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RESEARCH ARTICLE

MICROBIOLOGICAL PROFILE AND AIR QUALITY INDOOR EFFECTS IN THE OPERATION THEATRES OF A TERTIARY CARE HOSPITAL: AN IMPACT ON DISEASE BURDEN."

¹*Dr. Kirti Nirmal, ²Dr. Seema Gangar, ³Dr. Manoj Kumar Meena, ⁴Dr. Shukla Das and ⁵Dr. N.P Singh

¹Assistant Professor, Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden- East Delhi 110095; ²Senior Resident, Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden- East Delhi 110095; ³Assistant Professor, Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden- East Delhi 110095; ⁴Director Professor, Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden- East Delhi 110095; ⁵Director Professor & Head of Department, Department of Microbiology, University College of Medical Sciences and Guru Teg Bahadur Hospital, Dilshad Garden- East Delhi 110095

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*Corresponding Author:

Dr. Kirti Nirmal

ABSTRACT

An operation theatre (OT) is a very complex setup, which presents numerous challenges for both patients and health care providers. A safe OT environment decreases the susceptibility of patients to postoperative infections. The microbiological profile and bacterial colonization and the microbial contamination of air quality indoor effects in the operating theatres of a tertiary care hospital were studied. Bacterial species in the OTs were isolated and identified by standard conventional methods. Air quality surveillance of OTs was done by settle plate method. A total of 342 samples were collected from surfaces, articles and of the various OTs. Out of these, 32 (9.3%), samples showed bacterial growth. The predominant species isolated was Aerobic spore forming bacilli 20 (62%) followed by Coagulase-negative *Staphylococcus* (CONS) with 8 (25%) isolates followed by 3 (89.3%) *Micrococcus species* and one (3.1%) Methicillin sensitive *Staphylococcus aureus* were isolated. Analysis of the OTs air samples showed the least colony forming unit (cfu) rate of air (27cfu/m³) in ophthalmology OT and the highest rate of 133 cfu/m³ in general surgery OT was very high. The study shows that the frequent use of OTs, traffic is more, more patient load and infrequent cleaning between surgeries can cause high CFU counts.

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INTRODUCTION

In the middle of the 19th century, two pioneers of infection control Ignaz Semmelweiss and Joseph Lister revolutionized the science of surgery by introducing the concept of antiseptic procedures. They discovered that the incidence of wound infections could be drastically reduced by requiring hand disinfection and aseptic procedures resulting in reduction of mortality rates after surgery.^{1, 2} Hospital acquired infections (HAI) or Health care Associated Infection (HCAI) are infections acquired by the patient that are not present or incubating at the time of admission to a hospital. A variety of microorganism including bacteria, viruses, fungi and parasites proliferate in various indoor areas when the microorganism-laden materials are carried by means of soil, water, dust, decaying organic matter, airflow, construction materials, equipment, and any other vehicle. They can either colonize or cause infection, depending on the susceptibility of the host.³ The infections like catheter-associated urinary tract infections (CAUTI), central line-associated bloodstream infections (CLABSI), surgical site infections (SSIs),

ventilator-associated pneumonia (VAP), hospital-acquired pneumonia, and *Clostridium difficile* infections are common HAIs.³ Till now the occurrence of HCAI by multi-drug resistant (MDR) infective agents is a major concern. HCAI are a considerable economic burden to the society, up to 7% of patients in developed countries and 10% in developing acquires at least one HCAI. The environment of the hospital especially in operation theatres including air, water, surfaces and health-care workers plays a major role in controlling the source and transmission of infection to the patient. Therefore, the issue of HCAIs and their prevention strategies are taken seriously by almost every healthcare centre in last few decades. Various guidelines for infection tracking and surveillance systems have been established by healthcare institutes for prevention of HCAIs.⁴ CDC recommends targeted microbiological sampling of the environment (air, water and inanimate surface) for the following indications.⁵

- Epidemiological purpose like investigation of an outbreak

- Research purpose
- Monitor a potentially hazardous environmental condition- to confirm the presence of a hazardous chemical or biological agent, and validate the successful abatement of the hazard and for quality assurance to evaluate the effects of a change in infection-control practice or to ensure that equipment or systems perform according to specifications and expected outcomes.
- To evaluate the change in infection control practice.

AIR SURVEILLIANCE

Normally, the air outside houses contains a variable load of microorganisms, dust and particles. The number of bacteria can be 40–100 CFU (colony-forming units) per m³ (1000 L) air, however inside rooms it increases up-to 300 CFU or more depending on number of people present in the room and area of the room. These count further increases in healthcare settings where the patient load is high. The transmission can be either directly from a sick patient coughing or sneezing or indirectly via re-aerosols from bedding, cleaning and other activities in the room of a patient with infection. In critical areas like OTs, it is recommended not more than 100 CFU/m³ air to reduce risk of postoperative wound infection.^{6,7,8} Air serves as a vehicle for the transmission of various microorganisms as droplet or droplet nuclei.

Respiratory droplets and droplet nuclei are generated during coughing, sneezing or talking. Droplets are usually larger in size (>5µm) as compared to droplet nuclei (<5µm) and can transmit up to short distance (<3 feet) from the source of origin as they usually do not remain suspended in the air. However upto 10–20% of infections is airborne. Aerosols or droplet nuclei are residuals of droplets (<5µm) which subsequently dries and remain suspended indefinitely in air and thus can transmit disease to long distance along the air current.⁹ Droplet precautions and airborne precautions should be used in addition to standard precautions to prevent the spread of these infectious agents. Hand hygiene, use of appropriate personal protective equipment, respiratory hygiene, and cough etiquette, disinfection of surface, equipment and surrounding environment should be practiced. It is important to maintain good indoor air quality in critical areas and other areas of the healthcare institution to prevent or at least minimize nosocomial infections.

• Evaluation of the Air Quality – The monitoring of indoor air quality includes:

- Parametric monitoring
- Microbiological monitoring
- Parametric monitoring

Parametric monitoring consists of various physical parameters like periodic assessment of temperature, ventilation, humidity, direction/velocity/ pressure of air flow, number of air changes per hour (ACH), re-circulation of air or air-handling system, filter efficiency and other dust-control measures.¹¹ Along with these parameters properly constructed walls, floor, ceiling and number of persons present in room can greatly affect the quality of air in the room. As per CDC guidelines, the ventilation specifications that should be followed in specific areas of healthcare facilities such as airborne infection isolation (AII), protective environment (PE) rooms, critical care rooms, isolation anteroom and operating rooms are in Table 2. Heating, ventilation, and air conditioning (HVAC) systems maintain the indoor air temperature and humidity at comfortable, it remove contaminated air, facilitate air-handling requirements and minimize the risk for transmission of airborne pathogens from infected patients. Filtration is the primary means to remove particulates of approximate 1–5 µm in diameter from air. The air passes through two filter beds or banks. The first bank filters are usually low to medium efficiency filters (efficiencies of 20%–40%) and second filter bank usually consists of high efficiency filters. (Efficiencies of ≥ 90%).

This filter system is usually adequate for most of patient areas, however HEPA (efficiencies of 99.97% for removing particles ≥0.3 µm in diameter) filter bank are indicated for special care areas like PE rooms, OT etc. Ultraviolet Germicidal Irradiation (UVGI) is effective in reducing the transmission of airborne bacterial and viral infections but has minimal effect on fungal spores. UV lamps (low-pressure mercury vapor lamps) emit radiant energy of 253.7 nm are easily available. As per specific health-care zone, range of temperature standards are given, for cool temperature standards like in OTs, clean workrooms, and endoscopy rooms 68°F–73°F (20°C–23°C) are recommended, A warmer temperature of 70°F–75°F (21°C–24°C) is needed in most of the areas. For humidity, most commonly relative humidity is measured. The comfort range is 30%–60% relative humidity, whereas >60% relative humidity is perceived as uncomfortable, promote fungal growth. The other measures of humidity include specific humidity, dew point, and vapor pressure.¹² The ventilation rates for health-care facilities are expressed as room air changes per hour (ACH) and between 12 ACH–15 ACH, peak efficiency for particle removal in the air space takes place. Usually recirculated air mixed with fresh air is used in most of the areas of Health-care facilities. Along with the above mentioned parameters positive pressure (greater supply than exhaust air volume) and negative pressure rooms like OTs and airborne infection isolation (AII) rooms respectively should be maintained with respect to corridors and adjacent areas. The air should be introduced at the ceiling and exhausted near the floor. The CDC recommendations for engineered specifications for positive pressure room are described in table 3.

Microbiological monitoring: The indoor environment of critical rooms like OTs, transplant units etc may contain microorganisms, dust, aerosol, lint, skin squamous epithelial cells, and respiratory droplets. It is one of the risk factors for the development of post-surgical infections and had contributed to increased prevalence of hospital acquired infections. Airborne microbial level in OT air is directly proportional to the operating room traffic. Microbial contamination can be evaluated via air sampling either by active monitoring or by passive monitoring of the air.

Active monitoring: Active monitoring is used when the concentration of microorganisms is not very high eg. OTs, AII rooms etc. In this type of monitoring, the known volume of air passes through the air sampler and the quantity of the microorganisms is measured as colony forming units per cubic meter (CFU/ m³) of air. The particle collection device can be liquid or solid culture media or nitrocellulose membrane. eg. Sieve impact or, Slit to agar impact or, impingement in liquid air etc.

Passive monitoring: In this method a non-selective culture media (Blood agar) is exposed to the air undisturbed for a given amount of time known as settle plate method. Passive method depends on many factors like gravitational field, electrical gradient, thermal gradient and others, determines the way bioaerosols would settle down.

Settle plate method: This method is the most commonly used method in OTs. The non-selective media (usually Blood agar) in petri dish with 9 cm diameter is kept at various locations in the OT room one meter away from the walls, one meter above the floor and for one hour duration. The plates are then incubated in aerobic conditions at 37°C for 24 hour. The bacteria in room air that came in contact with the surface of culture plate as sediments can be counted as CFU/m³/ hour. As per index of microbial contamination (IMA) the maximum acceptable CFU/m³/ hour in OTs is ≤ 786.4 CFU/m³/ hour (≤5 CFU/9cm diameter plate/ hour) at rest or ≤ 3932.1 CFU/m³/ hour (≤25 CFU/9cm diameter plate/ hour) when operational. The advantage of this method is that it is a simple, inexpensive and does not require special equipment. Multiple plates can be used to assess the overall condition of the room. However the major disadvantage is detection of small particles, fungal spores or droplet nuclei suspended in the air and inability to quantify the volume of air sampled.

Active monitoring: Active monitoring is used when the concentration of microorganisms is not very high eg. OTs, All rooms etc.

going into an empty hole decreases therefore the most probable viable particle count is calculated by using Anderson Positive Hole Conversion.

Table 1. The examples of infectious agents that cause droplet or aerosol transmission are¹⁰

S.N.O	Droplet transmission	Aerosol transmission
1	<i>Corynebacterium diphtheriae</i> (Diphtheria, pharyngeal)	Mycobacterium tuberculosis (Tuberculosis)
2	<i>Haemophilus influenzae</i> type B (epiglottitis, pneumonia, meningitis)	Measles
3	<i>Neisseriameningitidis</i> (meningitis, pneumonia, sepsis)	Varicella-zoster virus (chickenpox, shingles)
4	<i>Bordetella pertussis</i> (whooping cough)	Aspergillus fungus (aspergillosis)
5	<i>Mycoplasma pneumoniae</i> (pneumonia)	Bacillus anthracis spores (Anthrax)
6	Group A Streptococcus (sore throat, scarlet fever)	
7	Viruses – SARS-CoV-2 virus (COVID-19), Influenza, Mumps, rhinovirus, Rubella, Adenovirus, Lassa, Ebola, Marburg etc.	

Table 2. Summary of ventilation specifications in selected areas of health-care facilities

Specifications	All room (includes bronchoscopy suites)	Protective Environment room	Critical care room§	Isolation anteroom	Operating room
Air pressure¶	Negative	Positive	Positive, negative or neutral	Positive or negative	Positive
Room air changes	≥6 ACH (for existing rooms); ≥12 ACH (for renovation or new construction)	≥12 ACH	≥6 ACH	≥10 ACH	≥15 ACH
Sealed**	Yes	Yes	No	Yes	Yes
Filtration supply	90% (dust-spot ASHRAE 52.1 1992)	99.97% (Fungal spore filter at point of use (HEPA at 99.97% of 0.3µm particles))	>90%	>90%	>90%
Recirculation	No (Recirculated air may be used if the exhaust air is first processed through a HEPA filter)	Yes	Yes	No	No

§ Positive pressure and HEPA filters may be preferred in some rooms in intensive care units (ICUs) caring for large numbers of immunocompromised patients.
 ¶ Clean-to-dirty: negative to an infectious patient, positive away from an immunocompromised patient.
 ** Minimized infiltration for ventilation control; pertains to windows, closed doors, and surface joints.

Table 3. Engineered specifications for positive pressure room

Engineering characteristics	Positive pressure areas (e.g., protective environments [PE])	Negative pressure areas (e.g., airborne infection isolation [AII])
Pressure differentials	> +2.5 Pa§ (0.01" water gauge)	> -2.5 Pa (0.01" water gauge)
Air changes per hour (ACH)	>12	≥12 (for renovation or new construction)
Filtration efficiency	Supply air: 99.97% @ 0.3 µm DOP (dioctylphthalate particles of 0.3 µm diameter) Return air : none required (If the patient requires both PE and AII, return air should be HEPA-filtered or otherwise exhausted to the outside)	Supply: 90% (dust spot test) Return: 99.97% @ 0.3 µm DOP (dioctylphthalate particles of 0.3 µm diameter); HEPA filtration of exhaust air from AII rooms should not be required, providing that the exhaust is properly located to prevent re-entry into the building.
Room airflow direction	Out to the adjacent area	In to the room
Clean-to-dirty airflow in room	Away from the patient (high-risk patient, immunosuppressed patient)	Towards the patient (airborne disease patient)
Ideal pressure differential	> + 8 Pa	> -2.5 Pa

§ Pa is the abbreviation for Pascal, a metric unit of measurement for pressure based on air velocity; 250 Pa equals 1.0 inch water gauge.

In this type of monitoring, the known volume of air is sucked through the air sampler at a sufficiently high velocity to cause any microorganisms in the sample to be impacted against a chosen medium or particle collection device. The quantity of the microorganisms is measured as colony forming units per cubic meter (CFU/ m³) of air. The particle collection device can be liquid or solid culture media or nitrocellulose membrane. Eg. Sieve impact or, Slit to agar impact or, Centrifugal sampler, impingement in liquid air etc.

Sieve impact or samplers –This is an impaction device which use for the acceleration of air to aspirate a known volume of air though numerous small holes (inlet orifices) on a perforated plate, the drawn air is then directed onto the surface of a settle or contact plate containing agar medium. This is an aggressive and effective method of air sampling.

The plate is then incubated at 37°C for 24 hr in aerobic conditions and the colonies are counted and result is expressed as CFU/m³. The number of colonies on the agar plate do not reflects the actual microbial load of the air as the number of the viable particles being impinged on the plate increases, the probability of the next particle

$Pr = N (1/N + 1/N-1 + 1/N-2 \dots\dots 1/N-r+1)$ Where Pr = Probable statistical total; r=

number of CFU on 9 cm petri dish; N= total number of holes in sampling head.

Slit sampler: In this device, a known volume of air is drawn by vacuum through a slit, which impinge the suspended particles across the surface of a rotating agar plate (150 x 20 mm). The speed of the plate rotation can be adjusted so that the whole surface of the plate is covered based on time (one hour). It is a simple and convenient device to use. The demerits of this device are that viable microbiological counts can vary for a number of reasons like skewing/inefficiency in collecting smaller particles, dehydration of the media because of vacuum etc.

Centrifugal Sampler impactor: The known volume of air is drawn into the sampling head by means of an impeller vane, due to the centrifugal force (4000-4200 RPM) as in a “tornado- likes piralling conical form the suspended particles are impacted onto a thin inward facing agar strip fitted around the circumference of the sampling head.

The exposed agar strips are then removed and incubated. This method is also used for isolation of fungal spores, thus incubated at 25°C for 7 days. The advantage of this method is that the device is simple, portable and battery operated, however it's difficult to count colonies by colony counter on strip as compared to standard petri dish.

Gel membrane filtration: The air is drawn through a sterile gelatin filter of approximate 300µm thickness with pores (pore size 0.2µm) mounted on a stand. The maximum retention of particles is seen as they get captured on the surface as well as within the filter pores. The filter is then removed and is either placed on agar in a petri dish using a no touch technique or can be dissolved in sterile solution for further evaluation.

Impingers: In this method, liquid media is used to entrap the air particles. The known volume of air drawn in by the suction pump towards the known volume of liquid media via a narrow tube inlet. On hitting the surface of liquid media, the particles get impinged into liquid media. The media is then used to culture and colony count.

Air Particle Counter: The device can detect particles in real time by use of laser technology. The air is drawn in the device and fluorescence is induced in the viable air particles with the help of laser technology. In addition to infectious aerosols, several crucial non-infectious, indoor air-quality issues should also be addressed by health-care facilities such as presence of sensitizing and allergenic agents and irritants in the workplace (e.g., ethylene oxide, glutaraldehyde, formaldehyde, hexachlorophene, and latex allergens).

CONCLUSION

Microbial contamination can be evaluated via air sampling of the environments under undisturbed conditions.² When air is sampled during or after human activity (e.g., walking and vacuuming), a higher number of airborne microorganisms may be detected. However, microbiologic sampling of air, water, and inanimate surfaces (i.e., environmental sampling) is an expensive and time-consuming process.¹ Regular microbiological surveillance of different hospital units, patients' surveillance by the infection control unit, formulation of rational antimicrobial use policy, and implementation of findings help reduce nosocomial infections.⁶ The present study was carried out to explore the profile of microorganisms in air culture at various units of a tertiary care hospital in Nepal

Appendix 1: Abbreviation

OTs: Operation Theatre

CONS: Coagulase Negative *Staphylococcus*

HAI: Hospital Acquired Infection

HCAI: Health Care Associated Infection

CAUTI: Catheter Associated Urinary Tract Infection

CLABSI: Central Line Associated Blood Stream Infection

SSI: Surgical Site Infection

VAP: Ventilator Associated Pneumoniae

MDR: Multi-drug resistant

CFU: Colony Forming Unit

AI: Airborne Infection Isolation

PE: Protective Equipment

HVAC: Heating, Ventilation and Air Conditioning

UVGI: Ultra violet Germicidal Irradiation

IMA: Index of Microbial Contamination.

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