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Original Research Article

Microbiological surveillance of operation theatre in a tertiary care hospital in North East India

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ABSTRACT

Background: Good hospital hygiene is an integral part of infection control programme. "Microbiological surveillance" provides data about the factors contributing to infection. Bacterial counts in operation theatres are influenced by number of individual present, ventilation and air flow methods. Purpose of the study is to find out prevalence rate of microorganisms in Operation Theatre, to find out the frequency of contamination from various sites in operation theatre.
Methods: The study was conducted in the department of microbiology, Regional institute of medical sciences, Imphal, Manipur, India. Air samples were taken by settle plate method in petri dishes containing blood agar and surface samples were taken by a sterile swab soaked in nutrient broth from all operation theatres. The samples were processed according to standard operative procedures.
Results: Least bacterial colony forming unit (CFU) was shown by ophthalmology OT 17 CFU/mm^3 and highest was shown by emergency OT 200 CFU/mm^3. Isolated organism was divided into normal flora (CONS, micrococci), contaminant (bacillus species) and pathogenic organism e.g. *Staphylococcus aureus*, *Acinetobacter spp.*, *Pseudomonas spp*. 15 (23.4%) swab samples out of a total of 64 swab samples were found to be growth positive. Out of that 4 CONS, 4 micrococci, 3 *Bacillus spp*, 2 *Acinetobacter spp*, 1 *Enterobacter spp*, 1 *Pseudomonas spp.* were isolated.
Conclusions: Strengthening surveillance and laboratory capacity will surely enhance infection prevention and control. Routine sampling is strongly recommended for increasing awareness to identify and control all possible sources and types of infections.

Keywords: Imphal, Microbiological surveillance, Operation theatre, Settle plate method

INTRODUCTION

Good hospital hygiene is an integral part of infection control programme. "Microbiological surveillance" provides data about the factors contributing to infection.[1] Bacterial counts in operation theatres are influenced by number of individual present, ventilation and air flow methods. In developing countries like India, where there are no uniform guidelines, many OTs are built and maintained according to the individual's knowledge level, availability of funds, technical staff, and equipments. The environments in the operation theatre are dynamic and subject to continuous change. Good infrastructures do not mean a safe environment as human make a greater difference in making the environment unsafe. Microbiologists should be aware of organisms, sites and populations as surveillance cultures should be chosen carefully to allow meaningful

interpretation of results. Environmental monitoring means the microbiological testing of air, surfaces and equipment to detect changing trends of microbial counts and micro-flora.[2] Surgical-site infection is the leading complication of surgery. Normal skin flora of patients or healthcare workers causes more than half all infections following clean surgery, but the importance of airborne bacteria in this setting remains controversial.[3]

Invasive procedures, high antibiotic usage and transmission of bacteria between patients due to inadequate infection control measures may explain why OTs and ICUs are "hot zones" for the emergence and spread of microbial resistance.[4] Lack of adherence to established standards and guidance can result in adverse patient outcomes in health-care facilities.[5] Biological contaminants occur in the air as aerosols and may include bacteria, fungi, viruses, and pollens. In this method, the larger bacteria carrying particles settle by gravity from the air to exposed surfaces (blood agar plates). It is recommended that for conventional operating theatres the bioload should not exceed 35 cfu/m^3 in an empty theatre or 180 cfu/m^3 during an operation.[6]

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.[7]

Purpose of the study is to find out prevalence rate of microorganisms in operation theatre, to find out the frequency of contamination from various sites in operation theatre and evaluation of antibiotics susceptibility patterns of organisms isolated.

METHODS

The study was conducted in the department of Microbiology, Regional institute of medical sciences, Imphal, Manipur, India. The theatre being sampled have been left vacant for more than one hour before sampling proceeds to avoid false-positive results due to recent theatre usage. The theatre doors are kept closed prior to and during the sampling period.

Collection and transport of specimens

Air and surface samples were taken from all operating theatres of a tertiary care hospital in Imphal. Blood agar plates, sterile swabs and nutrient broth were transported to operation theatres in sealed plastic bags.

Air sampling

Blood agar plates (3 for each OT) with known surface area were taken and marked with distinctive numbers, record of the position, time and duration of exposure for each plate is prepared. The blood agar plates were uncovered and keeping the lid over the plate at an inclined position. 2 plates kept at corners (1 near the door and 1 near the washroom) and 1 on the OT table of the operation theatre at a height of 1 meter above the ground. The uncovered plates were exposed for 15 to 30 minutes, then at once the lids were replaced. The plates were packed and taken to the microbiology laboratory. Incubate the plates aerobically for 24 hours at 37℃. After incubation, the colonies on each plate were counted and recorded as the number of bacteria carrying particles settling over the area of the plate in each period. For obtaining pure cultures Subcultures to be done on blood agar and MacConkey agar. Identification of isolates was done by gram staining and standard biochemical tests.[8] Antibiotic sensitivity tests done by Kirby Bauer Disc Diffusion Method under CLSI Guidelines.[9]

Air contamination standards

The level of bacterial contamination of air is usually expressed as the number of bacteria carrying particles per cubic meter (bcp/m^3) or bioload (B). In the settle-plate technique the number of microorganisms expressed as cfu/m3 is estimated using Koch's sedimentation method according to which,

$$\text{Cfu/m3} = \frac{\text{a X 1000}}{\text{P X t X 0.2}}$$

Where, a = the number of colonies on the petri plate, p = the surface measurement of the of the plate used in cm^2, t = the time of exposure of the petri plate in minutes. Recommended conventional operating theatres values:

- An empty theatre bioload should not exceed 35 cfu/m^3
- During an operation bioload should not exceed 180 cfu/m^3
- Ultra clean air operating theatres where cardiac and joint replacement surgeries in the centre of the empty theatre bioload should be less than 1 cfu/m^3
- During an operation, less than 10 cfu/m^3 and should not exceed 20 cfu/m^3 at the periphery.

Surface sampling

Sterile swabs moistened with nutrient broth were used for collecting samples from different surfaces. Samples were collected from operation table, wash basin, drug rack, instrument table, instruments, overhead lamp, door handle, etc. All samples were labelled properly and immediately transported to the microbiology laboratory. Swabs taken from different sites were inoculated on nutrient agar, blood agar and MacConkey agar. These culture plates were incubated at 37°C under aerobic conditions for 24 hours. After incubation, the colonies were counted and identification of isolates was performed by gram staining and standard biochemical tests.[8] Antibiotic sensitivity tests done by Kirby Bauer disc Diffusion method under CLSI guidelines.[9]

All isolates were divided in to three broad categories Fleischer et al. Normal flora e.g. coagulase negative *Staphylococcus* (CONS) Sandle et al contaminant e.g. *Bacillus spp*. Baird et al pathogen e.g. *Staphylococcus aureus*, *Klebsiella spp*., and *Pseudomonas aeruginosa* Desai et al.[2,10-12]

RESULTS

Air sampling by settle plate method

The bacterial CFU/m^3 counts of air from Ophthalmology OT was found to be least 17 CFU/m^3 air during pre-OT and 31 CFU/mm^3 during intra-OT, followed by ENT OT 18 CFU/mm^3 pre-OT and 56 CFU/mm^3 intra OT, urology OT 21 CFU/mm^3 pre-OT and 90 CFU/mm^3 intra-OT, orthopedics OT 77 CFU/mm^3 pre-OT and 95 CFU/mm^3 intra-OT. High bacterial CFU was found in obstetrics and gynaecology OT 62 CFU/mm^3 pre-OT and 185 CFU/mm^3 intra-OT, surgery OT 82 CFU/mm^3 pre-OT and 177 CFU/mm^3 intra-OT while highest in emergency OT 66 CFU/mm^3 in pre-OT and 200 intra-OT (Table 1).

Table 1: Bacterial count of air from various OTs (air sampling).

Name of the OT	Pre-OT (CFU/mm^3)	Intra OT (CFU/mm^3)
Surgery	82	177
Emergency	66	200
Obstetrics and gynecology	62	185
Ophthalmology	17	31
Urology	21	90
ENT	18	56
Orthopaedics	77	95

From surgery OT only bacillus was isolated in both pre-OT and intra OT, *Staphylococcus aureus* was isolated from emergency OT along with bacillus and CONS, CONS and micrococci were isolated from obstetrics and gynaecology OT, only bacillus was isolated from ophthalmology OT. Urology OT air surveillance yielded *Staphylococcus aureus*, *Acinetobacter species* along with *Bacillus species*, from ENT, OT, CONS and Bacillus were isolated, while *Staphylococcus aureus*, Bacillus and micrococci were isolated from orthopedics OT (Table 2).

Table 2: Various bacterial isolates from air sampling of different OTs.

Name of the OT	Organism isolated pre OT	Organism isolated intra OT
Surgery	*Bacillus species*	*Bacillus species*
Emergency	*Coagulase negative staphylococcus species* and *Bacillus species*	*Staphylococcus aureus* and *Bacillus species*
Obstetrics and gynaecology	*Coagulase negative staphylococcus species* and Micrococci	*Coagulase negative staphylococcus species*
Ophthalmology	*Bacillus species*	*Bacillus species*
Urology	*Staphylococcus aureus* and *Bacillus species*	*Staphylococcus aureus* and *Acinetobacter species*
ENT	*Coagulase negative staphylococcus species*	*Coagulase negative staphylococcus species* and *Bacillus species*
Orthopaedics	*Bacillus species* and micrococci	*Staphylococcus aureus*

Antibiotic sensitivity test was done for all pathogenic bacteria isolated. *Staphylococcus aureus* isolated from emergency OT was found to be sensitive for vancomycin (30µg), linezolid (30µg), cefoxitin (30µg), and gentamicin (10µg) while it was resistant for ciprofloxacin (5µg), cotrimoxazole (25 µg) and clindamycin (10µg).

Staphylococcus aureus and *Acinetobacter spp.* isolated from urology OT was sensitive to all the antibiotics tested, *Acinetobacter spp*. was tested for Imipenem (10µg), ciprofloxacin (5µg), gentamicin (10µg), ceftriaxone/sulbactam (30/15µg), ceftazidime (30µg), ceftazidime/clavulanic acid (30/10µg), cotrimoxazole (25µg). Moreover, *Staphylococcus aureus* isolated from orthopedics OT was also sensitive for all the tested antibiotics.

Surface sampling by swab method

Bacterial species were isolated from 15 (23.4%) out of total 64 swab samples taken from all OTs. CONS 6.25% and micrococci 6.25% (both normal flora) were the predominant isolates followed by bacillus 4.6% (contaminant). Pathogenic bacteria such as *Acinetobacter species* 3.12%, *Enterobacter species* 1.56% and *Pseudomonas species* 1.56% were also isolated (Table 3).

Surface and articles of surgery OT were found to be contaminated with *Bacillus species* (25%), *Enterobacter species* (12.5%) and micrococci (12.5%) were isolated from emergency OT. *Acinetobacter species* and micrococci (both 12.5%) were predominantly isolated from obstetrics and gynaecology OT articles / surfaces

whereas the ophthalmology OT surfaces shows growth of *Pseudomonas species* (12.5%), micrococci (12.5%) and *Bacillus* (12.5%). Urology OT showed least rate of contamination, only CONS (12.5%) was isolated whereas from ENT OT Acinetobacter species (12.5%) and CONS (25%) were isolated while only normal flora CONS (12.5%) and micrococci (12.5%) were isolated from orthopedics OT (Table 4). Total 4 potential pathogens were isolated from OTs, 2 *Acinetobacter spp*, 1 *Pseudomonas spp*, and 1 *Enterobacter spp*. These pathogens were isolated from emergency OT, obstetrics and gynaecology OT, ophthalmology OT and ENT OT.

Table 3: Various bacterial isolates from surface sampling.

Bacterial isolate	Number (% age)
Bacillus species	3 (4.6%)
Micrococci	4 (6.25%)
Coagulase negative staphylococcal species	4 (6.25%)
Acinetobacter species	2 (3.12%)
Enterobacter species	1 (1.56%)
Pseudomonas species	1 (1.56%)

Table 4: Organisms isolated from surface sampling of different OTs.

Name of the OT	Organism isolated
Surgery	*Bacillus species*
Emergency	*Enterobacter species* and micrococci
Obstetrics and gynaecology	*Acinetobacter species* and micrococci
Ophthalmology	*Pseudomonas species*, micrococci and *Bacillus species*
Urology	*Coagulase negative staphylococcal species*
ENT	*Acinetobacter species* and *Coagulase negative staphylococcal species*
Orthopaedics	*Coagulase negative staphylococcal species* and micrococci

Enterobacter spp. isolated from emergency OT washbasin and *Acinetobacter spp*. isolated from washbasin of obstetrics and gynaecology OT was found to be sensitive for imipenem (10µg), ciprofloxacin (5µg), ceftriaxone (30µg), gentamicin (10µg), while it is resistant for ceftazidime (30µg), ceftazidime/clavulanic acid (30/10µg), cotrimoxazole (25µg). Whereas, *Acinetobacter spp*. isolated from ENT OT was sensitive to all the above antibiotics while *Pseudomonas spp*. isolated from ophthalmology OT was sensitive for all antibiotics except cotrimoxazole (25µg).

DISCUSSION

Aerobic cultures on non-selective medium should not exceed 35 colonies forming units (CFU) of bacteria per cubic meter of air in an empty operation theatre and 180 CFU per cubic meter of air during an operation for a conventional theatre. Four OTs out of seven are exceeding the limit point in the study. Only three OTs like ophthalmology OT, ENT OT and urology OT are not exceeding the limit. Anjali K et al, Mir RF et al, Kiranmai et al and Javed et al also reported in their study that ophthalmology OT and ENT OT was the one with least air contamination.[13-16]

Many studies have been carried out in operation theatres to determine relationship between total bacterial air count in OT and risk of infection. 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.[17] According to Pasquarella et al microbiological quality of air may be considered as mirror of the hygienic condition of the operation theatres.[18] The quality of indoor air depends on external and internal sources, such as ventilation, cleaning procedures, the surgical team and their activity.[10]

In this study, the bacterial CFU was ranged between 17-82 CFU/mm^3 for pre-OT and 31-200 CFU/mm^3 for intra OT. Microbiological surveillance study in OTs of a tertiary care hospital at Lahore by Javed et al, and Mysore by Deepa et al, have reported a significantly higher bacterial air count in the range of 6500-15730CFU/m^3 and 628-1571 CFU/m^3 respectively.[16,19] Whereas, in contradiction the study conducted by Desai et al showed a low bacterial air count in the range of 20-75 CFU/m^3.[12] 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.

Settle plate method for air sampling showed highest percentage of occurrence of CONS and *Bacillus spp*. followed by micrococci while in the study done by Anjali et al *Bacillus spp*. and micrococci were predominant.[13] Moreover, among the pathogens *Staphylococcus aureus* and *Acinetobacter spp.* were isolated, in a study done by Qudiesat et al Staphylococcus aureus was isolated while in a study done by Kiranmai et al *E.coli, Klebsiella spp*. and *Enterobacter spp*. was isolated from air sampling.[15,20] Moreover, to the best of our knowledge this is the first environment microbiological surveillance study in India

in which antibiotic sensitivity test for isolated pathogenic bacteria was performed. A total of 64 swabs were collected from various surfaces and articles from different operation theatres. Out of which 15 (23.4%) samples showed growth on the culture media at 37ºC after 24 hours of incubation and remaining 49 samples showed no growth. Of these 15 isolates obtained from various OTs, micrococci and CONS were 4 (6.25%) each, 3 (4.6%) were Bacillus and remaining 4 were pathogenic bacteria while in a study done by Anjali et al 13.2% swab samples were growth positive out of which 5.8% were CONS, 4.4% were *Klebsiella spp*. and the remaining 3 were *Bacillus spp*.[13]

The instruments and articles which were sterilized by autoclave showed no growth whereas highly touched areas like door handles, OT light's showed growth of bacteria. Highest contamination was found on the surface of washbasin followed by door handles and overhead lamps while OT table and drug rack were found to be colonised with normal flora like CONS and micrococci. Moreover, in a study done by Kiranmai et al OT table and drug rack were most contaminated sites.[15]

Bacterial surface sampling of OT's was 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 spp.*, which are the contaminant.

CONCLUSION

Strengthening surveillance and laboratory capacity will surely enhance infection prevention and control. Routine sampling is strongly recommended for increasing awareness to identify and control all possible sources and types of infections. Settle plate's method for air and swabbing technique for surfaces are considered as crude methods but in a limited resource setup these methods are proved to be more valuable in detecting the contamination level. In future, more and more studies should be encouraged to find out prevalence of type of bacterial isolates and their antibiotic sensitivity pattern which will be very helpful as infection control measures.

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