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Short report

Air microbial sampling in operating theatres by active and passive methods: equation correlation from the GISIO-ISChIA study results and comparison with the EU GGMP recommendation, towards the definition of threshold values

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Abstract. *Background and aim:* The aim of this study was to calculate the equation of correlation between the microbial air contamination values obtained by active sampling (colony-forming units per cubic metre, CFU/m^3) and by passive sampling (Index of microbial air contamination, IMA), by using the data from the ISChIA study (Infezioni del Sito Chirurgico in Interventi di Artroprotesi, surgical site infections in arthroprosthesis), and to compare the values obtained with the recommended limits defined by the EU Guidelines to Good Manufacturing Practice (EU GGMP), 2008, for clean areas used to manufacture sterile medicinal products. *Methods:* Air sampling was performed during 335 elective prosthesis procedures. Correlation between CFU/m^3 and IMA values was evaluated using the Spearman test; $p<0.05$ was considered to indicate significance. This equation was used to calculate the IMA values corresponding to the CFU/m^3 recommended threshold values by EU GGMP for the different Grades of microbial contamination. *Results*: The following correlation equation was obtained: $y = 1.86 + 0.12x$, where "x" = CFU/m^3 value and "y" = IMA value. The relationships between CFU/m^3 and IMA values obtained from active and passive sampling during the ISChIA study showed to be in line to those suggested by the EU GGMP for pharmaceutical manufacturing, in particular for Grade C and D. *Conclusions:* This study shows that the EU GGMP relationship could be considered valid also for operating theatres. Both methods, active and passive samplings, can be used to evaluate microbial air quality and highlight critical situations; however, in particular during the activity, passive sampling estimating the risk posed by airborne microorganisms to the surgical wound, can be considered more relevant, and for its simplicity, economy and standardization, can be suggested for routine microbial monitoring. (www.actabiomedica.it)

Key words: Operating theatres, microbial contamination, air, active sampling, passive sampling, correlation

Introduction

Surgical site infection (SSI) is the most frequent and feared complication of surgery, associated with increased morbidity, mortality, and costs. Microbial contamination of surgery is a necessary precursor of SSI, and the ambient air of the operating theatres represents an important vehicle of SSI-causing microorganisms which can fall directly into the wound or can land on exposed surfaces and subsequently be transferred into the wound (1). To reduce microbial air contamination, heating, ventilation and air conditioning (HVAC) systems, turbulent airflow or unidirectional airflow, are used; however, effectiveness of the HVAC systems can be undermined by their poor management and by the incorrect behaviour of the surgical team. It is, therefore, essential to verify that the air quality corresponds to what is expected; in this context, air microbiological control can represent a useful tool to assess air quality in operating theatres, test the effectiveness of preventive measures and identify hazardous situations. Active and passive sampling can be used; the active method measures the concentration of viable and cultivable microorganisms in the air, expressed as colony forming units per cubic metre (CFU/m^3), while passive method measures the rate at which viable and cultivable microorganisms settle on surfaces (2-4). Passive method has been standardized by the Index of Microbial Air contamination, IMA (5,6). Within the ISChIA study (Infezioni del Sito Chirurgico in Interventi di Artroprotesi, surgical site infections in arthroprosthesis) a significant correlation (p<0.001) was found between microbial air contamination values measured by active sampling (CFU/m^3) and microbial air contamination values measured by passive sampling (IMA) (7,8). Aim of this study was to obtain the equation that correlates the CFU/m^3 and IMA values, and to compare the values obtained by applying this equation, with the relationships from the recommended limits defined by the EU Guidelines to Good Manufacturing Practice (EU GGMP) for clean areas used to manufacture sterile medicinal products (9).

Methods

The data of CFU/m^3 and IMA used for the equation of correlation derived from the air sampling during 335 elective prosthesis procedures, performed within the GISIO-ISChIA study (7,8). The number of CFU/m^3 was measured by using the Surface Air System Sampler (SAS, Pbi International, Milan, Italy) with 55 mm diameter RODAC plates containing Tryptic Soy Agar, flow rate of 180 L/min and suction volume set to 1000 L. The IMA values were obtained from the number of CFU that settled on 9 cm diameter settle plates left open to the air according to the 1/1/1 scheme - for 1 hour, 1 m from the floor, about 1 m from any obstacles. After sampling the plates were incubated at 36±1°C for 48 h. The analysis of the results was performed by using SPSS version 26 (IBM SPSS Inc., Chicago-IL). Correlation between CFU/m^3 and IMA values was evaluated using the Spearman test; $p<0.05$ was considered to indicate significance. This equation was used to calculate the IMA values corresponding to the CFU/m^3 recommended threshold values by EU GGMP for the different Grades of microbial contamination. To compare IMA values with CFU/plate EU GGMP values, one-quarter of the CFU value indicated by EU GGMP for each Grade after a 4-h exposure was used.

Results

The Spearman correlation coefficient between CFU/m^3 and IMA values was 0.698 ($p<0.001$). The following correlation equation was obtained: $y = 1.86 + 0.12x$, where "x" = CFU/m^3 value and "y" = IMA value. Table 1 shows the comparison between the EU GGMP values and the corresponding values obtained using this equation. In particular, at Grade B, corresponding to 10 CFU/m^3 and 5 CFU/4h (1.25 CFU/h), the IMA value was 3.06 (+144.80%); at Grade C, corresponding to 100 CFU/m3 and 50 CFU/4h (12.50 CFU/h), the IMA value was 13.86 (+10.88%); at Grade D, corresponding to 200 CFU/m3 and 100 CFU/4h (25 CFU/h), the IMA value was 25.86 (+3.44%).

Conclusions

The relationship between CFU/m^3 and IMA values obtained during the ISChIA study showed to be

Table 1. Comparison among EU GGMP values and GISIO-ISChIA study results.

Recommended limits for microbial contamination[a]				IMA values obtained applying the correlation equation derived from the GISIO-ISChIA study
Grade	Air sample CFU/m^3	Settle plates (diametre 90 mm) CFU/4 hours[b]	Settle plates (diametre 90 mm) CFU/1 hour[*]	
A	<1	<1	<1	-
B	10	5	1.25	3.06
C	100	50	12.5	13.86
D	200	100	25	25.86

Notes: (a) These are average values. (b) Individual settle plates may be exposed for less than 4 hours. *CFU on settle plates 90 mm in diameter after 1-h exposure, calculated as one-quarter of the CFU value indicated by EC GGMP after a 4-h exposure

in line to those suggested by the EU GGMP for pharmaceutical manufacturing. Therefore, the EU GGMP relationship could be considered valid also for operating theatres in operation. The highest correspondence was found at Grade D, which includes the value of 180 CFU/m^3 proposed as target values in turbulent ventilated operating theatres in operation (10-12). Referring to turbulent ventilated operating theatres, the equation obtained allows to confirm that the relationship of the threshold values of 180 CFU/m^3, by using active sampler, and 25 IMA, obtained by using settle plates, corresponding to grade D of EU GGMP, proposed independently for operating theatres, can be considered reasonable. However, modern turbulent HVAC systems are more efficient than in the past; in the GISIO-ISChIA study median values of 7 IMA and 54 CFU/m^3 in hip and knee arthroplasty were found (7,8), and also in other studies lower values of microbial air contamination were obtained (13,14). Therefore, the Grade C of EU GGMP, which showed a good correspondence with the IMA value from GISIO-ISChIA study, should be regarded as an acceptable standard for modern turbulent ventilated operating theatres. At Grade B a lack of correspondence between IMA value and EU GGMP value was found, which could be justified by the very low level of air microbial contamination; in this case, even a slight increase in CFU, determines a very evident percentage difference.

Both methods, active and passive, can be used to assess air quality in operating theatres; however, in particular in operational operating theatres, where the air sampling is performed to evaluate the risk of microbial surgical wound contamination, passive sampling, estimating the risk posed by airborne microorganisms to the surgical wound, can be considered more relevant, and for its simplicity, economy and standardization, can be suggested for routine microbial monitoring.

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