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Microbial air monitoring in operating theatres: experience at the University Hospital of Parma

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SUMMARY

Background: Microbial air monitoring in operating theatres has been a subject of interest and debate. No generally accepted sampling methods and threshold values are available.
Aim: To assess microbial air contamination in empty and working conventionally ventilated operating theatres over a three-year period at the University Hospital of Parma, Italy.
Methods: Air sampling was performed in 29 operating theatres. Both active and passive sampling methods were used to assess bacterial and fungal contamination.
Findings: In empty theatres, median bacterial values of 12 colony-forming units (cfu)/m^3 [interquartile range (IQR) 4–32] and 1 index of microbial air contamination (IMA) (IQR 0–3) were recorded. In working theatres, these values increased significantly ($P < 0.001$) to 80 cfu/m^3 (IQR 42–176) and 7 IMA (IQR 4–13). Maximum recorded values were 166 cfu/m^3 and 8 IMA for empty theatres, and 798 cfu/m^3 and 42 IMA for working theatres. Combining active and passive samplings, fungi were isolated in 39.13% of samples collected in empty theatres and 56.95% of samples collected in working theatres. Over the three-year study period, bacterial contamination decreased in both empty and working theatres, and the percentage of samples devoid of fungi increased. In working theatres, a significant correlation was found between the bacterial contamination values assessed using passive and active sampling methods ($P < 0.001$).
Conclusion: Microbiological monitoring is a useful tool for assessment of the contamination of operating theatres in order to improve air quality.

Introduction

Surgical site infection (SSI) is a major complication following surgery and is associated with increased morbidity and mortality, as well as increased costs.[1–4] Over the past decades, the role of air as a vehicle of infection has been the subject of much interest and debate.[3,5–13] Following a study by the Medical Research Council showing a correlation between microbial air contamination and SSI incidence in prosthetic joint surgery,[11] ultraclean operating theatres have been recommended for this type of surgery, while conventional theatres supplied by turbulent airflow systems are recommended for other types of surgery.[3,7] Guidelines for the design and ventilation of operating theatres have been published, and threshold values have been proposed for both ultraclean and

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conventional theatres.[14–20] However, there is no international consensus on tolerable limits of microbial air contamination, and there are no generally accepted methods and frequencies for air sampling. Moreover, the usefulness of microbiological air monitoring methods is controversial.[21–46]

A monitoring programme for operating theatres based on microbiological air sampling has been implemented at the University Hospital of Parma, involving both empty theatres (during commissioning and after major renovations) and working theatres. The main objectives of this study were: (1) to assess the microbial air contamination in empty and working operating theatres; (2) to propose local threshold limits for microbial air contamination in operating theatres; and (3) to assess any correlation between active and passive sampling methods.

Methods

Setting

Microbial monitoring was performed in 29 conventionally ventilated operating theatres, situated in nine different operating suites. In all theatres, air was supplied by a ventilation system designed to provide 15 air changes per hour. The system was equipped with high-efficiency particulate air filters, which can remove particles of >0.3 μm with an efficiency of 99.97%. Air pressure in the operating theatres was 5 Pa higher than in adjacent rooms.

Air sampling

Microbial air sampling was performed over a three year period (2008–2010) in the patient area of empty theatres during commissioning and after major renovations, and periodically during surgical activity. In addition, air samples were collected during surgery in the corridor adjacent to each operating theatre. Samples were collected using both active and passive sampling methods. Active sampling was performed using a DUOSAS 360 sampler (Pbi International, Milan Italy), with 55-mm diameter RODAC plates, a flow rate of 180 L/min, and suction volume set to 500 L in working theatres and 1000 L in empty theatres. Results were expressed as colony-forming units (cfu)/m^3. Settle plates (9 cm in diameter) were left open to the air according to the 1/1/1 scheme (for 1 h, 1 m from the floor, about 1 m from any obstacles) to determine the index of microbial air contamination (IMA).[44] Tryptic soy agar was used for total aerobic bacterial count, and Sabouraud dextrose agar with chloramphenicol was used for fungal isolation. Incubation parameters were 36 ± 1 °C for 48 h and 22 ± 1 °C for 120 h, respectively.

In order to propose a local standard for monitoring microbial air contamination in operating theatres, the median cfu/m^3 and IMA values were chosen as the target values and the 75th percentiles were chosen as the alert values. In the case of bacterial contamination in working theatres, the standard error of the mean was also considered.

Statistical analysis

Statistical Package for the Social Sciences Version 18 (SPSS Inc., Chicago, IL, USA) was used for statistical evaluation. Descriptive statistical analysis was performed to obtain mean, standard deviation, standard error of the mean, median and percentiles. The Mann–Whitney test was used to evaluate differences in microbial contamination between empty and working theatres, and differences in microbial contamination between working theatres and adjacent corridors. Differences between the results recorded over the three-year study period (2008–2010) were evaluated using analysis of variance. Chi-squared test for trend was used to assess the percentage of samples showing positive fungal isolation. The correlation between active and passive samples was investigated using Spearman's non-parametric test. *P*-values of ≤ 0.05 were considered to indicate significance.

Results

Table I shows the descriptive data, expressed in cfu/m^3 and IMA, obtained in all empty and working theatres for both bacteria and fungi. In empty theatres, median bacterial values of 12 cfu/m^3 and 1 IMA were recorded, while these values increased significantly ($P < 0.001$) in working theatres to 80 cfu/m^3 and 7 IMA. Values for bacterial contamination varied widely, reaching 166 cfu/m^3 and 8 IMA in empty theatres, and 798 cfu/m^3 and 42 IMA in working theatres. Bacterial contamination decreased over the three-year period, although this was only significant for IMA values for working theatres ($P = 0.031$) (Table II).

Combining active and/or passive samples, fungi were isolated in 39.13% of samples from empty theatres and 56.95% of

Table I
Total bacterial count (BC) and fungal count (FC) assessed by active [colony-forming units (cfu)/m^3] and passive [index of microbial air contamination (IMA)] sampling in empty and working operating theatres (OTs)

	cfu/m^3				IMA			
	Empty OTs		Working OTs		Empty OTs		Working OTs	
	BC ($N = 40$)	FC ($N = 39$)	BC ($N = 149$)	FC ($N = 151$)	BC ($N = 45$)	FC ($N = 45$)	BC ($N = 147$)	FC ($N = 147$)
Mean	26.9	6	140.14	5.09	1.64	0.07	9.45	0.27
Standard deviation	39.97	17.47	163.26	11.21	2.18	0.25	7.75	1.15
Minimum	0	0	0	0	0	0	0	0
25th percentile	4	0	42	0	0	0	4	0
Median	12	0	80	2	1	0	7	0
75th percentile	32	2	176	4	3	0	13	0
Maximum	166	78	798	72	8	1	42	13

Descriptive statistics refer to all observations.

Table II
Yearly variation in mean bacterial contamination in empty and working operating theatres (OTs). Values measured by active [colony-forming units (cfu)/m^3] and passive [index of microbial air contamination (IMA)] sampling

			Active sampling (cfu/m^3)			Passive sampling (IMA)		
			N	Mean	*P*	*N*	Mean	*P*
Empty OTs	Year	2008	17	40.47	0.121	21	2.10	0.439
		2009	14	22.86		15	1.27	
		2010	9	7.56		9	1.22	
Working OTs	Year	2008	42	149.67	0.649	40	10.03	0.031
		2009	59	148.08		59	10.97	
		2010	57	122.04		48	7.10	

samples from working theatres. For empty theatres, the active sampler alone was able to detect the presence of fungi in 36.84% of samplings; in 7.89% of samplings fungi were also recorded by the settle plates. For working theatres, the active sampler alone detected fungi in 40.81% of samplings; in 2.72% of cases, fungal contamination was only detected by the settle plates. In 13.60% of cases, both types of sampling were able to detect fungal contamination. Median fungal contamination values for empty theatres were 0 cfu/m^3 and 0 IMA. No significant increase was observed in working theatres compared with empty theatres. Maximum values were 72 cfu/m^3 and 13 IMA. A decrease in the percentage of samples showing fungal contamination was observed over the three-year period in both empty and working theatres for both sampling methods. The trend, however, was only significant for IMA values for working theatres ($P = 0.035$) (Table III).

For empty theatres, median bacterial contamination values ranged from 2 cfu/m^3 in general and ophthalmic day surgery to 24 cfu/m^3 in general and plastic surgery. IMA values ranged from 0 IMA (cardiac surgery, general and ophthalmic day surgery, otorhinolaryngology and maxillofacial surgery, orthopaedic surgery) to 10 IMA (gynaecological and obstetric surgery). In four of the nine departments (cardiac surgery, general and ophthalmic day surgery, otorhinolaryngology and maxillofacial surgery, orthopaedic surgery), no fungi were isolated in empty theatres during the three-year period. Tables IV and V show the median and the 25th and 75th percentiles for bacterial and fungal contamination in working theatres for cfu/m^3 and IMA, respectively. Median bacterial contamination values ranged from 36 cfu/m^3 and 3 IMA (both in cardiac surgery) to 192 cfu/m^3 and 12 IMA (gynaecological and obstetric surgery, and paediatric surgery, respectively).

Median bacterial contamination in corridors adjacent to the operating theatres ranged from 42 to 271 cfu/m^3 and from 9 to 30 IMA (Table VI). Four operating suites (cardiac surgery, neurological and ophthalmic surgery, otorhinolaryngology and maxillofacial surgery, orthopaedic surgery) showed a significant difference in bacterial contamination between the operating theatre and the corridor for both cfu/m^3 and IMA. In two cases (general and plastic surgery, paediatric surgery), no significant difference was observed for either active or passive samples, while in three cases (general and ophthalmic day surgery, gynaecological and obstetric surgery, urological and urgent surgery), a significant difference was observed for passive samples alone. In paediatric surgery, the same median bacterial contamination value (129 cfu/m^3) was recorded in the operating theatre and the corridor. In gynaecological and obstetric surgery, the median bacterial contamination value was higher in the operating theatre (192 cfu/m^3) than in the corridor (42 cfu/m^3).

Regarding fungal contamination, significant differences between the operating theatre and the corridor were only observed in both active and passive samples in gynaecological and obstetric surgery. In general and plastic surgery, neurological and ophthalmic surgery, otorhinolaryngology and maxillofacial surgery, and urological and urgent surgery, significant differences were only observed for active samples. In cardiac surgery, general and ophthalmic day surgery, orthopaedic surgery and paediatric surgery, no significant differences in fungal contamination were observed between the operating theatre and the corridor in either active or passive samples.

Based on the data obtained, for empty theatres, the proposed target values were 12 cfu/m^3 and 1 IMA for bacterial contamination and 0 cfu/m^3 and 0 IMA for fungal contamination. For working

Table III
Percentage of samples devoid of fungi during the three-year period in empty and working operating theatres (OTs)

			Active sampling (cfu/m^3)				Passive sampling (IMA)			
			No fungi		χ^2 test for trend		No fungi		χ^2 test for trend	
			N	%	χ^2	*P*	*N*	%	χ^2	*P*
Empty OTs	Year	2008	8	50.0%	1.27	0.260	19	90.5%	0.84	0.358
		2009	6	42.9%			14	93.3%		
		2010	7	77.8%			9	100.0%		
Working OTs	Year	2008	17	40.5%	1.74	0.186	31	77.5%	4.43	0.035
		2009	26	42.6%			47	79.7%		
		2010	26	54.2%			45	93.8%		

cfu, colony-forming units; IMA, index of microbial air contamination.

Table IV
Median and percentiles of total bacterial count and fungal count in the different operating suites during surgery. Values measured by active sampling (colony-forming units/m^3)

Operating suites	Bacterial count				Fungal count			
	N	25th percentile	Median	75th percentile	*N*	25th percentile	Median	75th percentile
Cardiac surgery	14	24	36	42	14	0	0	2
General and ophthalmic day surgery	11	56	64	108	11	0	0	2
General and plastic surgery	42	58	106	210	42	2	2	8
Gynaecological and obstetric surgery	17	54	192	374	19	2	8	28
Neurological and ophthalmic surgery	16	31	47	76	16	0	0	0
Orthopaedic surgery	12	22	59.5	81	12	0	1,5	3
Otorhinolaryngology and maxillofacial surgery	15	24	69	160	15	0	0	2
Paediatric surgery	6	66	129	261	6	0	2	4
Urological and urgent surgery	16	39	126	221	16	0	1	2

theatres, the proposed target values were 80 cfu/m^3 and 7 IMA for bacterial contamination, and 2 cfu/m^3 and 0 IMA for fungal contamination. Alert values for empty theatres were 32 cfu/m^3 and 3 IMA for bacterial contamination, and 2 cfu/m^3 and 0 IMA for fungal contamination. For working theatres, alert values were 176 cfu/m^3 and 13 IMA for bacterial contamination, and 4 cfu/m^3 and 0 IMA for fungal contamination. Considering the standard error of the mean, acceptable values for bacterial contamination during surgical activity were 180 cfu/m^3 and 12 IMA.

A significant correlation was found between bacterial contamination recorded during surgical activity via active sampling and that recorded via passive sampling ($P < 0.001$).

Discussion

Microbial contamination of the surgical site is a necessary precursor of SSI.[3] The origin of pathogens can be endogenous (from patient's skin, mucous membranes or hollow viscera) or exogenous. Airborne micro-organisms can enter surgical wounds via two pathways: they can fall directly into the wound, or they can land on exposed surfaces and subsequently be transferred to the wound.[13]

Although the role of air as a vehicle of SSI-causing micro-organisms is still being debated,[3,5–13] preventive measures aimed at minimizing the introduction, generation and retention of particles inside the operating theatre are recommended.[3] The operating theatre could be viewed as a controlled environment, and a system for monitoring bio-contamination that is capable of detecting adverse conditions should be developed.[47] In controlled environments, such as pharmacies, hospitals, food processing plants and, more generally, in all settings where hygiene is considered to be crucial and microbial air contamination is limited through specific devices, operators must be familiar with the methods used to measure airborne micro-organisms.[41] However, there is no consensus on the usefulness of microbiological monitoring, and operators are often unaware of the high contamination levels in their working environment, which become evident

Table V
Median and percentiles of total bacterial count and fungal count in the different operating suites during surgery. Values measured by passive sampling (index of microbial air contamination)

Operating suites	Bacterial count				Fungal count			
	N	25th percentile	Median	75th percentile	*N*	25th percentile	Median	75th percentile
Cardiac surgery	14	1	3	4	14	0	0	0
General and ophthalmic day surgery	11	4	6	12	11	0	0	0
General and plastic surgery	41	5	10	15	41	0	0	0
Gynaecological and obstetric surgery	19	6	8	18	19	0	0	1
Neurological and ophthalmic surgery	16	4	5.5	8	16	0	0	0
Orthopaedic surgery	12	3.5	8.5	14.5	12	0	0	0
Otorhinolaryngology and maxillofacial surgery	14	3	6	10	14	0	0	0
Paediatric surgery	5	4	12	15	5	0	0	0
Urological and urgent surgery	15	3	8	16	15	0	0	0

Table VI
Comparison between median bacterial counts for the operating theatres (OTs) and corridors in the different operating suites

Operating suites	cfu/m^3			IMA		
	Median OT	Median corridor	*P*	Median OT	Median corridor	*P*
Cardiac surgery	36.0	157.0	0.001	3.0	11.0	0.001
General and ophthalmic day surgery	64.0	88.0	0.518	6.0	11.0	0.039
General and plastic surgery	106.0	140.0	0.215	10.0	15.0	0.166
Gynaecological and obstetric surgery	192.0	42.0	0.069	8.0	30.0	0.003
Neurological and ophthalmic surgery	47.0	148.0	0.004	5.5	9.0	0.023
Orthopaedic surgery	59.5	271.0	<0.001	8.5	23.0	0.026
Otorhinolaryngology and maxillofacial surgery	69.0	161.0	0.027	6.0	13.0	0.024
Paediatric surgery	129.0	129.0	0.670	12.0	10.0	0.752
Urological and urgent surgery	126.0	142.0	0.278	8.0	26.0	0.001

cfu, colony-forming units; IMA, index of microbial air contamination.

during research studies. For example, an Italian multicentre study on the bacterial contamination of air coming out of ventilation systems showed microbial charges of up to 600 cfu/m^3.[48]

Data on microbial contamination in operating theatres are far from detailed. To date, only a few studies have focused on the long-term evaluation of microbial air quality in operating theatres, and comparison of the results is difficult. To the authors' knowledge, this is the first study to investigate microbial air contamination in operating theatres using standardized methods, providing a global evaluation of air quality over a long period of time, in both empty and working theatres, and using active and passive sampling to assess both bacterial and fungal contamination.

A high degree of variability in air microbial contamination was observed between the different operating suites and the different operating theatres. In some empty theatres, high levels of fungal contamination were observed. Careful analysis of the problem revealed faults in the structure and management of the ventilation system, which were subsequently corrected. This demonstrates the importance of monitoring air quality during the commissioning of an operating theatre and after all major structural renovations. Holton and Ridgway reported the results of a 10-year microbiological testing programme in operating theatres that exposed major faults in the ventilation systems.[32] The authors underlined the value of such microbiological involvement in theatre testing, and recommended close cooperation between the hospital engineering department and the hospital hygiene unit for effective monitoring of theatre commissioning and upgrading.

A wide variation in microbial contamination was observed during surgical activity in operating theatres that used similar forms of ventilation, suggesting that factors which strongly affect the quality of air (e.g. number of people in the operating theatre, their movements, number of door openings) were not well controlled. This study included the corridors adjacent to the operating theatres in order to highlight any differences in air quality which could indicate that air mixing had occurred between the operating theatre and the corridor. When no difference was found between a theatre and its corridor, it was assumed that there had been an inflow of contaminated air from the corridor (e.g. due to the repeated opening of doors). As expected, most of the operating theatres showed lower bacterial contamination values than the corridors. In some operating suites, no significant differences were observed between bacterial contamination inside the theatre and that in the adjacent corridor, suggesting that air from the two environments had mixed. This was probably due to the repeated opening of doors, leading to the passage of contaminated air from the corridor into the operating theatre that could not be compensated for by the heating, ventilation and air conditioning (HVAC) system. The frequency of door opening has been demonstrated to be a positive predictor of increased bacterial counts.[49,50] In gynaecological and obstetric surgery, the median bacterial value was higher in the operating theatre than in the corridor, whereas the IMA value was higher in the corridor than in the operating theatre. This discrepancy is difficult to explain and would require specific examination. At the present time, any explanation would necessarily be speculative and unsupported by scientific evidence.

The fact that no significant increase in fungal contamination was observed during surgical activity suggests that the major source of fungal contamination was inadequately filtered incoming air. This would be consistent with the recommendation given in HTM 03-01 that 'a high preponderance of fungal organisms may be an indication of inadequate filtration for the particular installation'.[15]

The decrease in bacterial and fungal contamination over the three-year period in both empty and working theatres demonstrates the usefulness of microbiological monitoring for improving air quality, and the educational value of discussing the results with operators. Eickhoff, when recommending against routine environmental sampling programmes on behalf of the American Hospital Association Advisory Committee on Infections in Hospitals, recognized the usefulness of environmental sampling for education and training of personnel.[23]

One of the aims of the present study was to propose a benchmark for microbial air contamination in operating theatres as a contribution to the global discussion on acceptable values. This is particularly important for active sampling, as it has been demonstrated that different samplers produce different results.[43] Table VII shows the proposed threshold values (target and alert levels) for microbial air contamination in operating theatres based on the data collected over the three-year study period. The values are compared with available standards.[14–18,51] The value of 32 cfu/m^3 for bacterial contamination in empty theatres, corresponding to the 75th percentile, is similar to the value initially recommended by Health Technical Memorandum 2025[18] and subsequently included in the Italian ISPESL guidelines in 1999[16] and 2009[17]

Table VII
Proposed benchmark (target value and alert value) for bacterial air contamination in empty and working operating theatres compared with contamination values recommended for operating theatres (Die Spitäler der Schweiz, H+, 2007; Health Technical Memorandum, HTM 2025, 1994; Health Technical Memorandum, HTM 03-01, 2007; Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro, ISPESL, 1999, 2009) and contamination classes suggested in the European Commission Guide to Good Manufacturing Practice (EC GMP, 2008)

Pharmaceutical industries				Operating theatres					
EC GMP				H+	HTM 2025	HTM 03-01	ISPESL	Proposed benchmark (target and alert values)	
Grade	Active sampling cfu/m^3	Passive sampling		Passive sampling IMA[c]	Active sampling cfu/m^3	Active sampling cfu/m^3	Active sampling cfu/m^3	Passive sampling IMA[c]	Active sampling cfu/m^3
		Settle plates[a]	Settle plates[b]						
A	<1	<1	<1	-	-	-	-	-	-
B	10	5	1.25	2	35	10	35	1[d,f],3[e,f]	12[d,f],32[e,f]
C	100	50	12.50	15	-	-	-	7[d,g],13[e,g]	80[d,g]
D	200	100	25	25	180	180	180	-	176[e,g]

cfu, colony-forming units; IMA, index of microbial air contamination.
[a] cfu on settle plates 90 mm in diameter after 4-h exposure.
[b] cfu on settle plates 90 mm in diameter after 1-h exposure, calculated as one-quarter of the cfu value indicated by EC GMP after a 4-h exposure.
[c] cfu on settle plates 90 mm in diameter after 1-h exposure.
[d] Target value: median.
[e] Alert value: 75th percentile.
[f] Empty operating theatres.
[g] Working operating theatres.

(35 cfu/m^3). The median (12 cfu/m^3) is close to the 10 cfu/m^3 limit recommended by HTM 03-01[15] and was reached by nearly half of the samples in the present study (47.5%), suggesting that the HTM 03-01 limit is easily achievable in a properly ventilated theatre. The authors consider 12 cfu/m^3 (very close to 10 cfu/m^3) to be the target level and 32 cfu/m^3 (very close to 35 cfu/m^3) to be the action level.

For IMA values, the 75th percentile corresponds to 3 IMA, which is similar to the target value of 2 IMA recommended by the Swiss Hospital Association[14] for working orthopaedic operating theatres, and is below the 5 IMA limit recommended for working cardiosurgery and neurosurgery operating theatres. However, IMA values <1 were observed in 48.9% of samples. The alert values for cfu/m^3 and IMA in working theatres, considering the 75th percentile (176 cfu/m^3 and 13 IMA) and the standard error of the mean (180 cfu/m^3 and 12 IMA), are comparable with the 180 cfu/m^3 limit recommended by HTM 03-01[15] and the ISPESL guidelines,[16,17] and the 15 IMA value recommended by the Swiss Hospital Association,[14] respectively. However, the median value of 80 cfu/m^3 is much lower than 180 cfu/m^3, and the median value of 7 IMA is lower than the Swiss Hospital Association's recommended target value (15 IMA) for general surgery. HTM 03-01 states that the number of airborne bacterial and/or fungal cfu averaged over any 5-min period would be unlikely to exceed 180 cfu/m^3. In the present study, it was only possible to perform punctiform sampling as it was difficult to get permission from the surgical team to leave the sampler for longer periods.

If one refers to the European Union's Guidelines to Good Manufacturing Practice,[51] which classify the different environments based on airborne particles and microbial contamination measured by active and passive sampling, it can be seen that many empty theatres fall into Class B (10 cfu/m^3, 5 cfu/9-cm-diameter settle plate/4 h, which is equivalent to 1.25 cfu/h). During surgical activity, more than half of the operating theatres showed Class C bacterial contamination (100 cfu/m^3, 50 cfu/9-cm-diameter settle plate/4 h, equivalent to 12.5 cfu/h). This appears to be an acceptable standard for modern conventionally ventilated operating theatres. It is interesting to note that most of the samples collected in the corridors showed bacterial contamination values of <180 cfu/m^3 and 15 IMA, which are the threshold values suggested for working theatres by HTM 03-01[15] and the Swiss Hospital Association,[14] respectively.

An additional remark should be made on fungal contamination, which cannot be estimated using a non-selective medium incubated at 37 °C for 48 h.[19] The authors' experience shows that fungi have difficulty growing on tryptic soy agar incubated according to such parameters, but grow easily on selective media. This is a point to consider when defining standards for microbial contamination for operating theatres. Relying solely on a generic nutrient agar poses the risk of evaluating a situation as acceptable when, in reality, it is not. Although fungi are not among the most common aetiological agents of SSI, they can cause severe infections.[12] In some cases, identical genotypes were observed in the *Aspergillus* strain isolated from the SSI and the environmental strain, demonstrating that the environmental strain had passed intraoperatively to the patient.[52,53]

The present study used both active and passive sampling methods as they have different purposes.[47] Active sampling provides information about the concentration of viable particles in the air, whereas passive sampling measures the rate at which viable particles settle on surfaces, thus providing a measure of the contribution of aerobiocontamination to the biocontamination of surfaces. In operating theatres, passive sampling estimates the risk posed by airborne micro-organisms to the surgical wound.[8,28,29] This study found a significant correlation between the two methods, confirming previous observations.[43] However, such correlation was not always

apparent, showing that several other factors can affect their relationship, such as particle size or ventilation parameters.[43] 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. Perdelli *et al.* found the greatest correlation when considering the average of four active samples collected during the exposure period of the settle plates.[54]

This study found that settle plates were less sensitive for the collection of fungi than active samplers. The settle plates only detected the presence of fungi in three cases without simultaneous detection by the active sampler. In a study by Asefa *et al.* at a food processing plant, the authors concluded that settle plates can provide important information on the dominant airborne fungal spores that can fall on to and contaminate food products.[55] This concept could be applied to surgical sites. In any case, the sensitivity of settle plates could be improved by increasing the exposure time, as suggested in the European Union's Guidelines to Good Manufacturing Practice, where threshold values are given for 9-cm-diameter settle plates exposed for 4 h.[51]

No attempt was made to correlate the different levels of microbial air contamination found in the theatres with postoperative infection rates. The aim of this study was to assess microbial air contamination in empty and working theatres, regardless of possible correlations with infection rates, and to determine an achievable contamination level that could be used as a standard for all operating theatres supplied with turbulent ventilation systems. Higher levels should be regarded as indicators of possible problems, either in the ventilation system or in the surgical team's behaviour. The importance of monitoring bacterial air counts in operating theatres remains a subject of debate. The US Centers for Disease Control and Prevention guidelines for the prevention of SSIs recommend that routine environmental samplings should not be performed in operating theatres.[3] Environmental surfaces and air inside operating theatres should only be sampled during epidemiological investigations. However, the same document recommends that operating theatres should be maintained at a positive pressure with respect to corridors and adjacent areas; there should be at least 15 air changes per hour; doors should be kept closed except as needed for passage and equipment, personnel and the patient; and the number of personnel entering the operating theatre should be limited to necessary personnel. All these recommendations are clearly aimed at keeping the microbial air charge low. The Healthcare Infection Society Working Party Report, when referring to conventionally ventilated operating theatres, states that microbiological air sampling of empty theatres should be performed either as part of an investigation into theatre-acquired infections with a possible airborne element, or after any changes that may affect airflow supply rates or distribution patterns. Provided that engineering parameters are satisfactory and monitored regularly, microbiological air sampling does not need to be carried out on a routine basis, unless mandated by local agreements.[19]

The authors advocate that air sampling should be performed in empty theatres (during commissioning and after any major refurbishment) as well as in working theatres, with a frequency that will have to be established at local level. Attention should be devoted to the problem before unacceptable situations emerge. Air sampling in empty theatres allows evaluation of the efficiency of the HVAC system. In fact, it must not be assumed that an HVAC system complies with the required standards solely on the basis that it was certified as so by the installer.[32,56] Since several factors other than basic ventilation parameters are important in determining air quality, assessment of microbial air contamination of the operating theatre during surgical activity would allow the hospital's hygiene unit and the surgical team to examine their practices and, if necessary, improve them. If the hospital's hygiene unit, for any reason, decides not to perform any microbiological air monitoring in the operating theatres, it must ensure that all factors which are known to be associated with increased microbial air contamination (e.g. efficiency of HVAC system, number of door openings, number of people in operating theatre, incorrectly worn surgical clothing) are strictly controlled. Whatever the decision, it is the hygiene unit's responsibility and moral duty to ensure that cleanliness levels are consistent with the ventilation system installed (unidirectional airflow or turbulent airflow) in all operating theatres. Whenever an HVAC system is installed, it is essential that poor management of the ventilation system or the incorrect behaviour of operators should not undermine the economic investment. In this regard, air microbiological control can be a useful tool to assess air quality, test the efficacy of preventive measures and identify hazardous situations. People operating at all stages of microbiological air monitoring should be skilled, fully motivated and appropriately trained. They should not interfere with any existing routine and should not be outside workers, but should be part of an internal process aimed at quality improvement. Results should be analysed properly and communicated effectively; most importantly, action should be taken in the case of anomalies. The involvement of frontline practitioners providing continuous feedback on the results is absolutely necessary. Everybody should participate in the common effort of improving air quality in operating theatres, so as not to waste economic resources and to prevent critical situations that could give rise to healthcare infections.

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