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Seven-Year Retrospective Study of Microbiological Surveillance of Air in Operation Theatres at a Tertiary Care Hospital

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ABSTRACT

Background: Operation theatres (OTs) are prone to microbial contamination, a leading cause of surgical site and nosocomial infections. *S. aureus* is a major pathogen, especially in implant-related infections. Contributing factors include poor hygiene, inadequate cleaning, improper attire, and overcrowding. In empty OTs, microbial limits should be <30 CFU/m³ for total bacteria and <1 colony of *S. aureus*. This study was conducted to evaluate the microbial contamination of air in the theatres of a hospital.

Methodology: The analysis of 7 years (2018-2024) of data that was obtained from the routine microbiological evaluation of multiple Theatres of the Dr Shankarrao Chavan Government Medical College, Nanded, Maharashtra. Settle plates of blood agar were kept open in different OTs one meter above the ground, one meter away from the wall for around one hour, (1:1:1) rule, incubated for 24 hours at 37°C, colonies confirmed by biochemical tests, catalase, coagulase, and microscopy.

Results: Out of 4402 total plates, 167 plates (3.7%) showed growth of *Staphylococcus aureus* (*S. aureus)* and other bacteria. Out of which 104 plates (62%) showed growth of *S. aureus*, and 63 plates (38%) showed growth of other bacteria. 4325 (98.3%) plates showed no growth. Maximum contamination was found in obstetrics and gynaecology OT(OBGY). Minimum colonies of S. aureus and other bacteria were found in the block room.

Conclusion: Our study shows the air quality of our OTs is quite satisfactory within permissible limits. The settle-plate method is a very cost-effective and useful method for the surveillance of air in OTs.

Key-words: Nosocomial-infection, Contamination of operating theatres (OTs), Settle-plate, Staphylococcus aureus, Surgical-site

INTRODUCTION

Contamination of operating theatres (OTs) is one of the major sources of surgical-site infection and nosocomial infection for patients [1,3]. These infections pose significant risks, especially for immunocompromised individuals and those undergoing invasive surgical procedures.

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Evaluation of the level of air microbial contamination in operation theatres is a basic step toward prevention, as it provides insight into the environmental hygiene and efficacy of current infection control protocols [1]. Surgical procedures, conducted within the controlled environment of OTs, represent a critical aspect of modern healthcare and require a sterile field to minimize the risk of infection [2]. However, maintaining sterility remains a persistent challenge due to various environmental and human-related factors.

Multiple reservoirs have been reported responsible for the contamination of OT, depending on the number of pathogens involved [4]. These reservoirs include airborne particles, inadequately sterilized instruments, healthcare personnel, and surfaces within the operating

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environment. *S. aureus* is one of the major pathogens associated with infection of implantable biomedical devices. Its prevalence has been attributed to factors such as improper cleaning procedures, inadequate OT dressings including contaminated footwear, unfiltered or poorly ventilated air, the presence of excessive personnel during surgery, and overall unhygienic practices within the facility [3]. In a standard empty OT, the acceptable microbial limits should not exceed more than 30 colonies of other bacteria and less than one colony of *S. aureus* to ensure a safe environment for surgical procedures [3,6].

Approximately 10% of all infections acquired in healthcare settings can have serious consequences in terms of increased patient mortality, morbidity, prolonged hospital stays, and substantial economic burden on both patients and healthcare institutions [2,3,5]. Despite these alarming figures, such infections are largely preventable through the adequate application of infection control practices [3]. The reduction of microbial contamination depends primarily on improved cleaning regimens, proper disinfection protocols, restricted entry during surgical hours, and regular fumigation of OT premises, which collectively ensure a consistently sterile surgical environment [3,5]. Routine microbial surveillance, staff training, and strict adherence to standard operating procedures can further strengthen infection control and significantly reduce the risk of postoperative complications.

MATERIALS AND METHODS

Study Design- Retrospective analysis of 2018-2024 of a tertiary care hospital and Dr Shankarrao Chavan Government Medical College, Nanded, Maharashtra, obtained from routine microbiological surveillance of multiple OTs. Air samples can be collected in two ways: 1) by active air sampler. 2) By passive air sampling (settle plates). The set plate method was used for the surveillance. It is a simple and inexpensive, economical readily available method. Settle plates of blood agar with sterility checked by incubating plates at 37°C for 24 hours were used. Plates were kept open in different post-cleaning and post-fumigated empty OTs. The 1 – 1 – 1 method i.e., one metre above the ground, one metre away from the wall and for One hour. Then plates were sealed and sent to the microbiology department and kept in an incubator for 24 hours at 37°C [6]. Colonies were counted and confirmed by hemolysis pattern, biochemical tests (catalase, coagulase), microscopy and cefoxitin sensitivity. The observed colonies of microbes were numbered and then they were converted into colony-forming units (CFU) per cubic metre of the air (cfu/m^3) using the Omeliansky formula [5,7].

$$N = \frac{5a \times 10^4}{bt}$$

Where:

N = number of microorganisms per cubic meter of air (cfu/m^3)

a = number of colonies counted on the Petri dish

b = surface area of Petri dish in cm^2

t = time of exposure in minutes

Study Setting and Conditions- Plates were kept open in different post-cleaning and post-fumigated empty OTs, ensuring standardized sampling conditions.

Inclusion Criteria

- Operation theatres that were closed (not in active surgical use) during the sampling time.
- OTs cleaned as per the hospital protocol before sampling.

Exclusion Criteria

- Operation theatres in use or undergoing emergency procedures during sampling.
- OTs where cleaning protocols were not followed.

Microbial Identification- Colonies were counted and confirmed by hemolysis pattern, biochemical tests (catalase, coagulase), microscopy, and cefoxitin sensitivity. The observed microbial colonies were then converted into colony-forming units (CFU) per cubic meter of air (CFU/m^3) using the Omeliansky formula.

Data Collection Method- Microbial colonies were identified from the samples collected from the OTs.

Statistical Analysis- The statistics such as mean and standard deviation were used to summarize CFU counts. The results were compared with standard permissible microbial limits in OTs.

RESULTS

Fig. 1 displays a three-tiered pie chart representing the overall contamination observed in OT air samples. Out of

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a total of 4402 plates, the vast majority—4235 plates (96%)—showed no microbial growth, while 167 plates (4%) exhibited contamination. Among the contaminated samples, 62% (104 plates) were positive for *S. aureus*, and the remaining 38% (63 plates) showed growth of other bacteria, including coagulase-negative *Staphylococcus* species (CoNS). Further subdivision of *S. aureus* isolates revealed that 11% (11 plates) were Methicillin-Resistant *Staphylococcus aureus* (MRSA), whereas 89% (93 plates) were Methicillin-Sensitive *Staphylococcus aureus* (MSSA).

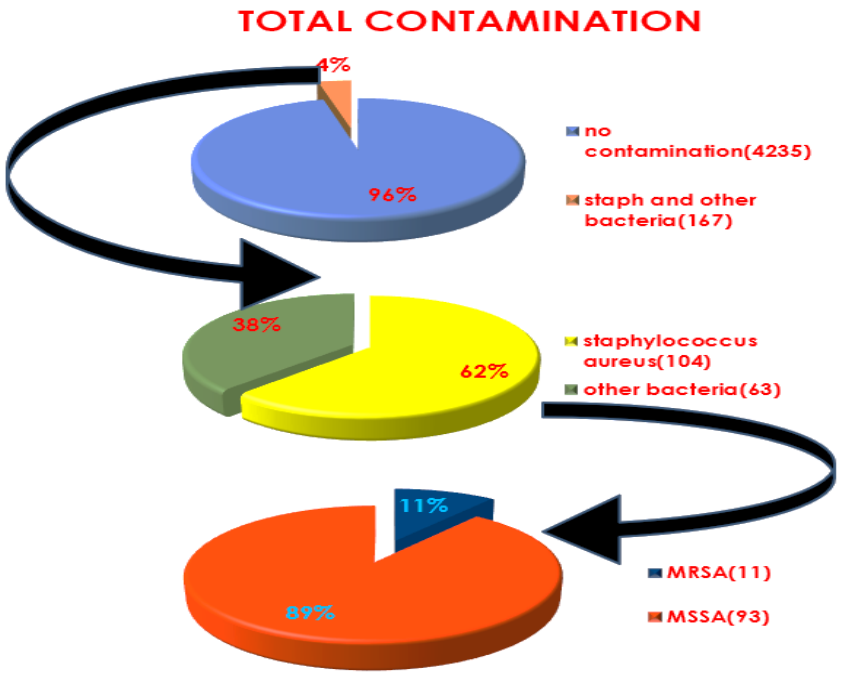

Fig. 1: Percentage of growth of organisms on settle plates

Three-tiered pie chart showing the distribution of contamination among 4402 OT air samples. A total of 4235 plates (96%) showed no microbial growth, while 167 plates (4%) were contaminated. Among these, *S. aureus* accounted for 62% (104/167) and other bacteria, including coagulase-negative *Staphylococcus* species, constituted 38% (63/167). Of the *S. aureus* isolates, 11% (11/104) were MRSA), and 89% (93/104) were MSSA. (Fig. 2).

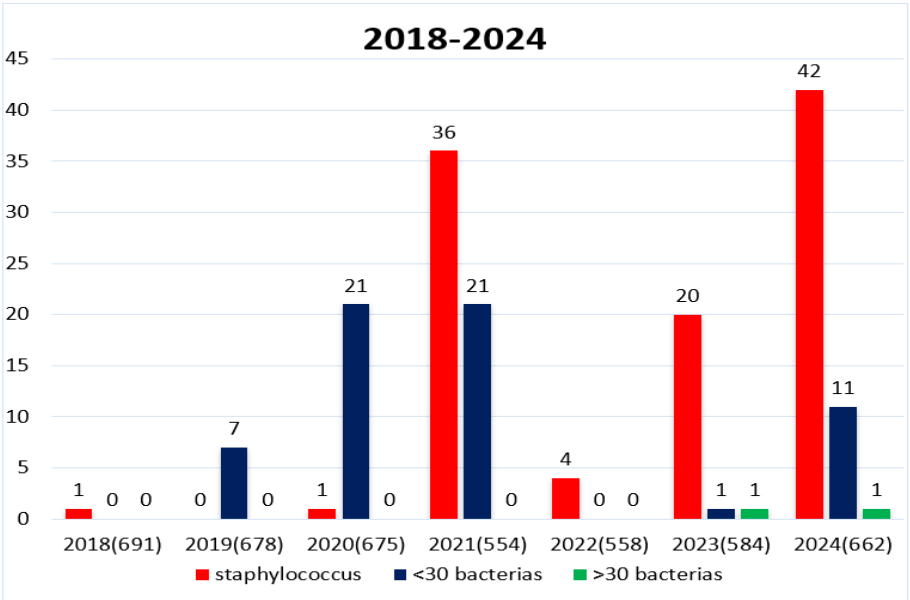

Fig. 2: yearly distribution of Orgaism Grown on Settle Plates

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Fig. 3 shows the distribution of microbial contamination across various operation theatres (OTs), categorized by organism type. The chart highlights that *S. aureus* was the predominant contaminant, especially in Gynaecology and Ophthalmology OTs, each recording 27 contaminated instances. General Surgery (16) and Orthopaedics (18) also showed notable staphylococcal presence. In contrast, ENT, Scopy OT, Ayurvedic OT, Pre-anesthesia, Septic OT, and the Block Room had minimal to no *Staphylococcus* contamination.

Other bacteria with colony count less than 30 CFU were also detected, most prominently in Gynaecology (15) and Ophthalmology (11) OTs, with moderate presence in General Surgery (10), Orthopaedics (9), and ENT (4). A minimal number of samples had other bacteria exceeding 30 CFU, limited to Gynaecology and Scopy OT (1 each). Overall, the data reveals that Gynaecology and Ophthalmology OTs carried the highest microbial burden, both in terms of *Staphylococcus* and other bacterial contaminants.

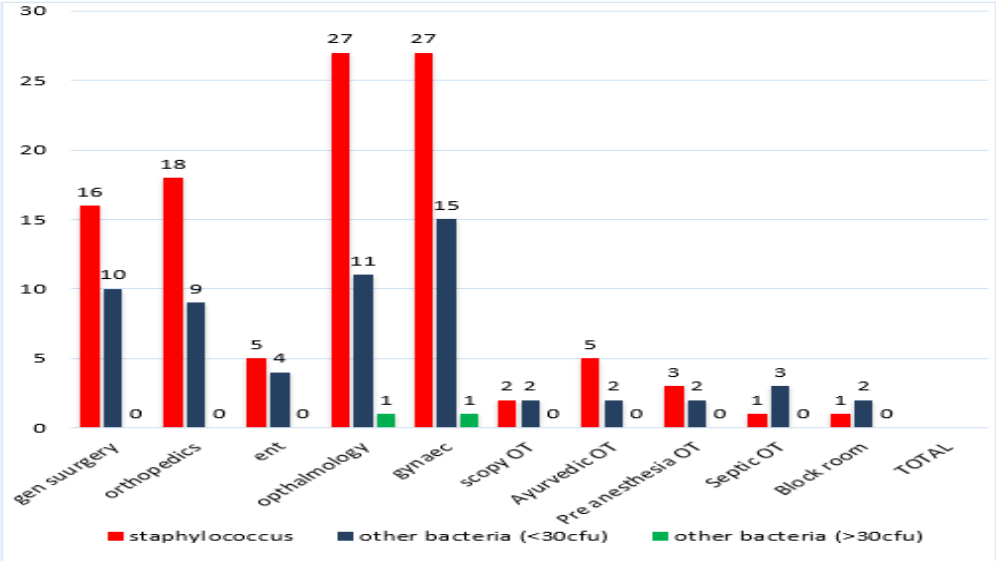

Fig. 3: OT-wise distribution of microorganisms grown in 7 years

Fig. 4 shows settle plates used to assess microbial contamination in the air. The first image (A) represents the control plate, which shows no visible microbial growth, indicating a sterile environment with minimal or no airborne contamination. In contrast, the second image (B) displays the test plate, which shows numerous distinct white colonies scattered across the surface. These colonies are the result of airborne microorganisms that settled onto the agar surface during the exposure period, suggesting the presence of microbial contamination in the test environment. This comparison between the control and test plates highlights the effectiveness of air quality monitoring and the importance of maintaining sterile conditions, particularly in sensitive areas such as operation theatres or clean rooms.

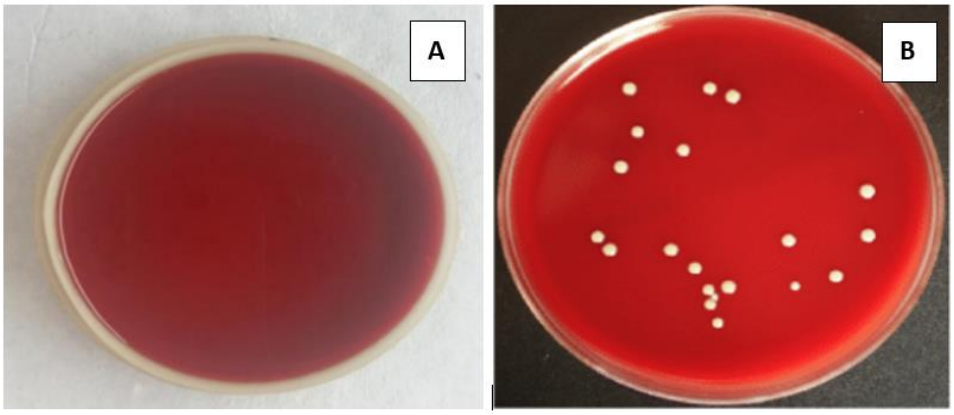

Fig. 4: Showing settle plates of control and test

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DISCUSSION

Maintaining optimal air quality in the operation theatre is crucial and plays a significant role in preventing hospital-acquired infections (HAIs) and surgical site infections (SSIs). It is a vital component of infection control that must not be overlooked. In our study, the majority of the bacterial growth observed (62%) was due to *S. aureus*, which is commonly transmitted through skin, anterior nares, boils, or cuts from both healthcare workers and patients [7].

The microbiological quality of air serves as a direct reflection of the hygiene standards maintained within the operating room environment [8]. The year 2021 recorded the highest number of contaminated plates, likely because of the post-COVID-19 pandemic period when patient influx into hospitals increased significantly.

Ophthalmology and Gynaecology OTs showed the highest contamination levels. This may be due to a lack of adherence to preventive measures such as mask and footwear usage by patients, a surge in the number of surgeries within a short timeframe, the occurrence of mucormycosis during the COVID period, and an increase in emergency procedures in Gynaecology OTs.

Variation in findings across different studies may be attributed to several factors, including the type of air sampling method used (active vs. passive), the timing of sample collection (during OT activity vs. inactivity), types of ventilation systems installed, the nature of disinfectants used, and sterilization protocols followed [9].

Distinguishing between endogenous (originating from the patient's flora) and exogenous (from healthcare staff, instruments, or environment) sources of microorganisms is often difficult, as endogenous organisms can become exogenous under certain conditions [3,4,9].

Numerous studies suggest that cocci show higher environmental resilience due to their thick peptidoglycan layer and protective pigments, enabling them to resist drying and heat stress [1,10]. Additionally, inadequate ventilation, especially when occupancy exceeds the room's capacity, facilitates microbial dispersion through skin, clothing, and hair, thus increasing the contamination risk [11,12].

An effective infection control program can enhance awareness and accountability among healthcare staff. Regular evaluation and adaptation of surveillance practices to suit the conditions in developing nations can significantly reduce HAI incidence—by up to one-third [2,13]. Rakh *et al.* [14] also emphasized that proper biomedical waste management plays a key role in minimizing microbial contamination and thereby reducing the occurrence of nosocomial infections.

Table 1: Comparison of our study findings with the other studies

	Our study	**Another study**
Most frequently isolated organism	*S. aureus* (62%)	- *S. aureus* (69%), f/b CoNS (60%) – Mathew et al. (2020) - *Staph aureus* (50%) – Rajani *et al.* [8]
Maximum growth in OT	Gynae (25.74%)	General surgery (37.5%) – Rajani *et al.* [8]
Minimum growth in OT	Block room (1.79%)	ENT OT (5%) – Rajani *et al.* [8]

CONCLUSIONS

The settle-plate method is very convenient, cost-effective, and useful for the surveillance of air in OTs. The study shows air quality of our OTs is satisfactory within permissible limits with very low bacterial contamination, though predominant contamination is by *S. aureus.* Limiting the entry of individuals, proper ventilation, cleanliness, OT dressing, hand hygiene, use of autoclaved instruments, proper disinfectant and proper OT fumigation can improve further air quality in OTs. It is essential to implement and strictly adhere to infection control protocols at every level of healthcare to ensure patient safety and minimize the risk of infections.

CONTRIBUTION OF AUTHORS

Research concept- Sanjaykumar More
Research design- Raymon Gupta, Anuja Samale, Komal Kulkarni
Supervision- Sanjaykumar More
Materials- Anuja Samale, Komal Kulkarni
Data collection- Anuja Samale, Komal Kulkarni
Data analysis and Interpretation- Sanjaykumar More
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Final approval- Sanjaykumar More

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REFERENCES

[1] Shraddha Naik, Ghogare HS, Bhalchandra MH. Estimation of microbial air contamination of operation theaters by settle plate method at tertiary care hospital, Aurangabad, India. Int J Curr Microbiol App Sci., 2018; 7(1): 1059-61. doi: 10.20546/ijcmas.2018.701.127.

[2] Islahi S, Rehman F, Alam M, Fatima A, Wahid S, Hasan F. Commencement and monitoring: microbiological surveillance in operation theaters at a tertiary care center in North India. Cureus, 2024; 16(4): e58690. doi: 10.7759/cureus.58690.

[3] Mathew D, Dadah A, Mohammed D. Bacterial contamination of operating theatres: a case study of a hospital in Northern Nigeria. Int J Sci Res., 2020; 15: 84-90.

[4] Okon KO, Osundi S, Dibal J, Ngbale T, Bello M, Akuhwa RT. Bacterial contamination of operating theatre and other specialized care units in a tertiary hospital in North Eastern Nigeria. Afr J Microbiol Res., 2012; 6(1): 3092-96.

[5] Shukla A, Srivastava S, Srivastava A, et al. Surveillance of microbiological environment of operation theaters. Cureus. 2021; 13(12): e20525. doi: 10.7759/cureus.20525.

[6] Yousuf R, Kausar R, Kadri SM. Bacteriological surveillance of operation theaters and other specialized care units of community hospitals across Kashmir valley, India. J Bacteriol Mycol Open Access. 2020; 8(2): 41-44. doi: 10.15406/jbmoa.2020.08.0024.

[7] Deepa S, Abishek MU, Venkatesha D. The air as harbinger of infections in critical care units. Med Sci., 2014; 8(28): 8-13.

[8] Rajni E, Sharma R. Estimation of microbial air contamination by settle plate method: are we within acceptable limit. Int J Res Med Sci., 2015; 3(3): 703-07.

[9] Najotra DK, Malhotra AS, Slathia P, Raina S, Dhar A. Microbiological surveillance of operation theatres: five year retrospective analysis from a tertiary care hospital in North India. Int J Appl Basic Med Res., 2017; 7(3): 165-68. doi: 10.4103/ijabmr.IJABMR_281_16.

[10] Kasdekar MM, Duthade MM, Damle AS, Khapurkuntikar MN, Iravane JA, et al. Air quality monitoring of operation theaters in Government Medical College and Hospital, Aurangabad. Int J Curr Microbiol App Sci., 2016; 5(6): 42-49. doi: 10.20546/ijcmas.2016.506.005.

[11] Abdel Hameed AA, Habeeballah T. Air microbial contamination at the Holly Mosque, Makkah, Saudi Arabia. Curr World Environ., 2013; 8: 2. doi: 10.12944/CWE.8.2.03.

[12] Gizaw Z, Gebrehiwot M, Yenew C. High bacterial load of indoor air in hospital wards: the case of University of Gondar teaching hospital, Northwest Ethiopia. Multidiscip Respir Med., 2016; 11: 24. doi: 10.1186/s40248-016-0061-4.

[13] Tesfaye T, Berhe Y, Gebreselassie K. Microbial contamination of operating theatre at Ayder referral hospital, Northern Ethiopia. Int J Pharma Sci Res., 2015; 6: 10.

[14] Rakh VA, Bagade VG, Kale SB, Baravakar JP, Shruti S. Study on the waste disposal practices in a tertiary care hospital in Western Maharashtra. SSR Inst Int J Life Sci., 2025; 11(2):7200-05.