

ORIGINAL ARTICLE

Effect of environmental and behavioural factors on  
microbiological air quality of operating rooms

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Abstract

**Introduction:** An operating theatre (OT) is a very complex setup, which presents numerous challenges for both patients and health care providers. A safe OT environment decreases the susceptibility of patients to postoperative infections. Therefore, a study was conducted to determine microbiological air quality for establishing baseline values and to assess concurrently the effect of environment and behavioural factors in 46 OTs of a tertiary care hospital.

**Materials & Methods:** The OTs were divided into two groups based on the type of air flow: laminar diffuser (Group I) or conventional diffuser (Group II). Two hundred and thirty-eight samples were collected with an active technique using a sieve impactor, sampling at 100 L/minute. Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) Version 20 (IBM Corp., Armonk, NY).

**Results:** In Group I OTs with the laminar diffuser, bacterial and fungal values during the lean period for environmental and behavioural factors were 47.78 colony forming units (CFM)/m<sup>3</sup> and 0.24 CFU/m<sup>3</sup>, respectively. The bacterial and fungal values during the surgical period were 98.01 CFU/m<sup>3</sup> and 0.40 CFU/m<sup>3</sup>, respectively. The same values for Group II OTs with the conventional diffuser were 52.83 CFU/m<sup>3</sup> and 0.39 CFU/m<sup>3</sup> during the lean period, and 80.06 CFU/m<sup>3</sup> and 0.40 CFU/m<sup>3</sup> during the surgical period.

**Discussion:** Both environmental and behavioural factors – temperature, humidity, percentage of fresh air in circulation, door type, controlled entry, minimal door opening and proper OT attire – were seen to contribute concurrently to maintaining air quality in operating units.

Keywords: *operating room; air quality; bacteria; fungi; environment; behaviour; India*

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Hospital-acquired infections lead to morbidity and mortality in patients. Surgical site infections are one such component of hospital-acquired infection. In India, the incidence of postoperative infections in various hospitals is relatively higher (10–25%) than in the United Kingdom and the United States. (1–3). The rate of surgical wound infections is strongly influenced by operating theatre (OT) practices and air quality (4). Airborne microorganisms may enter the patient's body during the intraoperative procedures and cause infections. Operating theatre planning and design flaws, contaminated OT air, faulty sterilisation and disinfection techniques and breaks in OT discipline contribute to the deterioration of environmental air quality in the OT air (4). The strategy for reducing intraoperative contamination involves putting environmental control and behavioural measures in place. Environmental control approaches include, among others, properly installing and

maintaining a heating ventilation and air conditioning (HVAC) system. A behavioural approach aims to reduce the number of airborne particles in the OT through disciplinary measures such as limiting the number and restricting to a minimum the movements of personnel in the OT (4). In patients undergoing orthopaedic implant surgery, adopting a range of measures such as limiting needless activity, having laminar flow, work-up in the preparation room rather than in the OT and the wearing of proper attire in the OT had a significantly positive effect on outcomes during the postoperative period (5). A combination of environmental controls and behavioural measures in the OT led to a reduction in the incidence of intraoperative bacterial contamination (4).

Background

Our tertiary care postgraduate hospital and research centre with 1,948 sanctioned beds contains six hospital buildings

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within the campus. Each building has an OT complex with different numbers of OTs in it. Each of these OT complexes has different types of infrastructural design features, HVAC systems and environmental controls. It is a well-known fact that among other things, microbiological air quality in the OT has a role in postoperative morbidity and mortality. Therefore, this study was conducted to create baseline values of bacterial and fungal concentrations as colony forming units/m<sup>3</sup> in the OT air of different OT complexes. Also, few studies have been conducted to assess concomitantly the effect of environmental factors (airflow, temperature, humidity and air supply) and behavioural factors (number of persons in OT, number of door openings, sliding vs hermetically sealing doors and proper attire of the surgical team) on microbiological quality of air. This extensive study will also focus on the effect of environmental and behavioural factors on microbiological air quality.

## Methods

This prospective study was carried out in 46 OTs of five different buildings with 50,000 annual surgeries. The OTs were divided into two groups based on the type of air flow:

Group I: laminar airflow diffuser with a high-efficiency particulate air (HEPA) filter (25 OTs);

Group II: conventional supply diffuser with an HEPA filter (21 OTs).

A total of 238 samples were collected over a period of 6 months for bacterial and fungal air contamination. Two samples were collected for each surgery: one preoperative sample and one intraoperative sample from the same OT.

Samples for OT microbiological air quality were collected with an active technique using a Air Petri Sampling Mark II (HiMedia Laboratories Pvt. Ltd, India) by allowing air to pass through the sieve impactor at the rate of 100 L/min for 5 min with a Petrie plate of 9 cm in diameter containing media, within 1 m of the operating table and placed at the height of 1 m. Media used for sample inoculation was 5% sheep blood agar for bacteria and Sabouraud dextrose agar for fungi.

The exposed plates were incubated aerobically at 37°C for 24–48 h for bacterial growth, and 7 days for fungal growth. The level of airborne contamination for bacteria and fungi was calculated as colony forming units (CFU)/m<sup>3</sup> based on colony counts. Various environmental and behavioural factors were also recorded at the time of OT air sampling.

## Statistical analysis

Dependent variables were fungal and bacterial counts, whilst independent variables were temperature, relative humidity, type of air flow, number of personnel present and frequency of door opening. The Statistical Package for Social Sciences (SPSS) Version 20 (IBM Corp., Armonk, NY) was used for statistical analysis. The results

were analysed using correlation analysis, t-test, one-way analysis of variance, with  $P < 0.05$  taken as a significant.

## Results

Two hundred and thirty-eight air samples were collected from 46 functional OTs. The mean bacterial count in Group I was 72.54 CFU/m<sup>3</sup>, whilst in Group II, it was 67.01 CFU/m<sup>3</sup>. The mean fungal count was 0.31 CFU/m<sup>3</sup> in Group I and 0.39 CFU/m<sup>3</sup> in Group II.

The mean bacterial and fungal counts of 118 samples collected during the preoperative period and 120 samples collected during the intraoperative period are recorded in Tables 1 and 2. The intraoperative concentration of bacterial microorganisms was significantly higher ( $P < 0.05$ ) as compared to the preoperative period in both Group I and Group II. No significant difference was seen in fungal counts in Group I and Group II.

## Environmental controls

### Airflow and HEPA filters

In Group I OTs with the HEPA filter laminar flow HVAC system, mean bacterial counts were 47.78 CFU/m<sup>3</sup> and 98.01 CFU/m<sup>3</sup> during the preoperative and intraoperative periods, respectively. The mean fungal counts were 0.24 CFU/m<sup>3</sup> and 0.40 CFU/m<sup>3</sup> during the preoperative and intraoperative periods, in Group I OTs. In Group II OTs with the conventional airflow HEPA filter, mean bacterial counts were higher than Group I in the preoperative period and lower than Group I in intraoperative period. The fungal counts did not show any significant variation (Table 1).

### Temperature

The samples were collected at temperatures ranging from 16°C to 28°C in two different groups of OTs during the preoperative and intraoperative periods. There was an increase in the colony count (CFU/m<sup>3</sup>) with increased temperature in both groups during the preoperative and intraoperative periods (Tables 3 and 4). The increase in the microorganism concentration is significant ( $P < 0.05$ ) during the intraoperative period with respect to the preoperative period in Group I (Table 3).

### Humidity

There was an increase in bioload with a rise in humidity (40–69%) during the intraoperative period in both groups, but not statistically significant (Tables 3 and 4).

## Behavioural controls

### Effect of number of persons in the OTs

The bacterial counts were maximum when the number of persons exceeded 10 during the intraoperative period. The fungal counts did not show much variation with

**Table 1.** Bacterial and fungal counts in different operating theatre complexes during the preoperative and intraoperative periods

Group	Number of samples		Preoperative period (n = 118 samples)		Intraoperative period (n = 120 samples)		P value
			Mean colony forming units (CFU)/m <sup>3</sup>	95% CI	Mean colony forming units (CFU)/m <sup>3</sup>	95% CI	
Group I: HEPA filter, laminar flow	142	Bacterial	47.78	38.32–57.23	98.01	86.29–109.74	<0.00
		Fungal	0.24	0.13–0.34	0.40	0.21–0.59	0.137
Group II: HEPA filter, conventional flow	96	Bacterial	52.83	43.59–62.06	80.06	68.11–92.01	<0.001
		Fungal	0.39	0.16–0.32	0.40	0.13–0.67	0.961
Total	238	Bacterial	49.74	43.02–56.47	90.53	82.03–99.02	<0.00
		Fungal	0.29	0.18–40	0.40	0.24–0.55	0.286

HEPA: high efficiency particulate air; CI: confidence interval.

**Table 2.** Bacterial and fungal counts in individual operating theatre units during the preoperative and intraoperative periods

Group	Areas (no. of operating theatres)	Fresh air (%)	No. of samples	Bacterial colony counts, CFU/m <sup>3</sup>		Fungal colony counts, CFU/m <sup>3</sup>	
				Preoperative	Intraoperative	Preoperative	Intraoperative
Group I: HEPA filter, laminar flow	Cardiothoracic and vascular surgery (4)	25	24	45.50	88.33	0.25	0.17
	Ophthalmology (5)	25	28	44.43	84.43	0.14	0.14
	Paediatric (4)	25	24	86.08	122.75	0.58	1.08
	Trauma (5)	1 AHU – 100 4 AHU – 25	30	45.06	110.43	0.13	0.50
	Emergency complex (7)	2 AHU – 100 1 AHU – 25	36	28.78	88.89	0.17	0.22
Group II: HEPA filter, conventional flow	Main operation theatre complex (18)	All AHU – 100	86	41.80	59.20	0.40	0.00
	Emergency orthopaedics (3)	25	10	54.17	82.38	0.39	0.44

CFU: colony forming units; HEPA: high efficiency particulate air; AHU: air handling unit.

respect to number of persons during the preoperative and intraoperative periods (Tables 3 and 4).

#### Number of persons with mask below nose

The bacterial counts increased with the increase in the number of persons having a mask below the nose in Groups I and II (Tables 3 and 4).

#### Number of times door opened

As door opening increased during the surgical period, so did the bacterial CFU/m<sup>3</sup>. The increase in the bacterial count in the intraoperative period in Group I is significant with respect to the preoperative period. Also, the increased bacterial count during the intraoperative period of Group I is significant with respect to the bacterial count in intraoperative period of Group II (Tables 3 and 4).

Type of door (hermetically sealing vs swing door): The bacterial and fungal CFU/m<sup>3</sup> for OTs with hermetically sealed doors were lower than for OTs with swing

doors in Group I, although not statistically significant (Table 3).

## Discussion

Surgical-site contamination by airborne particles is ascribable in 30% of cases to direct settling of the particles on the wound, and in 70% of cases to settling on the instruments and surgeon's hands followed by transfer to the wound (6). In order to reduce airborne bacteria, it is important to control both environmental and behavioural factors. This study was conducted to establish baseline values for different OTs and also to record the effect of environmental and behavioural factors on the microbiological air quality in different types of OTs.

Establishing baseline values: the OTs were divided into Group I (laminar air diffuser) and Group II (conventional air diffuser). The OTs in both groups had more than 20 air changes per hour. The cardiovascular and thoracic surgery (four OTs), ophthalmology (five OTs) and paediatric

**Table 3.** Bacterial and fungal counts during the preoperative and intraoperative periods in Group I operating theatres

Variable	Preoperative period				Intraoperative period			
	Mean colony forming (CFU) units/m <sup>3</sup> , bacterial	SD	Mean colony forming units (CFU)/m <sup>3</sup> , fungal	SD	Mean colony forming (CFU) units/m <sup>3</sup> , bacterial	SD	Mean colony forming units (CFU)/m <sup>3</sup> , fungal	SD
Temperature (°C)								
16.1–20	38.80	27.95	0.30	0.67	79.44	52.56	0	0
20.1–24	38.25	31.29	0.15	0.36	89.46	43.10	0.23	0.48
24.1–28	95.67	49.63	0.54	0.52	120.77	51.99	0.86	1.16
P	<0.000		<0.03		<0.02		<0.00	
Humidity (%)								
30–39	80.00	27.40	0.33	0.57	39.00	56.44	0.00	
40–49	36.72	43.99	0.33	0.48	88.23	40.84	0.35	0.60
50–59	58.64	38.80	0.20	0.50	94.75	58.84	0.41	1.01
60–69	39.65	36.13	0.21	0.42	113.53	44.31	0.61	0.76
70–79	53.66	53.87	0.00	0.00	109.80	49.17	0.20	0.42
Number of persons in operating theatres								
1–5	48.46	40.51	0.24	0.46	65.4	46.17	0	0
6–10	24	22.62	0	0	99.38	50.15	0.46	0.84
>10					118	10.92	0	0
Number of persons with mask below nose								
0	34.94	26.16	0.17	0.42	87.50	41.62	0.17	0.37
1	67.92	45.07	0.31	0.48	103.15	59.51	0.56	1.08
2	110.6	50.92	0.6	0.54	111.62	38.84	0.61	0.76
P	<0.00		<0.05					
Door opening								
0–3	46.26	37.52	0.24	0.47	95.38	42.68	0.29	0.64
4–7	59.44	52.20	0.22	0.44	96.89	48.17	0.39	0.57
8–11	44.40	53.56	0.2	0.44	119.00	83.99	1.00	2.23
P							<0.01	
Door type								
Hermetic	44.92	27.63	0.19	0.49	86.23	46.55	0.15	0.36
Sliding	49.39	46.07	0.26	0.44	104.98	49.87	0.55	0.95

SD: standard deviation.

(four OTs) centre in Group I were receiving 25% fresh air and 75% return air. The emergency complex with seven OTs had two air handling units (AHUs) supplying 100% fresh air, whilst one AHU supplied 25% fresh air and 75% return air. Similarly, in the trauma OT, one AHU supplied 100% fresh air, whilst the remaining four supplied 25% fresh air and 75% return air. The highest bacterial counts during the preoperative period in Group I were seen in the paediatric centre OT complex, whilst the lowest were in the emergency OT complex. A possible reason could be that the paediatric OT complex was being maintained at a higher temperature (25.02 °C mean) for obvious reasons. On the other hand, the emergency OT HVAC systems had been recently renovated, and three out of the seven OTs also had 100% fresh air being supplied instead of 25% fresh air, which could be the reason for the lowest

bacterial counts during the preoperative period (5). During the intraoperative period, the count was again maximum in paediatric OT as expected, and lowest in ophthalmology OT units. The bioload during the intraoperative period should have been lowest in the emergency OT units as was the case during the preoperative period, but these OTs were being used by multiple departments and involved a lot of staff and patient movements. The ophthalmology OT deals with just one speciality, takes elective cases for a limited number of hours per day and has controlled entry of personnel.

In Group II, the main OT complex had the lowest CFU/m<sup>3</sup> during the intraoperative period. This OT complex with 18 OTs has 100% fresh air supply and a conventional diffuser. The fungal colony counts did not show much variation in Group II.

**Table 4.** Bacterial and fungal counts during the preoperative and intraoperative periods in Group II operating theatres

Variable	Preoperative period				Intraoperative period			
	Mean colony forming (CFU) units/m <sup>3</sup> , bacterial	SD	Mean colony forming units (CFU)/m <sup>3</sup> , fungal	SD	Mean colony forming (CFU) units/m <sup>3</sup> , bacterial	SD	Mean colony forming units (CFU)/m <sup>3</sup> , fungal	SD
Temperature (°C)								
16.1–20	15.50	10.60	0.0	0.00	71.50	28.99	0.00	0.00
20.1–24	48.59	28.36	0.34	0.71	79.37	45.06	0.44	1.02
24.1–28	70.33	33.22	0.58	0.99	86.57	26.20	2.29	0.48
P	<0.02							
Humidity (%)								
30–39	72.60	30.85	0.83	0.37				
40–49	51.71	39.85	0.49	0.13	76.90	54.43	0.30	0.73
50–59	56.00	30.08	0.33	11	72.66	21.19	0.00	0.00
60–69	46.61	23.07	0.70	0.16	82.85	27.51	61	1.24
70–79					84.50	52.31	0.16	0.40
P	<0.00							
Number of persons in operating theatres								
1–5	48.83	31.98	0.43	0.81	21		0.00	1.15
6–10	65.55	25.28	0.27	0.64	70.45	32.23	0.48	1.19
>10					103.56	50.35	0.25	0.44
P	0.01							
Number of persons with mask below nose								
0	44.18	31.86	0.23	0.42	74.00	29.21	0.25	0.86
1	59.73	34.17	0.73	1.10	68.29	40.31	0.64	1.49
2	63.56	17.95	0.22	0.66	86.75	53.26	0.44	0.51
3					81.20	34.09	0.20	0.44
4					121.67	17.95	0.00	0.00
P								
Door opening								
0–3	44.00	31.30	0.41	0.82	75.64	30.28	0.07	0.26
4–7	67.88	24.94	0.35	0.70	81.00	45.91	0.41	0.78
8–11					95.00	66.46	2.50	3.53
P	<0.01						<0.002	
Door type								
Sliding	52.83	31.09	0.39	0.77	80.06	42.06	0.40	0.94

SD: standard deviation.

In our study, bacterial count values exceeded the generally accepted values for conventional OTs during the preoperative period, but it was within accepted levels during the intraoperative period. Most reports suggest that an acceptable bacterial limit for a working OT is below 180 CFU/m<sup>3</sup> (7–10). Microbial monitoring in 29 conventionally ventilated OTs was performed, and it was found that bacterial contamination values in working OTs varied widely (8). The mean bacterial contamination was from 122 to 149.7 CFU/m<sup>3</sup>; however, 6–27% of the samples had more than 180 CFU/m<sup>3</sup> and maximum values of 798 CFU/m<sup>3</sup>. A report mentions that airborne bacterial counts range from 87 to 585 CFU/m<sup>3</sup> (11). Another study

recommends that for conventional OTs, the bioload should not exceed 35 CFU/m<sup>3</sup> in an empty theatre or 180 CFU/m<sup>3</sup> during an operation (12).

#### Environmental factors

Airborne microbial counts for bacterial and fungal contaminations were found in lower concentration when the temperature was maintained at 16–20°C and 20–24°C, respectively, as compared to temperatures above this range in both Group I and Group II HVAC systems, but the increase in counts with increased temperature was less in Group II, which had conventional air flow and 100% fresh air. It is clear that temperature influences viral, bacterial

and fungal particles, but it emerges that 100% fresh air has a diluting effect on concentration of microorganisms (13, 14). No significant correlation could be found with respect to the relative humidity. Our study was similar to a previous report that found no significant correlation between relative humidity and bacterial concentrations (15). Also, not much variation was seen in the fungal CFU/m<sup>3</sup>.

#### Behavioural factors

The number of persons in the OTs had appreciable impact on the bacterial and fungal counts during the preoperative period and during the surgery. It was found that bacterial counts were minimum in the morning before the start of OTs, with higher bacterial counts recorded as the ongoing activities and the number of personnel increased during the intraoperative period. These phenomena are well explained in studies that mention that the rate of infection is proportional to the duration of surgery and the number of personnel in the room, but inversely proportional to the air changes/hour due to its dilution effect and laminar flow (16, 17). Several reports noted that the number of staff and their activities also influence microorganism concentrations in the air of OTs (18–20). The microbial level in the OT air is directly proportional to the number of people moving about in the room (21). The number of persons and the number of persons with a mask below nose in the OTs during the surgery, along with door opening and closing, added to the increase in bacterial and fungal counts. The same has been corroborated in the literature (22).

#### Conclusion

It is concluded that CFU/m<sup>3</sup> values for bacterial and fungal counts are more than the conventionally accepted values for preoperative periods but fall within the accepted values for intraoperative period counts. The limitation of this study is that although Group I OTs have laminar air flow, they cannot be labelled specifically in the clean room category. However, for developing countries with an older infrastructure, these values of bacterial and fungal counts will serve as a baseline during the microbiological evaluation. The different values of colony counts noted in similar types of HVAC system with similar percentage of fresh air circulation during the preoperative and intraoperative periods – with very low counts on one end of the spectrum and very high counts on the other end – suggest that low air contamination can be achieved with concomitant control over the environmental and behavioural factors responsible for air contamination.

Therefore, it may be considered that 20–24°C temperature with a 45–55% humidity, hermetically sealing doors, percentage of fresh air, controlled entry of people entering the OT, minimal door opening and regular maintenance and cleaning of HVAC system are required to maintain the microbiologic air quality of OTs.

#### Conflict of interest and funding

The authors declare that there is no conflict of interest. The authors have not received any funding or benefits from industry or elsewhere to conduct this study.

#### Ethics approval

The protocol for this work has been approved by the ethics committee of Post Graduate Institute of Medical Education & Research, Chandigarh (letter no. INT/IEC/2015/781, dated December 11, 2015).

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