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Original Research Article

Surveillance of operation theatres of a tertiary care hospital by settle plate method and surface swab technique

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ABSTRACT

Background: Infection control by environmental monitoring plays a significant role in reducing the morbidity and mortality of patients who are victims of hospital associated infections. Many developing countries have reported high postoperative infection rate in their hospital, so regular monitoring of pathogens in the air sample is one of the preventive measure for post-operative infections. This study was carried out with an interest to look for the organisms and the frequency of isolation in air sample and surface swabs before and after fumigation.

Materials and Methods: Study was conducted over a period of 12 months from August 2018 to August 2019. Total of 6 OT, two staff room, one washing room were included for settle plate and surface swab method of monitoring for air contaminants and pathogens.

Results: Total of 252 settle plate samples were collected over a period of 12 months before and after fumigation, bacillus was major (59%) contaminant followed by pathogens like *Pseudomonas* (14%), CONS (10%), *Klebsiella* (7%), *E.coli* (5%), *Micrococci* (3%) and *Staphylococcus.aureus* (2%). Surface swab method- Fungus isolated from AC filter were *Aspergillus* (78%), *Mucor* (14%), *Rhizopus* (6%), *Pencillin* (2%). Swabs collected from OTs were tested for growth of *Clostridium.tetani*. Growth was observed in the month of October, February and May. Advised fumigation and through cleaning of OTs with high level disinfectant whenever grown was observed before and after fumigation, results found to be satisfactory and advised to continue with procedures when growth found <10 colonies on settle plate and no growth of CT in RCMB.

Conclusion: Regular monitoring of pathogens in the air is one of the preventive measures for post-operative infection. Settle plate method and surface swab method is useful for observing the aerobic and anaerobic growth in operation theaters.

1. Introduction

Postoperative infection remains a major cause of morbidity amongst patients undergoing surgery. Maintenance of strict a sepsis is essential if postoperative infections and their consequences are to be minimized.[1]

Operating room (OR) air is persistent source of postoperative infections. In the late 18th century, the surgeon Joseph Lister used carbolic spray to disinfect the OR air for reducing the mortality rate from postoperative infection.[2]

Postoperative infections can be caused by a contaminated surfaces, contaminated environment, unsterile equipment, infected personnel and contaminated disinfectants.[2]

Biological contaminants occur in the air as aerosols and may include bacteria, fungi & viruses.[3] Factors which affect the contamination in air are number of persons present in theatre, type of ventilation, rate of air exchange, quality of air provided & types of organisms.[4–6] Monitoring the environment by the microbiological testing of surfaces and equipment's is useful to detect types and counts of microbial

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flora.[7]

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.[2]

Most of the contaminants are harmless saprophytes and commensals, only 0.01% of bacteria are pathogens. Common pathogens isolated in OT are *Staphylococcus.aureus*, *Coagulase negative Staphylococcus, Pseudomona.aeuroginosa* and fungus.[2]

Organisms isolated and their sensitivity pattern indicates the seriousness of the infection which can guide the infection control programme effectively. Microbiologic surveillance is required to know the efficiency of disinfection methods and fumigation technique.

This study was done to analyze air contamination by settle plate method and surface swab method.

2. Aim and Objectives

1. Microbiological surveillance of OT by settle plate method & surface swab method for anaerobic & aerobic infection.
2. To compare the utility of surface swab method & settle plate method for routine microbiological screening of OT.
3. To assess the trend and quality of air maintained between two subsequent fumigations.

3. Materials and Methods

3.1. Study design

Prospective observational study.

3.2. Study period

Months, from August 2018 to August 2019.

3.3. Inclusion criteria

Major OT of MIMS, Mandya.

3.4. Exclusion criteria

Minor OT, all ICUs of MIMS, Mandya.

3.5. Method of data collection

4. Methods

Over a period of 12 months, environmental Bacteria Carrying Particle (BCP) load were studied weekly and surface samples monthly. Once in four month fungal growth was detected in air conditioning filters.

Surface swabs were collected and plates for settle plate were kept after before and after fumigation. high-level disinfection were used every Saturday by formaldehyde gas generated by addition of KMnO4 and 40% liquid formalin, additional fumigation were done whenever an obviously infected case was operated.

The ORs were sealed off for 24-36 hours following formaldehyde fumigation before the next surgery. Liquid ammonia solution was used to neutralize the irritant effects of formaldehyde two hours before surgery.[8]

4.1. Settle plate methods

Sheep blood agar plates (10cm size) after labeling with appropriate date and time were exposed with lid opened for 30 minutes at different areas like washing room, OT table (head end and foot end), OT floor, doctors room and nurse room before and after fumigation of OT.[9] Plates were closed and sealed, transported to the laboratory and incubated at 37ºC for 48hrs. Growth was observed, colony forming units (CFU) were counted. The acceptable limit of CFU was calculated by using the formula based on the colony count, area of the plate exposed, and the duration of exposure and it is fixed as 10 CFU instead of 12 to certify the OT is safe for surgery.[10] Approximately 180 bacteria per cubic meter of air correspond to 10 colonies settling on a plate. The operating rooms are said to carry out operative procedures only when the bacterial load is less than 180 per cubic meter/<10 colonies grow on blood agar (BA). Detection of even a single colony of *S.aureus* is considered a risk for infection.[11]

4.2. Surface swab

Surface swabs were collected from the operation table at the head end, foot end, over head lamp, the wall near the electrical switch, the floor, Anesthesia machine, washing area, Fan and Dustbin. The swabs were inoculated in Robertson's cooked meat broth (RCMB) for 7 days at 37°C, smears from the bottles were stained with Gram's and examined for *Clostridium.tetani* spores. Results were recorded.[10]

Swabs were collected from filters of air-conditioning units and streaked on Sabourauds's dextrose agar without antibiotics to isolate fungi with an interval of one month. If fungi growth was observed then air conditioner filter was cleaned using chlorine dioxide (sporicidal disinfectant).[10]

Commercially available spore strips impregnated with spores of *Bacillus.sterothermophillus* were used to check the efficacy of sterilization by autoclave. After autoclave strips were removed and aseptically transferred to RCMB which was incubated at 56ºC for 5 days. Broth was examined for signs of turbidity intermittently. Efficacy of autoclave was checked once in a month.[10]

Table 1: Showing CFU before fumigation

Name of the OT	No of CFU/Plate before fumigation												Interpretations
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	
Ortho OT –I	28	10	37	21	12	28	19	39	11	15	26	38	Advised fumigation and through cleaning with high level disinfectant
Ortho OT –II	21	28	34	10	16	28	21	37	10	13	29	23	
Surgery OT	29	26	14	27	25	29	16	30	28	19	27	23	
ENT OT	28	19	34	13	35	27	12	10	32	35	28	18	
Septic OT	24	10	31	29	16	24	36	32	22	27	38	19	
OBG OT	16	28	15	33	14	17	25	35	30	27	26	18	
Washing room	16	29	32	29	37	26	21	16	23	12	16	20	
Doctors room	29	39	22	29	25	20	23	30	22	10	21	30	
Nurse room	20	36	28	26	15	23	27	28	20	25	18	27	

Table 2: Showing the CFU after fumigation

Name of the OT	No of CFU/Plate after fumigation												Interpretations
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	
Ortho OT –I	0	0	2	1	0	0	0	0	0	7	1		Advised repeat fumigation only when CFU >10
Ortho OT –II	0	4	0	0	0	0	1	0	0	0	0	0	
Surgery OT	0	0	0	0	0	0	0	1	0	0	0	0	
ENT OT	0	0	0	0	0	0	0	0	0	0	0	0	
Septic OT	5	0	0	1	0	2	0	3	0	0	0	0	
OBG OT	3	0	3	0	0	6	0	0	1	0	0	0	
Washing room	0	0	4	0	3	0	0	1	0	2	2	0	
Doctors room	0	0	1	0	0	0	0	0	2	0	0	0	
Nurse room	0	0	0	3	0	5	0	0	0	0	1	0	

Table 3: List of bacteriaisolated from settle plate

Organisms (Bacteria)	Percentage (%)
Bacillus.subtilis	59%
Pseudomonas.spp	14%
CONS	10%
Klebsiella spp	7%
E.coli	5%
Micrococci	3%
Staphylococcu. aureus	2%

Table 4: List of fungus isolated from AC filter

Organisms (Fungus)	Percentage (%)
Aspergillus	78%
Mucor	14%
Rhizopus	6%
Penicillin.	2%

Table 5: Showing presences of Clostridium.tetani growth before fumigation

Name of the OT	Before fumigation presences of CT growth												Interpretations
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	
Ortho OT –I	N	N	N	N	N	N	N	N	N	N	N	N	Advised fumigation and thorough cleaning with disinfectant When growth was observed.
Ortho OT –II	N	N	Y	N	N	N	N	N	N	N	N	N	
Surgery OT	N	N	N	N	N	N	N	N	N	Y	N	N	
ENT OT	N	N	N	N	N	N	N	N	N	N	N	N	
Septic OT	N	N	Y	N	N	N	Y	N	N	N	N	N	
OBG OT	N	N	N	N	N	N	N	N	N	N	N	N	
Washing room	N	N	N	N	N	N	N	N	N	N	N	N	
Doctors room	N	N	N	N	N	N	N	N	N	N	N	N	
Nurse room	N	N	N	N	N	N	N	N	N	N	N	N	

*N-No growth
*Y-growth observed

5. Results

5.1. Settle plate methods

Total of 252 settle plate samples were collected from six OT, two staff room and one washing room over a period of 12 months.

Table 1 : Explains about the observed colony count before and (Table 2) after fumigations in Operation theaters.

Bacterial and fungal growth was observed in blood agar plate, bacteria's were identified according to CLSI guidelines. In our study we isolated Organisms like *Coagulase negative Staphylococcoci, Staphylococcus aureus, Micrococci, Bacillus, E.coli, Pseudomonas* and *Klebsiella*. Growth of Mucor and Aspergillus was observed twice in settle plate.

5.2. Surface swab

Over a period of one year, we observed morphologically resembling *Clostridium.tetani* like organisms in the month of October, February and May by surface swab smear stained with Gram's as shown in (Table 5).

After fumigation *Clostridium.tetani* growth was not observed in operation theaters.

Fungal growth observed in air conditioner filters such as *Aspergillus, Mucor, Rhizopus* and *Penicillin.*

6. Discussion

This study was carried out with an interest to look for the organisms and frequency of isolates in air sample and surface swabs before and after fumigation. In this present study we isolated organisms like *Coagulase negative Staphylococcoci, Staphylococcus aureus, Micrococci, Bacillus subtilis, E.coli, Pseudomonas* and *Klebsiella* from settle plate kept open in OTs and staff rooms. *Bacillus subtilis* is the major contaminant found in OTs, Pathogen like *Pseudomonas* found to be major organism and *Staphylococcus* found to be least isolated.

In comparison to our study, similar organisms were isolated in a study conducted by Dr. Krunal Shah et al. They observed *Bacillus subtilis* 55%, *Pseudomonas* 38%, CONS 30%, *Klebsiella* 25%, *E.coli* 10%, *Stapyloccocus.aureus* 8%. In addition to our study they also isolated Proteus *mirabilis and Acinetobacter spp*. Study conducted by Dr. E. Rajni Sabharwal et al., showed similar organisms as observed in this present study. [12,13]

7. Conclusion

Monitoring both aerobic and anaerobic organisms in air by settle plate and surface swab method can provide a simple and cost effective way of detecting the

contamination. Surface swab with RCMB culture can only yield *Clostridium.tetani* were as settle plate can detect aerobic pathogens as well fungus in air.

Settle plate is more useful because aerobic postoperative infections are more common than anaerobic postoperative infections. Surface swab technique is mandatory in places where civil work is undertaken and in orthopedics due to RTA (road traffic accidents) cases & trauma cases. OT which undergoes both the methods is beneficial in detecting pathogen and prevent from hospital associated infections.

8. Source of Funding

None.

9. Conflict of Interest

None.

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