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Original Research Article

A PROSPECTIVE OBSERVATIONAL STUDY ON THE MICROBIAL FLORA OF AIR, SURFACES AND EQUIPMENT OF OPERATION THEATRES, CENTRAL STERILE SERVICE DEPARTMENT AND LABOR ROOM

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Abstract

Background: Hospital-associated infections are an important source of morbidity and mortality with postoperative surgical site infections (SSI) being the second most common cause after urinary tract infections. Environmental monitoring by the microbiological testing of surfaces, equipment and air is useful to detect changing trends of types and counts of microbial flora. Aim of study is to identify bacterial colonization of the surfaces, equipment and indoor air of operation theatres (OTs), labor ward (LW), and Central Sterile Service Department (CSSD). **Materials and Methods:** The study was conducted in the Department of Microbiology, Nandha Medical College and Hospital, Erode, Tamilnadu, India. Air samples were taken by settle plate method in petri dishes containing blood agar and Sabouraud's dextrose agar (SDA). Surface samples were taken by a sterile swab soaked in saline from all operation theatres, LW and CSSD. The samples were processed according to standard operative procedures. **Results:** A total of 1242 samples were collected, of which 1109 were surface swabs and 133 were settle plate samples. Out of 1109 surface swab samples, 139 (12.5%) and out of 133 settle plates, 40 (30.1%) were growth positive. Among 139 surface swabs, 81(58%) Bacillus species, 33(24%) CONS, 15(11%) *Staphylococcus aureus* and 10(7%) *Enterococcus* were isolated. Out of 40 air samples, 17 (42.5%) Bacillus species, 4 (10%) Micrococci, 6 (15%) *Staphylococcus aureus*, 4(10%) *Pseudomonas spp*. and 3 (7.5%) Klebsiella spp. were isolated. Fungal spp. 6(15%) isolated were Aspergillus fumigatus, Aspergillus niger, Rhizopus and Mucor. **Conclusion:** Surfaces and air in various health facilities were found contaminated with different types of bacteria including potential pathogens that pose a great risk to patients. Hospital infection control program need to be improved to control health care associated infections and for better management of patients.

INTRODUCTION

In developed countries, even with sophisticated treatments and technologies, 5-10% of patients continues to account for complications and more than 25 percent of such patients in developing countries, have been found to acquire health care associated infections after admissions to acute-care hospitals.[1] In Indian scenario an average incidence is10% to 30%, but may be still higher in ICUs. Such health care associated infections add to the morbidity, mortality, and costs that one might expect from the underlying illness alone.

Microbial contamination of hospital environment, especially the operating theatre and other specialized units had continued to increase prevalence of health care associated infections especially surgical site infection (SSI).[2] The environment in the operation theatre is dynamic and subject to continuous change. Invasive procedures, high antibiotic usage and transmission of bacteria between patients due to inadequate infection control measures explain why OTs are "hot zones" for the spread of antibiotic resistant organisms.[3] Environmental monitoring means the microbiological testing of air, surfaces and equipment in order to detect changing trends of microbial counts and micro-flora.[4, 5]

Microbiological surveillance is done usually by the surface swabs and Petri plate gravitational settling method. Evaluation of the quality of air in operating theatres can be performed routinely by

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microbiological sampling and particle counting. The quality of indoor air depends on external and internal sources such as ventilation, cleaning procedures, the surgical team and their activities. [6]
Air contamination is usually expressed as Bacteria Carrying Particles per cubic meter (BCP/m3) or Colony Forming Units per cubic meter (CFU/m3) of air measured with an air sampler.[7] It is recommended that for conventional operating theatres the bio load should not exceed 35 CFU/m3in an empty theatre with less than one colony of Clostridium perfringens or S. aureus or 180 CFU/m3during an operation.[8] It is also suggested that for ultra-clean operating theatres the bio load should be less than 1.0 CFU/m3in the center of an empty theatre and less than 10 CFU/m3during an operation and should not exceed 20 CFU/m3at the periphery.[3,5]
The present study was conducted to determine the bacterial colonization of the surfaces and equipment and to identify the level of microbial contamination of air in operating theaters of hospital, CSSD and labor room.

MATERIALS AND METHODS

The prospective observational study was carried between January 2023 to July 2023 in the Bacteriology laboratory, Department of Microbiology, Nandha Medical College and Hospital, Erode, Tamilnadu, India. Sampling methods used in the study were surface swabbing and gravitational settling plate method. The samples were collected from 11 operating theatres, labor room (LR), and Central Sterile Service Department (CSSD). Labor ward includes clean labor room, septic labor room, Eclampsia room and neonatal resuscitation room.
The OT was sterilized by fumigation with high level disinfectant. The surfaces and equipment were disinfected with 70% alcohol based solutions like ethyl alcohol or isopropyl alcohol, 1% hypochlorite solution and Bacillocid Special.[9]The ORs were sealed off for 24-36 hours following fumigation before the next surgery. The samples were taken before the entry of surgery and support team. The theatre doors are kept closed prior to and during the sampling period. Sterile gloves, masks, and sterile gown were worn for collection to prevent the contamination of media and OT surface being swabbed. [10, 11]
Samples from surfaces and equipment
A total of 1109 samples were collected from 9 OTs, 4 labor rooms and CSSD. Sterile swabs soaked in saline were used to collect the samples. Samples were collected from different sites and equipment (instrument trolley, operation table, lights, Boyle's apparatus, wall, floor, AC vent, Door and Handle, wash basin) of nine OTs of the hospital. [8] In CSSD samples were collected from ETO sterilizer, ETO room wall, Steam sterilizer, steel trolley, store room wall, sterile things rack, ceiling table, steel cupboard, dispatch counter and A/C. In clean and septic labor rooms, eclampsia room samples were collected as in OT but in neonatal resuscitation room extra samples were collected from baby warmer, baby trolley and phototherapy machine.
All the swabs were labeled properly and immediately inoculated in Robertson's cooked meat broth (RCMB) and incubated for 7 days at 37°C. Smears from the bottles showing turbidity were stained with Gram's and examined for *Clostridium tetani* spores. *Clostridium tetani* were gram positive, slender bacillus with rounded ends, and somewhat "drumstick", like appearance. Results were recorded. [7,12] A loopful of turbid broth was sub cultured on Blood agar (Himedia) and MacConkey agar (Himedia). After incubation at 37-degree C for 24 & 48 hours, the isolated colonies were identified by using standard procedure. [13]
Air sampling
Air sampling was done by gravitational settle plate method. During air sampling procedure, sterile gloves, surgical masks and protective gowns were used to prevent contamination of agar plates. Plates were checked visually for any bacterial growth before use. Blood agar and SDA (Himedia) plates (10cm size) after labeling with appropriate date and time were exposed with lid opened at different areas in OT's, LRs and CSSD, one meter above ground, one meter away from the wall for one hour(1, 1, 1).[14] Plates were closed and sealed, transported to the laboratory. Blood agar plates were incubated at 37ºC for 48 hrs under aerobic conditions. SDA plates were incubated for 7 days at 25 to 27°C in the BOD incubator. [6, 14]
After incubation, plates were observed for growth and CFU/m3 were estimated using Omeliansky formula according to which
CFU / m3 = a x 1000 / p x t x 0. [2]
a = no of colonies on the Petri plates,
p =the surface measurement of the plate used in cm2
t = the time of the exposure of the Petri plates in minutes.
Final identification of bacterial isolates were done by following standard bacteriological techniques, and fungal identification was done by Lacto Phenol Cotton Blue (LCB) mount. [15-17]

RESULTS

A total of 1242 samples were collected, of which 1109 were surface swabs and 133 were settle plate samples. Of these, 139 (12.5%) surface swabs samples were culture positive and 970 (87.5%) were culture negative as shown in Figure 1
Surface swab
Among the 1109 swabs collected, morphologically resembling *Clostridium tetani* like organisms were found in the in the months of January, March, April and June as shown in Table 1. They were mainly found in the major OT 1 & 2, Labor room and

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Neonatal resuscitation room. After fumigation *Clostridium tetani* growth was not observed in the above areas.

Table 1: Clostridium tetani growth before fumigation

Name of OT	Jan	Feb	March	April	May	June	July	Interpretations
Major OT - 1	G	NG	NG	NG	NG	NG	NG	Recommended fumigation and through cleaning with surface disinfectant when growth was observed
Major OT - 2	NG	NG	NG	NG	NG	NG	NG	
Major OT - 3	G	NG	NG	NG	NG	NG	NG	
Major OT - 4	NG	NG	NG	NG	NG	NG	NG	
Emergency OT	NG	NG	NG	NG	NG	G	NG	
Ortho OT	NG	NG	NG	NG	NG	NG	NG	
ENT OT	NG	NG	NG	NG	NG	NG	NG	
Ophthalmology OT	NG	NG	NG	NG	NG	NG	NG	
Septic OT	NG	NG	NG	NG	NG	NG	NG	
Minor OT - 1	NG	NG	NG	NG	NG	NG	NG	
Minor OT - 2	NG	NG	NG	NG	NG	NG	NG	
Clean labour room	NG	NG	G	NG	NG	G	NG	
Septic labour room	NG	NG	NG	NG	NG	NG	NG	
Eclampsia room	NG	NG	NG	NG	NG	NG	NG	
Neo resusc room*	NG	NG	NG	G	NG	NG	NG	
CSSD	NG	NG	NG	NG	NG	NG	NG	

*Neonatal resuscitation room
*NG - No growth
*G – Growth

Table 2 shows the various sites in OTs, CSSD and labor rooms having bacterial growth. The bacterial contamination was found to be high in door and door handle, floor, bed and in A/C vent. Least contamination was found in wall and light. Surgical OT and emergency OT were found to have high bacterial growth when compared to other OTs.

Table 2: Bacterial growth from surfaces/equipment of various operation theatres, CSSD and labour rooms

Name of OT	Sampled sites							
	Wall	Floor	Light	Bed	Door	Trolley	Boyle's apparatus	A/C vent
Major OT - 1	+	+	-	-	+	-	-	-
Major OT - 2	-	+	-	+	+	-	-	+
Major OT - 3	-	-	-	-	-	-	-	-
Major OT - 4	-	-	-	-	-	-	-	-
Emergency OT	+	-	+	+	+	-	-	-
Ortho OT	-	-	-	-	-	-	-	-
ENT OT	-	-	-	-	-	-	-	-
Ophthamology OT	-	-	-	-	-	-	-	-
Septic OT	-	-	-	-	-	-	-	-
Minor OT - 1	-	-	-	-	-	-	-	-
Minor OT - 2	-	+	+	+	-	-	-	-
Clean labour room	-	+	-	-	+	-	-	+
Septic labour room	-	-	-	-	-	-	-	-
Eclampsia room	-	-	-	-	-	-	-	-
Neo resusc room*	-	-	-	-	+	-	-	+
CSSD	-	-	-	-	-	-	-	-

Among the 139 culture positive, Bacillus spp. was the most common bacterial isolate followed by Coagulase negative Staphylococcus (CONS) as shown in Fig.2. The list of bacterial species isolated were shown in Table-3.

Table 3: List of bacteria isolated from surface swab samples

S.NO	Bacterial isolates	Percentage (%)
1.	Bacillus spp	58%
2.	CONS	24%
3.	Staphylococcus aureus	11%
4.	Enterococcus	7%

Gravitational settle plate method

Out of 133settle plates observed from OTs, Labor room and CSSD, 40 (30.1%) plates were showing growth of organisms. Among 40 positives samples, Emergency OT and OT 4 both shows11 and 6 settle plates of maximum positives respectively.OT1 and Septic OT shows 5, other OTs shows a range of 1 to 4. Clean labor room shows positive for one settle plate as shown in Table 4.

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Table 4: CFU rate of air in OTs, LR and CSSD (Settle plate method)

S.NO	Name of OT	Positive Samples	Percentage of Positivity (%)
1.	Major OT - 1	5	12.5
2.	Major OT - 2	3	7.5
3.	Major OT - 3	1	2.5
4.	Major OT - 4	6	15
5.	Emergency OT	11	27.5
6.	Ortho OT	3	7.5
7.	Septic OT	5	12.5
8.	Minor OT - 1	4	10
9.	Minor OT - 2	1	2.5
10.	Cleanlabor room	1	2.5
TOTAL		40	100

In our study by settle plate method, we isolated Bacillus spp as the predominant organism (42.5%) which is a contaminant.42.5% of isolates were pathogens and 15% were fungi respectively. The distribution of commensals, pathogens and fungi were shown in Table 5.

Table 5: Distribution of bacteria and fungi isolated from settle plate

S.NO	Organism Isolated	Number of plates	Percentage (%)
1.	Bacillus spp.	17	42.5%
2.	Pseudomonas spp.	4	10%
3.	Staphylococcus aureus	6	15%
4.	Micrococci	4	10%
5.	Klebsiella spp.	3	7.5%
6.	Aspergillus fumigatus	2	5%
7.	Aspergillus niger	1	2.5%
8.	Rhizopus	2	5%
9.	Mucor	1	2.5%
TOTAL		40	100%

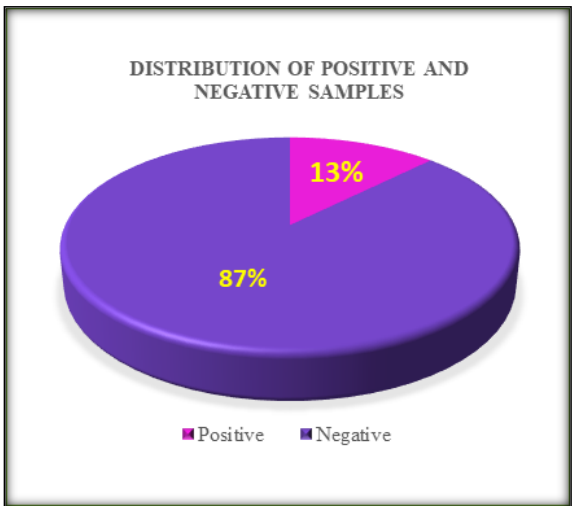

Figure 1: shows the percentage of positive and negative samples collected by surface swab method

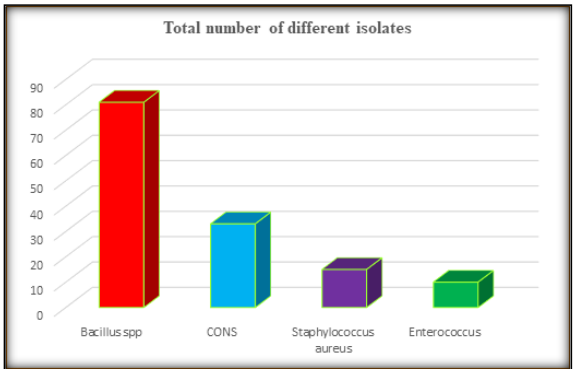

Figure 2: Distribution of bacteria isolated from surface swab samples

DISCUSSION

The clinical implication of microbial contamination in OT is a hot topic for patient's safety, clinicians and hospital administrators as post-operative infections can cause burden in terms of extra cost of medication, prolonged hospitalization, re-operations, increases the morbidity and emotional stress etc.[3] The HAI rates in a Hospital will an indicator of quality of the service provided.Hence,it becomes necessary to implement a proper surveillance mechanism to monitor these rates and to implement a working model to minimize it.

In our study we have identified and isolated *Clostridium tetani* from OT 1&2, and in LR. As in accordance with Sumangala B, ET al. [7] Since ours is a newly constructed medical college, *Clostridium tetani* would have been present in surfaces but after thorough cleaning with surface disinfectants and fumigation they were not isolated afterwards.

It was a routine practice in our theatres to do thorough cleaning on Saturday and fumigation on Sunday on weekly basis. Frequent entry in to OT and touching the door handles and OT tables by the staff without wearing gloves and direct contact with the patients might be the possible cause of high contamination in our study. Therefore, hand hygiene is mandatory in preventing cross-infections in sterile zones like OTs, CSSD and LR.

In the present study, we have collected 1109 surface swabs with a bacterial contamination rate of 12.5% (n = 139) were obtained from 9 OTs, CSSD and LR, The various studies showed positivity rate which

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varied from 6.4% to 45.8% as shown in the table. The variation in the results could be due to infection control practices, housekeeping protocols, duration of the study, time of sample collection (before or during surgery), sample size, condition of the OT (before or after fumigation).

S.NO	Authors	Percentage of positivity
1.	Vanlalruati et al.,[1]	6.4%
2.	Roma A et al.[18]	7.07%
3.	*Dr* Krunal Shahet al.,[19]	8.79 %
4.	Yadav et al.[16]	23.4%
5.	Patel Purav et al.,[20]	34.62%
6.	Meenakshi et al.[21]	45.8%

In the present study, Bacillus spp. 81 (58%) an environmental contaminant was the predominant organism. This is in accordance with other studies Shukla ET al. (45.4%).[22] Desai ET al.(50%).[23] Kiranmai ET al. (75%).[8] Javed ET al. (77%)[5] and Najotra ET al. (87.6%).[11] Coagulase negative Staphylococci isolates were 33 (24%) which is in accordance with the study by Laxmi et al.(17.35%), [10] The most common pathogens causing skin and soft tissue infections such as *Staphylococcus aureus* (11%) and *Enterococcus* (7%) were isolated, showing similar results to Vanlalruati et al.[1]

The microbiological quality of air reflects the hygienic condition of operating room. The quality of indoor air depends on external and internal sources, such as ventilation, cleaning procedures, the surgical team and their activity. Since air is an important vehicle for transmission of many pathogenic organisms, it is important to detect the number of bacteria carrying particles in operation theatres.

In our air sample analysis, we found that maximum contamination was found in Surgical OT, Emergency OT and septic OT. Pritikumari, PET al. [24] also had similar findings. Higher air contamination rates in these OTs observed could be due to many factors including unplanned emergency surgeries leading to decreased adherence to restricted entry in that area. The most common isolate from air samples was Bacillus spp (42.5%) followed by Gram positive organisms (25%) and Gram negative bacteria (17.5%).This finding of higher rate of Bacillus isolation was comparable to a study by Sumangala B ET al. [7] Many studies have suggested that higher concentrations of these cocci can be attributed to the lower susceptibility of these organisms to environmental stresses, due to the presence of pigments and higher peptidoglycan contents in their cell walls, which provides protection from drying and heat stress Kasdekar, M.M.ET al.[25]

Airborne GNB were few in number and the commonest isolates from the air samples were *Pseudomonas spp.* and *Klebsiella spp.* The lower concentrations of these GNB may be attributed to their susceptibility to environmental stress due to lower peptidoglycan contents, leading to their injury and/or cell death Suchithra ETal.[26]The fungi such as *Aspergillus spp* (7.5%), *Rhizopus* (5%) and *Mucor* (2.5%) were isolated from air samples. Though these fungi were seen in few numbers, their mere existence in the hospital air is of concern as many of these spp are known to cause opportunistic infections, especially in immunocompromised patients. *Aspergillus spp* also causes allergic reactions as well as pulmonary infections. Their presence in hospital air needs attention.

CONCLUSION

This study suggested that surfaces and indoor air of all OTs, Labor room and CSSD were mainly infected mostly by non-pathological Microorganisms. Aerobic spore-forming Bacilli were the most common isolate. Normal flora and pathogens though present were less grown. Fungi were only isolated from air which could be from A/C let. To prevent air contamination, positive pressure maintenance and proper maintenance of quality of HEPA filters is recommended. There should be minimum occupancy of persons at any time inside the OT. The hospital infection control committee should implement the infection control program by carrying out targeted surveillance of all critical areas in the hospital, staff education and accountability, supervise and monitor cleanliness and hygienic practices, oversee sterilization and disinfection, monitor the use and quality control of disinfectants and finally Waste management. This can reduce the incidence of HAIs by around one-third. This data can be used to set regional standards for levels of acceptable microbial population and can also be used to suggest suitable guidelines in order to decrease the microbial rates in surfaces, equipment and indoor air.

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