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## Original Research Article

## Microbiological surveillance of operation theatre and its implications as a hospital infection indicator in a tertiary care hospital, Gujarat, India

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## ABSTRACT

**Introduction:** Microbial contamination in operating theatres is a critical concern. Effective infection control practices are essential for minimizing microbial exposure and ensuring patient safety during surgical procedures. In addition to aerobic organisms, anaerobic pathogens, particularly *Clostridia spp.*, have been recognized for their potential to cause infections, especially in surgical wounds and abscesses.

**Aims and Objectives:** This study aims to evaluate the levels of microbial contamination in various operating theatres, assess the effectiveness of current infection control practices, and provide evidence-based recommendations for enhancing infection prevention strategies.

**Materials and Methods:** A total of 1140 surface samples and 540 air samples were collected from six operating theatres, including surgical, septic, orthopaedics, obstetrics, gynaecology, and gastroenterology OTs. The samples included air cultures and surface swabs from various locations. Microbial identification was performed using standard culture techniques, including anaerobic culture methods for the isolation of *Clostridia spp.*

**Results:** The results indicated significant microbial contamination levels across the operating theatres. The septic OT exhibited the highest isolation rate (15.42%), while the obstetric OT had the lowest (7.64%). Surface contamination was highest on floors (36.57%), and air culture surveillance revealed the septic OT had the highest CFU/m<sup>3</sup> (251). Notably, *Escherichia coli*, *Pseudomonas spp.* and *Clostridia spp.* were among the dominant pathogens isolated, particularly in the gastroenterology and septic OTs.

**Conclusion:** This study underscores the importance of microbiological surveillance and effective infection control practices in operating theatres. The evaluation of current practices identified gaps that need addressing, such as adherence to cleaning protocols and staff training. Implementing regular audits and enhancing training programs for healthcare workers can significantly reduce microbial contamination and improve patient outcomes. The inclusion of anaerobic organisms, particularly *Clostridia spp.*, highlights the need for comprehensive monitoring of all potential pathogens in surgical environments.

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## 1. Introduction

One such problem in contemporary healthcare is microbial contamination within operating theatres, stretching from

increased morbidity to extended hospital stays and raised healthcare costs, constituting a profound burden on both patients and the health system.<sup>1</sup> The WHO has emphasized precautions to prevent infections and implement control measures within surgical settings to address these issues.<sup>2</sup>

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The operating room environment is a very complex and dynamic ecology characterized by various factors, which can influence the microbial growth, air quality, surface cleanliness, and the practices of surgical staff.<sup>3</sup> Airborne pathogens pose a particularly great degree of concern, as they can directly contaminate the surgical site throughout procedures.<sup>4</sup> Surfaces throughout the operation theatre, such as floors, walls, surgical tables, anaesthesia equipment, and surgical trolley, may serve as reservoirs for pathogenic microorganisms and complicate control of infection.<sup>5</sup>

Microbiological surveillance in the operating theatres is one of the most important strategies for determining potential sources of contamination and putting infection control strategies into effect. Regular monitoring establishes the environment conducive to surgical site infections and helps in formulating necessary interventions.<sup>6</sup> Prior studies have shown that the increased levels of microbial contamination in the surgical environment necessitates strict microbiological monitoring.<sup>7,8</sup>

With that, the study conducted a deep and detailed microbiological survey in the operating theatres in one of the tertiary healthcare hospitals in Gujarat, India, wherein the samples from different sites of the operation theatre were taken and analysed systematically for types of microorganisms over 18-month period. By systematically collecting and analysing samples from various sites within the operating theatre, this research seeks to identify the types of microorganisms present, including anaerobic organisms such as *Clostridia spp.*, and assess their relationship with postoperative infections. This study tried to contribute to better understanding of infection control behaviour in surgical settings and to improve curbing practices for reducing the risk of nosocomial infections such as surgical site infections.

## 2. Aims and Objectives

The primary aim of this study is to conduct a microbiological surveillance of operating theatres in a tertiary care hospital in Gujarat, India, to evaluate microbial contamination levels and their implications.

Objectives include characterizing the types of microorganisms present in the operating theatre environment, including both aerobic and anaerobic pathogens, and quantifying microbial contamination across various sites within the operating theatre. Additionally, the study seeks to analyze adherence to infection control protocols and identify areas for improvement. By achieving these objectives, the study aspires to improve understanding of microbial dynamics and inform best practices for infection control in surgical settings.

## 3. Materials and Methods

### 3.1. Study design

A prospective study design was adopted for the study during an 18-month period in assessing the levels of microbiological contamination in operating theatres of a tertiary care hospital in Gujarat, India.

### 3.2. Inclusion criteria

The samples were collected from designated Operation theatres on Monday morning under sterile conditions.

### 3.3. Exclusion criteria

1. Non-designated operating rooms.
2. If OT was used for surgery or opened before 12 hours, the samples from that OT were not collected.
3. If there was any contamination of samples during collection or transportation process, the samples were discarded.

### 3.4. Data collection methods

A total of 1140 surface samples and 540 air samples were collected from 6 operating theatres. Sampling was conducted at regular intervals to ensure a comprehensive assessment of microbial contamination. All OTs have been cleaned with soap and water, mopped, and subjected to fumigation with quaternary ammonium compounds. They were then closed overnight. The following morning, the OTs were opened, and samples were collected in sterile gowns and gloves, masks over mouths, and covers over noses.

1. Air surveillance was done once in a month (1st Monday). Air samples were collected using 5 settle plates (4 at corners of OT, 1 at centre of OT, 1 meter above the floor level). All plates were exposed for 30 minutes to capture airborne micro-organisms.
2. Surface samples: Were taken every 1st and 3rd Monday. Samples were taken from various locations, like floors, walls, surgical trolley, anaesthesia trolleys, and operating tables, using sterile swabs moistened with sterile saline for aerobic organisms. For anaerobic organisms Amies transport swab was used. In Septic OT additional samples were collected from AC units and Suction apparatus.

### 3.5. Laboratory diagnosis

The samples were labelled and transported to the microbiology lab in a sterile condition for processing. Laboratory diagnosis depended upon the following methods:

Microscopy was done to establish the presence of microbial cells. Notably, Gram staining was adopted

to differentiate the Gram-positive from the Gram-negative bacteria making it highly suitable for preliminary examinations of the microbial composition.

1. Culture Techniques: The samples were streaked on various culture media for the growth of different microorganisms:

- (a) Blood Agar: It is an enriched medium used to culture fastidious organisms and to assess hemolytic activity where applicable, which is typical of some pathogenic bacteria.
- (b) MacConkey Agar: This selective medium was used to isolate and differentiate Gram-negative bacteria particularly Enterobacteriaceae based on lactose fermentation.
- (c) Sabouraud's Dextrose Agar: This selective medium was used for the isolation of fungal pathogens.
- (d) Anaerobic Culture Techniques: Specific anaerobic culture methods like Gaspack and McIntosh and Filde's anaerobic jar were employed to isolate *Clostridia spp.* from the samples, ensuring that anaerobic conditions were maintained during the incubation process.

The plates were incubated at 37°C for 24-48 hours, after which colony counts were done, and colonies were identified by their morphological characteristics, biochemical tests and VITEK-2.

### 3.6. Statistical analysis

Microsoft Excel and SPSS software (version 25) were employed to conduct the data analysis. The microbial counts were summarized and the prevalence of specific organisms was declared using descriptive statistics. The results were presented in tabular formats to facilitate simple interpretation and comparison. This study has endeavoured to offer a comprehensive understanding of the microbial status of the operating theatre and its relevance to infection control procedures by employing these other difficult methods.

## 4. Results

Microbiological surveillance of operating theatres in a tertiary care hospital in Gujarat, India, led to a significant finding concerning levels of microbial contamination and types of organisms present. A total of 1140 surface samples, from various locations, namely, floors, walls, surgical trolley, anaesthesia trolleys, and operating tables, were collected from 6 operating theatres. Air samples were collected from 540 sites. A summary of the results follows.

### 4.1. Microbial contamination levels (In various operation theatres)

The analysis revealed varying levels of microbial contamination across different operating theatres. The overall distribution of microbial counts is illustrated in Table 1.

As shown in Table 1, we found that Highest Contamination Level was seen in Septic OT (15.42%), followed by Gastroenterology OT (13.43%) whereas the least contamination was seen in Obstetric OT (7.64%).

### 4.2. Microbial contamination levels (On various surfaces)

The analysis of microbial contamination levels on different surfaces within the operating theatres is summarized in Table 2.

As shown in Table 2, we found that Highest Contamination Level was seen on Floor (36.57%).

Followed by Anaesthesia Trolley (32.87%) while the Lowest Contamination Level was seen at Surgical Trolley (18.98%).

### 4.3. Air culture surveillance (CFU/m<sup>3</sup>) of operation theatres

The results of air culture surveillance in terms of colony-forming units per cubic meter (CFU/m<sup>3</sup>) are presented in Table 3.

As shown in Table 3, Highest Contamination Level was found in Septic OT (251 CFU/m<sup>3</sup>) followed by Gastroenterology OT (187 CFU/m<sup>3</sup>), and surgery OT (14.94%) while Gynaecology OT showed the least contamination (106 CFU/m<sup>3</sup>).

### 4.4. Isolation rates of organisms from various operation theatres

The isolation rates of various bacterial species from different operating theatres are summarized in Table 4.

As shown in Table 4, and Figures 1, 2, 3, 4 and 5 the most Dominant Species in Surgical OT was CoNS (13) while *Pseudomonas spp* was most dominant in Septic OT (25) and Orthopaedic OT (20). *E.Coli* dominated Obstetric OT (15) and gynaecology OT (17) while *Bacillus spp* were dominantly present in Gastroenterology OT (21). Overall the dominant species were CONS (76), *Pseudomonas* (75), *Bacillus* (64), *E coli* (60) and *S.aureus* (42) while least present were *Serratia* (5) and *Micrococcus species* (11), *Enerobacter spp* (16) and *Acinetobacter spp* (18).

#### 4.5. Prevalence of organisms in air cultures of various OTs

The prevalence of organisms isolated from air cultures in different operating theatres is presented in Table 5.

As shown in Table 5 and Figure 3, the *Bacillus* spp were the most Dominant Species in Air culture in Surgical OT (55), septic OT (87), Gynaecology OT(28), Obstetric OT (27) and Gastroenterology OT (45). CoNS was most dominant in Orthopaedic OT (41). Overall the most dominant species were *Bacillus* spp (271), and *Pseudomonas*(219) while the least present were *Serratia* (10) and *Aspergillus* spp(6) and *Clostridia* spp (4).

#### 4.6. Prevalence of different organisms in Air Culture

The overall prevalence of different types of organisms isolated from air cultures is summarized in Table 6.



**Figure 1:** Colonies of *S. aureus* on nutrient agar



**Figure 2:** Colonies of CoNS on MacConkey agar

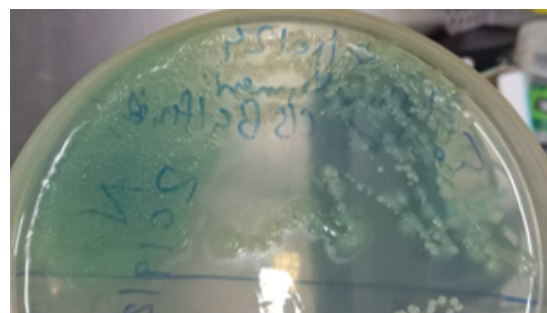
Gram-positive organisms were the most prevalent (60.23%), followed by Gram-negative organisms (38.74%). The contribution of fungal species and anaerobes was less than 1% (Table 6). The implications for patient safety and the critical function of microbiological surveillance



**Figure 3:** Colonies of *Bacillus* Spp on nutrient agar



**Figure 4:** Colonies of *E.Coli* on MacConkey agar



**Figure 5:** Colonies of *Pseudomonas* spp on nutrient agar



**Figure 6:** Colonies of *Aspergillus* Spp on sabouraud dextrose agar

**Table 1:** Microbial contamination levels (In Various OTs)

Name of OT	Total swabs	Number of positive swabs	Isolation rate (%)
Surgical OT	180	21	11.67
Septic OT	240	37	15.42
Orthopaedics OT	180	19	10.56
Obstetric OT	144	11	7.64
Gynaecology OT	180	21	11.67
Gastroenterology OT	216	29	13.43
Total	1140	138	12.11

**Table 2:** Microbial contamination levels (On Various Surfaces)

Surface	Total swabs	Number of isolated pathogens	Isolation rates (%)
Floor	216	79	36.57
Wall	216	65	30.09
Surgical trolley	216	41	18.98
Anaesthesia trolley	216	71	32.87
Operating table	180	35	19.44
Other surfaces	96	27	28.13
Total swabs	1140	318	27.89

**Table 3:** Air culture surveillance (CFU/m<sup>3</sup>) of OTs

Operation theatre	Total Sites	CFU/m <sup>3</sup>	Positivity rate of CFUs (%)
Surgical OT	90	147	14.94
Septic OT	90	251	25.51
Orthopaedics OT	90	162	16.46
Obstetric OT	90	131	13.31
Gynaecology OT	90	106	10.77
Gastroenterology OT	90	187	19.00

**Table 4:** Isolation rates of organisms from various OTs:

Bacterial species	Surgical OT	Septic OT	Orthopaedics OT	Obstetric OT	Gynaecology OT	Gastroenterology OT	Total (Species)
<i>Bacillus spp</i>	10	8	7	11	7	21	64
<i>Pseudomonas spp</i>	6	25	20	4	5	15	75
CoNS*	13	20	15	5	5	18	76
<i>S.aureus</i>	5	8	7	7	5	10	42
<i>Escherichia coli</i>	2	6	3	15	17	17	60
<i>Micrococcus spp</i>	1	5	0	0	1	4	11
<i>Acinetobacter spp</i>	3	8	0	0	2	5	18
<i>Enterobacter spp</i>	6	2	0	1	1	6	16
<i>Serratia spp</i>	2	0	1	0	2	0	5
Total (OT Wise)	48	82	53	43	45	96	367

\*CoNS: Coagulase negative staphylococcus.

in operating theatres are suggested by these results. The necessity for rigorous infection control measures is emphasized by the identification of a variety of pathogens, particularly in high-risk areas such as septic and gastroenterology operating theatres. The development of standardized protocols for environmental monitoring and the exploration of innovative disinfection methods to improve patient safety in surgical settings should be the primary focus of future research.

## 5. Discussion

Results from this study provide vital insights into microbial contaminations levels within operating theatres. Findings indicate a high presence of organisms with notable prevalence in various operating theatres on various surfaces.

**Table 5:** Prevalence of organisms in Aircultures of various OTs

Species	Surgical OT	Septic OT	Orthopaedics OT	Obstetric OT	Gynaecology OT	Gastroenterology OT	Total Specie wise
<i>Bacillus spp</i>	55	87	29	27	28	45	271
<i>Pseudomonas spp</i>	31	77	33	21	15	42	219
CoNS*	17	36	41	23	11	22	150
<i>S.aureus</i>	15	21	25	22	16	25	124
<i>Escherichia coli</i>	12	8	16	24	19	27	106
<i>Micrococcus spp</i>	4	3	7	7	1	19	41
<i>Acinetobacter spp</i>	0	7	6	2	2	1	18
<i>Enterobacter spp</i>	4	7	5	4	2	2	24
<i>Serratia spp:</i>	5	3	0	1	0	1	10
<i>Aspergillus spp</i>	2	1	0	0	1	2	6
<i>Clostridia spp</i>	2	1	0	0	0	1	4
Total(OT wise)	147	251	162	131	106	187	973

\*CoNS: Coagulase negative staphylococcus.

**Table 6:** Prevalence of different organisms in air culture.

Type of organism	Numbers	Percentage (%)
Gram positive organisms	586	60.23
Gram negative organisms	377	38.74
Fungal spp	6	0.71
<i>Clostridia spp</i>	4	0.41

### 5.1. Prevalence of organisms in various operating theatres

The summary includes isolation rates of organisms from different operating theatres in Table 4. A total of 96 isolates was observed in the gastroenterology OT, while septic OT had 82, with *bacillus spp.* (21 isolates) and *Pseudomonas spp.* (25 isolates) as the predominant pathogens. This supports the observations of Atata et al.,<sup>9</sup> who provided a link between environmental contamination and nosocomial infections, particularly in surgical locations. The surgical operating theatre also showed significant isolation rates, with *Bacillus spp.* (10 isolates) and Coagulase-Negative Staphylococci (CoNS) (13 isolates) being most common, which indicates the need for stringent infection control efforts. Coagulase-negative Staphylococci reside within the normal skin flora and may accidentally introduce contaminations into a surgical site or an incision at the time of surgery.

In the orthopaedics operating theatre, the presence of *Escherichia coli* (3 isolates) and *Serratia spp.* (1 isolate) raises concerns, particularly as *E. coli* is known to be a common pathogen associated with SSIs in abdominal surgeries. The obstetric and gynaecology operating theatres had lower isolation rates, with *E. coli* being the most prevalent organism in both (15 and 17 isolates, respectively), highlighting the risk of infections stemming from gastrointestinal flora during surgical procedures.<sup>9</sup>

### 5.2. Microbial contamination levels on different surfaces

epicts the changing levels of microbial contamination of various surfaces in the operating theatre. The floor surfaces shared the highest percentage of positivity (36.57%), then the anaesthesia trolley (32.87%) and walls (30.09%). All these work were in accordance with Fijan et al.,<sup>[31]</sup> putting emphasis on the fact that hospital textiles and other surfaces could act as a reservoir for bacteria pathogens of healthcare-associated infections. The high level of *Bacillus spp.*, the most frequently isolated organism from floor surfaces, corroborates the findings of Laxmi et al.,<sup>10</sup> indicating that *Bacillus species* commonly inhabit hospital environmental conditions and can aid in its nuisances. The high contamination rates on surfaces show that these places need regular and thorough cleaning to minimize cross-contamination. Surgical Trolley recorded a relatively low positivity (18.98%), which might be a result of better cleaning protocols or because they come into less contact with contaminated materials compared to other surfaces.<sup>11</sup>

### 5.3. Air culture surveillance

On the other hand, air culture results in Table 3 revealed that septic operating theatres had the highest concentrations of CFU/m<sup>3</sup> (251) and positivity rate (25.51%), which is markedly higher than the surgical operating theatre that had an established CFU/m<sup>3</sup> (147) and positivity rate (14.94%). The high airborne bacterial concentration in the septic theatre is quite alarming for its potential to elevate the risk of

SSIs through airborne settling of bacteria.<sup>12</sup> These findings corroborated with those reported by Tang and Wan,<sup>13–15</sup> for concentrations of airborne bacteria in operating theatres appeared to correlate positively to an increase in the infection rates. The presenting *Pseudomonas spp.* in the air samples corroborates the work of Robakowska et al.,<sup>16</sup> who identified the air as an important vector for nosocomial infections. On the other hand, it remains maintained by Dreyfus et al.<sup>17</sup> that the isolation of *Staphylococcus aureus*, particularly around the surgical tables, was in complete accordance with their findings, establishing a direct correlation with the environmental contamination in SSI cases during orthopedic surgeries. The presence of *Pseudomonas spp.* in air samples confirmed the work of Paprocka et al.<sup>18</sup> who expressed that *Pseudomonas aeruginosa* remained one of the most frequently isolated opportunistic pathogens in healthcare facilities and in most settings' susceptible patients.

#### 5.4. Prevalence of organisms in air cultures

ummarizes the prevalence of organisms in varying operating theatre air cultures. *Bacillus spp.* was the most frequently isolated organism in air samples taken from the septic operating theatre (87 isolates), while *Pseudomonas* was second (77 isolates). This high prevalence of *Bacillus spp.* in the air is noteworthy in that *Bacillus spp.* is associated with environmental contamination in healthcare environments.<sup>18,19</sup> Additionally, the presence of *Staphylococcus aureus* (21 isolates) and *E. coli* (8 isolates) amplified the susceptibility of the surgical environment to airborne transmission.

#### 5.5. Prevalence of aspergillus spp.

Despite their lower frequency of detection, the presence of *Aspergillus* species raises concerns about fungal infections, particularly in immunocompromised patients. Calò et al.<sup>20</sup> underscored the significance of environmental monitoring for fungal pathogens in surgical settings, which is consistent with this discovery.

Consequently, the findings of this investigation emphasize the importance of implementing rigorous infection control protocols in operating rooms. In order to mitigate microbial contamination it is imperative to adhere to established cleansing protocols and conduct routine microbiological surveillance. The results are consistent with the World Health Organization's recommendations for comprehensive infection prevention strategies in surgical environments, as stated in.<sup>1,2</sup>

#### 5.6. Prevalence of anaerobes

The presence of *Clostridia spp.*, with four positive samples identified, is particularly concerning due to its association with anaerobic infections, which can lead to severe

complications in surgical wounds, especially in cases involving deep tissue infections or abscesses. *Clostridia spp.* are known for their ability to produce toxins and cause conditions such as gas gangrene, which can significantly impact patient outcomes following surgical procedures. Our findings coincide with observations made by Al-Ameer et al.<sup>21</sup> The isolation of *Clostridia spp.* highlights the need for comprehensive monitoring of anaerobic organisms in surgical settings, as their presence can indicate potential risks for postoperative infections.

### 6. Recommendations for Best Practice

Based on the study conclusions and an analysis of current practices in infection control, several new recommendations for best practice may be made:

#### 6.1. Improved cleaning procedures

It is essential to have strict cleaning and disinfection procedures for all surfaces and equipment in operating theatres to ensure high touch points are cleaned more often, e.g., surgical trolleys and anaesthesia equipment, to bring down the microbial load.<sup>22</sup>

#### 6.2. Regular microbiological monitoring of contamination levels

It is necessary that the levels of microbial contamination in air and surface samples be monitored at regular intervals to check out for risks of infection from given sources and conclude on the effectiveness of the clean-up procedures that have been observed.<sup>22</sup>

#### 6.3. Infection control training

Continuous training programs should be established to train all health care workers on infection prevention strategies, including hand hygiene, application of personal protective equipment, and contamination care. Past studies have shown that increased awareness and compliance lead to significant reductions in infection rates.<sup>22</sup>

#### 6.4. Implementation of process mapping

Process mapping methodologies may be used in the identification of critical control points in infection prevention practice and targeted interventions to reduce associated surgical procedure risks.<sup>23</sup>

#### 6.5. Adherence to guidelines

Healthcare facilities should ensure compliance and adherence to set infection control guidelines and protocols, including the WHO and other relevant health organization recommendations. Regular assessment for such adherence can lead to improvement in the general practice of infection

control.<sup>24</sup>

In their application, such recommendations will enhance the infection control practices within the health care institutions, which will, in turn, lower the risk of SSIs and improve patient outcomes.

## 7. Conclusion

This study underscores the importance of microbiological surveillance in operating rooms and the correlations with patient safety. The evaluation of infection control practices is imperative to finding gaps and areas for improvement in the maintenance of sterile conditions within operating theatres: evaluation of adherence to institutional cleaning and disinfection protocol and compliance with hand hygiene practices among surgical teams and evaluation of the efficacy of usage of personal protective equipment. Continuous audits and feedback should be put in place in ensuring the upward trend in compliance with infection control measures in health care facilities. In addition, further training programs should be established to upgrade the knowledge and skills of health workers regarding infection control strategies.

The identification of various pathogens, especially from hyper-risks zones, such as septic patients and gastroenterology operating theatres, warrants strict infection control measures. Strengthening training and compliance would yield a step-change to reduce SSIs and enhance patient outcome further. Future studies can prioritize developing standardized protocols for environmental monitoring and explore innovations on disinfection techniques to further improve patient safety in surgical settings.

## 8. Ethical Approval

The research was conducted in accordance with ethical guidelines and received clearance from the institutional ethical committee prior to commencement. (IEC number: PUIECHR/PIMSR/00/081734/6205)

## 9. Source of Funding

None.

## 10. Conflict of Interest

None.

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