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RESEARCH ARTICLE

ENVIRONMENTAL MICROBIOLOGICAL SURVEILLANCE OF OPERATION THEATRES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Microbial contamination of air/surfaces/articles in operation theatre (OT) is a major cause of surgical site and nosocomial infections. Approximately 10% of nosocomial infections have serious outcomes leading to longer duration of hospital stay and cost burden. Currently there is no uniform consensus on either the standards for surveillance, methodology for monitoring or the levels of acceptable contamination and the risk of nosocomial infections borne by OT. The present study was conducted with an aim to isolate and identify the microbial contamination of the air, surfaces and equipments of the OT of a tertiary care post graduate teaching hospital. Air quality surveillance of OT's was done by settle plate method and note of bacterial CFU/ m^3 count was made. For surface sampling, wet swabs were taken from different sites and equipments. Bacterial species were isolated and identified by conventional methods. In the settle plate technique, mean CFU/m^3 was recorded for 8 different OT's. Ophthalmology OT recorded least bacterial CFU rate of air(114 CFU/m3) followed by ENT OT(166 CFU/m3) and in Gynaecology and Obstretics OT(255CFU/m3), highest bacterial CFU rate was noted. Out of total 68 surface and articles sampled from different OTs, only 9(13.2%) showed growth of bacteria. The most common isolate was Coagulase negative Staphylococcus species (5.8%) followed by Bacillus and Klebsiella species (4.4%) each.

INTRODUCTION

Microbial contamination of air/surfaces/articles in OTs is a major cause of surgical site and nosocomial infections. Approximately 10% of the nosocomial infections have serious outcomes leading to longer duration of hospital stay and cost burden. Air bioload present in the form of aerosols may contain bacteria, viruses, yeasts, moulds and fungal spores. The most important source for generating aerosol is infected patient, activities of medical staff and visitors load. Once aerosolized, subsequent loss of water helps the pathogen to remain suspended and carried to considerable distance. Microbiological contamination of air in the OTs is a major risk factor for surgical site infection (SSI) (Fleischer *et al.,* 2006). SSI delays wound healing, prolongs hospitalization, increases morbidity and the overall costs. 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. Environmental monitoring means the microbiological testing of air, surface and equipment in order to detect changing trends of microbial counts and micro-flora (Baird, 1996). It helps in monitoring the capability of the air filters used in the OT's and also helps in assessing quality and making timely changes in measures that need to be adopted in order to maintain air quality in these areas. Though at present, there is no international consenses on the methods, types of samples (settle plate versus volumetric air sampling), frequencies of sampling and tolerable limits of bioburden in OTs. It is recommended that for conventional operating theatres, the bio load should not exceed 35 CFU/m^3 in an empty theatre or 180 CFU/m^3 during an operation. It is also suggested that for ultra-clean operating theatres the bioload should be less than 1.0 CFU/m^3 in the centre of an empty theatre and less than 10 CFU/m^3 during an operation and should not exceed 20 CFU/m^3 at the periphery (Baird, 1996).

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MATERIALS AND METHODS

The study was carried out at the Department of Microbiology, Geetanjali Medical College and Hospital, Udaipur Rajasthan, for a period of three months during the year 2014.

Sample Collection and Transport

Air and surface samples were taken randomly without prior discussion with the cleaning staff and adequate care was taken to ensure that there was no trafficking in these areas while the

sampling procedures were completed. Standard operating procedures (SOP) were followed at the operation theatres to monitor the bacterial load by the settle plate method (Charnley and Efthekhar, 1969). Air sampling was done by settle plate method. Blood Agar and Sabourd's Dextrose Agar plates were taken to the operation theaters in sealed plastic bags. The plates were labeled with sample number, site within theatre, time and date of sample collection. One plate each was kept at the center of operation theatre and the four corners of the operation theatre at about 1 meter above the ground, 1 meter from the wall and exposed for 1 hour (Baird, 1996). Surface sampling was done by soaking a swab in nutrient broth which was rolled over to the surfaces of equipments, instruments trolley, operation tables at the head end, Over head lamp, monitor, an aesthesia table, IV infusion pumps, crash cart, door handles. A total of 68 surface samples were labeled properly and transported immediately to the microbiology laboratory for processing.

Processing Of Samples

Air sampling – Exposed plates were sent to laboratory in sealed plastic bags. Blood agar plates were incubated at 37°C for 24 hours and SDA plates were incubated at 25°C for 7 days. After incubation, the colonies were counted and isolates were identified by using conventional methods. The resultant colonies were counted and converted into colony forming unit per cubic meter of air (CFU/m3) using Omeliansky formula (Abdel Hameed, 2013).

$N=5a \times 10^4 (bt)^{-1}$
N=colony forming unit per cubic meter of air (CFU/m^3)
a=number of colonies per petridish
b=surface area of petridish in cm^2
t=time exposure (minutes)

Surface sampling - Swabs collected from various surfaces were inoculated by streaking on Blood Agar and MacConkey Agar plates. These culture plates were incubated at 37^0C for 24 hours under aerobic condition. After incubation, the isolates were identified by conventional methods. All isolates were divided in to three broad categories: (Fleischer *et al.,* 2006) Normal flora e.g. Coagulase Negative Staphylococcus (CoNS) (Sandle, 2006) Contaminant e.g. Bacillus species (Baird, 1996) Pathogen e.g. Staphylococcus aureus, Klebsiella species, and Pseudomonas aeruginosa (Desai *et al.,* 2012).

RESULTS

Table 1. Bacterial Count Of Air From Various Ot's : (Air Sampling)

Name of OT	CFU/m^3
Orthopedics	00
Neuro surgery	00
Cardio-thoracic vascular surgery [CTVS]	00
General surgery	204
Urology	218
Ophthalmology	114
ENT	166
Gynaecology and Obstretics	255

Table 2. Various Bacterial Isolates From Ot's In Air Sampling

Name of OT	Organism isolated
Orthopedics	No Bacterial growth
Neuro surgery	No Bacterial growth
Cardio-thoracic vascular surgery	No Bacterial growth
General surgery	Coagulase negative Staphylococcus species and Bacillus species
Urology	Coagulase negative Staphylococcus species
Ophthalmology	Coagulase negative Staphylococcus species
ENT	Coagulase negative Staphylococcus species and Bacillus species
Gynaecology and Obstretics	Coagulase negative Staphylococcus species

Table 3. Various bacterial isolates from ots in surface sampling

Bacterial isolates	Number (%) n/68
Coagulase Negative Staphylococcus species	4 (5.8 %)
Klebsiella species	3 (4.4 %)
Bacillus species	3 (4.4 %)
Total	10 (14.6%)

Table 3. Various Bacterial Isolates From OT's in Surface Sampling

Name of OT	Organism isolated
Orthopedics	No Bacterial growth
Neuro surgery	No Bacterial growth
Cardio-thoracic vascular surgery	No Bacterial growth
General surgery	Coagulase negative Staphylococcus species
Urology	No Bacterial growth
Ophthalmology	No Bacterial growth
ENT	Coagulase negative Staphylococcus species
Gynaecology and Obstretics	Klebsiella species, Coagulase negative Staphylococcus species and Bacillus species

DISCUSSION

The clinical implication of microbial contamination in OT is a hot topic for patients safety, clinicians and hospital administrators as post operative infections can cause burden in terms of extra cost of medication, prolonged hospitalization, re-operations, etc. Prevention measures that need to be practiced to avoid such critical situations rest not only with the operating personnel but also with the entire infection control team. The role of the clinical microbiologist becomes crucial, as they help in identifying and give suggestion for controlling some of the microbial contamination. In this context, monitoring and microbiological surveillance can serve as warning systems for change in the type and count of microbial flora.

Counting microbes in the air is not a easy task, but through air and surface sampling it is possible to evaluate bacterial contamination of environment (Davis *et al.,* 1999). Many different methods are in use which can be divided into four groups: counts of colony forming units per cubic meter of air (CFU/m^3); counts of CFU on settle plates; counts under a microscope; and measurement of a chemical component of the microbial cells per cubic meter of air. At the moment, the only effective means of quantifying airborne microbes is limited to the count of CFU. The CFU count is the most important parameter, as it measures the live micro-organisms which can multiply. Air samples can be collected in two ways: by active air samplers or by passive air sampling (the settle plates). Both methods are widely used (Pasquarella *et al.,* 2004). From table

I of our study, the bacterial CFU counts of air from all OTs ranged from 114 CFU/m^3 (Ophthalmology OT) to 255 CFU/m^3 (Gynaecology and Obstretics OT). From table II of our study, Coagulase Negative Staphylococci (CoNS) was isolated from air samples from all operation theatres except Orthopedics, Neurosurgery and Cardio-thoracic vascular surgery OT. Bacillus species were present in the air of ENT and General surgery operation theatres. No fungus were isolated from any OTs.

Microorganisms that cause infections in OT include bacteria, fungi and viruses (Qudiesat *et al.,* 2009). It has been observed that counts in the range of 700-1800/m^3 were related to significant risk of infection and the risk was slight when they were below 180/m^3 (Parker, 1978). A 13-fold reduction in airborne bacteria in the OTs would reduce wound contamination by around 50% (Genet *et al.,* 2011). In our study, bacterial count of air is in a range of 114-255 CFU/m^3. Microbiological surveillance study in OTs of a tertiary care hospital at Lahore (Javed *et al.,* 2008) and Mysore (Deepa *et al.,* 2014) have reported a significantly higher bacterial air count in the range of 6500-15730CFU/m^3 and 628-1571CFU/m^3 respectively. This is in contradiction with the study conducted by Desai *et al*. 2012, in which a low bacterial air count in the range of 20-75 CFU/m^3 was reported. The reason for such variations many be attributed to the method employed for surveillance (active air sampling or passive air sampling), time of sampling, disinfectant used and mechanical ventilation of OTs.

Air sampling of all OTs showed that they were free from pathogens. CoNS were isolated from the air sample of all OTs except, orthopedics, CTVS, and Neurosurgical OT. Highest prevalence of CoNS was in Gynaecology and Obstretic OT (40%), General surgery OT (30%) and lowest prevalence was from ophthalmology (5.5%). These findings are concurrent with various studies conducted by Javed *et al*. 2008 and Desai *et al.* 2012.

From table III of our study, a total of 68 swabs were collected from various surfaces and articles from different operation theatres. Out of which 9 [13.2%] samples showed growth on the culture media at 37^0C after 24 hours of incubation and remaining 45 samples showed no growth. Nine swab samples yielded 10 isolates. Of these 10 isolates obtained from various OTs, 4(5.8%) were Coagulase Negative Staphylococcus species, 3 (4.4%) were Klebsiella species and the remaining 3 were Bacillus species.

From table IV of our study, we observe Coagulase Negative Staphylococci (CoNS) was isolated from swabs of operation theatres of general surgery, ENT and Obstetric and Gynaecology. Klebsiella and Bacillus species were present in the swabs of Obstertric and Gynaecology operation theatres. No fungus were isolated from any OT's. In the present study a total of 68 swabs collected from various surfaces and articles in the OTs. Out of this only 9 of them showed the growth. The instruments and articles which were sterilized by autoclave showed no growth whereas highly touched areas like door handles, IV stand and OT light's showed growth of bacteria. In our study, floor, roof and upper part of the wall were not surveyed, because it may cause confusion during the interpretation of results and are not in direct contact with patients, hence they do not contribute in the prevention of SSI infection (Stockley *et al.,* 2006). Bacterial surface sampling of OT's were observed to be colonized with CoNS, which was the most predominant organism isolated from various surfaces and articles. The reason might be due to the shedding of CoNS from skin of health care workers and patients and easy cross transmission in between the patient and the health care worker or vice versa. This was followed by Bacillus species, which are considered to be the contaminant.

Limitation of Our Present Study

In our study settle plate method for air sampling was used, though it may be regarded as a crude measure of airborne contamination. In places without other facilities it can still provide a simple and cost effective way of enumerating the air contamination rate at multiple points. With limited sources in our hospital, settle method and environmental sampling has given a valuable report. Settle plates are inexpensive and easy to use and require no special equipment. They are useful for qualitative analysis of airborne microorganisms and the data they produce may detect underlying trends in airborne contamination and provide early warning of problems.

Conclusion

Harboring of potential pathogens in OTs of hospital can pose a great risk to patients. Monitoring the bioload of air helps in assessing the capability of air filters used in the OT's and also helps in assessing quality and making timely changes in measures that need to be adopted. Our study was conducted to gain knowledge regarding the air quality and the quantity of airborne pathogens in OT. In our study, bacterial air sampling in all OTs showed a much less colony count ranging from 114-255CFU/m^3. 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 indoor air. Strengthening surveillance and laboratory capacity will surely enhance infection prevention and control. Routine sampling is strongly recommended so that we can become more aware and alert to identify and control all possible sources and types of infections. Hence in future, more and more studies should be undertaken to find out prevalence of type of bacterial isolates and also comparative studies on the types of routine sampling for detection of microbiological quality of air of OT along with infection control measures which will be very helpful in controlling SSI.

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