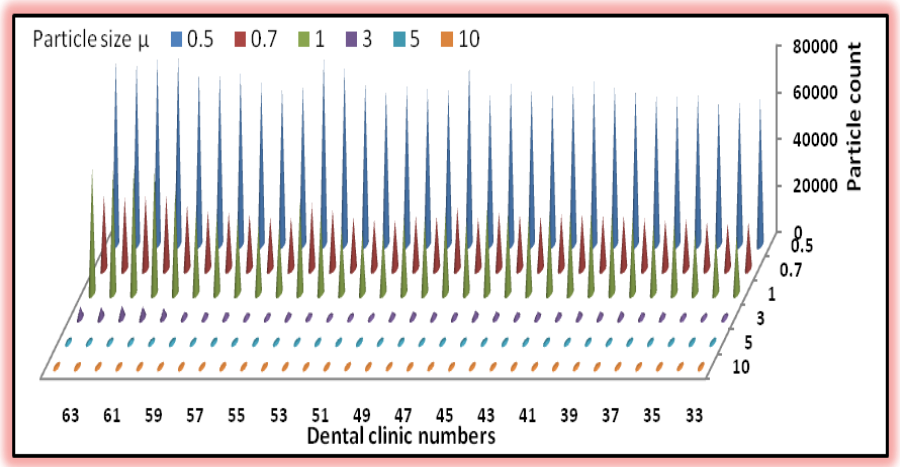
In previous studies Ghadimi E *et al.*, (2013) reported that particle concentrations was released into the dental airborne and increased during dental procedures such as aerosolized saliva. [38] Van Landuyt *et al.*, (2014) concluded that amount of <0.5 µm particles was higher than the amounts of >1 µm particles during dental drilling procedures. [39]

According to the European Commission Guide the particles count 0.5 and 5 µ in male and female DCs during manned and non-manned conditions were classified as grade C for all DCs except male DC number C29 the particle count size 0.5 µ was 119556 and DC number 5 in male during non-manned conditions was 3361, which classified as a grade D.



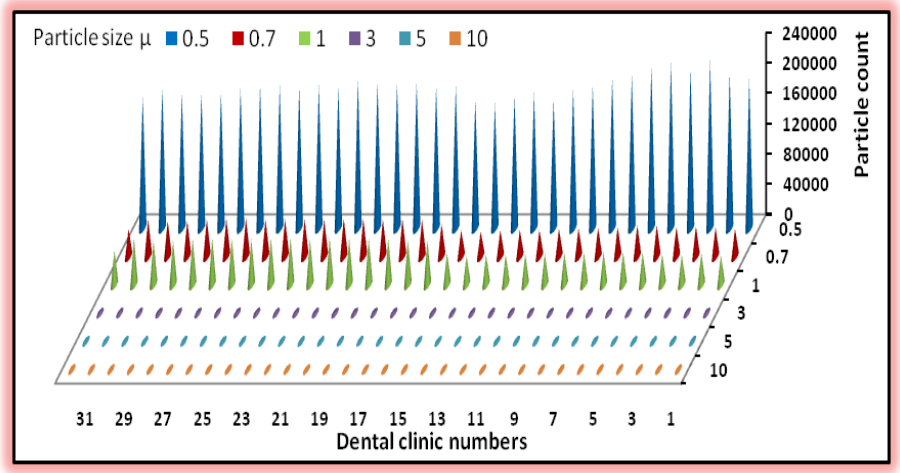
- Rh. was 60-62 % and Temp. was 20-22°C.

Figure 3: Mean of particles count in male DCs during non-manned conditions.



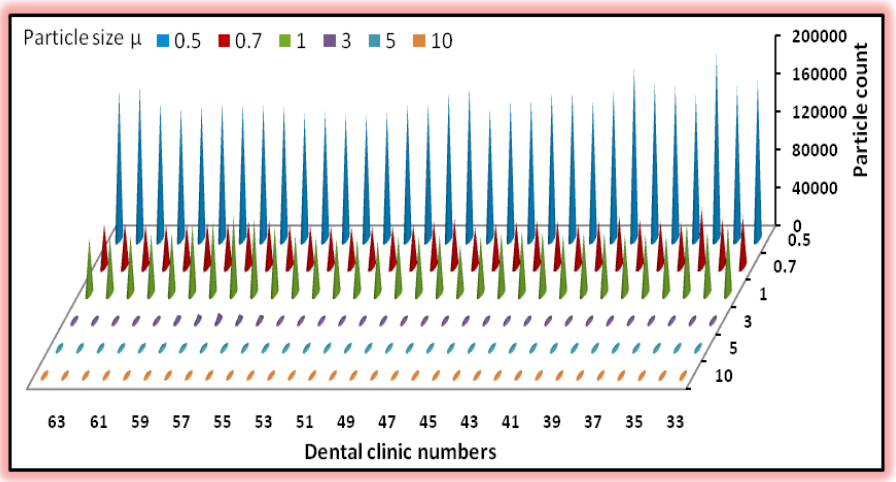
- Rh. was 55-53 % and Temp. was 16-18°C.

Figure 4: Mean of particles count in female DCs during non-manned conditions.



- Rh. was 60-62 % and Temp. was 20-22°C.

Figure 5: Mean of particles count in male DCs during manned conditions.



- Rh. was 55-53 % and Temp. was 16-18°C.

Figure 6: Mean of particles count in female DCs during manned conditions.

Table 3: Microbial percentage measured in male and female DCs during the manned and non-manned conditions by using the settle, air sampling plates and during the non-manned condition by using the contact surface plate.

Dental clinic	C1-C32					C33-C64				
Microbial isolates	SP %		ASP %		CSP %	SP %		ASP %		CSP %
Sampling condition	Manned	Non-manned	Manned	Non-manned	Non-manned	Manned	Non-manned	Manned	Non-manned	Non-manned
Streptococcus haemolyticus	6	10	8	8	2	2	1	6	4	1
Streptococcus pyogenes	14	11	10	9	4	4	2	3	4	3
Streptococcus pneumoniae	0.1	–	0.1	–	–	0.2	–	0.1	–	–
Micrococcus luteus	16	12	15	14	5	14	10	5	3	4
Micrococcus lylae	10	10	11	13	2	11	10	4	4	5
Micrococcus antarcticus	2	2	1	3	3	9	7.4	6	5	1
Micrococcus flavus	1	7	2	1	2	3	2	3	3	3
Micrococcus endophyticus	3	4	1	1	2	2	1	2	5	4
Staphylococcus aureus	3	2	3	2	3	2	2	2	2	2
Staphylococcus warneri	4	3	2	3	1	2	4	5	4	3
Staphylococcus epidermidis	8	4	5	2	2	1	6	3	3	3
Staphylococcus haemolyticus	2	3	1.2	2	2	1	2	2	4	2
Staphylococcus saprophyticus	2	3	2	2	1	–	2	1	2	2
Kocuria kristinae	–	–	2	1	3	2	4	3	3	3
Kocuria rosea	13	11	13	9.9	4	9	8	8	4	2
Kocuria varians	–	2	1	3	–	2	5	7	2	5
Bacillus cereus	2	4	5	8	20	14	2	2	16	19
Bacillus clausii	7	6	11.1	10	14	11	9	11.9	14	20
Bacillus firmus	4	2	4	3	15	9.8	10	14	11	9.2
Bacillus stearothermophilus	1	3	1.2	4	10	–	11	11	5	6
Pseudomonas aeruginosa	0.3	0.2	0.1	0.1	0.1	0.2	–	0.1	0.1	0.2
Escherichia coli	0.3	0.2	–	0.2	–	0.1	0.1	0.2	0.1	–
Penicillium notatum	0.3	0.2	0.2	0.2	1	0.2	–	0.1	0.5	0.4
Penicillium chrysogenum	0.1	–	0.3	0.1	0.5	0.1	0.4	–	0.3	0.5
Penicillium digitatum	0.2	0.1	0.1	0.1	1	0.2	–	0.2	0.2	0.2
Aspergillus flavus	0.2	0.2	0.2	0.1	1	–	0.3	0.1	0.1	0.5
Aspergillus nidulans	0.2	–	0.1	0.1	–	0.1	0.4	0.1	0.1	0.2
Aspergillus parasiticus	0.2	0.1	0.1	0.1	0.4	–	0.2	0.1	0.2	0.5
Aspergillus ochraceus	–	–	0.1	–	–	0.1	–	–	0.2	0.1
Aspergillus niger	0.1	–	0.2	0.1	1	–	0.2	0.1	0.2	0.2

SP: settle plates, ASP: air sampling plate and CSP: Contact surface plate.

The predominant airborne percentage of bacterial Gram positive, negative and fungal isolates were measured in male and female DCs during the manned

and non-manned condition by using the settle plate, air sampling plates and during the non-manned condition by using the contact surface plate method. All data were collected in Table 3.

Decraene V *et al.,* 2008 had isolated the *M. luteus* and *S. epidermidis* at high concentration, while the oral flora such as *Actinomyces* spp., *Streptococcus viridans, Haemophilus* spp., *Neisseria* spp. and *Lactobacillus* spp. at low concentration by using a settle plate in a UK DC. [35] Osorio R *et al.,* (1995) detected the dominant airborne microbes that *Streptococcus* sp were accounted between 73-82% in DCs both before and after clinical activity. [40] In a similar study in Japan DCs the airborne *Micrococcus* sp, *Streptococcus* sp and *Corynebacterium* sp were reported as 23, 22, 21% respectively. [41]

CONCLUSION

In this research the air and surface quantity of the DCs was found to be good, and acceptable within grades C and D for the air and surface. The importance of this research was that it recommended the usage of the regular environmental microbial monitoring in DC to prevent the transmission of diseases between the healthcare staff and patients, especially when dealing with immunocompromised patients. However, determining and identifying the risk factors are associated with microbial contamination and critical situations which require corrective intervention in some DCs and developing appropriate systems for environmental controlling the associated infections.

ETHICAL DISCLOSURES

The authors announce that no experiments were performed on voluntaries or animals and no data were collected from patient in this research. The authors have obtained the written approval of the Educational Dental Hospital, Umm Al-Qura University, Makkah, Saudi Arabia to doing this study.

ACKNOWLEDGEMENTS

The authors would like to thank Institution of Scientific Research and Revival of Islamic Heritage at Umm Al-Qura University (project # 43409044) for the financial support. The authors are grateful to Educational Dental Hospital staff for their valuable contribution in the work.

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How to cite this article: Maher YA, Jastania RA, Beyari MM et al. Variability in airborne bacterial and fungal population in educational dental hospital, Umm Al-Qura University. Int J Health Sci Res. 2017; 7(2):165-175.

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