Aerosol Bioburden and Antimicrobial Resistance in Orthopaedic Operating Unit in a Tertiary Hospital in Thailand

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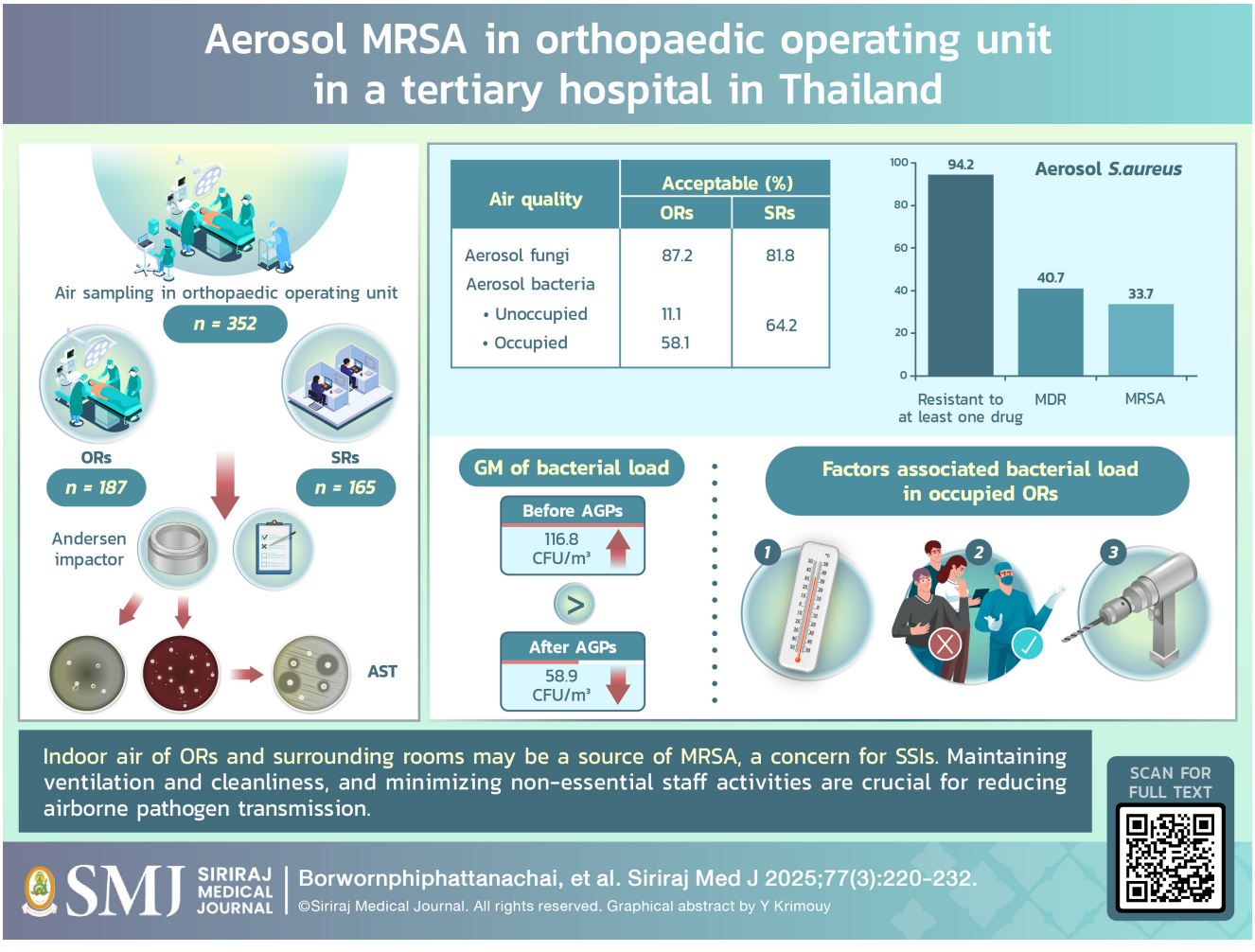

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ABSTRACT

Objective: This study aimed to determine the microbial indoor air quality and factors associated with bacterial air contamination in the orthopaedic operating unit.

Materials and Methods: Conducted in seven operating rooms (ORs) and six surrounding rooms (SRs) in an orthopaedic operating unit. A total of 352 air samples were collected using an Andersen air sampler. Fungal and bacterial counts were determined as air quality indicators. Antimicrobial resistance (AMR) of predominant bacteria and factors influencing microbial air quality were analyzed.

Results: Most air samples in the ORs (87.2%) and SRs (81.8%) contained acceptable fungal counts. However, unoccupied ORs (11.1%), occupied ORs (58.1%), and SRs (64.2%) had fewer samples with acceptable bacterial levels. The geometric mean (GM) bacterial load in the ORs before aerosol-generating procedures (AGPs) was 116.8±1.8 CFU/m^3, higher than after AGPs (58.9±2.1 CFU/m^3). After controlling potentially confounding factors, the factors influencing bacterial loads occupied OR were temperature before AGPs (0.164 CFU/m^3, 95%CI 0.017-0.311, p=0.029), the number of staff after AGPs (0.082 CFU/m^3, 95%CI 0.019-0.144, p=0.011), and using saw/drill device after AGPs (0.701 CFU/m^3, 95%CI -1.326-0.076, p=0.029). Predominant bacteria were Gram-positive cocci (90.8%), of which 20.5% were *S. aureus*. Most *S. aureus* (94.2%) were resistant to at least one drug, with 40.7% being multidrug-resistant. Additionally, 33.7% were methicillin-resistant *S. aureus* (MRSA).

Conclusion: The indoor air of ORs and SRs may be a source of AMR bacteria, particularly MRSA, a concern for surgical site infections. Maintaining ventilation, cleanliness, and minimizing non-essential staff activities are crucial for reducing airborne pathogen transmission.

Keywords: Aerosol *S. aureus*; orthopaedic operating rooms; MRSA; antimicrobial resistance (Siriraj Med J 2025; 77: 220-232)

INTRODUCTION

The indoor air quality of healthcare facilities or medical centres can be affected by numerous biological pollutants, giving rise to concerns about infection control and healthcare workers' health and well-being.[1,2] Biological contaminants, such as bacteria, fungi, and viruses, may be present in a wide range of healthcare environments and also infected patients, and can be transferred through room air, which could cause healthcare-associated infections (HAIs). Generally, exposure to these bioaerosols is associated with severe effects on people with a weakened immune system, not only patients but also healthcare workers and visitors.[2,3]

Surgical site infections (SSIs) are costly HAIs that can involve prolonged hospitalization, readmission, reoperation, and increased diagnostic and medical costs.[4,5] In Thailand, one study found that the reported incidence of SSIs in a university hospital between 2007 and 2016 showed a decreasing trend, with an average incidence of 2.98%; however, severe problems were observed, including significantly increased hospital costs of around US $5,509 and extra hospital stays of 24 days compared with non-SSI patients.[6] SSIs are generally most evident in orthopaedic surgery[7], especially operations with aerosol-generating procedures (AGPs), such as hip and knee arthroplasties[8], hip fracture surgery[9], internal fixation of fractures[10], and spinal surgery.[11] The incidence of orthopaedic SSIs has been reported to be as low as 1.9% and up to 22.7% in different studies, and can cause diverse adverse effects on patients, including health implications, long-term disability, financial loss, and increased mental stress.[4,12] Therefore, SSI prevention is a priority in healthcare facilities, with especially strong attention needed in orthopaedic surgery units, and consequently, SSI prevention is included as a surgery quality indicator in many facilities.[13]

The microbial air quality in the orthopaedic operating room (OR) is considered to represent an associated risk for SSIs, particularly for clean operations.[14-18] One report stated that airborne transmission accounted for 20%–24% of SSIs.[19] The microbes in bioaerosols can fall directly into a wound, or may land on the exposed surfaces of instruments or the staffs' hands, and then may be transferred into the wound.[20] Implementing unidirectional airflow could ensure an acceptable bacterial load and lower the risk of SSIs compared with in the case of turbulent (mixing) airflow in orthopaedic ORs.[21] Many factors can affect the presence, concentrations, and diversity of bioaerosols in hospital environments, including the season, temperature, humidity, working activities, frequency of door closing and opening, behaviors of the surgical staff, and the surgical procedures.[20,22,23] Moreover, microbial contamination on the floor, walls,

and high-touch surfaces in orthopaedic ORs may be important areas of microbial air contamination.[24,25] There is also evidence that airborne particles could be dispersed from staff walking on a contaminated floor in an orthopaedic OR.[26] Many previous studies have reported different varying levels of microbial contamination during surgical activities, consistently finding the lowest values under "at rest" conditions and the highest values under "in operation" conditions. Additionally, the indoor air quality before the beginning of operations reflects the effectiveness of the ventilation system and cleanliness in OR.[18,22] However, the duration of the surgical activities, both pre-incision and post-incision, can increase risk factors for microbial air contamination in OR.[17,25]

The most common microorganisms involved in SSIs after orthopaedic procedures are *Staphylococcus aureus*, including methicillin-resistant *S. aureus*, coagulase-negative Staphylococci (CoNS), including methicillin-resistant CoNS, and *Escherichia coli*. These pathogens are responsible for several SSIs and usually originate from the patient's commensal flora and from exogenous microbial contamination in the air environment of ORs.[27] Moreover, there is much evidence that these aerosol bacteria have the potential for prolonged survival on the surfaces of items, equipment, and in the air in ORs.[24,25,28-30]

In 2018, the Ministry of Public Health of Thailand promoted policies and practices referring to "2P safety goals" (patient safety and personal safety) for ensuring a sustainable healthcare system that corresponds to the World Health Organization's Strategy for Patient Safety.[31] Orthopaedic ORs are important hospital areas that require a clean environment and good hygiene practices for preventing not only serious SSIs but also healthcare workers' exposure to infectious agents that may be found both on inanimate surfaces and equipment and in the indoor air environments occupied by colonized persons or infected patients. As indoor air quality is critical to patient safety in hospitals, the World Health Organization (WHO) recommended maximum guideline values for hospital areas at 100 CFU/m^3 for bacteria and 50 CFU/m^3 for fungi.[32] Microbial air monitoring in orthopaedic ORs and nearby areas is an important control measure for ensuring the safety of both patients and staff. Despite most centers being aware of the issue, the determination of microbial air contamination in orthopaedic ORs is still rarely performed. This study aimed to assess the air microbial load in orthopaedic ORs and SRs in operating units in a tertiary hospital, as well as the burden of antimicrobial-resistant (AMR) aerosol bacteria, and the factors associated with bacterial air contamination in orthopaedic ORs.

MATERIALS AND METHODS

Study design and sampling

This laboratory-based cross-sectional study was conducted with air samples collected in ORs and SRs between July and September 2022 in orthopaedic operating units of a tertiary hospital, in Bangkok, Thailand. For the ORs, 187 air samples were collected from seven selected ORs, with the samples collected in both unoccupied and occupied ORs. In the unoccupied ORs, the air samples were collected before working hours (7.00 am to 8.30 am). In the occupied ORs, the air samples were collected during working hours (9.30 am and 3.00 pm), and were collected before the incisional (before the AGPs) and wound-closing (after the AGPs) stages in each operation. For the SRs, 165 air samples were collected from six types of SRs, namely scrub rooms (SCs), induction rooms (IDs), sterile storeroom (ST), X-ray room (XR), specimen room (SP), and office room (OF). For these, the air samples were collected before working hours between 7.00 am to 8.30 am (unoccupied SRs) and after the AGPs in the ORs (occupied SRs).

The HVAC system in the ORs utilizes high-efficiency particulate air (HEPA) filters, ensuring optimal air quality. These ventilation systems achieve a minimum of 20 air changes per hour (ACH) while providing 100% fresh air. Vertical laminar airflow systems effectively direct particle-free air over the aseptic surgical field. Regular maintenance of air conditioning equipment is performed, and filter units are replaced on time. Each OR shares similar structure designs, featuring two entry points: one from the corridor and another from the scrub room, with an additional door opening to the sterile storeroom. During the workday in ORs, the floor, equipment, and environmental surfaces are cleaned and decontaminated before each case. Throughout surgical procedures, the OR doors remain closed, except when extra equipment is needed or staff members must pass through. Following the last surgical case of the day, routine cleaning is conducted. The SRs are equipped with a turbulent airflow HVAC system providing 6 ACH, which does not utilize HEPA filters.

Air sampling and data collection

Air samples were collected using a single-stage Andersen air sampler (Bio-Stage Single-stage Impactor, SKC, Inc., USA) at a flow rate of 28.3 litres per minute (LPM) for 5 minutes, as recommended in the NIOSH-0800 instructions.[33] To achieve sampling the breathing zone, the air sampler was placed one metre above the floor level and one metre away from the surgical table in the ORs, while in the SRs, the air sampler was placed one

metre above the floor level and at the centre of each room. For each air sampling site, one air sample was collected onto blood agar (BA) for bacterial cultivation, and another onto potato dextrose agar (PDA) for fungal cultivation. The bacterial and fungal sampling plates were incubated at 37 °C for 24–48 hours and at 25 °C for 5–7 days, respectively. Total bacterial and fungal colonies were counted manually, and the number of the microbial count was calculated and reported as colony-forming units per cubic metre (CFU/m^3), as described in a previous study.[34]

Bacterial colonies were presumptively identified by their morphology and Gram stain, and then biochemical tests were conducted for suspected *Staphylococcus* spp., which have been found to be the dominant type. The identified *S. aureus* was subjected to antimicrobial susceptibility tests using the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid, UK) against eleven antibiotics, with the test organism adjusted to 0.5 McFarland turbidity standards. The susceptibility test results were interpreted according to the Clinical Laboratory Standard Institute (CLSI).[35] *S. aureus* ATCC25923 was used as a control bacteria strain for quality control for the susceptibility tests.

Determination of the factors affecting the bacterial load in the occupied operating rooms

During the air sampling, the air bacterial load-related factors in the orthopaedic ORs were recorded on the sample collecting form. These factors comprised the physical factors (temperature, and relative humidity), operative procedure-related factors (e.g., types of procedure, perioperative activities, size of incision, types of AGPs, operative time of the procedure, type of surgical devices, extra special instruments), number of staff, and frequency of door opening during surgery.

Statistical analyses

IBM SPSS (SPSS Inc., Chicago, USA) was used for all the statistical analyses. Descriptive statistics were employed to explore the physical and microbial air quality, and microbial load in the air samples, and are reported in terms of the percentage, mean ± standard deviation (SD), median, and GM ± geometric standard deviation (GSD). The Mann–Whitney test, independent t-test, and ANOVA were used to compare the air quality and microbial loads among the different sets of data. Univariate and multivariate linear regression analyses were used to analyze the relationship between the bacterial load (continuous variable, measured in CFU/m^3) and the associated factors by controlling for potential confounding variables (temperature, relative humidity, number of staff, frequency of door opening, and operative time as continuous variables; perioperative activities, surgical devices, cloth, size of incision as categorical variables). Statistical significance was set at $p<0.05$.

RESULTS

Air quality in the orthopaedic operating unit

A total of 352 indoor air samples were collected: 187 samples in ORs and 165 samples in SRs. The mean temperature and relative humidity (RH) during air sampling differed between the ORs and SRs, with values of 21.1±1.4 °C and 54.6±4.0% in the ORs, and 22.3±1.1 °C and 57.2±4.7% in the SRs, respectively. The mean temperature in the unoccupied ORs was highest at 23.4±2.0 °C, which was significantly higher than before and after the AGPs ($p<0.05$). Similarly, the mean temperature in the unoccupied SRs (23.0±1.1 °C) was significantly higher than in the occupied SRs (21.9±0.9 °C) ($p<0.05$). Additionally, the mean RH in the unoccupied SRs was significantly lower (56.0±5.7%) than in the occupied SRs (57.8±4.0%) ($p<0.05$). In the overall comparison, the mean air temperature and RH in the ORs were significantly lower than in the SRs ($p<0.05$) (Table 1).

The air quality assessment in the ORs showed that the median aerosol fungal count in the unoccupied ORs, and in the ORs before AGPs and after AGPs were 3.5, 0, and 0 CFU/m^3, respectively. Of these, the percentages of air samples with an acceptable fungal count (<10 CFU/m^3) were 77.8%, 86.3%, and 91.3% in the unoccupied ORs, and in the ORs before AGPs and after AGPs, respectively. Using the bacterial count as an indicator, a significantly higher median bacterial count was evidenced before AGPs (120.1 CFU/m^3) compared with that after AGPs (65.4 CFU/m^3) and in the unoccupied ORs (28.3 CFU/m^3). These indicated there was a lower percentage of acceptable aerosol bacterial counts before AGPs (36.3%) compared with after AGPs (80.0%). However, the lowest percentage of the acceptable bacterial count (11.1%) was detected in the unoccupied ORs, where a lower acceptable level of <10 CFU/m^3 is recommended compared with <100 CFU/m^3 for occupied ORs, as detailed in Table 1.

Regarding the microbial air quality in the SRs, the median fungal count in the unoccupied SRs (21.2 CFU/m^3) was significantly higher than that in the occupied SRs' air samples (14.1 CFU/m^3). The percentages of acceptable fungal counts were 78.2% for the unoccupied SRs and 83.6% for the occupied SRs. According to the aerosol bacterial count assessment, the median bacterial counts in the unoccupied SRs (91.9 CFU/m^3) and occupied SRs (84.8 CFU/m^3) detected at an acceptable level were 60.0% and 66.4%, respectively.

TABLE 1. Air quality measurement in orthopaedic operating units.

Variable/ Air quality indicator	Operating rooms (N= 187)			*p*-value	Surrounding rooms (N=165)		*p*-value
	Unoccupied (n=27)	Occupied Before AGPs (n=80)	After AGPs (n=80)		Unoccupied (n=55)	Occupied (n=110)	
Temperature (ºC)							
Mean (SD)	23.4 (2.0) **	20.9 (0.9)	20.6 (0.8)	<0.001	23.0 (1.1) *	21.9 (0.9)	0.014
MIN-MAX	18.1-25.3	18.5-23.7	18.4-22.7		20.1-25.3	19.4-24.4	
Relative humidity (%)							
Mean (SD)	54.8 (5.1)	54.4 (3.8)	54.8 (3.8)	0.804	56.0 (5.7) *	57.8 (4.0)	0.02
MIN-MAX	47.0-66.0	46.0-65.0	46.0-64.0		48.0-79.0	50.0-69.0	
Bacterial count (CFU/m³)							
Acceptable#, N (%)	3 (11.1)	29 (36.3)	64 (80.0)		33 (60.0)	73 (66.4)	
Median	28.3[a]	120.1[b]	65.4[c]	<0.001	91.9	84.8	0.692
25th percentile	21.2	86.6	35.3		56.5	49.5	
75th percentile	63.6	169.6	97.2		130.7	114.8	
MIN-MAX	7.1-212.0	14.1-515.9	7.1-282.7		7.1-438.2	0-2572	
Fungal count (CFU/m³)							
Acceptable#, N (%)	21 (77.8)	69 (86.3)	73 (91.3)		43 (78.2)	92 (83.6)	
Median	3.5	0.0	0.0	0.157	21.2	14.1	0.033
25th percentile	0.0	0.0	0.0		7.1	7.1	
75th percentile	7.1	7.1	7.1		42.4	28.3	
MIN-MAX	0-63.6	0-215.6	0-148.4		0-212.0	0-848.1	

**ANOVA test, p<0.05 compared between unoccupied, before AGPs, and after AGPs; * t-test, p<0.05 compared unoccupied and occupied; [a,b,c] Mann-Whitney test, p<0.05

#European Union Good Manufacturing Practice (EU GMP) for cleanroom recommended microbial count "acceptable" in unoccupied operating room ≤ 10 CFU/m³ and occupied operating room ≤ 100 CFU/m³ for bacteria and ≤10 CFU/m³ for fungal count, while in surrounding rooms bacterial count ≤ 100 CFU/m³ and fungal count ≤ 50 CFU/m³ for unoccupied and occupied rooms.

Abbreviations: AGPs, aerosol-generating procedures; GM, geometric mean; GSD, geometric standard deviation,

Aerosol microbial load in the ORs

The microbial load in the air samples that were fungal positive (n=55) and bacterial positive (n=160) collected in the occupied ORs was analyzed and the GM of the microbial load was compared for different AGP procedures, as shown in Table 2. The GM of the fungal load in the occupied ORs (before and after AGPs) showed non-significant differences in each surgical procedure (p>0.05). However, the overall GM of the bacterial load in the occupied ORs collected before AGPs (116.8±1.9 CFU/m³) was significantly higher than after AGPs (58.9±2.1 CFU/m³) (p<0.05). Specifically, the GM of the bacterial load in samples collected before AGPs was significantly higher than that after AGPs for each of the AGPs (p<0.05), of which the highest aerosol bacterial load (152.5±1.5 CFU/m³) was found in the air sampled before the spinal laminectomy procedure, as shown in Table 2.

Aerosol microbial load in SRs

The microbial load in the occupied SRs was analyzed and compared between different types of SRs. A total of 87 air samples collected in the occupied SRs were found to be fungal positive with a GM of 20.2 CFU/m³. The highest GM fungal load was observed in the sterile storeroom (44.7±3.4 CFU/m³). In comparison, the GM fungal loads in the sterile storeroom and office room were significantly higher than in the induction

TABLE 2. Microbial load in air sample collected in occupied ORs with different operating procedures.

Microbial load	Total		TKA	SL	ALR	IFI	*P*-value
Fungal load (CFU/m³)							
Before AGPs							
No. of positive samples (%)	30	(100)	8 (26.7)	7 (23.3)	9 (30.0)	6 (20.0)	
GM (GSD)	10.7	(2.4)	7.4 (1.8)	14.5 (4.0)	11.1 (1.8)	11.4 (2.2)	0.532
After AGPs							
No. of positive samples (%)	25	(100)	7 (28.0)	10 (40.0)	5 (20.0)	3 (12.0)	
GM (GSD)	9.7	(2.3)	7.5 (2.5)	8.8 (1.6)	11.2 (2.0)	19.5 (5.8)	0.421
Bacterial load (CFU/m³)							
Before AGPs							
No. of positive samples (%)	80	(100)	20 (25.0)	20 (25.0)	20 (25.0)	20 (25.0)	
GM (GSD)	116.8*	(1.9)	140.9 (1.6)*	152.5 (1.5)*	106.4 (1.6)*	81.5 (2.5)*	0.005
After AGPs							
No. of positive samples (%)	80	(100)	20 (25.0)	20 (25.0)	20 (25.0)	20 (25.0)	
GM (GSD)	58.9	(2.1)	59.6 (2.2)	75.3 (1.9)	61.4 (2.2)	43.7 (1.9)	0.128

* t-test, p<0.05 compared before and after AGPs each procedure

Abbreviations: AGPs, aerosol-generating procedures; GM, geometric mean; GSD, geometric standard deviation; ALR, arthroscopic ligament repair; IFI, internal fixation with implant; SL, spinal laminectomy; TKA, total knee arthroplasty.

rooms, scrub rooms, and x-ray room (p<0.05). Among the bacterial-positive air samples collected in the occupied SRs (n=109), the highest GM bacterial load was observed in the scrub rooms (93.4±2.1 CFU/m³), followed by the sterile storeroom (89.8±2.6 CFU/m³), specimen room (85.9±1.3 CFU/m³), and induction rooms ((77.5±2.0 CFU/m³), with a non-significant statistical difference. However, these rooms contained a significantly higher bacterial load than the X-ray room and office room (p<0.05) (Table 3).

Factors influencing the indoor air bacterial load in the ORs

The independent factors influencing bacterial loads during surgeries were the indoor air temperature before AGPs (0.164 CFU/m³, 95%CI 0.017-0.311, p=0.029), indoor air temperature after AGPs (0.218 CFU/m³, 95%CI 0.011-0.425, p=0.039), number of staff (0.075 CFU/m³, 95%CI 0.006-0.144, p=0.033), used saw/drill devices (0.526 CFU/m³, 95%CI-0.873-0.179, p=0.003), used midas/burr equipment(0.420 CFU/m³, 95%CI 0.031-0.809, p=0.035), and size of incision (0.343 CFU/m³, 95%CI 0.033-0.654, p=0.030). After controlling for potentially confounding factors for the bacterial load in the ORs using multiple linear regression analysis, the result exhibited a statistically significant correlation between the aerosol bacterial loads before AGPs and temperature (R^2=4.8%, p=0.029). Moreover, the aerosol bacterial load after AGPs was correlated with the number of staff, and use of saw/drill devices (coefficient of determination, R^2=15.70% p=0.031) (Table 4).

Dominant aerosol bacterial and antimicrobial resistance in the orthopaedic operating unit

A total of 925 bacterial colonies were isolated and identified from the blood agar air samples. Gram-positive cocci were dominantly observed, accounting for 90.8% of the species (840/925). Of these, CoNS accounted for 49.8% (418/840), followed by *Micrococcus* spp. (29.8%, 250/840), and *S. aureus* (20.5%, 172/840). The antimicrobial susceptibility tests were performed on 172 *S. aureus* isolates that were isolated from the ORs (n=98) and SRs (n=74). The results indicated high rates of AMR among the *S. aureus* isolates, of which, 94.2% (162/172) of the isolates demonstrated resistance to at least one tested antimicrobial agent, with high rates of resistance to erythromycin (73.8%, 127/172) and penicillin (61.0%,105/172), as detailed in Table 5. Furthermore, 40.7% (70/172) and 33.7% (58/172) of the AMR aerosol *S. aureus* were defined as multidrug-resistant strains and methicillin-resistant *S. aureus* (MRSA).

TABLE 3. Microbial load in air sample collected from occupied SRs.

Microbial load	Total	SC	ID	ST	XR	SP	OF	P-value
Fungal load (CFU/m³)								
No. of positive samples (%)	87 (100)	29 (33.3)	22 (25.3)	24 (27.6)	4 (4.6)	4 (4.6)	4 (4.6)	
GM (GSD)	20.2 (2.9)	12.7 (2.2)	15.5 (2.2)	44.7 (3.4)	10.0 (1.5)	24.3 (1.9)	36.6 (3.6)	<0.001
Bacterial load (CFU/m³)								
No.of positive samples (%)	109 (100)	39 (35.8)	27 (24.8)	32 (29.3)	4 (3.7)	3 (2.7)	4 (3.7)	
GM (GSD)	81.8 (2.3)	93.4 (2.1)	77.5 (2.0)	89.8 (2.6)	31.7 (1.4)	85.9 (1.3)	23.4 (2.6)	0.01

Abbreviations: AGPs, aerosol-generating procedures; GM, geometric mean; GSD, geometric standard deviation; ID, induction room; OF, office room; SC, scrub room; SP, specimen room; ST, sterile storeroom; XR, x-ray room

TABLE 4. The factors associated with bacterial load in occupied operating rooms using linear regression analysis.

	Univariate analysis			Multivariate analysis#		
Variables	**Estimate**	**95% CI**	**p-value**	**Estimate**	**95%CI**	**p-value**
Bacterial load of before AGPs (CFU/m³)						
Temperature (ºC)	0.164	0.017-0.311	0.029	0.164	0.017-0.311	0.029*
Relative humidity (%)	0.022	-0.015-0.059	0.244			
General anesthesia	-0.066	-0.348-0.216	0.642			
Regional anesthesia	0.103	-0.204-0.410	0.507			
Number of staff (n)	-0.011	-0.070-0.049	0.724			
Hair removal	-0.262	-0.685-0.161	0.221			
Bacterial load of after AGPs (CFU/m³)						
Temperature (ºC)	0.218	0.011-0.425	0.039	0.177	-0.018-0.372	0.075
Relative humidity (%)	0.032	-0.011-0.075	0.140	0.036	-0.005-0.076	0.087
Number of staff (n)	0.075	0.006-0.144	0.033	0.082	0.019-0.144	0.011*
Saw/drill devices	-0.526	-0.873-0.179	0.003	-0.701	-1.326-0.076	0.029*
Midas/burr equipment	0.420	0.031-0.809	0.035	-0.401	-1.087-0.286	0.248
Bovie	-0.277	-0.951-0.396	0.415			
Irrigation	-0.462	-1.130-0.206	0.173	-0.623	-1.281-0.035	0.063
Fluoroscope	-0.193	-0.521-0.135	0.246			
Frequency of door opening	-0.019	-0.042-0.004	0.098	-0.010	-0.032-0.012	0.387
Operative time (mins)	-0.001	-0.003-0.001	0.355			
Size of incision	0.343	0.033-0.654	0.030	0.256	-0.040-0.552	0.089
Type of gown	0.072	-0.300-0.444	0.701			

#The risk factors with P-value <0.2 by univariate analysis were included into multivariate analysis;
*significant with P-value <0.05
Abbreviations: AGPs, aerosol-generating procedures; CI, confidence interval

TABLE 5. Antimicrobial drug resistance of *S. aureus* isolates from orthopaedic operating unit (n=172).

Antimicrobial agent	PEN	FOX	GEN	ERY	TET	CIP	CLI	LIN	CHL	RIF	SXT
Number of isolates (%)											
Resistance	105 (61.0)	58 (33.7)	8 (4.7)	127 (73.8)	23 (13.4)	14 (8.1)	75 (43.6)	2 (1.2)	7 (4.1)	7 (4.1)	14 (8.1)
Susceptible	67 (39.0)	114 (66.3)	164 (95.3)	45 (26.2)	149 (86.6)	158 (91.9)	97 (56.4)	170 (98.8)	165 (95.9)	165 (95.9)	158 (91.9)

Abbreviations: FOX, cefoxitin; CIP, ciprofloxacin; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; LIN, linezolid; PEN, penicillin; RIF, rifampin; TET, tetracycline; SXT, trimethoprim/sulfamethoxazole

DISCUSSION

This study evaluated the aerosol bioburden in ORs and SRs located in operating units of a tertiary hospital using culturable fungus and bacteria as indicators. Because of the budget and time constraints of this study, the total air sample of 187 was determined by randomly collecting, ensuring coverage of all four types of interested AGPs performed in the seven operating rooms in the study location. In the study of aerosol fungus in the ORs, the air contamination was found to have low fungal counts in both the unoccupied ORs (median=3.5 CFU/m^3) and occupied ORs (median=0 CFU/m^3). Of these samples, 87.2% of the total air samples in the ORs had acceptable fungal counts that were considered very clean according to the standard recommended by EU good manufacturing practice (GMP) for cleanrooms (≤ 10 CFU/m^3). Of note, the air fungal count was the highest at 215.6 CFU/m^3 in the air sample collected before the AGPs for a spinal laminectomy procedure. In our observation, this high fungal count may be affected by the activity occurring before the AGPs, such as the more complex patient preparations, use of certain instrumental techniques, and the involvement of a large surgical team for the operation. In addition, the complicated internal fixation with implant procedure resulted in the highest fungal count of 148.4 CFU/m^3 in the air samples collected after the AGPs. However, the aerosol fungal loads during surgeries (before and after the AGPs) of each procedure did not show statistically significant differences. Generally, indoor fungal spores can originate from outdoor air exposure and are uncommonly found in closed and cleaned areas such as OR. In this study, a low fungal load was observed in the ORs, except for occasional high fungal counts in a few air samples collected while company technicians were in the ORs preparing special surgical instruments. The high fungal load observed in this study may not have been affected by routine activities and showed no significant difference before and after AGPs. This observation is evidence to recommend limiting non-healthcare personnel in ORs. In some parts of the SRs, the median fungal count in the unoccupied SRs (21.2 CFU/m^3) was significantly higher than that of the occupied SRs (14.1 CFU/m^3). A high percentage of 81.8% of the total air samples had an acceptable fungal count ≤ 50 CFU/m^3, which is considered very clean according to the standard recommended by EU GMP for cleanrooms. In occupied SRs, it was observed that the GM aerosol fungal load in each SR was lower than that for the unoccupied SRs (data not shown), except for the occupied sterile storeroom, which had the highest fungal load (44.7±3.4 CFU/m^3). The higher aerosol fungal load in the sterile storeroom during working hours may be affected by the greater number of people entering the storeroom compared with before working hours. This study also observed a higher relative humidity (RH) in the occupied sterile storeroom (56.9%) compared with the unoccupied room (54.5%). The higher RH may serve as the optimal fungal growth condition as it was previously reported that the concentration of fungi was correlated with the temperature and humidity.[36] Moreover, the high fungal load in the air samples may be due to the resistance of fungal spores that can survive and grow under stress conditions, such as dehydrated and moisturized ambient air, as well as under UV radiation.[19]

In comparison to fungal contamination, the aerosol bacterial counts in the ORs showed heavier contamination. Only 58.1% of the air samples collected from the occupied ORs had acceptable bacterial levels ≤ 100 CFU/m^3 and could be considered clean according to the standard recommended by EU GMP for cleanrooms. Furthermore, 11.1% of the air samples collected from the unoccupied ORs had a very low acceptable bacterial count of ≤10

CFU/m^3, which is considered very clean according to the standard recommended by the EU GMP for cleanrooms. This study revealed that the overall bacterial load in air samples collected before AGPs was significantly higher than after AGPs. High GM aerosol bacterial loads were observed before the spinal laminectomy procedure (152.5 CFU/m^3), total knee arthroplasty (140.9 CFU/m^3), and arthroscopic ligament repair (106.4 CFU/m^3). These results indicate that the indoor air bacterial load during the pre-incisional period of patient preparation was higher than the standard recommendation (≤100 CFU/m^3). This high bacterial load may be affected by the activity of the surgical teams, in particular their many movements and use of several instruments, especially surgical teams in critical operations. There is evidence that the behaviours of the surgical team during patient preparation before AGPs, such as surgical planning, staff postures, door opening, removing cloth sheets, and handling surgical tools, are associated with increasing the bacteria load in the air.[37] Staff, visitors, and patients are potential sources of aerosol bacteria as they shed the aerosolized bacteria from the skin and respiratory tract via walking, talking, and coughing.[22,38] Crowd traffic during the pre-operation stage can also harm the indoor air quality by interrupting the airflow and ventilation in ORs, potentially resulting in a heavy aerosol bacterial load, as demonstrated in a previous study.[22] This study also investigated the bacterial load after different procedures and found the spinal laminectomy procedure had the highest GM of 75.3 CFU/m^3, followed by arthroscopic ligament repair (61.4 CFU/m^3), total knee arthroplasty (59.6 CFU/m^3), and internal fixation with implantation (43.7 CFU/m^3). During operations, aerosol bacteria may settle down directly on the surgical wound, or surrounding surfaces, which may then be indirectly transmitted to the wound by the surgeon's hands and/or devices.[14] It was noted that the GMs for the bacterial loads after these AGPs were lower than the recommended threshold. In the present study, lower activities and lower numbers of surgical staff were observed during the air sampling after AGPs. This indicated that decreasing staff activities during operation may be an effective measure for lowering the aerosol bacteria for improving patient safety. However, keeping a highly efficient laminar airflow is also a crucial measure for preventing SSIs.[21]

Orthopaedic surgery involves making a large incision into deep tissue or organ space and presents specific concerns for postoperative SSI due the significant factors that must be considered for airborne pollutants that may impact patient safety. Differences in aerosol bacterial loads have been reported to be associated with the highest levels of activity among surgical teams in spinal laminectomy.[25]

Regarding the aerosol bacterial contamination in SRs, 64.2% of the total air samples were acceptable with a bacterial count of ≤100 CFU/m^3, which is considered clean according to the standard recommended by EU GMP for cleanrooms. The lowest bacterial load of 36.1 CFU/m^3 was observed in the unoccupied X-ray room, while the unoccupied specimen room had the highest bacterial load of 189.7 CFU/m^3. The higher aerosol bacterial load in the specimen room before working hours may be affected by its smaller volume than other areas, and need to keep the door closed to protect the aerosolized volatile substances, which results in poor air ventilation and a high RH (59.0%), as also recorded in this study. This study also revealed that the bacterial loads in the occupied scrub rooms (93.4 CFU/m^3) and sterile storeroom (89.9 CFU/m^3) were higher than during no-staff activities in the early morning. There was evidence that the microbial indoor air quality may be affected by external and internal sources, including the numbers of staff, crowds of visitors, polluted outdoor air, and poor ventilation, which can all increase the aerosol bacterial density.[36,39] This study was only a short-term assessment of indoor air quality and aerosol microbial contamination in a closed OR area located in the central city without seasonal factor, that many studies have reported that seasonal variation showed no significant difference in fluctuations of bacterial density in OR.[38,40,41]

This study investigated the factors associated with the indoor air bacterial load using linear regression analysis. The univariate analysis and multivariate analysis showed that the temperature during perioperative periods (before AGPs) had a significant positive correlation with the bacterial load in ORs (p<0.05). This finding agrees with previous studies that reported a correlation between the temperature and indoor air bacterial load in surgical rooms[42], and surgical and medical wards.[43] After adjusting potential confounding factors, the results revealed that the temperature and number of staff were significantly correlated with an increase in bacterial load before and after AGPs, respectively, whereas the use of saw-drill instruments showed significant correlation with a decrease in bacterial load after AGPs. Among these associated factors, several reports have evidenced that the temperature and the number of staff can negatively affect the indoor air bacterial load.[37,42,44] Several staff members and their behaviors, including opening and closing the OR doors, foot traffic, and entering or exiting the room, led to a changed airflow pattern and created pressure differences in ORs, which caused microbial particle contaminants

to enter the ORs.[45] The correlation between an open or deep incision site and the concentrated bacterial load in this study may be due to the requirement for more sewing of tissue and muscle layers, and the need for more assistant staff for these complicated operations.[39] These results confirmed that several staff members in the operation room are at potential risk of indoor air bacterial load. Although saws and drills are surgical devices that generate an aerosol, larger- and medium-sized particles are predominantly produced by oscillating saws and drills, while lesser small-sized particles are generated by oscillating saws (28%–40%), high-speed air-powered drills (17%), and high-speed drills with irrigation (9%).[46,47] In a simulation of AGPs in the OR, the dispersal of body fluid was observed to have the highest particle count during bone cutting. These particles fly up and hover in the air for a short period and then slowly move under the direction of laminar airflow. Ensuring laminar airflow with a HEPA filter may be a crucial control measure for preventing occupational contact with infectious body fluids during AGPs in ORs.[48] According to several studies conducted in operating rooms, a general correlation exists between the total microbial count and the risk of infection. However, there are discrepancies in findings among different studies, which can be attributed to several factors, including the surveillance method (active or passive air sampling), sampling time (during surgery or at rest), ventilation system of the ORs, adequacy of room cleaning, and type of disinfectants used.[48]

This study found that 90.8% of the isolated bacteria present in indoor air environments were Gram-positive bacteria. Of these, 49.8 % were CoNS, which arose from the normal flora of the skin and mucous membranes, and 20.5% were *S. aureus*, which is a common SSI causative bacteria. Gram-positive bacteria possess a thick cell wall and exhibit higher resistance compared to Gram-negative bacteria, enabling them to survive under adverse environmental conditions and in closed environments.[45] Gram-positive bacteria, particularly *Staphylococcus* spp., are more frequently found in hospital indoor air and are considered as a bacterial indicator of indoor air pollution.[34,36] Reasonable explanations for the predominantly widespread nature of Gram-positive bacteria in hospital environments are their resistance to dry conditions and frequent transmission through a variety of reservoirs, including human (skin, nasal cavity, cloth, and boils of healthcare workers, patients, and visitors), and environmental surfaces (high touch surfaces, floors, walls, ceilings, and doors).[49]

The WHO declared *S. aureus* and CoNS species as major contaminants in the air of patients rooms, floors, and other surfaces in ORs. Based on our findings, CoNS was more frequently found as an aerosol than *S. aureus*, which is consistent with a study by Shaw LF *et al.*[37] However, studies in Ethiopia[44], Iran[36], and Nigeria[42] showed that *S. aureus* was predominant, followed by CoNS. *S. aureus* and CoNS are potential pathogens that can cause skin and soft tissue infections and are recognized as important causes of SSIs. Therefore, the presence of these pathogens, particularly *S. aureus*, in OR indoor air could indicate a significant risk of SSI from airborne bacteria. There is evidence that these airborne particles with viable bacteria released from the surgical team members and patients can settle onto surfaces, including surgical wounds and instruments.[18,21,46] Therefore, the OR should be regularly cleaned and disinfected, including all surfaces, after every operation. Integrated hygienic control measures in ORs and the medical operatives are essential, not only wearing personal protective equipment but also following environmental cleaning guidelines, including a hand hygiene regimen, and limiting the number of personnel in the room as these may generate bioaerosols in the indoor air.

According to the reported SSIs causative bacteria after orthopaedic surgeries, *S. aureus* accounts for a high prevalence, including a high level antimicrobial resistance rate.[51] The observed high resistance of *S. aureus* to penicillin (61%) and erythromycin (73.8%) in our study aligns with global trends, possibly attributed to the penicillin-resistant strains.[52] Moreover, this study also found MRSA that exhibited resistance to cefoxitin (58/172, 33.7%) and was distributed in the indoor air in both the ORs and SRs. This finding is at a higher percentage than that reported in another hospital in Thailand, where MRSA was observed in 2.3% of indoor air samples and on 0.5% of surface samples.[53] The frequency of MRSA in the nasal carriage of surgical patients was also reported to be 0.3%–9.4%.[54] In general, MRSA has a lower prevalence in non-human isolates than in human isolates. However, several studies have reported a high prevalence of MRSA among environmental surfaces, and in sand/water samples. A previous study found that 44.7% of *S.aureus* isolated from hospital surfaces were identified as MRSA, that was higher than aerosol isolates observed in this study.[51] In contrast, some studies indicated a low prevalence of MRSA ranging from 0.3%–3.5% in non-human samples of non-hospitals. In global epidemiology, MRSA is classified in the group 2 priority list of antibiotic-resistant bacteria, with varying prevalence rates globally, including 19.1% in Africa, 31.9% in the Americas, 17.4% in the Mediterranean, 67.9% in Europe, 27.3% in Southeast Asia, and 43.2% in the Western

Pacific.[47] Also, the prevalence of MRSA colonizers was revealed to be 12.0% in India, 15.6% in Africa, and 1.6% in Europe[55] among healthcare workers. Several reasons may explain the variation in the prevalence of MRSA in non-human samples, including differences in the sampling period, sample size, sampling site, sampling techniques, isolation method, single enrichment step, frequency of MRSA in different samples, or geographical locations.[56]

The high prevalence of MRSA and the emergence of MDR in OR settings require careful attention and is an alarm that the surveillance of *S. aureus* and MRSA colonizers in surgical patients and healthcare workers, especially surgical teams, should be included in infection control measures. Mastering these methods necessitates a strong focus on hospital hygiene training, including hand hygiene and recommendations for surgical attire (such as shirt, trousers, mask, cap, and gloves), the use of personal protective equipment, and adherence to regulatory requirements.[51] The hospital's standard manual cleaning protocol for OR involves using a combined cleaner and disinfectant.[57] Using portable air cleaning technologies[58], and ultraviolet (UV) light, aims to ensure clean air and reduce viable airborne microorganisms in the OR. However, its disadvantages include high costs, potential gas irritation, and degradation and discoloration of certain surface materials, which may pose risks in the OR environment.[57] Thus, we recommend extending the determination of the susceptibility profile to all bacteria associated with air contamination and developing their genetic characteristics so that we can trace back or identify the MRSA reservoir and their mode of transmission.

CONCLUSION

These findings support that the indoor air in ORs and surrounding areas may serve as potential sources of AMR bacteria, posing a critical concern for SSI as a postoperative complication. The overall bacterial contamination rate in the air within the OR areas suggests ineffective cleaning and decontamination practices, disturbed airflow ventilation, and high activity levels. Maintaining a well-controlled ventilation system, ensuring environmental cleanliness, and minimizing non-essential staff activities are crucial for reducing the risk of airborne transmission of pathogens among surgical patients and healthcare workers. Applying integrated hygienic control measures in operative procedures with high compliance and also the surveillance of MRSA colonizers are essential for ensuring the safety of surgical patients and reducing the risk of SSIs.

Data Availability Statement

The data supporting this study are available upon request from the corresponding author.

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DECLARATION

All authors declare that they have no personal or professional conflict of interest.

Registration Number of Clinical Trial

None

Author Contributions

KB: Project administration, Sample collection and analysis, Data analysis, Writing original draft manuscript and editing. MR: Conceptualization and research design, Manuscript review and criticize. YU: Sample collection and analysis, Manuscript review and editing. JN: Research design, Data analysis, Manuscript review and editing. FU: Conceptualization and research design, Academic and methodology supervision, Manuscript criticism and revision.

Ethical Approval Statement

The present study was approved by the Standard Operating Procedures of Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University (MUPH 87/2022).

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