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Major Article

Is there a correlation between airborne bacterial load measured by volumetric air sampling and fluorescent particle counting?

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Background: Microbiological air sampling in operating rooms, based on colony-forming unit (CFU) counts, is time-consuming and costly. Novel technologies, such as particle counters that detect fluorescent bioparticles (FBPs), may offer a simpler method for estimating airborne bacterial loads. This study aimed to assess the correlation between CFU and FBP counts under realistic operating room conditions.

Methods: During 22 primary hip arthroplasty procedures at Uppsala university hospital, CFUs were measured using a Sartorius MD8 air sampler placed adjacent to the surgical field. Simultaneously, the Bio Aerosol Measuring System (BAMS), recorded FBPs sized 0.5-25 µm. FBP counts were analyzed during CFU sampling periods and using each CFU sampling interval as an individual data point, yielding 115 paired comparisons. Correlations were assessed using Spearman's rank correlation coefficient.

Results: No significant correlation was observed between FBP and CFU counts. Comparing FBP with CFU counts during Sartorius sampling intervals, rho was −0.31 (P = .23). Using individual CFU sampling intervals, the correlation for particles ≥ 3 µm rho was = −0.10 (P = .35).

Conclusions: Analyses revealed no correlation between FBP and CFU counts in operation room air quality assessments, suggesting that BAMS-based FBP measurement is not a suitable alternative to established microbiological methods.

BACKGROUND

One risk factor for surgical site infections (SSI) is the number of airborne bacteria in the operating room (OR).[1] Lidwell et al demonstrated that increased colony-forming unit (CFU) numbers were associated with a higher rate of periprosthetic joint infection in total joint replacement surgery.[2] In their recent systematic review, Seth Caous et al also suggested that there appears to be a correlation between air contamination and the risk of SSI.[3] An increased number of airborne bacteria is correlated with a higher number of staff members,[4] type of clothing systems,[5] increased surgical team activity[6] and the number of door openings.[7,8]

Quantification of airborne bacteria within the OR is typically achieved by counting CFUs/m³ of air, using an active air sampler with the measurement point placed in the sterile surgical field.[1,9] Passive air sampling using settle plates is a not as widely used but a possible alternative.[10] To minimize the risk of wound contamination from airborne bacteria, the CFU/m³ number should be below 10 during ongoing surgery, which is defined as ultraclean air.[11] In Sweden, the recommended limit for infection-prone surgery such as implant surgery is 5 CFU/m³ during ongoing surgery.[9]

Since 1999 (revised in 2015), the ISO (International Organization for Standardization) classification (14644-2015) has been the international standard used to design, limit, construct, validate and

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Ethics approval: Because this is a prospective observational study without any intervention, informed consent from patients was not required by the Swedish ethical review authority (2022-06282-01).

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operate a cleanroom.[12] According to ISO 14644-2015, the air cleanliness in a room is measured by sampling airborne particles using a real-time particle counter (PC). PCs can detect particles using a photo detection technique. A more modern type of PC can also detect size, count and differentiate fluorescent bioparticles (FBPs) by identifying traces of biological substances, such as NADH and riboflavin, which are associated with living organisms, including bacteria. This refined detection method suggests that FBP measurements may offer a viable alternative to the current primary method of airborne bacteria detection, which relies on CFU sampling.[13]

The literature on the correlation between CFU counts and airborne particles is scarce and inconsistent, with only a few recent studies attempting to compare CFUs with fluorescing bioparticles, including the systematic review by Stålfelt et al (2023) concluding there is no necessary evidence for a correlation between CFUs and FBPs.[14-16] Several studies have suggested that fluorescent PCs cannot be directly compared with microbiological sampling methods, as they detect FBPs that are not necessarily of microbial origin.[17,18]

In another recent study from 2025, using a unidirectional airflow operating theater, Stålfelt et al found no significant differences between CFUs and FBPs measured close to the surgical field, nor between CFUs close to the surgery field and nonfluorescent particles 1 m away. However, a strong and statistically significant correlation was observed when FBPs were measured close to the surgical field and nonfluorescent particles measured in the periphery, suggesting a relationship between near-field and peripheral particles even under unidirectional airflow.[19]

The current study aimed to investigate the correlation between CFUs and FBPs in OR air under realistic conditions, with each device placed according to its intended use. The goal was to assess whether their relationship remains consistent despite differences in sampling locations.

METHODS

Study design

This was a prospective, observational study comparing 2 methods of measuring air quality in the OR during primary hip arthroplasty surgery: the standard method, which involves measuring CFU/m³ with the Sartorius MD8 air sampler (model number 16746, Sartorius AG), and the index method, which measures FBP/50 dm³ using the novel Bio Aerosol Measuring System (BAMS, Zecon AB).

Measuring devices

The Sartorius MD8 draws 1,000 L of air through a gelatine membrane filter with a pore size of 3 µm for 10 minutes (100 L/min). The gelatine membrane is supplied together with the device. The Standard Institute of Sweden recommends that samples should be collected using a filter positioned < 0.5 m from the surgical field (Fig. 1).[20,21]

FBP measurements were obtained using a BAMS, which is compliant with ISO-21501-4 (Fig. 2).[22] BAMS uses one 405 nm laser beam, which is directed at particles drawn in at a rate of 5 L of air/minute (50 L/10 min). When the laser beam hits a particle, it generates scattered light. If the particle does not contain biomaterial, the scattered light remains at the same wavelength and is detected by a photodiode. However, if the particle contains biomolecules, such as NADPH or riboflavin, it emits scattered light and fluorescence at a different wavelength (488-700 nm). This phenomenon is detected by one photomultiplier tube.

In contrast to CFU measurement, which requires incubation, fluorescence-based PCs provide real-time monitoring of airborne biomaterial particles, displayed simultaneously on a screen in the OR (Fig. 2). All data were stored locally on the device and backed up to a secure online storage service.

Fig. 1. Air sampler Sartorius MD8 (Sartorius AG).

Fig. 2. Bio Aerosol Measuring System (BAMS, Zecon AB) and the screen installed on the wall of the operating room.

Settings

The study encompassed 22 primary hip arthroplasty procedures performed within the same OR at Uppsala university hospital between April 2023 and June 2024.

The ventilation system in the OR used unidirectional airflow with an Allander ceiling (HEPA filters) (Supplementary Fig. A.1),[23] achieving airflow of 1,607 L/s and 50 air changes/hour. Conditioned air is supplied evenly across the entire ceiling surface and flows downward in parallel layers, minimizing turbulence. Four exhaust air devices were placed in each corner of the OR. All surgical staff members wore disposable 100% polypropylene surgical clothes (Clean Air Suit, Mölnlycke Health Care AB).

The filter connected to the Sartorius MD8 was placed in the sterile environment close to the surgery field. The OR was equipped with a built-in monitoring BAMS that had been previously installed and placed along a wall of the same OR, approximately 3.0 m from the surgical field and at the same height as the operating table (Supplementary Fig. A2). The number of particles registered was displayed instantly on the screen.

The Sartorius MD8 drew air through the gelatine filter membrane repeatedly during the time of surgery, with pauses of 1 to 5 minutes when changing the filter. The filter was changed by the operating nurse and handed over to a nonsterile staff member.

Laboratory settings

The filters from the Sartorius air sampling, which have a collection capacity for particles ≥ 3 µm, were placed on Tryptic Soy Agar plates (90 mm diameter Petri dishes) and incubated for 96 hours at 22 °C, followed by 72 hours at 37 °C in an aerobic environment, according to local hospital culture procedures. The number of CFUs was then counted manually. Plates exposed to nonsterile surfaces during sampling or exhibiting condensation during incubation were considered contaminated and subsequently discarded.

Data collection

All 22 surgeries were elective primary hip arthroplasties scheduled on dates when both sampling devices were available. The sampling data were not linked to patient identity. Each surgery was assigned a unique ID number.

Sartorius MD8 sampling was initiated with the connected filter as close as possible to the skin incision and continued for 10 minutes each. Depending on the time of surgery, three to ten 10-minute intervals were conducted during the surgeries (Supplementary Table A.1).

The BAMS device sampled data from the skin incision to the end of surgery. The BAMS registered particle sizes ranged from 0.5 to 25 µm, with sizes ≥ 3.0 µm used in this study.

Statistics

Data from 17 of 22 surgeries were included in the study. Data from 3 surgeries were omitted due to missing BAMS data. Two further surgical cases were excluded from the analysis due to filter or agar plate contamination.

Given the non-normal distribution of the data, Spearman's rank correlation coefficient (rho) was employed for all association analyses. Spearman's method was chosen as it does not assume normality and is suitable for monotonic relationships.

The number of CFUs was compared to the number of FBPs. The FBP sizes were grouped into 5 groups [3.0 µm [3.0-5.0 µm), 5.0 µm [5.0-10.0 µm), 10.0 µm [10.0-25 µm], ≥ 3 µm, and ≥ 5 µm].

FBP counts were analyzed and log-transformed, thereafter compared to CFU counts using 2 distinct methods:

- FBP and CFU counts from all CFU sampling periods during each surgery were summed up, treating each surgery as a separate observation (Supplementary Fig. A.3). Spearman's rank correlation (rho) was used for the analysis.
- FBP counts were summed during each 10-minute interval (in total 115). The measurements were pooled from all surgeries and treated as independent observations in the analysis, comparing the number of CFUs with the FBP count collected during the same 10-minute interval. This procedure was performed to assess the short-term association between the number of CFUs and FBP counts. Spearman's rank correlation (rho) was used for the analysis. To account for measurements originating from the same surgery, logistic mixed effects models were also used. In the model CFU was recoded as a binary variable, at least one bacterial colony (CFU > 0) or none (CFU = 0), and defined as the outcome variable. The model included FBPs as fixed effect variables and Surgery ID was treated as a random intercept.

RESULTS

Analysis of FBPs collected concurrently with CFU sampling for each surgery revealed no statistically significant correlation with CFU counts (Table 1, Fig. 3).

In total, 115 CFU analyses using each 10-minute interval as an individual data point were collected and compared to the corresponding FBP counts registered by BAMS for the same 10-min intervals. No significant correlations were noted using Spearman's correlation (Fig. 4, Supplementary Table A.2), this was confirmed by the logistic mixed models (data not shown).

DISCUSSION

Our study found no statistically significant correlations between the CFU counts measured by the Sartorius MD8 and the number of FBPs registered by the BAMS. Additionally, no differences were observed across various particle size groupings in the analyses.

Table 1
Total CFU and FBP counts for each of the 17 surgeries

Surgery ID	CFU	Intervals (10 min)	FBP 3 µm	FBP 5 µm	FBP 10 µm	FBP ≥ 3 µm	FBP ≥ 5 µm
1	2	6	185	71	14	270	85
2	22	6	32	12	1	45	13
3	65	6	120	50	8	178	58
4	0	5	528	151	23	702	174
5	66	6	153	57	9	219	66
6	154	6	131	39	8	178	47
7	62	9	227	83	17	327	100
8	19	9	436	149	21	606	170
9	179	8	396	149	28	573	177
10	0	10	306	104	25	435	129
11	16	9	843	297	50	1,190	347
12	3	4	214	62	10	286	72
13	0	4	146	47	8	201	55
14	50	4	90	30	5	125	35
15	0	10	696	261	63	1,020	324
16	49	10	506	234	63	803	297
17	13	3	182	59	15	256	74
rho*			-0.34	-0.25	-0.36	-0.31	-0.26
P-value†			.20	.35	.16	.23	.31

NOTE. Values represent the sum of all CFU and FBP measurements collected during each surgery. CFU were collected in 10-min sampling intervals, and FBP were measured simultaneously. Spearman's rank correlation coefficient (rho) showed no significant correlation between FBP and CFU counts for any of the particle size groups (µm); 3.0 µm [3.0-5.0 µm), 5.0 µm [5.0-10.0 µm), 10.0 µm [10.0-25 µm], ≥ 3, ≥ 5 (untransformed data).

CFU, colony-forming units; *FBP*, fluorescent bioparticles.

*Spearman rank correlation coefficients (rho) between CFU and FBP counts.

†*P*-values correspond to Spearman correlation.

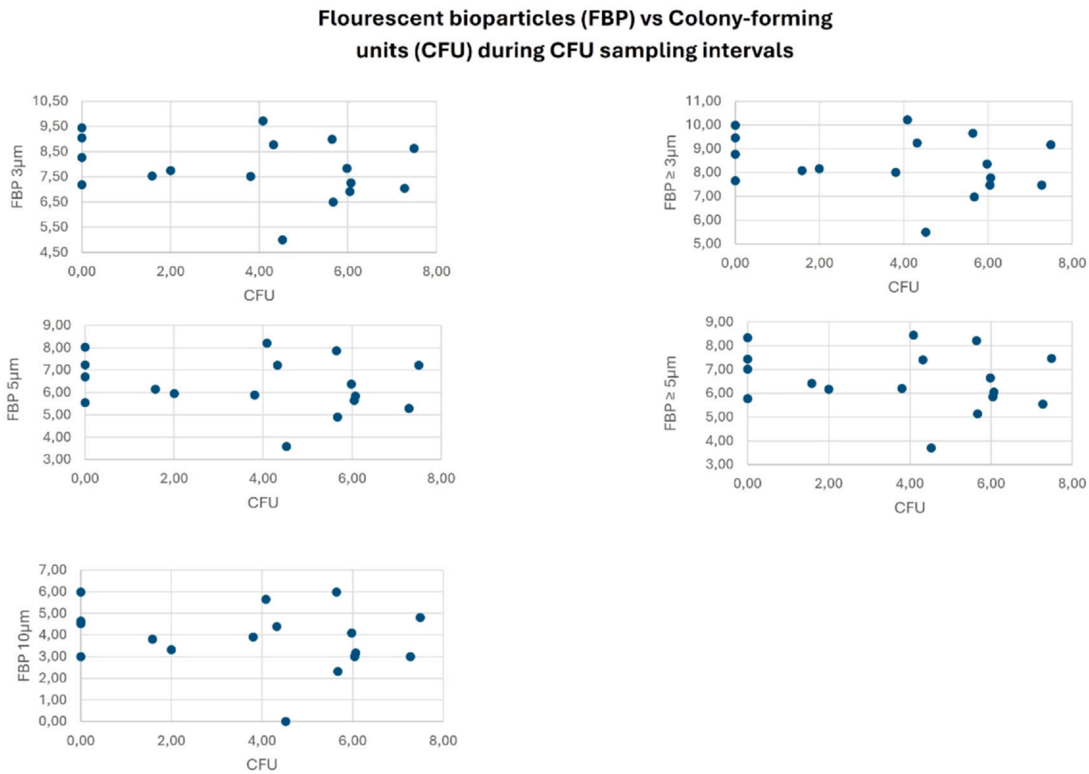

Fig. 3. Correlation of FBP and CFU counts per surgery: Log₂-transformed FBP data plotted against log₂-transformed (CFU +1) counts, each point represents one surgery. Particle sizes were grouped as follows: 3.0 μm [3.0-5.0 μm), 5.0 μm [5.0-10.0 μm), 10.0 μm [10.0-25 μm], ≥ 3 μm, and ≥ 5 μm. CFU counts were compared with FBPs collected during CFU sampling intervals.

Earlier studies have yielded inconclusive results, as summarized in the systematic review by Stålfelt et al, which included 10 studies using nonfluorescent PCs and one using a FBP counter.[16] The authors concluded that there is no clear correlation between airborne aerobic CFU counts and airborne particles, suggesting that it may not be feasible to replace traditional CFU measurement methods with particle measurement in ORs.

Although PCs have been in use for several decades, only a few studies have explored the role of FBPs in surgical settings. The first such study, by Dai et al, reported a statistically significant correlation between FBPs and airborne bacteria (Pearson correlation = 0.76).[14] However, this study is limited by the small quantity of data collected, as it was conducted in a single OR over an 11-hour period during 3 surgeries. This study generated only 23 comparisons, whereas our study generated 115 comparisons across 17 surgeries of the same type (hip arthroplasties).

A recent study, comparing aerobic CFUs and FBPs, was reported by Larsson et al. They showed a statistically significant correlation between airborne aerobic CFU counts and airborne FBP numbers,[15] following adjustments for FBP distortion caused by electrocautery-generated particles. Also, while Larsson et al used the same measuring devices as in the present study, their placement in the OR differed. Larsson et al positioned both devices next to each other in the nonsterile field, whereas our study sampled CFUs directly from the surgical field. In the present study, our interest was to investigate whether the currently used CFU sampling method performed in the sterile surgery field, correlates to particle measurements obtained in a more practical, safer and less intrusive location of the operating theater. The placement of the instruments was chosen to reflect how they are intended to be used in clinical practice.[24,25] The Sartorius sampler is designed specifically for measurements within the sterile field, whereas the BAMS device is not suitable for sterile environments and must be positioned in a nonsterile area. We therefore configured the setup to mirror these real conditions and to simulate their likely future positions in an OR. According to ISO standard 14698-1:2003, the microbiological air sampler should be placed in the critical zone.[26] Additionally, the ventilation in the OR in our study (Allander ceiling with unidirectional air flow) differed from that in the Larsson et al study (turbulent mixed airflow).[23] These differences can explain some of the discrepancies between the 2 studies.

The particle size ranges reported in previous studies show variability. We chose to define our particle groups as presented, partly because the gelatine membrane filters used in the Sartorius device have a permeability threshold of 3 µm. In addition, we aimed to investigate larger particle sizes, as suggested by Noble et al, who described how bacteria can become airborne by attaching to particles. Noble et al observed that bacteria, which typically measure between 0.5 and 5 µm preferentially adhere to particles of 4-20 µm in size.[27] These findings indicate that airborne bacterial particles below 3 µm are likely to play a minor role in infection, since their small size limits deposition on wounds and surgical instruments.

In an annotation by Whyte et al, the authors discuss that the efficiency of volumetric samplers is unlikely to reach 100%, partly because they collect air samples only over short periods.[10] Consequently, episodes of poor air quality occurring between sampling intervals may go undetected when using interval-based air sampling methods, as is necessary for active CFU sampling. Building on this discussion, the present study aimed to evaluate the potential for FBP measurements to replace CFU sampling given their ability to provide continuous air sampling, along with the additional advantages of simplicity and cost-effectiveness.

Several factors may account for the inconsistencies observed in studies correlation of CFU and FBP. Differences in study design, the

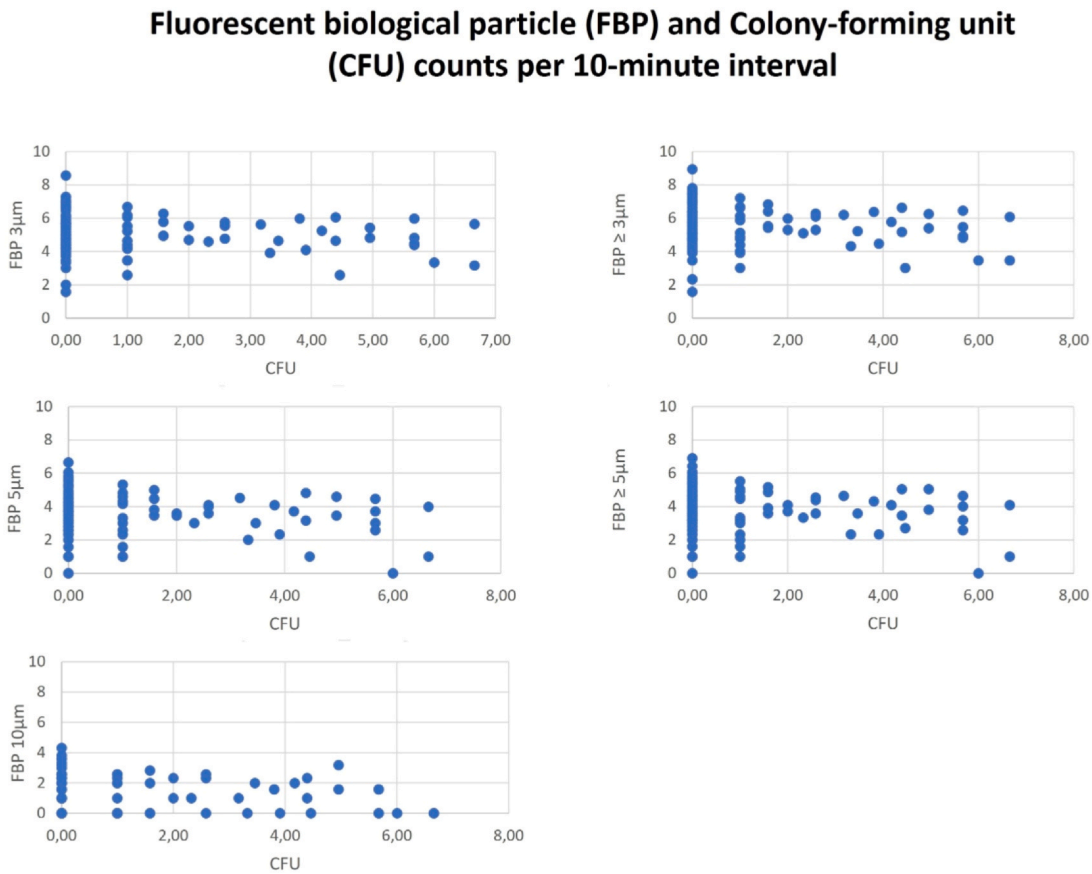

Fig. 4. Correlation of FBP and CFU counts per 10-min interval: Log₂-transformed FBP data plotted against log₂-transformed (CFU +1) counts during each 10-min interval treated as an individual attempt. Each point represents a single 10-min sampling period. FBP sizes grouped as follows: 3.0 µm [3.0-5.0 µm), 5.0 µm [5.0-10.0 µm), 10.0 µm [10.0-25 µm], ≥ 3 µm, and ≥ 5 µm.

measuring devices used, the placement of the devices, and the ventilation systems all contribute to variability. The observed discrepancies may also be attributable to random variation, underscoring the importance of replication using larger sample sizes.

The correlation between CFU levels and infection risk has recently been debated and questioned.[28,29] Additionally, the inconsistencies observed may be attributed to the differing types of particles used (biofluorescent vs nonfluorescent) and variations in particle sizes across studies. An important consideration during correlation analysis is the disparate parameters measured by the 2 devices: the Sartorius MD8 detects airborne bacteria as CFUs, whereas the BAMS measures FBPs based on biological molecular traces. Assessment of operation room cleanliness has long relied on the CFU measurement as the accepted benchmark, and its use has been well established for decades.[28] However, CFU counts are dependent on sample handling and the conditions necessary for bacterial growth. Some bacteria may not survive to the point of colony formation, whereas others may fail to grow on the media used. Furthermore, bacterial aggregation into colonies may lead to an underestimation of the true bacterial population.[29] In a subset of surgeries in this study, CFU counts exceeded the ultraclean air threshold, however, no clinical data were collected regarding the eventual occurrence of postoperative infections. Accordingly, in a manuscript included in a thesis by Persson, no correlation between airborne CFU levels and the risk of SSI was found.[30] Based on this, it becomes relevant to consider whether CFU measurements are suitable as a reference point or “gold standard,” when evaluating new methods for assessing cleanliness in ORs.

Particle measurement in accordance with ISO standards has been employed since 1999 to classify cleanrooms in contamination-sensitive environments. ISO classification is applied in industries such as aerospace, microelectronics, and medical device manufacturing.[31] However, these standards are primarily based on studies using total numbers of particles, which raises uncertainty about their ability to carry infectious material. With the introduction of more modern PCs capable of detecting FBPs, the potential for this technology to serve as an alternative to traditional CFU measurements is expected to rise in the future. FBP numbers can be monitored in real time, providing continuous data collection during surgical procedures at a relatively low cost once the system is installed. Clean room standards, originally developed for industrial applications focused on particle deposition, may not be directly applicable to ORs, where the presence of airborne bacteria is of primary importance. In contrast, CFU measurements are often more expensive, can only be performed periodically, and require several days for results to become available. Measuring CFU using passive air sampling with settle plates is a less expensive alternative.[10,32]

The number of studies in this field remains limited, and methodological variations, such as differences in equipment setup and study design, make direct comparisons challenging.

Strengths and limitations

A strength of this study is that the devices were placed according to their intended use, with the Sartorius measuring point positioned in the sterile field and the BAMS in the nonsterile area, reflecting reallife operating conditions. This setup allowed assessment of whether CFU and FBP levels correspond proportionally under realistic conditions, although it may have introduced some influence from personnel movement and spatial variation in airborne particles.

A key strength of our study is that all measurements were conducted during the same type of surgical procedure (total hip arthroplasty), consistent staff attire, limited staff in the OR and restricted door access. This approach guarantees comparability across cases. Our study, as well as that of Larsson et al, has a larger number of samples compared to earlier studies.

CFUs were directly sampled from the surgical field, providing a direct measure of microbial contamination in the critical zone as recommended by the ISO-21501-4.[22] In contrast, FBPs were measured in the surrounding air of the OR, allowing for a meaningful comparison between microbiological contamination in the surgical field and particle numbers in the broader operating environment.

Studies indicate that the application of electrocautery in surgery leads to an increase in the concentration of airborne particles.[15,33] Our study lacked consistent documentation of electrocautery use during surgery, which can also be considered a limitation. Another limitation of this study is the variability of airflow within the critical zone stemming from the unidirectional airflow system and the BAMS device's location outside the Allander ceiling. This variability may influence the study's findings. However, as previously discussed, Stålfelt et al demonstrated that particle levels inside and outside the surgical field can remain correlated even in a unidirectional airflow OR.[19]

CONCLUSIONS

In conclusion, our study did not find any correlations between airborne aerobic CFUs measured near the sterile surgery field and FBP counts measured at a distance. This supports previous literature findings on inconsistent relationships between CFUs and particle counts. Based on our findings, the BAMS device cannot be considered a replacement for conventional microbiological air sampling in assessing the quality of OR air. Future research should focus on determining whether elevated FBP levels are independently associated with an increased risk of SSI, rather than assuming that such a relation is mediated through a correlation with CFUs.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at doi:10.1016/j.ajic.2026.04.016.

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