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## ORIGINAL ARTICLE

Comparison of two air sampling methods to monitor operating room air quality and assessment of air quality in two operating rooms with different ventilation systems in the national hospital of Sri Lanka

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## Abstract

Air contamination in the operating room (OR) is an important contributor for surgical site infections. Air quality should be assessed during microbiological commissioning of new ORs and as required thereafter. Despite many modern methods of sampling air, developing countries mostly depended on conventional methods.

This was studied in two ORs of the National Hospital of Sri Lanka (NHSL) with different ventilation system; a conventional ventilation (CV) and a laminar air flow (LAF). Both ORs were sampled simultaneously by two different methods, the settle plate and sampler, when empty and during use for a defined time period. Laboratory work was done in the Medical Research Institute.

The two methods of sampling showed moderate but highly significant correlation. The OR with CV was significantly more contaminated than LAF when empty as well as during use by both methods. Overall, the difference in contamination was more significant when sampled by the sampler. Differences in contamination in empty and in-use ORs were significant in both ORs, but significance is less in LAF rooms.

The consistent and significant correlation between settle plate and sampler showed that the settle plate is an acceptable method. As expected, the LAF theatre showed less contamination while empty and also during use. Air contamination differences were more significant when sampled with sampler indicating that it is a more sensitive method. Both CV and LAF ORs of the NHSL did not meet the contamination standards for empty theatres but met the standards for in-use indicating that the theatre etiquette was acceptable.

Keywords: air quality, indoor; ventilation; operating rooms; Sri Lanka

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## Introduction

Operating room (OR) air is an ever-present potential source of postoperative infections. In the late 18<sup>th</sup> century, the English surgeon Joseph Lister used carbolic spray to disinfect the OR air reducing the mortality rate from postoperative infection dramatically.<sup>1</sup> Air quality in ORs should be assessed during microbiological commissioning of new ORs and whenever required thereafter.<sup>2</sup> OR ventilations are very much variable from country to country, within a country and even within a hospital as per requirement and availability. Ventilation systems are classified as conventional plenum ventilation (CV), laminar flow ventilation (LAF), wall mounted air conditioners and free-standing air conditioners.<sup>3</sup>

Air contamination is expressed either as Bacteria Carrying Particles per cubic meter (BCP/m<sup>3</sup>) or Colony Forming Units per cubic meter (CFU/m<sup>3</sup>) of air measured with an air sampler. During surgery the ORs with CV were found to have between 50-100 CFU/m<sup>3</sup> if well maintained but can go up to 500 CFU/m.<sup>3,4,5</sup> Each person in the room disperses at least 10,000 CFU/min at rest and up to 50,000 CFU/min with activity.<sup>6</sup> It has been demonstrated that conventional cotton surgical gowns did little to reduce the dispersion rate but a disposable gown made of non-woven fabric would reduce the dispersion by 30% in the conventionally ventilated system and about 65% in the laminar flow system.<sup>7</sup> The number of airborne particles increases in a logarithmic progression with the entry of people and onset of activity in the rooms.8

For clean procedures, Whyte and colleagues (1982) stated that bacterial contamination of the wound in the OR is caused by bacteria from the patient in 2% and by bacteria in the air of the OR in 98%.<sup>5</sup> In the latter, 30% reaches the wound directly via the air and 70% reaches the wound via hands of the surgical personnel or through the instruments used.<sup>5</sup> Most of the contaminants are harmless saprophytes and commensals, and even when carriers or infected patients are present, usually less than 1%, and commonly only 0.01 – 1% of the airborne bacteria are pathogens.

The bacterial counts observed in the theatres varied from 50-500 CFU  $/m^3$  in CV to 2-20 CFU $/m^3$  in ultra

clean air system and one or fewer when the special suits were worn.<sup>9</sup> A well maintained CV theatre has about 400 – 500 CFU/m<sup>3</sup> during surgery.<sup>10</sup> The risk of a large number of CFUs coming in contact with the open wound increases with time. 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. There should be at least 20-30 air exchanges/ hour for recirculated air.<sup>11</sup>

For a CV theatre, the bioload should not exceed 10 bacterial and/or fungal CFU/m<sup>3</sup> while empty and unless there are unusually high number of personnel or extensive activity in the room, the number of airborne bacteria and/or fungal CFUs averaged over any five minutes period should not exceed 180/m<sup>3</sup> during use.<sup>12</sup> For an ultra-clean (LAF with HEPA filter) theatre, it should be less than 1 CFU/m<sup>3</sup> when empty and less than 10 CFU/m<sup>3</sup> during use at the centre of the OR and on average, air sampled within 300mm of the wound should not contain more than 10CFU/m<sup>3</sup>.<sup>12</sup>

Air sampling can be done by many methods ranging from simple to complex. The methods available at present are sedimentation (settle plate), impactors (slit samplers, sieve samplers, centrifugal samplers and impingement samplers) and gelatin membrane filtration.<sup>4</sup>

The National Hospital of Sri Lanka (NHSL) has different surgical specialties and orthopaedic unit is one of the major units. Of the many ORs in the orthopaedic unit, two ORs with different ventilation system were selected for the study. The OR with CV system was being used for routine surgery and the one with LAF was used for major surgeries like hip and knee surgeries. The ORs were used only for day surgery and thoroughly cleaned at the end of each session. The ORs remain closed at night with the ventilation systems running continuously. The ORs did not have any record of regular maintenances or monitoring of air exchange rates and the users were not aware if the ORs were properly commissioned before putting to use. The operators were not informed of the number of air exchanges for both systems and whether the ventilation systems were actually functioning as expected. Therefore, this study to compare two

sampling methods and evaluation of the air quality in the two ORs was very timely and rationale.

### Methodology

The study was carried out between January-March 2009. Air samples were collected from two ORs of orthopaedic unit of the NHSL; one with CV system and other with LAF. Air was sampled simultaneously by two methods; the traditional settle plate method and a commercial slit sampler. Sampling was done in the early morning before start of surgery (empty OR) and during surgery (in-use OR after about an hour of starting the session) every Tuesdays and Thursdays for eight weeks.

### Method for settle plate

This was done by following procedures described in Mackie and McCartney: Practical Medical Microbiology<sup>13</sup> with slight modification. Multiple blood agar plates were placed at different locations (the operating table, near the doors and the corners) in the OR to be sampled. The ideal recommendation is the 1, 1, 1 method where plates should be placed at different locations in the OR one meter away from the side walls, one meter above the floor and for a duration of one hour. Plates were placed on tables and stools about a meters height and 1 meter from the side walls but due to time constraints, they were exposed for 10 minutes only. After exposure, plates were immediately taken to the laboratory and incubated aerobically at 35-37°C for 48 hours. The visible colonies were counted, using hand lens when necessary. The mean number of CFUs of all plates at different areas was taken. The colonies were identified using basic microbiological tests.

In order to get the CFU values in CFU/m<sup>3</sup> for comparison with the commercial sampler, first the settling rate was calculated. For a 90mm plate (surface area  $\approx 63.6 \text{ cm}^2$ ) exposed for 10 minutes, the settling rate was calculated and expressed as CFU/m<sup>2</sup>/min.

### Method for commercial air sampler

A slit sampler from HiMedia Laboratories, India, was used for this as per the descriptions in the user's manual for 'HiAirflow 90'. The most probable number of microorganisms in the volume of air sampled was calculated from the 'Conversion Table HiAirflow 90' with the manual. This gave the probable number of CFU/m<sup>3</sup>.

### The relation between CFU/m<sup>2</sup>/min and CFU/m<sup>3</sup>

After getting the CFU/m<sup>2</sup>/min from the number of colonies with 10 minutes exposure, the CFU/m<sup>3</sup> of air was calculated using the formula of Parker.<sup>13</sup> Parker stated that 'the number of particles settling on 1m<sup>2</sup>/ min is equal to the number of such particles in 0.3m<sup>3</sup> of air'. The conversion of CFU/m<sup>2</sup>/min for the settle plate into CFU/m<sup>3</sup> using this formula makes it directly comparable to the commercial sampler values in CFU/m<sup>3</sup>.

### **Quality Control**

All the blood agar plates were pre-incubated overnight at 37°C to exclude any contamination during media preparation. Those plates that grew organisms in the pre-incubation were not used for the sampling.

#### Data analysis

Data was analyzed using SPSS version 17. Correlation between two air quality measurement methods was evaluated using Person's correlation coefficient. Student's t-test was used for comparison of air quality in different ORs. Paired t-test was used for the comparison of empty and in-use ORs.

## Ethical clearance

Ethical clearance was obtained from the ethical boards of Medical Research Institute (MRI), Colombo for laboratory work in the Department of Bacteriology and the National Hospital of Sri Lanka (NHSL), Colombo for sampling from the two ORs.

### Results

For the eight weeks duration of the study, there were 16 sampling days, 32 sampling sessions each in the CV and LAF rooms while empty and in-use. With samples taken by both methods simultaneously at all sessions, there was a total of 64 sampling sessions both in CV and LAF. The summary of all observations for CFU on a 90mm plate and the CFU/m<sup>3</sup> in the CV and LAF ORs is shown in Table I.

# Comparison of two air sampling methods; traditional settle plate method and a commercial sampler

CFUs observed in the settle plate and the commercial sampler showed a positive correlation between the two methods (Figure 1). For the number of CFUs on a 90mm plate, a statistically significant (p < 0.001) moderate correlation with an 'r' value of 0.606 was observed.

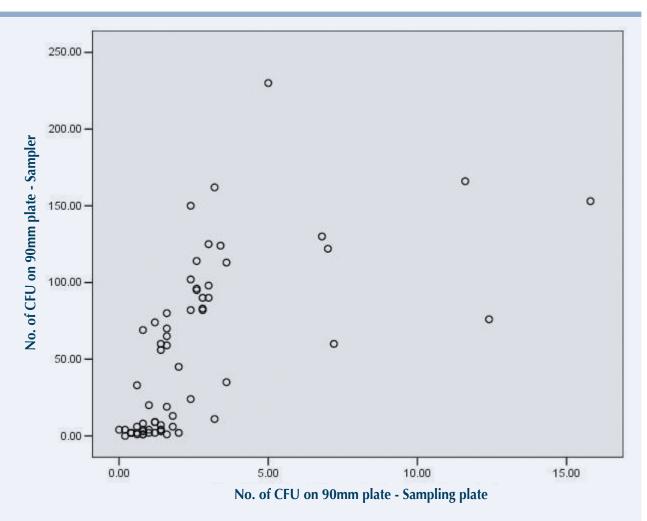


Figure 1. Scatter plot of colony forming units (CFU) in 90mm plates in sampler and settle plates

For the CFU/m<sup>3</sup>, the actual contamination per volume of air, the two methods also had a moderate (r = 0.592) and significant (p <0.001) positive correlation. Since the correlation was high, sub analysis was done to see the correlation in different cut off points; ORs and time of measurements, but it did not show significant differences.

# Assessment of air quality in the two ORs of the NHSL and measure of contamination by two methods in both the ORs

# Quality of air in the two ORs of the orthopaedic unit, NHSL

The air contamination level of the two ORs while empty and during use was compared with the standard guidelines. With a contamination level of 79 CFU/ m<sup>3</sup> and 4.38 CFU/m<sup>3</sup> respectively, both the CV and LAF theatres did not meet the international standard requirement of empty theatres (Table I). However, both the ORs met the standards for in-use ORs with the contamination levels of 157.94 CFU/m<sup>3</sup> and 19.19 CFU/m<sup>3</sup> for CV and LAF respectively (Table I).

# The difference of air contamination in the OR with CV and LAF

Air contamination observed by settle plate while empty in the two ORs appears to be different with mean CFU/plate of 2.650 and 0.825 in the CV and LAF respectively (Table II). This difference was statistically significant (p= 0.014). Contamination as measured by settle plate while the OR was in-use also showed similar results, with a p value of 0.001.

Similar significant differences in contamination were observed with the commercial sampler while empty and in-use, with p values < 0.001 in both situations.

Overall, the significance was higher when the sampling was done by the commercial air sampler than settle plate.

# Table I. Mean CFU on 90mm plates and CFU/m<sup>3</sup> for settle plate-sampler in empty and in-use operating rooms of National Hospital, Sri Lanka

		Mean CFU/ CFU per m <sup>3</sup>		
	Method	CV	LAF	
	No. of CFU on 90mm plate- Sampler	68.44	2.50	
Empty OR	No. of CFU on 90mm plate- Settle plate	2.65	0.83	
	Probable no. of CFU/m <sup>3</sup> of air- Sampler	79.00	4.38	
	Probable no. of CFU/m <sup>3</sup> of air- Settle plate	141.82	43.00	
	No. of CFU on 90mm plate- Sampler	123.56	16.06	
In-use OR	No. of CFU on 90mm plate- Settle plate	5.09	1.43	
	Probable no. of CFU/m <sup>3</sup> of air- Sampler	157.94	19.19	
	Probable no. of CFU/m <sup>3</sup> of air- Settle plate	265.09	76.21	

# Comparison of air contamination in the two ORs while empty and in-use as measured by the commercial sampler

For this the paired t-test was used to test for significance. Only the result of the commercial sampler was used for this purpose since it was found to be more sensitive from findings above. As shown in Table III, air contamination was very high in the CV theatre when in-use compared to empty OR and this difference was highly significant (p < 0.001). Similarly in the LAF theatre, the contamination difference in empty and in-use OR was significantly high (p=0.027) but not as high as that in the CV.

# Table II. Differences in air contamination in two ORs while empty and in-use as measured by the two methods simultaneously

Sampling method	Operating room	Mean No. of CFU/plate	Std. deviation	Std. Error Mean	
	CV (empty)	2.650	2.7271	0.6818	t=2.621 df =30
Cottle plate	LAF (empty)	0.825	0.5651	0.1413	p = 0.014
Settle plate -	CV (in-use)	5.088	3.8623	0.9656	t= 3.726 df = 30
	LAF (in-use)	1.425	0.7335	0.1834	p = 0.001
	CV (empty)	68.44	25.266	6.317	t=10.41 df = 30
Commercial	LAF (empty)	2.50	1.549	.387	p = <0.001
Sampler	CV (in-use)	123.56	42.593	10.648	t= 8.941 df = 30
	LAF (in-use)	16.06	22.338	5.585	p = <0.001

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Operating room	Mean CFU/plate	Std. Error of Mean	Std. Deviation	Paired samples test (paired t test)
CV (empty)	68.44	6.32	25.27	T= -5.284
CV (in-use)	123.56	10.65	42.59	Df=15
				Sig. (2 tailed) = 0.000
LAF (empty)	2.50	.39	1.55	T= -2.452
LAF (in-use)	16.06	5.58	22.34	Df=15
				Sig. (2 tailed) =0.027

# Table III. Comparison of air contamination in the two ORs while empty and in-use as measured by commercial sampler

# Differences in contamination levels at different areas of the ORs

The differences in level of air contamination at different areas of the OR in both CV and LAF ORs were assessed. In the CV OR the average CFU were 0.64, 1.48 and 2.09 at the operating table, near the door and at the corners respectively. In the LAF room, the average CFU were 3.03, 3.17 and 4.76 respectively.

Both differences observed between areas and between ORs were significant. Since these were significant, Post hoc test (Tukey HSD) was conducted to assess the differences between areas and ORs. CFU in CV theatre was significantly high in all three areas considered (p <0.001). CFU in corner was significantly higher than the operating table (P <0.05). Though the number of colonies in the door area was apparently less than that observed in the corners and that of operating table less than the door, observed difference between corner and door and table and door was not statistically significant. In both CV and LAF rooms, the CFU was gradually increasing from operating table to corner.

CV room air contamination in each area was more than twice compared to LAF room. Differences between rooms were more obvious during use of the ORs.

Between areas and between room differences were significant in two-way ANOVA. Since these were significant, Post hoc test (Tukey HSD) was conducted to assess the differences between places and theatres. CFU in LAF was significantly high in all three places considered (p <0.001). CFU in the corner was significantly higher than the operating table (P <0.05). Here too, though the number of colonies in the door area was less than that observed in the corners and that of table less than the door, observed difference between corner and door and table and door was not statistically significant (p >0.05).

# Common isolates from the ORs during the study

In this study, the common isolates identified were Coagulase negative staphylococci, *Micrococcus* species, aerobic spore bearers, *Acinetobacter* species, coliforms, *Staphylococcus aureus*, *Candida*/yeasts and *Aspergillus* species in decreasing order.

# Discussion

The fact that both the ORs did not have any record of commissioning, regular maintenances, and monitoring of air exchange rates was unacceptable as per practice guidelines but such occurrences are common in developing countries. However, the overall finding that the LAF theatre had better air quality than the CV theatre during empty and during operation as tested by both methods gave some assurance that the ventilation systems in both the ORs were functioning. Henceforth, it should be mandated that an OR should firstly meet all engineering standards and then be microbiologically commissioned to ensure it meets the standards for the specific ventilation system installed.

The two air sampling methods showed moderate correlation by Pearson's correlation test and the correlation, though moderate, was highly significant. This shows that the settle plate method can be a good substitute to the commercial samplers especially in developing countries. Although a significant correlation was seen in the two sampling methods, the commercial sampler gave more consistent results at every sampling session. The settle plate method had some outlier readings and occasionally gave inconsistent results. Therefore, while using the settle plate method, it would be more reliable to repeat for few sessions with multiple agar plates and taking the mean instead of depending on single reading with fewer plates.

In the two orthopaedic theatres of the NHSL, both CV and LAF rooms did not meet the recommended contamination standards for empty theatres. However, both the theatres met the in-use standard despite unrestricted access to all categories of OR staff and trainees. This may indicate that the OR attire and theatre etiquette was acceptable among the OR staff.

The differences in contamination in empty and in-use ORs were highly significant as measured by both the methods. Contamination differences in empty and inuse ORs were less significant in the LAF room which indicates that LAF rooms get less contaminated during use.

The differences in contaminations were highly significant when the sampling was done by the commercial sampler in empty and in-use rooms for both the theatres. This indicates that the slit sampler is a more sensitive method than the settle plate method in detecting the differences in microbial contamination of air and should be preferred where available.

Among the different areas of the OR, the corner of the rooms seemed to be the most contaminated. This justifies recommending not to keep sterile articles closest to the walls of the rooms but as near as possible to the centre of the ORs. In addition, sterile items should be opened only at the time of use to minimize the time of exposure to the potentially contaminated OR air.

### Limitations

Since there was very little time before they opened the OR and started the surgery it was really a challenge to sample the empty OR in the morning. This gave very limited time thereby forcing empty OR sampling only for 10 minutes which was acceptable but not the ideal 1, 1, 1 method. In addition the engineering specifications of the ORs were not known and the functionality of the ventilations systems has not been monitored.

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