Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2015; 3(8):703-707 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Estimation of microbial air contamination by settle plate method: are we within acceptable limit

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Abstract: Microbiological quality of air can be considered as a mirror of hygienic conditions of any place, especially operating theatres. Nowadays the evaluation of the level of air microbial contamination in places at risk is considered to be a basic step towards prevention. The objective of this study was to determine the bacterial load and antibiotic susceptibility pattern of various isolates in operating room's indoor air. The index of microbial air contamination was based on the count of the microbial fallout on to Petri dishes left open to the air according to the 1/1/1 scheme (for 1 h, 1m from the floor, at least 1m away from walls or any obstacle). A total of 86 samples taken repeatedly from ten different operation theatres were processed and the isolates were *Staphylococcus aureus*, Coagulase negative Staphylococcus, *Micrococcus* spp and *Bacillus* spp. Total bacterial load from all the OTs were within acceptable standard limits. Not even a single strain of MRSA was detected. Thus, air quality of an operation theatre area is an important issue which cannot be neglected, especially in reference to the hospital acquired infections. Recording the susceptibility pattern of the isolates to commonly used antibiotics in the area not only helps to select appropriate antibiotics for empirical therapy but also helps to design suitable hospital infection prevention protocols in an effort to minimize the incidence of costly surgical site infections.
Keywords: operation theatre air quality, settle plate method, hospital acquired infections

INTRODUCTION

The first practical class for both under graduates and post graduates in microbiology in every medical college is to exhibit the ubiquitous nature of micro organisms in atmosphere and the difference in their concentration depending upon the environmental conditions and locations. The concentration and quality of micro organisms in atmosphere can have a direct bearing on human health and environment. In recent years air microbiology has gained a lot of attention. In fact, it would not be an overstatement that microbiological quality of air can be considered as a mirror of hygienic conditions of any place, especially, operating theatres. Many studies have been carried out on this topic, and nowadays the evaluation of the level of air microbial contamination in places at risk is considered to be a basic step towards prevention [1-7]. However, there still are problems to be solved relating to methodology, monitoring, data interpretation and maximum acceptable levels of contamination.

Counting microbes in the air is not an easy task. Many different methods are in use, which can be divided into four groups; the count of colony forming units (CFU) per cubic meter of air (cfu/m^3); the count of CFU on settle plates; measurement of a chemical component of the microbial cells/m^3 of air and the count under the microscope. At the moment, the only effective means of quantifying airborne microbes is limited to the count of CFU [8]. 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 and have their own set of advantages and disadvantages.

For the purpose of active air sampling, air samplers are used, which collect a known volume of air blown on to a nutrient medium by different techniques. Standards for air control are based on the measurement of cfu/m^3. Use of active air samplers is however laden with a lot of drawbacks like they are expensive, heavy, and noisy, need continuous calibration and are difficult to sterilize. The advantage however is that the results are expressed as number of cfu per cubic metre (cfu/m^3) of air, which correlates well with most official guidelines.

Passive air sampling is performed using settle plates. Petri dishes containing solid nutrient medium are left open to air for a given period of time. Microbes carried by inert particles fall onto the surface of the nutrient, with an average deposition rate of 0.46 cm/s

being reported. After incubation at 36±1°C they grow into colonies in a number proportional to the level of microbial contamination of the air. Despite a few limitations, the settle plate method is still widely used as a simple and inexpensive way to qualitatively assess the environments over prolonged exposure times. They are sterile, economical and readily available. Many sites can be checked at the same time. The chief advantage, in context of operation theatres, is that settle plates reflect the bacterial load nearest the operative site without creating any turbulence. Charnley has commented that the settle plate reproduces the circumstances of infection by dust particles sedimenting into the wound better than a slit sampler [9]. Friberg et al. have also suggested that settle plates showing bacterial surface contamination are a more practical and relevant indicator of actual wound contamination rather than air counts [10].

The present study was therefore conducted to evaluate the air quality in the operation theatres of a tertiary care hospital in Jaipur, Rajasthan. The ethical clearance was obtained from the University ethical review board.

MATERIAL AND METHOD

The objective of this study was to determine the bacterial load and antibiotic susceptibility pattern of isolates in operating room's indoor air of Mahatma Gandhi Hospital (MGH). The study was conducted from March to August 2013 in MGH. Mahatma Gandhi Medical College and Hospital is a 1000 bedded tertiary care hospital situated in Jaipur, India and offers different specialized medical services for people living in this part of the state and also nearby areas. There are 10 operation theatres in the hospital. About 5 major operations (both emergency and elective) are conducted on a daily basis. Air sample collections were done during the working hours while the surgery was on and on an average twenty people were present in each room including the doctors, students, nurses and ward boys.

Sampling

The evaluation of bacterial contamination in an operating theater was performed by using settle plate method. For sterility testing, Petri dishes containing 5% sheep blood agar media were preincubated overnight under the conditions that matched incubation of air samples to be taken. This allowed the plates with contaminants to be discarded. The sterile plates were then transported to operation theatres in sealed plastic bags. The plates were labeled with sample number, site within theatre, time and date of sample collection. During air sampling sterile gloves, mouth mask and protective gown were worn to prevent self contamination of the blood agar plate. Five plates were kept in a single OT, four in the corners and one in the centre, at the time when the surgery was in progress. The index of microbial air contamination was based on the count of the microbial fallout on to petri dishes left open to the air according to the 1/1/1 scheme (for 1 h, 1m from the floor, at least 1m away from walls or any obstacle). After this exposure, the plates were covered with their lids and taken to laboratory in sealed plastic bags and incubated at 37^{o}C for 24 hours. The concentration of airborne bacteria was expressed as colony forming units per cubic meter (cfu/m^3).

The culture plates that showed discrete macroscopic colonies were counted using plate colony counter. The colonies were assessed for the growth of potential pathogenic bacteria initially by colony characteristics, haemolysis pattern and microscopic examination of Gram stained smears. Final identification was done following standard bacteriological techniques [11]. The antimicrobial susceptibility testing was done for every potential pathogenic bacteria isolate by Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines [12].

Finally, the data was interpreted according to scientifically determined baseline values initially suggested by Fisher in the 1970s and now widely adopted by the European Cooperation for Accreditation of Laboratories [8].

RESULTS

A total of 86 samples taken repeatedly from ten different operation theatres over a period of six months were processed and the isolates were *Staphylococcus aureus*, Coagulase negative Staphylococcus, *Micrococcus* spp and *Bacillus* spp. Total bacterial load from all the OTs were within acceptable standard limits (Table-1). There was no *Streptococcus pyogenes* or any Gram negative isolate found.

Staphylococcus aureus was isolated from the air samples obtained from surgery, trauma, orthopedics and gynaecology OTs. Surgical OT showed 50% prevalence of *Staphylococcus aureus* in the air which is highest among all the OTs (Table 2). Coagulase negative Staphylococci (CoNS) were isolated from the air samples from all the OTs with the lowest prevalence in Neurosurgery (16.66%) and CTVS (25%). Lowest isolation rate for *Micrococcus* spp was found to be from CTVS (Cardio thoracic and vascular surgery) OT. *Bacillus* spp. was present in the air of the various OTs with lowest prevalence being 33.3% in the Neurosurgery, urology and new OT. Air samples obtained from Surgery and Gynecology & Obstetrics showed 100% positivity for *Bacillus* spp.

All the *Staphylococcus aureus* isolates were susceptible to most of the drugs except ampicillin and ciprofloxacin (Table-3). CoNS isolates were found to be more resistant to various drugs in comparison to *Staphylococcus aureus* isolates. Amongst CoNS isolates, 50% were sensitive to ciprofloxacin, 55% to

ampicillin, 56% to tetracycline and 62% to erythromycin. All the *Staphylococcus aureus* and CoNS isolates were found susceptible to vancomycin. Not even a single strain of MRSA was detected in our study.

Table-1: Total aerobic bacterial load of indoor air from OTs against the standard in MGH; March-August 2013

S.No	Name of OT	Aerobic colony count/hr (mean value)	STANDARD-OPTIMAL
1.	ENT	5	0-25
2.	Neurosurgery	2	0-5
3.	Eye	8	0-25
4.	Urology	5	0-25
5.	Gynae & Obs.	17	0-25
6.	Orthopaedics	3	0-5
7.	Surgery	15	0-25
8.	Trauma	10	0-25
9.	CTVS	3	0-5
10.	New OT	5	0-25

Table-2: Number of various bacterial isolates from different OTs

S.No	Name of OT	Total No. of samples	Isolated Organisms							
			CoNS		*Staphylococcus aureus*		*Micrococcus* spp.		*Bacillus*	
			No.	% age	No.	% age	No.	% age	No.	% age
1.	ENT	10	3	30	Nil	0	10	100	6	60
2.	Neurosurgery	6	1	16.66	Nil	0	4	66.66	2	33.33
3.	Eye	8	4	50	Nil	0	3	37.5	5	62.5
4.	Urology	6	2	33.33	Nil	0	2	33.33	2	33.33
5.	Gynae & Obs.	10	6	60	3	30	8	80	10	100
6.	Orthopaedics	10	5	50	3	30	6	60	5	50
7.	Surgery	12	10	83.33	6	50	12	100	12	100
8.	Trauma	10	6	60	4	40	10	100	7	70
9.	CTVS	8	2	25	Nil	0	2	25	3	37.5
10.	New	6	3	50	Nil	0	2	33.33	2	33.33

Table-3: Antibiotic sensitivity pattern of GPCs isolated from various OTs in MGMCH

S.No	Organism (n)	Antimicrobial agents (% sensitivity)								
		AMP	Va	Cf	Cn	T	E	CD	Co	Cxm
1.	*Staphylococcus aureus* (16)	60	100	66.6	100	100	100	87.5	80	100
2.	CoNS (42)	55	100	50	100	56	62	72	66	100

* AMP: Ampicillin; Va: Vancomycin; Cf: Ciprofloxacin; Cn: cefoxitin; T: Tetracycline E: Erythromycin CD: Clindamycin; Co: Cotrimoxazole; Cxm: Cefuroxime

DISCUSSION

In any hospital setting, nosocomial infections are important clinical indicators of quality of patient care and infection control. [13] Surgical site infection (SSI) is the second most common health care associated infection, next only to hospital acquired urinary tract infection [14]. The factors determining the prevalence of SSI could be patient related, surgeon related or related to the overall contamination level of hospital environment like indoor air [15]. While patient and surgeon related factors like the presence of co-morbid conditions, pre operative preparation, surgeon's expertise, insertion of implants, adequacy and timing of anti microbial prophylaxis are very important factors, there is no undermining the fact that the microbiological quality of operation theatre air has a huge bearing on the outcome of any surgical procedure. The microbiological quality of air can indeed be considered as a mirror of the hygienic condition of operating room [2, 16]. Recording the susceptibility pattern of isolates to commonly used antibiotics in the area helps to select appropriate antibiotics for empirical therapy. This also helps to design suitable hospital infection prevention protocols in an effort to minimize the incidence of costly SSI. Thus the current study was planned with the objective of determining the bacterial load and antibiotic susceptibility pattern of isolates in operating room's indoor air of Mahatma Gandhi Medical Hospital (MGH).

From the era when Louis Pasteur first used nutrient medium exposed to air to collect living micro organisms and Robert Koch used settle plates to measure indoor microbial air contamination, air microbiology has grown in leaps and bounds. Settle plates are used in different environments for evaluating microbial air contamination. The first attempt to standardize the settle plates was made in the 1970's by Fisher. [10] He developed the 1/1/1 schedule as a standard for measuring the microbial air contamination in hospital environments at bio-risk wherein the result was expressed as total microbial count.

In this study, mean aerobic colony counts in all the OT's were within acceptable limits. *Bacillus* spp., *Micrococcus* spp., *Staphylococcus aureus* and CoNS were the various isolates in the operating theatre areas. Such encouraging results may be a result of fact that this is a new institution, only a decade old, with good architecture and ventilation systems in place, combined with a major emphasis on basic cleaning practices and hand washing.

Unlike another study conducted in a specialized hospital in Ethiopia which revealed a very alarming level of resistance of *Staphylococcus* isolates to methicillin, not even a single isolate of MRSA was detected in our study [16]. Neither was there any *Streptococcus pyogenes* or Gram negative isolate unlike that found in other studies conducted on similar lines in Lahore, Nigeria and Taiwan [4, 17, 18]. Surgical OT however showed 50% prevalence of *Staphylococcus aureus* in the air which was highest amongst all the OTs. Despite the bacterial load being within acceptable limits, this was still a point of concern for which various rectifying measures were taken. The operation theatre (OT) staff was asked to maintain strict discipline in the OT which included reducing people traffic, regular servicing of the ventilation system and stringent cleaning of the premises.

The main routes of microbial entry into an open clean surgical wound are from the patient's skin, from the surgeon's hand or by airborne microbes setting into the wound or onto instruments that will be used in the wound. While most of these parameters can be adequately taken care of by CSSD, it is the responsibility of the microbiologist to keep a regular check on the level of microbial air contamination. Most of the microbes in theatre air fare from staff and a few are from the patient. If theatre ventilation is effective air should not be a source of infection transmission between patients, regardless of whether the procedure is "dirty" or clean. Appropriate staff dress and discipline can minimize the spread of bacteria from healthcare personnel and reduce airborne microbial contamination. Reducing people traffic in the operation theatre would go a long way in achieving desired results.

In conclusion, air quality of an operation theatre area is an important issue which cannot be neglected, especially in reference to the hospital acquired infections. It is a fast evolving field with various schools of thought emerging. While some advocate routine screening of the microbiological quality of indoor air, there is a larger proportion of specialists claiming that routine basic cleaning if done stringently suffices in most situations.

Despite the fact that there are no universal guidelines supporting the concept of regular monitoring and sampling, it was developed as an in house policy. Nevertheless, till the time we have clear cut Standard Operating Procedures and guidelines, the issue demands continuous surveillance by the infection control teams to provide a safe environment for not only the surgical patients but also a safe working environment for hospital employees.

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