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Statistical analysis of microbial air quality of laboratory animal house

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Abstract

Indoor air quality is becoming an increasingly important issue for occupational and public health aspect. The hygienic conditions of the environment could be determined by microbial air quality which may correlate with the disease occurrence also. The aim of this study was to assess and statistically analyze the microbial air quality of laboratory animal house using active air sampling method. The study was carried out in Disease Free Small Animal House (DFSAH), LUVAS, Hisar for one week in November, 2016 and repeated for one week in December, 2016. Air sampling was carried out in colony areas of guinea pig, rabbit, rat and mice, galleries, kitchen and washing room; exposing media plates for 30 seconds. Then plates were incubated at 37 °C for 24 hr., allowing visible colonies to develop and subsequently counted. The number of visible colonies gives an estimate of the number of colony forming units (CFU)/ft³ of air. From the current study, it could be concluded that there was statistically significant difference in bacterial counts in air samples (n=70) taken at 12PM (4.81±3.58) and at 4PM (2.93±2.10) (p value <0.001). However, there was no statistically significant difference in bacterial counts of air samples taken at Gallery and Guinea pig colony area at different times. Further, one-way ANOVA comparison of microbial air quality of different locations of laboratory animal house showed no statistically significant difference (p value >0.05). This study was an attempt to accurately reflect the difference of microbial air quality at different locations and time. The main purpose was to monitor the integral air quality of DFSAH, LUVAS and suggest areas for improvement of air quality. To ensure better health status and disease prevention in laboratory animals as well as animal handlers, indoor air quality should be monitored.

Keywords: Active air sampling, colony forming units

Introduction

Air plays an important role as a reservoir of various disease causing micro-organisms (bacteria, viruses and fungi) which effect human as well animal health (Wathes, C. M., 1994) ^[14]. Micro-organisms can originate from animal itself, health care personnel and environmental sources. The environmental matrices *i.e.* air, water and contact surfaces play major role as reservoirs of microorganisms (Tseng, C.C., 2010) ^[10]. The presence of bio-aerosols in environment can compromise normal activities. Infectious aerosols tend to be extremely small and can, therefore, remain suspended and viable in the air stream over long periods of time, resulting in extremely high risk of airborne infection in confined places.

Several methods have been developed for determination of microbiological air quality, active air sampling is one of them. Active sampling uses air samplers which have been designed to draw in a pre-set volume of air onto a culture plate. After incubation at a specific temperature, colony forming units (CFU)/m³ or (CFU)/ft³ of air is calculated from the number of colonies on the culture plate. This method is often applicable when the air microbial concentration is low, as in health-care settings (Blomquist G., 1994 and Pasquarella, *et al.*, 2008)^[1, 10]. In the current study, microbial air quality of laboratory animal house at different locations and time was determined using air sampler. The main purpose was to monitor the indoor air quality of DFSAH, LUVAS and suggest air quality improvement zones.

Materials and Methods

The study was carried out in Disease Free Small Animal House (DFSAH), LUVAS, Hisar for determination of the microbial air quality by using air sampler (Himedia; LA030). The sampler had capacity of 4800 liters with flow rate of 300 liters/min; based on sieve impaction particle capture mechanism. The experiment was conducted in two-fold; for one week in November, 2016 and again for one week in December, 2016. For each repetition, air sampling was carried out at seven locations of animal house *i.e.* colony areas of guinea pig, rabbit,

rat and mice, galleries, kitchen and washing room at 12 PM and 4PM; using Trypticase soya agar (TSA) media plates and exposing the plates for a time period of 30 seconds. Then plates were incubated at 37 °C for 24 hr., allowing visible colonies to develop and subsequently counted. The number of visible colonies gives an estimate of the number of colony forming units (CFU)/m³ or (CFU)/ft³ of air. After incubation, the total number of colony forming units for the bacteria were recorded and converted to CFU/ ft³. Paired t-test (STATATM; Stata Corp) was used for analyzing the difference between microbial counts at 12 PM and 4PM at different locations. One way ANOVA was carried out to assess the difference in microbial quality of air at different locations within the house. The statistical significance was expressed in term of p-value and the critical level was set at 0.05.

To secure the quality of the study, aseptic techniques like utilization of safety clothes; sterilization of sampling utensils; proper incubation of samples *etc.* were applied. Field blanks were also used to check the presence of cross contamination of media plates.

Results

After incubation of media plates, the bacterial load was enumerated as colony forming units and converted to CFU/ft³. Paired t-test (STATATM; Stata Corp) and one way- ANOVA was used for analyzing the difference (mean \pm s.d.) between microbial counts at 12 PM and 4PM at different locations. The statistical significance was expressed in term of p-value and the critical level was set at 0.05 (Table 1).

From the current study, it was found that CFU/ft³ was less than 50 in air samples of all locations at 12PM and 4PM in laboratory animal house in two consecutive months.

As per the study, it was found that there was statistically significant difference in bacterial counts in Mice colony area samples (n=10) at 12PM (5.55 ± 3.34) and at 4PM (2.53 ± 1.28) (p value=0.006) in two consecutive months. The difference in microbial count in air samples of Mice colony area in November month (n=5) at 12PM (5.55 ± 2.50) and at 4PM (2.04 ± 0.43) (p value=0.035) was also found statistically

significant.

As per the current study, the bacterial counts in air samples of Rabbit colony area in December month (n=5), at 12PM (2.23±0.39) and at 4PM (1.40±0.35) (p value=0.007) showed statistically significant difference. Likewise, statistically significant difference found in bacterial counts of air samples taken during two consecutive months in Rat colony area (n=10) at 12PM (6.96±2.83) and at 4PM (4.30±2.23) (p value=0.001). The difference in bacterial counts was also significant in November month (n=5) in Rat colony area at 12PM (6.45±1.79) and at 4PM (3.13±0.51) (p value=0.009). Similarly, statistically significant difference found in total bacterial counts of air samples of two consecutive months in Kitchen area (n=10) at 12PM (3.68 ± 1.88) and at 4PM (1.93 ± 1.20) (p value=0.014). The difference in bacterial counts was also statistically significant in November month Kitchen area samples (n=5) taken at 12PM (3.28 ± 1.07) and at 4PM (1.51±1.60) (p value=0.008). The statistically significant difference in bacterial counts was also found in December month (n=5) in Washing room at 12PM (8.49±1.61) and at 4PM (6.26 ± 1.06) (p value=0.010). It was observed that there was statistically significant difference in bacterial counts in Washing room air samples (n=10) at 12PM (9.30 ± 3.74) and at $4PM (5.08\pm1.49) (p value=0.009) in two consecutive months.$ From the current study, it could be concluded that there was statistically significant difference in bacterial counts in air samples (n=70) taken at 12PM (4.81±3.58) and at 4PM (2.93 ± 2.10) (p value <0.001). There difference in bacterial counts in air samples taken at 12PM (4.64±3.65) and at 4PM (2.48±1.71) in November month (n=35) and at 12PM (4.98±3.55) and at 4PM (3.38±2.37) (p value <0.001) in December month (n=35) was also statistically significant. However, there was no statistically significant difference in

However, there was no statistically significant difference in bacterial counts of air samples taken at 12PM and 4PM in Gallery and Guinea pig colony area.

Further, one-way ANOVA comparison of microbial air quality of different locations of laboratory animal house showed no statistically significant difference (p value >0.05) (Table 2).

 Table 1: T-test results comparing air quality of laboratory animal house at different times (n=no. of observations).

Location (n)	Month (n)	cfu / ft ³ (Mean ±s.d.)		df	t statistics	p-value
		12 P.M.	4 P.M.			
Guinea pig colony (10)		5.32±2.14	4.43±1.23	9	1.169	0.273
	November (5)	4.34±1.19	4.15±1.69	4	0.187	0.860
	December (5)	6.30±2.53	4.72±0.61	4	1.377	0.240
Mice colony (10)		5.55±3.34	2.53±1.28	9	3.610	0.006
	November (5)	5.55 ± 2.50	2.04±0.43	4	3.132	0.035
	December (5)	5.55±4.34	3.02±1.71	4	1.900	0.130
Rabbit colony (10)		2.17±0.55	1.75±1.67	9	0.691	0.507
	November (5)	2.12±0.72	2.11±2.41	4	0.005	0.996
	December (5)	2.23±0.39	1.40±0.35	4	5.078	0.007
Rat colony (10)		6.96±2.83	4.30±2.23	9	4.784	0.001
	November (5)	6.45±1.79	3.13±0.51	4	4.738	0.009
	December (5)	7.47±3.77	5.47±2.74	4	2.422	0.073
Gallery (10)		0.70±0.32	0.47±0.25	9	1.746	0.115
	November (5)	0.64±0.41	0.53±0.34	4	0.510	0.637
	December (5)	0.75±0.23	0.42 ± 0.16	4	2.425	0.072
Kitchen (10)		3.68±1.88	1.93 ± 1.20	9	3.045	0.014
	November (5)	3.28±1.07	1.51±1.60	4	4.837	0.008
	December (5)	4.07±2.53	2.34±0.47	4	1.488	0.211
Washing room (10)		9.30±3.74	5.08±1.49	9	3.283	0.009
	November (5)	10.11±5.22	3.89±0.60	4	2.727	0.053
	December (5)	8.49±1.61	6.26±1.06	4	4.577	0.010
Total (70)		4.81±3.58	2.93±2.10	69	5.922	< 0.001

	November (35)	4.64±3.65	2.48±1.71	34	3.949	< 0.001	
	December (35)	4.98±3.55	3.38 ± 2.37	34	4.924	< 0.001	
Note- cfu /ft ³ -Colony forming units per cubic feet, s.d standard deviation; d.f - degree of freedom							

Table 2: One-way ANOVA comparison of air quality of different locations of laboratory animal house (n=no. of observations).

	cfu / ft^3 (Mean ± s.d.)						
	Total (n=70)		Novembe	er (n=35)	December (n=35)		
	12 P.M.	4 P.M.	12 P.M.	4 P.M.	12 PM	4 PM	
	4.81±3.58	2.93 ± 2.10	4.64 ± 3.65	2.48 ± 1.71	4.98±3.55	3.38±2.37	
df	1,68	1,68	1,33	1,33	1,33	1,33	
F-value	2.93	0	3.1	0.74	0.41	0.48	
p-value	0.092	0.952	0.087	0.397	0.524	0.492	

Note- cfu/ft³-Colony forming units per cubic feet, s.d.- standard deviation; d.f -degree of freedom

Discussion

Though there are no uniform international standards available on levels and acceptable maximum bacterial loads in indoor air, the work conducted by a WHO expert group on assessment of health risks of biological agents in indoor environments suggested that total microbial load should not exceed 1000 CFU/m3 (Nevalainen, A. and Morawaska, L., 2009)^[8] whereas other scholars considered that 750 CFU/m³ should be the limit for bacteria (Francisco, R.A.N. and Luiz, F.G.S., 2000; Cappitelli, et al., 2009)^[4, 2]. Airborne microbial concentrations ranging from 4500 to 10,000 CFU/m³ also have been suggested as the upper limit for ubiquitous bacterial aerosols (Nevalainen, A., 1989)^[9]. The sanitary standards of European Commission for non-industrial premises consider less than 50 CFU/m³ as 'very low' bacterial load, 50-100 CFU/m³ as 'low', 100-500 CFU/m³ as 'intermediate', 500-2000 CFU/m³ as 'high' and above 2000 CFU/m³ as 'very high' load (Commission of European Communities, 2016)^[3].

On the basis of this study, it could be speculated that the microbial air quality of the laboratory animal house could be considered 'Good' as CFU/ft³ was <50. As all other factors were kept constant including cleaning time, frequency of cleaning, ventilation *etc.*, the difference in behavior of different laboratory animals and type of bedding material contributes to the variation of bacterial load at different time interval in colony areas.

It has been reported that lower temperature during winter months and increased ventilation in summer months to reduce high temperatures may contribute to lesser bacterial load. However, some other authors reported higher value of number of microorganisms during the warm season (Kiekhaefer, *et al.*, 1995)^[5].

In a study conducted in 32 turbulent air flow operating theatres of University Hospital in Southern Italy, active sampling was carried out using the Surface Air System and passive sampling with settle plate. The mean TVC (total viable count) at rest (in the morning before the beginning of surgical activity) was 12.4 CFU/m³ and 722.5 CFU/m²/h for active and passive samplings respectively. The mean in operational TVC was 93.8 CFU/m3 (s.d.=52.69; range=22-256) and 10496.5 CFU/m²/h (s.d.=7460.5; range=1415.5-25479.7) for active and passive samplings respectively. Statistical analysis confirmed that the two methods correlate in a comparable way with air quality (Napoli, et al., 2012)^[7]. In an another study, the means of bacterial and fungal counts in 138 indoor air samples (72 from dental treatment units, 48 from dental supporting units and offices and 18 from patient waiting area) collected before and during dental works for 6 days (Monday to Saturday) was significantly increased during

dental procedures when compared with those collected before dental works (p<0.001), whereas, those were not significantly different in the dental supporting units and offices (p >0.05) (Luksamijarulkul, *et al.*, 2009)^[6].

Currently, since air sampling protocols are not standardized, it is difficult to compare the results from different studies (Pasquarella, *et al.*, 2008)^[10]. Different indoor environments have different levels of bio-contamination, different kinds of airflow, different numbers of people working in them who use different kinds of personal protective equipment, all factors which affect the results of both the sampling and the comparison between methods (Pasquarella, *et al.*, 2000)^[11].

Conclusions

A number of studies have been conducted but have not given consistent results due to the different air samplers used, different places sampled *i.e.* operating rooms, dental clinics etc. and the different parameters applied *i.e.* volume of air sampled, sampling point, sampling time protocol, *etc.* The determination of microbial quality of air in laboratory animal house is a significant parameter to assess the environmental quality and to prevent disease in laboratory animals as well as animal handlers.

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