

Original Research Article

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Evaluation of Microbial Contamination using various Air Sampling Techniques in Operation Theatres

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ABSTRACT

Microbial contamination of the Operation theatres (OT's) continues to increase the prevalence of nosocomial infections. Our study evaluates the microbial contamination using various air sampling techniques in OT's and find a suitable technique to assess air quality. A total 151 air samples were collected from various OT's of our tertiary care centre. 76 samples were collected by an active method using an air slit sampler and 75 samples were collected by passive settle plate method using 5% Sheep blood agar in 9cm petri-plates. The mean microbial count of the both active and passive sampling method in OT's was 16.439 colony forming units (CFU)/m³ and 13.480 CFU/m³ respectively. In most OT's CFU/m³ were in normal limits. The labor OT at rest, exceeded the maximum limit value of active method <35 CFU/m³ and of the passive method <5CFU/9cm/h. The Oncology OT also exceeded the maximum limit value of the active method <180CFU/m³ and passive method <25 CFU/9cm/h. The most common isolate in both methods was *Coagulase negative Staphylococcus* (CoNS). The present study demonstrates that the active sampling method is better than the passive method to judge the microbial air quality in our tertiary care centre and the most common isolate was CoNS.

Keywords

Air sampler,
Operation theatres,
Air contamination,
mean microbial
count

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Introduction

The OT complex is the heart of any major surgical hospital. A good operating theatre design meets the functional needs of a theatre and must pay careful consideration to traffic patterns, the number and configuration of nearby operating rooms, the space required for staff, administration and storage, provisions

for sterile processing and systems to control air borne contaminants (Sammy A.Benna *et al.*, 2012). Modern operating theatres have a conventional plenum ventilation with filtered air where particles of 5 mm in size are removed. Few countries have set bacterial threshold limits for conventionally ventilated operating rooms, although most recommend 20 air changes per hour to obtain 50±150

colony forming units/m³ of air. There are no standardized methods for bacterial air sampling or its frequency.

In today's operating environment, more than half of clean surgical-site infection pathogens originate from normal skin flora of patients or staff. 1-3 bacteria on skin squames, lint and other dust get into the air in the operating theatre and by turbulent air currents that deposit on surfaces. To reduce prolonged morbidity and healthcare costs associated with these infections, airborne bacteria and other sources of contamination must be reduced to a minimum by the choice of staff theatre dress, patient preparation and draping, education of personnel and design and controlled ventilation by using High Efficiency Particulate Air (HEPA) in operating theatres (S Dharan *et al.*, 2002). The dose of contaminating micro-organisms required to produce infection is very low when foreign material is present at the site. As few as 10 CFU are estimated to be the safe bacterial content per cubic meter of air in an operating theatre where prosthetic replacement arthroplasties are performed (MLV French *et al.*, 1980; L Arrowsmith *et al.*, 1985; C.Napoli *et al.*, 2012).

Many developing countries have reported high post operative infection rate in their hospitals, i.e., 15% from Brazil 11% from Mumbai, 21.66% from Karnataka, 5% in USA and Europe despite having a well maintained condition and controlled ventilation system (CCVS) (MA Melhado *et al.*, 2006). A study by Rumpa Saha *et al.* found the presence of Methicillin sensitive *Staphylococcus aureus* (MSSA), Methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and fungi in theatre environment (Rumpa Saha *et al.*, 2017).

K Singh suggested first cleaning by using surface disinfectants and periodic fumigation

of the OT's as a preventive measure (K Singh *et al.*, 2013). J Jawaed on the contrary found total bacteriological count in air was high but surfaces showed less bacterial counts (J Jawaed *et al.*, 2008). C Pasquarella *et al.* also assessed microbial air contamination by active and passive sampling methods and concluded beyond doubt that microbiological monitoring is a useful tool for assessment of the contamination of operating theatres in order to improve air quality (C Pasquarella *et al.*, 2012).

Materials and Methods

Air sampling can be done by many methods ranging from simple to complex (ISO 14644-1 1999; The United States Pharmacopeia Chapter 2011). The present prospective analytical study was conducted from January to December 2018 after ethical clearance in various OT's of a tertiary care hospital. The culture plates were incubated and analyzed in the department of Microbiology. The sample size (Sammy A.Benna *et al.*, 2012) was 151 calculated by sample size formula... $N = Z^2 (1 - \alpha/2) \times p(1-p) / \alpha^2$ Where, $Z = 1.96$, estimated proportional (p) = 0.11, estimation error (α) = 0.05. All air samples collected from the operation theatres by both active and passive methods were included in the study. Air samples from all ICU's, post operative wards and other wards were not included.

(ISO 14644-1 1999; The United States Pharmacopeia Chapter 2011; EU GMP Guideline:1997; W.Whyte, *et al.*, 2007; A. A Anderson *et al.*, 1958).

Active method

A single sample was collected from each OT by both methods simultaneously. The air sampler "Single stage slit type air sampler" called Hi Air Flow LA 474, from Hi media Laboratories Pvt Ltd, India was checked for

cleanness before use by following the manufacturer's instructions. The theatre to be sampled was left vacant and doors shut for a minimum of 15 minutes, to 1 hour. A sterile 5% sheep blood agar plate was placed after pre-sterility testing by overnight incubation at 37°C, into the air sampler under aseptic precautions. The air sampler equipment was placed in the middle of the theatre table at the height of 2-5 feet and secured on a trolley. It was then switched on manually before leaving the room. The sampling equipment determines the volume of air sample. Sampling volume needs to be more than 250 L and optimally around 1600 L. Once sampling was completed, the agar plate was removed aseptically, labeled and sent to the microbiology laboratory for incubation and subsequent processing.

Passive method

Simultaneously settle plates which are standard petri-dishes of 9cm diameter containing culture media were placed on tables or a stool about a meters height and a meter from the side walls and left open for 10 minutes, each plate covering all the 4 corners and centre both at rest and during activity. After exposure, the plates are covered with their lids. Then sent to the microbiology laboratory and incubated at 37°C for 18-24 hours. After incubation, the total number of CFU was noted manually and results were expressed in CFU (Colony Forming Units) / Plate / time or in CFU / meter square / hour (T Tshokey *et al.*, 2016).

The above two methods were done in the OT both at rest and also during movement of OT personnel like operating surgeons, assistants and nurses. In order to get the CFU values in CFU/m³ for comparison with the commercial sampler, first the settling rate was calculated. For a 90mm plate (surface area ≈ 63.6 cm²) exposed for 10 minutes, the settling rate was

calculated and expressed as CFU/m² /min (T Tshokey *et al.*, 2016).

To correlate the CFU's present on the agar plate to the most probable number (MPN) of microorganism / m³ of air sampled using the Air Sampler the following formula was used as per manufacturers instructions (LA474 Air slit sampler, Hi-media Kit insert)

$$Pr = N [1/N + 1/(N-1) + 1/(N-2) + \dots + 1/(N-r+1)]$$

Where, Pr – Most probable number of microorganisms in the volume of air sampled
N – Number of holes on microflow sampling head

R – Number of CFUs on the agar plates after incubation.

In active method

The sampled volume number: 1600L [1.6m³]

Number of colonies formed are:126

The calculated corrected number of colonies [using feller table]: 152

Most probable number of colonies in air sample= Pr/N
= 152/1.6
= 95 CFU/m³

In passive method

Number of colonies formed are : 30 CFU/m²/min..

The relation between CFU/m²/min and CFU/m³ will be analysed after getting the CFU/m²/min from CFU/m³ After getting the CFU/m²/min from the number of colonies with 10 minutes exposure, the CFU/m³ of air was calculated using the formula of Parker.

Parker stated that 'the number of particles settling on $1\text{m}^2/\text{min}$ is equal to the number of such particles in 0.3m^3 of air. The conversion of $\text{CFU}/\text{m}^2/\text{min}$ for the settle plate into CFU/m^3 using this formula makes it directly comparable to the commercial sampler values in CFU/m^3 .

$$1\text{m}^2/\text{min} = 0.3\text{m}^3\text{ of air}$$

$$\begin{aligned}\text{Most probable number of colonies in air sample} &= 30 \times 0.3 \\ &= 9\text{ CFU}/\text{m}^3\end{aligned}$$

Identification of the different types of the colonies in the OT air sampling plate

(Mackie and Mc Cartney Practical Medical Microbiology. 2008)

The smears from the colonies (Bacterial / Fungal) that grew on the culture plate were identified by standard microbiological techniques. Isolates that were Gram positive oval yeast like budding cells were speciated by the germ tube test and the use of Chrome agar and cleanliness class interpretations were done as depicted in Table:1.

Results and Discussion

A total of 151 air samples was collected from the Main OT Complex, Cardiac OT, Oncology, Ophthalmology & Labor OT of the hospital. 76 samples were collected by active method of which 48 samples were taken at rest and 28 samples were taken during activity. 75 samples are collected by passive method of which 60 samples were taken at rest and 15 samples were taken during activity. In active method 50% samples showed growth and 50% showed no growth. In the passive method 33.3% samples showed growth and 66.7% no growth was seen as shown in Table 2 & Fig:1.

There was a statically significant correlation

between the results obtained by the active and passive air sampling methods based on the organism present and absent. The mean value of at rest and in activity by both methods and organisms isolated are depicted in Table 3,4 & 5

The mean microbial count of the both active and passive sampling method in OT's was $16.439\text{ CFU}/\text{m}^3$ and $13.480\text{ CFU}/\text{m}^3$ respectively. In most of our OT's the CFU/m^3 were within normal limits. The labor OT at rest, exceeded the maximum limit value of active method [$<35\text{ CFU}/\text{m}^3$] and of the passive method [$<5\text{CFU}/9\text{cm}/\text{h}$]. The Oncology OT also exceeded the maximum limit value of the active method [$<180\text{CFU}/\text{m}^3$] and passive method [$<25\text{ CFU}/9\text{cm}/\text{h}$]. Both the above reference values are suggested by the UK Health Department Memorandum (S Dharan *et al.*, 2002).

The most common isolate in both the air sampling methods was *Coagulase negative Staphylococcus* (CoNS) and all the Gram positive and Gram negative isolates were considered potential pathogens for surgical site infections. On the whole the significance was higher when the sampling was done by the commercial air sampler than by the settle plate method.

The currently available methods in air sampling are sedimentation (settle plate), impactors (slit samplers, sieve samplers, centrifugal samplers and impingement samplers) and gelatin membrane filtration (MLV French *et al.*, 1980; L Arrowsmith *et al.*, 1985).

Settle plates allow biological particles to "sediment" on it, but Impactors on the other hand draws air into the sampler which then directs the air stream at the collection agar.⁴ The most innovative air sampling technology is the gelatin membrane filtration method

where air is sampled at a programmable flow rate and passes through a gelatin membrane filter, which captures the microbes. This is the only sampling method with an absolute retention rate and the only method that also reliably captures airborne viruses (L Arrowsmith *et al.*, 1985).

When selecting an air sampling method, it is important to consider the benefits and problems connected with each method and to determine which sampler is best suited for specific needs and requirements (L Arrowsmith *et al.*, 1985).

Data indicates that prevention of postoperative infection is dependent on several factors including effective theatre design, sterilisation and disinfection procedures, good surgical techniques, bacterial contamination of theatre air, discipline which includes restricting the movement in OT's (C.Napoli *et al.*, 2012).

Currently, the lack of set guidelines for air sampling makes evaluation of results from different studies complicated. Regular monitoring of the OT environment having an adequate controlled ventilation system of HEPA filters will go a long way in ensuring clean air in the OT (I.Oren *et al.*, 2001) The same is true even for fungal air contamination (WJ Sayer *et al.*, 1972)

The European Union's Guidelines to good manufacturing practice, classify the different environments based on airborne practice and microbial contamination measured by active and passive sampling. It can be seen that many empty theatres fall in to Class B [10CFU/m³, 5CFU / 9cm / 4 hr =1.25 CFU/h], More than half of the OT's showed class C bacterial contamination [100 CFU/m³, 50 CFU/9cm/4 hr = 12 CFU/h] (C Pasquarella *et al.*, 2012). In the present study majority of our OT's showed Class B bacterial contamination by both methods except the Oncology OT and

Labor OT which showed class C bacterial contamination in active method and Class D [100CFU/ CFU/9cm/4 hr = 25 CFU/h] bacterial contamination in passive method. There is a statically significant correlation between the results obtained by the two sampling methods. Such correlation was not always apparent, showing the several other factors can affect their relationships such as particle size or ventilation parameters. It should also be considered that active sampling methods often work in a very narrow spatial and temporal range compared with the longer exposure times of settle plates.

Our present study revealed the presence of a number of organism. *Coagulase negative Staphylococcus* (CoNS) being more prevalent than *Methicillin resistant Staphylococcus* (MRCoNS) at rest. *Klebsiella pneumoniae* & *Acinetobacter baumannii* were the only two Gram negative organisms and fungi by sampling at rest of the Main OT complex. A study by Napoli from Italy documented that both techniques can be used for the bioburden surveillance but passive sampling is best for quantitative evaluation of hospital airborne bacteria (C.Napoli *et al.*, 2012).

Our study found that settle plates were less sensitive for the collection of fungi than active samplers. The sensitivity of the settle plate could be improved by increasing the exposure time, as suggested by the European Union guidelines to Good manufacturing Practice where threshold values are given for 9cm diameter settle exposed for 4 hours (C Pasquarella *et al.*, 2012).

T. Shokey's study also showed that the differences in contaminations were highly significant when the sampling was done by the commercial sampler in empty or in-use operation theatre. The settle plate method had some outlier readings and occasionally gave inconsistent results.

Table.1 Interpretation- Clean Room Classification according to cleanliness class (JIS B 9920-1998) (ISO 14644-1 1999)

Class 5 & 6	Special operating room(Transplant theatres, Cardio thoracic theatres),Special treatment room (burns and leukemia treatment rooms)	0-10 CFU/m³
Class 6 & 7	Recovery room (ICU,CCU,NICU)	10-100 CFU/m ³
Class 7 & 8	Regular operating rooms, all laboratories, Pharmaceutical rooms, rooms for infectious diseases	100- 200 CFU/m ³

Table.2 Comparative results of active and passive air sampling in OT's

		Method		
		Active	Passive	Total
Organism	Absent	38	50	88
		50.0%	66.7%	58.3%
	Present	38	25	63
		50.0%	33.3%	41.7%
Total		76	75	151
		100.0%	100.0%	100.0%

Chi square test: $X^2=4.312$ p=.038, significant

Table.3 The mean value of both air sampling methods

METHOD	N	Mean[CFU/m ³]	Standard Deviation
Active	38	16.439	20.35
Passive	25	13.480	18.07

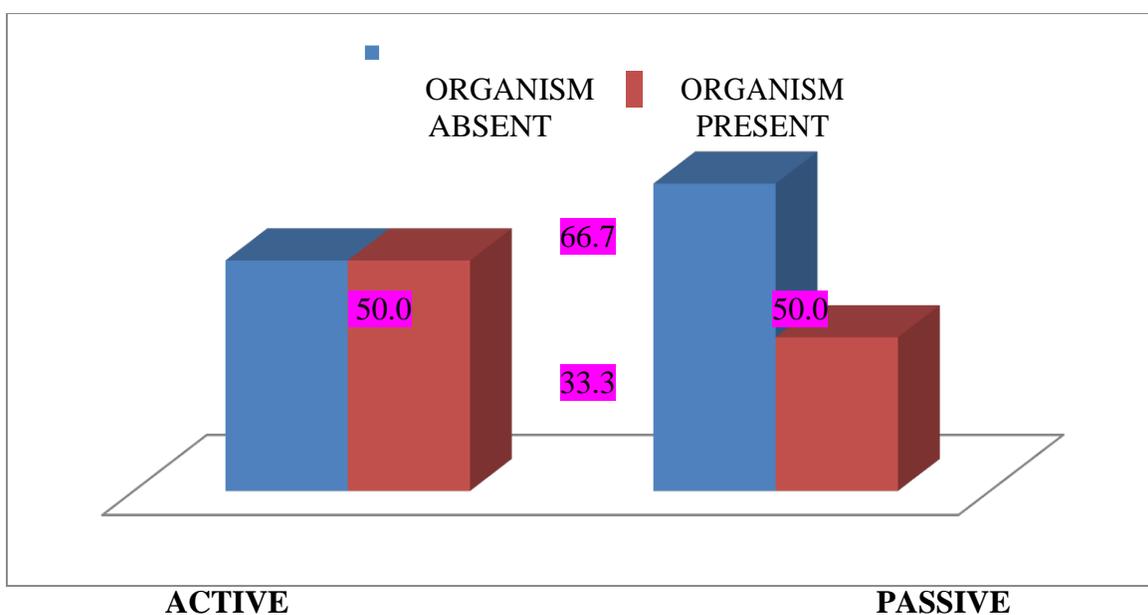
Table.4 The mean value at rest and in activity by both air sampling methods

		Method	Mean value CFU/m ³
A	rest	1. Active method	18.20
		2. Passive method	10.00
I	activity	1. Active method	16.32
		2. Passive method	19.80

Table.5 Organisms isolated by the active & passive sampling method

Identified organisms	Active method	%	Passive method	%
	Sample size		Sample size	
<i>Methicillin resistant Coagulase negative Staphylococcus</i>	4	5.3 %	5	6.7%
<i>Coagulase negative Staphylococcus</i>	23	30.3%	15	20%
<i>Micrococcus</i>	6	7.9%	1	1.3%
<i>Klebsiella pneumoniae</i>	1	1.3%	2	2.7%
<i>Acinetobacter baumannii</i>	3	3.9%	0	0%
<i>Candida albicans</i>	2	2.6%	2	1.3%
<i>Candida krusei</i>	2	2.6%	2	1.3%

Fig.1 Percentage of growth by both methods of air sampling



Thus, while using the settle plate method, it would be more reliable to repeat for few sessions with multiple agar plates and take a mean reading (T Tshokey *et al.*, 2016).

It is clear from our study that despite varying opinions about both active and passive methods of sampling by various researchers, the slit sampler is at an advantage being a more sensitive method than the settle plate

method and thus is a useful tool for any healthcare facility.

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