

Microbiological Surveillance of Hospital Environment in Chevella, India

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Abstract

Microbiological air contamination in operation theatres (OTs) and labor rooms (LRs) is a major risk factor for surgical site infection. Routine monitoring in vulnerable areas such as OTs and LRs should always be performed as part of infection control to evaluate the contamination by microorganisms and monitor for the presence of nosocomial agents. The present study is aimed to isolate and identify various pathogens in a hospital environment. The research was performed in the Bacteriology lab, Microbiology Department, Dr. Patnam Mahender Reddy Institute of Medical Sciences, Chevella, Ranga Reddy district, Hyderabad, India between November 2017 and November 2020 for a period of 3 years. Surface swabbing and settle plate technique were two sampling techniques used in the analysis. A total of 3492 samples were collected from various hospital surface sites and 5 OTs and LR sites and equipment via the swab technique, while a Petri plate gravitational settling (passive) sampling method was selected for the collection of air samples and all these samples were properly transferred to the microbiology laboratory and processed by standard microbiological protocols. A total of 3492 surface swabs were taken from 5 OTs, LR sites and equipment from the hospital. Out of these 294 (8.42%) were culture positive and 3198 (91.58%) were culture negative. Among 294 microbial isolates, the highest number was reported from *Bacillus* spp. 212 (72.11%) and least number was from *Pseudomonas* spp. 6 (2.04%). Through air sampling methods, bacterial isolates were isolated from OTs and LR of various clinical departments and it was found that the highest bacterial count was reported from general surgery (677 CFU/m³) followed by orthopaedics (585 CFU/m³). Most of the microbial isolates isolated from OTs and LR of clinical departments found be species belonging to *Bacillus* spp. and Coagulase-negative Staphylococci (CoNS). In developing countries, routine indoor air quality management in healthcare facilities needs to be constantly monitored and appropriate measures are taken to detect and prevent acquired infections in hospitals. Settle plate methods for air and surface swabbing, also in resource-limited settings, are very useful, simple and cost-effective techniques for OT and LR monitoring.

Keywords: Operation theaters, labor rooms, microbiological surveillance, hospital acquired infection, air sampling

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INTRODUCTION

The primary cause of incidence and death in surgical patients is hospital-acquired infections like surgical site infections^{1,2}. Hospital acquired infections are a worldwide issue. Over 1.4 million individuals around the world are thought to be infected in hospitals at any given moment, with 80000 deaths every year. Regardless of the world region, the hospital environment is a reservoir of pathogenic microorganisms to a greater or lesser extent. Nosocomial infections impact 5 to 10% of all patients admitted to healthcare facilities in developed countries. They affect 25% of hospitalised patients in underdeveloped nations³. As a result, it is evident that monitoring the hospital environment is an important part of preventing nosocomial infections. A significant risk factor for surgical site infection (SSI) is microbiological air pollution in the operation theaters (OTs) and labor rooms (LR)⁴.

Bacteria, viruses, yeasts, moulds and fungal spores can contain air bio-loads present in the form of aerosols. Infected patients, medical personnel movements and visitor loads are the most significant sources of aerosol generation⁵. Environmental monitoring means air, surface and equipment microbial testing that can predict the microbial load⁶. An analysis of the spectrum of microbial contaminants that seem to be responsible for nosocomial infections indicates that nearly all of them are potentially airborne. Several of these infections, however, are likely to be caused by direct exposure and equipment contact⁷. As a matter of infection control, routine surveillance in sensitive areas such as OTs and LR should always be conducted to assess microbial contamination and search for the existence of nosocomial agents. Microbiological sampling and particle counting^{2,8} will regularly be used to determine air quality in operating theatres. A reliable contingency planning is provided by settle plates as it analyses the harmful component of the airborne crowd that drops on a critical site, such as surgical cutting or operating theatre instruments^{9,10}. The monitoring of airborne pathogens in health care settings is vital not just for protection of the patient, but also for the hospital⁴.

As a core of a successful hospital management should follow strict infection prevention control and basic hygiene. The

combination of air and surface disinfection may be an optimal and cost-effective solution to reducing the rate of infection⁴. In order to eliminate nosocomial infections, hospital environmental control practices play an important role⁹. Hence the present study was aimed to perform a microbiological surveillance in a hospital for microbiological monitoring of OTs and LR, isolating and classifying the microorganisms present.

MATERIAL AND METHODS

The research was performed in the Bacteriology lab, Microbiology Department, Dr.Patnam Mahender Reddy Institute of Medical Sciences, Chevella, Ranga Reddy district, Hyderabad, India between November 2017 and November 2020 for a period of 3 years. Fumigation with formaldehyde solution and 1% hypochlorite solution were used for disinfection. The surfaces and equipment were disinfected with 70% alcohol based solutions like ethyl alcohol or isopropyl alcohol, 1% hypochlorite solution and 2% lysoformin solution. The OT was sterilized by fumigation with 1:1 formalin and water. Upon adequate sterilization and disinfection of the OTs, surface samples were taken prior to the entry of the surgical and service staff wearing sterile gloves, masks and sterile gowns to prevent contamination of media and the surface being swabbed^{11,12}. Surface swabbing and settle plate technique were two sampling techniques used in the analysis.

Samples from surfaces (swab method)

A total of 3492 samples were obtained from 5 OTs and LR sites and equipment. Sterile swabs soaked in nutrient broth were rolled over to the surface of equipment: operation table head end, foot end, instrument trolley, over head lamp, Boyle's apparatus, suction apparatus, IV infusion stand, crash cart, door handles, microscopes and TV^{4,6,11,12}. The samples were also collected from hospital floors, side walls and AC vents. They were placed back into the broth after collection. All the samples were carefully marked and transferred to the Microbiology laboratory directly and incubated at 37°C for 4h.

Swabs taken from different sites were streaked on to 5% sheep Blood agar (SBA) and Mac Conkey agar plates (Himedia company Limited) and incubated at 37°C for 24h under aerobic conditions.

Identification of isolates was done by Gram staining and standard biochemical tests like coagulase test, IMViC tests (indole, methyl red, Voges-Proskauer, citrate utilization), urease test, oxidase test, catalase test, nitrate reduction test, triple sugar iron agar test¹¹⁻¹⁴.

Air sampling (settle plate method)

A total of 648 air samples were collected. Media-containing plates are exposed to the atmosphere face-up to collect gravity-settling particles¹⁵. For bacterial sampling, SBA and MCA were used, while for fungal sampling, Sabouraud Dextrose agar (SDA) was used¹⁵. Media plates were transported to 5 separate OTs and LR in sealed plastic bags. The plates were labelled with sample number and date of sample collection. The plates were kept open in the OTs and LR at a height of 1 m above the ground and 1 m from the walls and exposed for 1h schedule 1/1/1^{4,6,11,12}. Upon exposure, the plates were covered with their lids and brought to the laboratory in sealed bags. The bacterial sampling Petri plates were aerobically incubated for 24 to 48h at 37°C, while the fungal plates were incubated for 7 days at 25 to 27°C in the BOD incubator^{8,15,16}. These plates were observed for the presence of growth after incubation and the number of colonies per plate was counted. In addition, Omeliansky's formula expressed this colony forming unit (CFU) per cubic meter of air (CFU/m³)^{11,12}.

$$N = 5a \times 10^4 (bt)^{-1}$$

Where, N = colony forming unit per cubic meter of air (CFU/m³)

a = number of colony forming unit per Petri plate (CFU)

b = surface area of Petri plate (cm²)

t = time exposure (min) .

Final identification was done by following standard bacteriological techniques, and fungal identification was done by Lacto Phenol Cotton

Table 1. Distribution of samples with and without growth

| Culture | Number of samples | Percentage (%) |
|----------------|-------------------|----------------|
| With growth | 294 | 8.42 |
| Without growth | 3198 | 91.58 |
| Total | 3492 | 100.00 |

Blue (LCB) mount^{14,16}. In OTs and LR, even a single colony of *Staphylococcus aureus* and fungus was considered unsatisfactory¹¹. All isolates were categorized into 3 broad categories². Normal flora, e.g. Coagulase-negative Staphylococci (CoNS), Contaminant e.g. *Bacillus* spp., Pathogen e.g. *Klebsiella* spp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Statistical analysis

Each experiment was conducted in triplicates and the results were expressed in terms of means and standard deviation.

RESULTS

A total of 3492 surface swabs were taken from 5 OTs and LR from hospital Dr. Patnam Mahender Reddy Institute of Medical Sciences. Of these 294 samples (8.42%) showed growth with the formation of colonies and 3198 samples (91.58%) did not (Table 1).

Table 2 depicted that, among 294 microbial isolates, highest number was reported from *Bacillus* spp. 212 (72.11%) and least number was from *Pseudomonas* spp. 6 (2.04%).

Table 3 showed that, among the 294 microbial isolates, isolated from various sites and equipment, *Bacillus* spp. was found to be the most common isolate followed by CoNS. Floors, crash cart, IV infusion stand were found to be more contaminated as shown in Table 3.

Through air sampling methods, bacterial isolates were isolated from OTs and LR of various clinical departments and it was found that highest microbial count 677 CFU/m³ was reported from general surgery. The microbial species isolated were CoNS, *Bacillus* spp. and *Aspergillus niger*.

Table 2. Various bacterial isolates from surface sampling

| Isolate | Number of samples | Percentage (%) |
|---|-------------------|----------------|
| Coagulase-negative Staphylococci (CoNS) | 51 | 17.35 |
| <i>Bacillus</i> spp. | 212 | 72.11 |
| <i>Staphylococcus aureus</i> | 11 | 3.74 |
| <i>Pseudomonas</i> spp. | 6 | 2.04 |
| Other | 14 | 4.76 |
| Total | 294 | 100.00 |

Table 3. Bacteria isolated from various sites and equipment

| Surface samples | Bacteria identified | | | | |
|--------------------------|----------------------|------|------------------------------|-------------------------|-------|
| | <i>Bacillus</i> spp. | CoNS | <i>Staphylococcus aureus</i> | <i>Pseudomonas</i> spp. | Other |
| Operation table head end | 4 | 0 | 0 | 0 | 0 |
| Foot end | 9 | 0 | 0 | 0 | 0 |
| Instrument trolley | 11 | 6 | 1 | 0 | 0 |
| Over head lamp | 16 | 5 | 1 | 0 | 0 |
| Boyle's apparatus | 12 | 6 | 1 | 0 | 0 |
| Suction apparatus | 16 | 4 | 3 | 1 | 1 |
| IV infusion Stand | 22 | 8 | 1 | 0 | 2 |
| crash cart | 29 | 3 | 1 | 0 | 2 |
| Door handles | 13 | 9 | 2 | 1 | 1 |
| Microscopes | 16 | 4 | 1 | 1 | 1 |
| TV | 8 | 2 | 0 | 1 | 1 |
| Floors | 33 | 3 | 0 | 2 | 4 |
| Side walls | 14 | 0 | 0 | 0 | 1 |
| AC vents | 9 | 1 | 0 | 0 | 1 |
| Total | 212 | 51 | 11 | 6 | 14 |

The second highest microbial count was reported from orthopaedics 585 CFU/m³ (Table 4).

DISCUSSION

Postoperative infections can be produced by a contaminated environment, unsterile equipment, contaminated surfaces, and infected personnel as well as contaminated disinfectants. Infectious diseases exacerbate hospitalization, produce long-term disabilities, raise antimicrobial resistance, create extra financial pressures for health care systems, and cause needless fatalities. Of almost all of the procedures and protocols, the much more important is supervision by Hospital infection control team certainly needed for environmental disinfection and instrument

sterilization. The Infection Control Team and the Infection Control Committee are responsible for coordinating the Infection Control Program for the prevention and control of infection in patients and personnel. In order to detect and monitor the microbial infection, clinical microbiologists play a critical role.

In the present analysis, 3492 surface swabs with a bacterial contamination rate of 8.42% (n = 294) were obtained from 5 OTs and LR, which is very low compared to previous studies in which the positivity rate varied from 23.4% Yadav et al.¹⁴ to 45.8% Meenakshi et al.¹¹ In this study, *Bacillus* spp. 212 (72.11%) an environmental contaminant was the predominant organism. This is in accordance with other studies Desai et al.²

Table 4. Various isolates from air sampling and their colony forming unit count from OTs and LR

| Sampling site | CFU/m ³ ± standard deviation | Microbial isolates |
|----------------------------|---|--|
| General surgery | 677±3.6 | CoNS, <i>Bacillus</i> spp. <i>Aspergillus niger</i> |
| Orthopaedics | 585±4.16 | CoNS, <i>Bacillus</i> spp. |
| Ophthalmology | 78±2.51 | CoNS, <i>Bacillus</i> spp. |
| ENT (Ear, Nose, Throat) | 182±2.01 | CoNS, <i>Bacillus</i> spp. |
| Gynaecology and obstetrics | 416±2.15 | CoNS <i>Bacillus</i> spp. <i>Aspergillus flavus</i> |
| Labour room | 520±2.08 | CoNS <i>Bacillus</i> spp. |

(64%), Javed et al.⁸ (77%), Najotra et al.¹² (87.6%), Kiranmai et al.¹³ (75%). 11(3.74%) isolates of *Staphylococcus aureus* were isolated during the 3 year period of surveillance in the present study, which is very low, still a potential pathogen and an important cause of skin and soft tissue infections. This is in accordance with the study of Najotra et al.¹² (2.9%). 6 (2.04%) of *Pseudomonas* spp. were isolated which is in accordance with the study of Yadav et al.¹⁴ (1.56%). In our study, the bacterial CFU counts of air from all OTs and LR ranged from 78 CFU/m³ (Ophthalmology OT) to 677 CFU/m³ (General surgery OT). Ophthalmology OT (78 CFU/m³) was having the least bacterial counts which is in accordance with studies Najotra et al.¹² (27%), Anjali et al.⁴ (114%), Yadav et al.¹⁴ (17%), Kiranmai et al.¹³ (16%). *Aspergillus flavus* was isolated from Gynaecology and Obstetrics OT and *Aspergillus niger* from General surgery OT. *Aspergillus* spp. is known to cause opportunistic infections especially in immunocompromised patients. Microbiological surveillance study of Javed et al.¹⁴ at Lahore, India have reported a significantly higher bacterial air count in the range of 6500-15730 CFU/m³ and Deepa et al.¹⁶ in their study from Karnataka, India have reported bacterial count of 628-1571 CFU/m³. This is in contradiction to our study.

Microbiological quality of air may be considered as mirror of the hygienic condition of the operation theatres.¹² Frequent opening of doors, crowding of the operating room increases the risk of accidental contamination of sterile areas and instruments¹⁷. *Bacillus* spp. and CoNS was isolated from all OTs. Coagulase-negative Staphylococci (CoNS) was isolated from LR. *Bacillus* spp. being a contaminant and CoNS being a skin commensal, shedding of CoNS from skin of health care workers and patients might poses an increased chance of contamination. Lack of education and negligence of patient also contributes to cross infection¹⁶. Regular surveillance of OTs and LR with infection control measures helps in controlling nosocomial infections¹³.

CONCLUSION

Regular microbiological surveillance of OT is essential to detect and control contamination. Implementing stringent and frequent disinfection procedures, installing high efficiency filtration systems, training of health care workers on best

hygiene practices, restrictions of entry into sterile areas could help in minimizing the nosocomial infections.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHORS' CONTRIBUTION

RVL performed the research work, drafted the manuscript, compiled information from the literature, and designed the tables. AR gathered information from the literature. SV and PVL managed the statistics and manuscript corrections.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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