ORIGINAL RESEARCH

LITTLE ANIMALCULES IN THE INDOOR AIR OF MATERNAL AND NEONATAL UNITS OF A TEACHING HOSPITAL: DEMYSTIFIED

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ABSTRACT

Background: Hospitals are major hoards of a vast array of infectious bioaerosols that pave way for adverse health outcomes among the patients and staff. Neonates and pregnant women are more vulnerable for such hospital acquired infections (HAIs) and allergies. Aims and objectives: This study was designed to determine the quality and quantity of aerobic bacteria and fungi in indoor air of neonatal and maternal wards and Operation Theatres (OTs). Methods: Conventional settle plate method was used to sample indoor air of maternal, neonatal wards and obstetric OTs. Results: Among the 60 air samples processed, 53 (88.33%) were culture positive for aerobic bacteria and 30 (50%) yielded fungi. Common isolates were Micrococcus spp (44%), Bacillus spp (34%), Aspergillus flavus (50%) and Aspergillus niger (30%). The aerobic bacterial and fungal concentration was found as 26-1048 CFU/m³ and 26-105 CFU/m³ respectively. The mean microbial concentration was significantly influenced by mopping and cleaning. (p<.05) Conclusions: OTs and wards of obstetric and neonatal department were free of fungal contamination. Labour and septic labour wards had high microbial load. Periodic reinforcement of environmental safety guidelines and strict adherence to infection prevention & control (IPC) protocols are need of the hour for every hospital.

Keywords: aerobic bacteria, bioaerosols, contamination, fungi, hospital indoor air

INTRODUCTION

Hospital indoor bio-aerosols contain bacteria, viruses, microbial spores, pollen and chemicals which account for 5% to 34% of indoor air contamination.¹,² Patients and staff especially neonates and pregnant women inhaling contaminated bio-aerosols are highly vulnerable to allergies and infectious diseases. Opportunistic pathogens like Staphylococcus spp, Klebsiella spp, Pseudomonas spp, Acinetobacter spp, Escherichia coli, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus and Mucor spp are the foremost causes of hospital acquired infections (HAIs). The external factors that contribute to indoor air contamination are the quality of air entering the space, bed occupancy rate, degree of ventilation, aerosol generating activities (including coughing, sneezing) of patients, staff & attenders and disinfection protocols.
About 4% to 56% of neonatal deaths around the world is due to HAIs owing to their immature immune system.\(^3,4\) Settle plate method is the simple and cost effective method,\(^5\) used to assess the quality of air. Hospital indoor air surveillance could generate reliable results for infection control and policy making.\(^6\) Hence this study was designed to determine bacterial and fungal concentration in the indoor air of critical areas such as maternal and neonatal units by conventional settle plate method.

**MATERIAL & METHODS**

This cross sectional study was carried out in the Departments of Microbiology, Obstetrics & Gynaecology (OG) and Paediatrics, of a teaching Hospital in South India from June to August 2022.

**Inclusion criteria:** Critical units like Special Neonatal Care, Neonatal Resuscitation, Labor, Obstetric Intensive Care Unit, Obstetric OTs (elective & emergency), Caesarean Post Operative, Postnatal, Antenatal and Pediatric Wards

**Exclusion criteria:** Surgery OTs, Orthopedics, Ophthalmology, ENT, Medicine, Dermatology wards etc.

**Sample size:** 15 units belonging to maternal and neonatal specialty.

**Study plan:** Institutional Ethics Committee clearance was obtained. Permission was obtained from all the departments concerned. Written informed consent was obtained from the staff nurses of the concerned unit.

**Pre- Sample collection protocol:** Culture media were prepared according to Standard operative procedure and underwent quality check.

**Air sampling:** Each sampling event from ward/OT was given a unique laboratory ID Number. Using a structured proforma, the name of the unit, environmental parameters, number of occupants, timing in relation to cleaning activities were collected. Bio medical waste segregation, type of toilets were also noted. Settle plate method was used. The sample collection was performed following 1 1 1 rule ie, 1 metre from the wall, 1 metre above the floor and at the centre of the room.\(^1\) 30 minutes exposure sampling was carried out. Pre-labeled, 90mm Petri plates containing culture media (nutrient agar, 5% sheep blood agar and Sabouraud dextrose agar \{SDA\}) were exposed in each of the study unit for 30 minutes. This was repeated at various timings after disinfection of equipments & floor, for 2 days a week. Public access was restricted during sampling.

**Sample transportation:** The collected samples were wrapped, placed in box and transported immediately to the microbiology laboratory for processing.

**Isolation of microbes:** The agar plates were incubated at 37°C for 24-48 hours and at 28°C for 7 days, for bacterial and fungal culture respectively. After incubation, number of colony forming units CFU/plate were enumerated and CFU/mm\(^3\) (Omeliansky formula) were found.

**Identification of isolates:** Standard bacteriological procedures were used to identify bacterial isolates. Antimicrobial susceptibility test for pathogens was done by Kirby Bauer disc diffusion method as per CLSI 2018 guidelines. Fungal species were identified by standard mycological techniques.

**Quality assurance:** Standard media, reagents and controls were used. Proper handling of the sterile media & collected samples were practiced.

**Statistical analysis:** Descriptive statistics for the data such as frequency and percentage of isolates was performed. The presence of statistically significant differences in the quality and
quantity of the culture isolates in relation to various parameters were analyzed by performing Student t test.

RESULTS
A total of 60 air sampling sessions were conducted at 15 units of the study hospital. Among the 60 samples, 14 (23.33%) were from Operation theatres (OT) of OG department. The rest were from obstetric post operative (16.66%), neonatal ICUs (15%), paediatric intensive care unit (PICU)(8.33%), gynaecology postoperative (6.66%), antenatal (6.66%), labour (5%), obstetric ICU (5%), septic labour ward (3.33%), postnatal (3.33%), and neonatal resuscitation rooms (3.33%). Out of the 60 sampling events, 36 (60%) were performed in the forenoon. During the sampling sessions, the temperature and humidity of the sampling sites ranged from 20°C to 38°C and 29% to 68% respectively.

Out of the 60 study samples, 7 (11.66%) were culture negative while the rest (53/60) were culture positive (88.33%). A total of 103 bacterial strains were isolated.(Table1, Fig.1) Micrococcus spp was the predominant isolate (44.66%). Staphylococcus aureus (1/103) was found to be Methicillin sensitive (MSSA). All the pathogenic isolates of Gram positive cocci (S.aureus, CoNS) were susceptible to cotrimoxazole, ciprofloxacin, cefoxitin, gentamicin, erythromycin and clindamycin. All the enteric Gram negative bacilli were susceptible to cotrimoxazole, ciprofloxacin, gentamicin, amikacin, ceftazidime and ceftazidime plus clavulanic acid. Similar pattern was found for Pseudomonas aeruginosa except inherent resistance to cotrimoxazole.

A total of 40 fungal isolates were obtained out of the 30 fungal culture positive samples. Fungal culture positivity was 50%. The predominant isolate was (20/40) Aspergillus flavus (50%), followed by (12/40) Aspergillus niger (30%), Aspergillus fumigatus (7.5%), non albicans candida spp (7.5%), Mucor spp (2.5%) and Penicillium spp (2.5%).(Fig. 2)

Index of Microbial Air (IMA) contamination: Majority (50.94%) of the samples had good bacterial IMA. On the other hand, all of the samples had very good fungal IMA. (Table 2) Bacterial contamination / load was found to be very low (105 CFU/m³) in Obstetric OTs, followed by an intermediate level in antenatal, postoperative and paediatric wards. Only one (1.88%) sample from Special neonatal ICU before moping, had 1048 bacterial CFU/m³. (Table 3) The mean bacterial load was lowest (116 CFU/m³) in Obstetric OTs and was highest (524 CFU/m³) in labour and septic labour wards. (Fig. 3)

Applying the unpaired Student t test, various categories of independent & continuous variables of microbial air contamination (timing of sampling, temperature, humidity, total occupants number, ventilation type and cleaning) were correlated with mean bacterial and fungal loads and p values were found. (Table 4) The mean bacterial contamination level was significantly influenced by mopping and cleaning activities (p<.05) while that of fungi was significantly influenced by all the parameters analyzed except timing of sampling. (p<.05)

The samplings done from OG and Paediatrics department were 44 (73.33%) and 16 (26.66%) respectively. Forenoon sampling from OG units was 63.63%. Highest mean bacterial and fungal loads were found in afternoon samples of Obstetric units. (Fig. 4)
Distribution of Bacterial isolates among the study samples

- Micrococcus spp: 46
- Bacillus spp: 36
- Klebsiella oxytoca: 7
- Pseudomonas aeruginosa: 5
- Escherichia coli: 4
- CoNS: 4
- Staphylococcus aureus: 1

Fig 1: Aerobic bacterial isolates among the study samples N=103

Fungal isolates distribution among the study samples

- Aspergillus flavus: 20
- Aspergillus niger: 12
- Aspergillus fumigatus: 3
- Non albicans candida spp: 1
- Mucor spp: 1
- Penicillium spp: 1

Fig 2: Fungal isolates distribution among the study samples
Fig 3: Mean bacterial CFU/m³ in various sampling sites

Fig. 4: Department, timing distribution of microbial load in the air samples

Table 1: Aerobic bacterial isolates from the study samples N=103

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Number of isolates</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus spp</td>
<td>46</td>
<td>44.66</td>
</tr>
<tr>
<td>Bacillus spp</td>
<td>36</td>
<td>34.95</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>07</td>
<td>06.79</td>
</tr>
<tr>
<td>Bacterial species</td>
<td>CFU/plate</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>-----------</td>
<td>---------------</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>05</td>
<td>04.85</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>04</td>
<td>03.88</td>
</tr>
<tr>
<td>Coagulase negative staphylococcus spp</td>
<td>04</td>
<td>03.88</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>01</td>
<td>00.97</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>103</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 2:  Categories of Bacterial and fungal CFU/plate and Index of Microbial Air contamination (IMA)

<table>
<thead>
<tr>
<th>Category of Bacterial CFU/plate</th>
<th>Bacterial IMA</th>
<th>Frequency (%)</th>
<th>Fungal IMA</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-10</td>
<td>Very good - Good</td>
<td>19 (35.84%)</td>
<td>1</td>
<td>16 (53.33%)</td>
</tr>
<tr>
<td>11-20</td>
<td>Good</td>
<td>27 (50.94%)</td>
<td>2</td>
<td>10 (13.33%)</td>
</tr>
<tr>
<td>21-30</td>
<td>Good-Fair</td>
<td>05 (09.43%)</td>
<td>3</td>
<td>03 (10%)</td>
</tr>
<tr>
<td>31-40</td>
<td>Fair</td>
<td>02 (03.77%)</td>
<td>4</td>
<td>01 (03.33%)</td>
</tr>
</tbody>
</table>

Table 3:  Bacterial load (CFU/m³) and unit wise distribution N=53

<table>
<thead>
<tr>
<th>Range of Bacterial CFU/m³</th>
<th>Grade of bacterial load</th>
<th>Units with the bacterial CFU range category</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>105</td>
<td>Very low-low</td>
<td>OG OTs, TAT OT, special NICUs</td>
<td>03 (05.66%)</td>
</tr>
<tr>
<td>106-210</td>
<td>Intermediate</td>
<td>Antenatal, labour, paediatric wards</td>
<td>10 (18.86%)</td>
</tr>
<tr>
<td>211-340</td>
<td>Intermediate</td>
<td>PICUs, Obst postoperative wards</td>
<td>14 (26.41%)</td>
</tr>
<tr>
<td>341-472</td>
<td>Intermediate</td>
<td>Obst postoperative wards, septic labour ward</td>
<td>17 (32.07%)</td>
</tr>
<tr>
<td>473-603</td>
<td>Intermediate-high</td>
<td>Septic labour, antenatal ward</td>
<td>05 (09.43%)</td>
</tr>
<tr>
<td>604-865</td>
<td>High</td>
<td>Labour ward, septic labour ward</td>
<td>03 (05.66%)</td>
</tr>
<tr>
<td>866-1048</td>
<td>High</td>
<td>Special neonatal ICU</td>
<td>01 (01.88%)</td>
</tr>
</tbody>
</table>

Table 4: Unpaired Student t test results of the study p≤.05 statistically significant

<table>
<thead>
<tr>
<th>Environmental parameter</th>
<th>Parameter category</th>
<th>No. in each category</th>
<th>Mean Bacterial CFU/m³, SD</th>
<th>Mean Fungal CFU/m³, SD</th>
<th>p value bacterial load</th>
<th>p value fungal load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timing of sampling</td>
<td>Forenoon</td>
<td>36</td>
<td>289.6, 259</td>
<td>17.3, 27</td>
<td>.1675</td>
<td>.1585</td>
</tr>
<tr>
<td></td>
<td>Afternoon</td>
<td>24</td>
<td>370, 133</td>
<td>27, 23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>&gt;30⁰C</td>
<td>33</td>
<td>306, 136</td>
<td>24, 27.6</td>
<td>.5526</td>
<td>.0341</td>
</tr>
<tr>
<td></td>
<td>≤30⁰C</td>
<td>27</td>
<td>340, 293</td>
<td>17, 23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5: The bacterial load (CFU/m³) in indoor air of present study Vs other studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Country, year</th>
<th>Bacterial load in indoor air CFU/m³</th>
<th>Places/wards</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>India 2022</td>
<td>105-1048</td>
<td>Paediatrics, OG wards, ICUs, OTs</td>
<td>Within permissible range</td>
</tr>
<tr>
<td>Bhatia and Vishwakarma, (20)</td>
<td>India 2010</td>
<td>150-1170</td>
<td>ICUs, Paediatrics, general wards</td>
<td>In concordance with the present study</td>
</tr>
<tr>
<td>Gizaw et al,(1)</td>
<td>Ethiopia 2016</td>
<td>480-1468</td>
<td>Medicine, physiotherapy wards</td>
<td></td>
</tr>
<tr>
<td>Laxmi et al,(21)</td>
<td>India 2017-20</td>
<td>78-677</td>
<td>Ophthalmology, general surgery OT</td>
<td></td>
</tr>
<tr>
<td>Sudharsanam et al,(5)</td>
<td>India 2008</td>
<td>65.5-1179</td>
<td>OTs, ICUs,orthopaedics, dialysis,postoperative, labour, neonatal wards</td>
<td></td>
</tr>
<tr>
<td>Deepa et al,(11)</td>
<td>India 2013</td>
<td>688-4766</td>
<td>OTs, ICUs</td>
<td>Discordant with the present study</td>
</tr>
<tr>
<td>Ashuro et al,(7)</td>
<td>Ethiopia 2021</td>
<td>300-2200</td>
<td>OG, paediatrics, surgery, medicine, orthopaedics wards</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

Microbiological quality of indoor air reflects the microbial burden, hygienic conditions and disinfection activities of the hospitals. As, most of the microbes are capable of long survival on surfaces and are resistant to disinfectants, their presence has an impact on the health of the occupants. Moreover, their profile changes every now and then. Hence it is important to detect their nature and number in indoor air of high risk units and to explore their changing trends in relation to many factors.

In this study, settle plate method was used and assessed the microbiological quality of air from various wards, ICUs and OTs of Paediatric (26.6%) and OG department (73.3%). Similarly, many authors,(7,8,9,10,11,12) utilized this method, since it indicates the risk of surgical site infections among post-operative patients.

Culture negativity in air samples: In the present study, 11.66% and 50% samples yielded no growth of bacteria and fungi respectively. This is in concordance with the report by Jalili et al.
who found bacterial and fungal culture negativity as 4.5% and 9.1% respectively.\textsuperscript{(13)} Deepa et al, India reported 26.78% culture negativity in their study.\textsuperscript{(11)}

Culture positivity in air samples: Overall bacterial culture positivity in this study was 88.33% which is comparable to the same which was 96.7% in the study by Nasiri et al from Iran.\textsuperscript{(3)} (Andersen one stage viable impactor). The variations among the results could probably be explained by the difference in methodologies, status of disinfection procedures & precautions and extent of adherence to aseptic protocols in selected high risk areas of hospitals.

Low bacterial & absent fungal contamination: The quantitative study of microbes demonstrated that the lowest bacterial load (4.42 CFU/plate or 116 CFU/m\(^3\)) was found in OG OTs and one Special Neonatal ICU. Fungal contamination (0 CFU/plate) was nil in the same. This is in agreement with the results of Singh et al,\textsuperscript{(14)} and Sudharsanam et al,\textsuperscript{(5)} who found bacterial CFU as 17 - 82 and 105 - 156 CFU/m\(^3\) respectively in the OTs. Ashuro et al, found lowest bacterial load (300 CFU/m\(^3\)) in neonatal ICUs.\textsuperscript{(7)} The concentration of microorganisms in OTs was considerably lower than in other areas. This could possibly be explained by the installation of new filters, low occupant density and high sanitary standards in OTs, when compared to other hospital areas sampled.

Index of Microbial Air contamination (IMA) in wards: Bacterial IMA was 4-40 CFU/plate in the wards, according to current study. This is comparable with the observation of Lakshmi SP et al,\textsuperscript{(15)} from this setting in 2008-2009, who found the same as 13-57 CFU/plate. This is also in agreement with that of Awosika et al,\textsuperscript{(16)} Shanmugaraj et al,\textsuperscript{(17)} and Sudharsanam et al,\textsuperscript{(18)} who found the same as 1-25, 40-74 and 45–150 CFU/plate respectively. Mwambete et al,\textsuperscript{(19)} observed aerobic bacteria in the range of 19-383 CFU/plate in their study which is much higher than that of this study. This variation could probably be explained by the factors influencing the indoor air quality of hospitals in various geographic locations and seasons.

According to the present study, the bacterial load of samples ranged from 105-865 CFU/m\(^3\) with the least contamination in special Neonatal ICUs. Only one sample from special neonatal ICU before cleaning had 1048 bacterial CFU/m\(^3\). This is comparable to that of other studies. (Table 5) Overall mean bacterial concentration of this study was 321.7 CFU/m\(^3\)/30 minutes and that of wards alone (excluding OTs) was 384.3 CFU/m\(^3\)/30minutes. This is in concordance with the observation by Napoli et al, who found the same as 722.5 CFU/m\(^2\)/hour.\textsuperscript{(10)} The mean bacterial load of this study was lower than that of Gizaw et al,\textsuperscript{(1)} and Ashuro et al,\textsuperscript{(7)} who reported the same as 878 CFU/m\(^3\) and 1585 CFU/m\(^3\) respectively. The results of each study were distinct because of the variations in cleanliness, indoor air quality, Infection Prevention and Control (IPC) practices, temperature and humidity, number & nature of occupants, traffic flow control mechanisms, exact duration of exposure of settle plates, nature of services & activities in each hospital and locations of sampling.

High mean bacterial load (524.1CFU/m\(^3\)) was found in labour & septic labour wards. This is similar to the observation by Laxmi et al,\textsuperscript{(21)} (2017-2020), who found the same in labour ward as 520 ± 2 CFU/m\(^3\). In discordance with this, Nasiri et al,\textsuperscript{(3)} reported mean microbial load of 93.3 ± 31.3 CFU/m\(^3\) in labour wards. Patel Pritikumari et al,\textsuperscript{(22)} from New Delhi, had found highest air contamination in the emergency OTs and emergency care service OTs. This could possibly be explained by the association of factors related to disinfection as well as facing unplanned emergencies, admission of expectant mothers with severe anaemia, leucopaenia, infections in utero, premature/prolonged ruptured membranes, accidental haemorrhage, chorio amnionitis and intrapartum sepsis which lead to increased contamination of air in the emergency services. Post natal wards had the highest microbial load (2195 CFU/m\(^3\)) in the study by Ashuro et al,\textsuperscript{(7)} also.
The failure of adherence to restricted entry protocols could favour microbial contamination in obstetric units.

Bacterial isolates in the air samples: All ICUs, wards were found to be colonized with contaminants as well as potential pathogens in this study. This is in concordance with the result of Meenakshi et al.,(12) Micrococcus & Bacillus spp comprised of 79.61% in the present study which is in concordance with the observation (71%) by Ashuro et al.,(7) Lakshmi SP et al.,(15) and Pereira et al.,(23) also observed high rate of Micrococcus and Bacillus spp. This preponderance of Gram positive organisms is due to their resistance to drying and disinfectants partly conferred by pigment production.

Micrococcus spp (44.66%), formed the majority which is similar to the result of Sudharsanam et al.,(5) In the same way, Nasiri et al.,(3) Patel Pritikumari et al.,(22) and Singh et al.,(16) had reported Micrococci isolation rate as 59.2%, 56% and 27.02% respectively. Bacillus spp (34.95%), was the second common isolate which is in concordance with (34.2%) that of Nasiri et al.,(3) Similar results had been found by Singh et al.,(16) Meenakshi et al.,(12) Bohra et al.,(24) and Patel Pritikumari et al.,(22) who had reported Bacillus isolation as 40.5%, 46%, 48.27% and 75% respectively. A study by Anjali et al.,(9) also found high isolation rate of Bacillus spp. This could be because of similar ecological niche of the strains. In contrast to this, in the studies by Kiranmai et al.,(25) and Desai et al.,(26) Bacillus spp was the predominant isolate over Micrococcus spp.

Staphylococcus aureus isolated in the current study comprised 0.97% which is in agreement with that of Bohra et al. (1.72%).(24) The strain of Staphyloccocus aureus was Methicillin sensitive (MSSA). This is similar to the result of Lakshmi SP et al.,(15)The isolation of Coagulase Negative Staphylococcus spp was 3.88% which is similar to the result of Mwambete et al.,(19) (5.33%) and Anjali et al., (5.8%).(9)

Isolation rate of Gram negative pathogenic bacilli of the present study was 15.54% which is in congruence with the study by Meenakshi et al. (10%),(12) Klebsiella oxytoca isolates comprised 6.79% which is in concordance with that of Kiranmai et al who reported the same as 4.4%.(25) Klebsiella spp was the predominant isolate in the study by Sudharsanam et al.,(5) also. Pseudomonas aeruginosa isolation rate was 4.85% which is comparable to the results of Jalili et al.,(13) (9.1%) and Desai et al. (12.5%).(26) Escherichia coli isolation rate was 3.88% which is in concordance with the observation of Jalili et al.,(13) (2.3%). Presence of Escherichia coli in air reflects the degree of purity of water used for floor cleaning and other activities in the hospital.

Air samples with no fungal growth: The present study found absence of fungal elements in all (100%) air samples from OTs (free of fungal contamination). This is similar to the observation by Sudharsanam et al.,(5) and Awosika et al.,(16) and Anjali et al.,(9)

Fungal IMA: This study found low fungal load (0-4 CFU/plate) which is in concordance with that of Awosika et al.,(16) and Sudharsanam et al.,(18) and Cabo Verde et al.,(27) who reported the same as 0-5, 0-13 and 0-1.2 CFU/plate respectively. The low fungal loads found in the certain areas of hospital could possibly be explained by better hygiene, bio medical waste management, infection control activities, closed type ventilation and susceptibility of fungi to biocides used.

In the current study the indoor air fungal load in ICUs and wards ranged from 26 to 105 CFU/m³. This is in congruence with the results of Bhatia et al.,(20) Montazeri et al.,(28) Saadoun et al.,(29) and Ortiz et al.,(30) who reported the same as 12-100, 32-110, 88-259 and 1-266 CFU/m³ respectively. The similarities could possibly be explained by similarities in methods and infection control activities. However, Cabo Verde et al., (Portugal, 2013-2014) found fungal load as 27-933 CFU/m³ in the emergency service areas.(27)

1936
Fungal isolates: Aspergillus spp comprised of 87.5% of fungal isolates. This is in agreement with the finding of Saadoun et al, who concluded that Aspergillus spp constituted 78-85% of the isolated fungi in air samples\(^{(29)}\) (A.fumigatus, A. niger, A.flavus, A.glaucus and A.terreus). Montazeri et al\(^{(28)}\) found the same as 77.1%. Pereira et al\(^{(23)}\) and Bhatia et al\(^{(20)}\) also found that Aspergillus spp was the commonest fungus in air samples. This results could probably be explained by the ubiquitous nature of Aspergillus spores in the air {due to resistance to water scarcity} and the pigmented nature of their conidia that provide them with resistance to UV rays and common cleaning chemicals. Sudharsanam et al also isolated Aspergillus spp (44.44%) from air of Orthopaedic and postoperative wards.\(^{(5)}\)

Candida non albicans spp isolated was 7.5% which is comparable to the same (3.6%) by Saadoun et al\(^{(29)}\). However, in the study by Sudharsanam et al, Candida non albicans spp isolation rate was 33.3%,\(^{(5)}\) (labour ward). This could probably be the result of higher fungal counts in hospital units during autumn than during summer which in turn may be related to occupant’s illness, behaviour, density, temperature and level of humidity.

According to this study, septic labour and labour ward had high microbial load which is in concordance with the finding of Sudharsanam et al.\(^{(5)}\)

In the present study, there was no statistically significant temporal variation in airborne microbial loads with respect to sample collection timing. This is similar to the observation by Pereira et al,\(^{(23)}\) Nasiri et al,\(^{(3)}\) Valentina and Umadevi,\(^{(31)}\) and Sudharsanam et al.\(^{(5)}\)

Statistical significance of Cleaning and microbial load: According to the current study, there were statistically significant differences in indoor bacterial and fungal concentrations in the samples collected before and after cleaning activities. This is similar to the observation by Mwambete et al.\(^{(19)}\)

Statistical significance of environmental factors & fungal load: According to this study, indoor fungal load had statistically significant association with high temperature, high humidity and higher number of occupants. \((p<.05)\) The association between the same and open type ventilation was extremely statistically significant \((p < .0001)\). This observations are in agreement with those of Nasiri et al,\(^{(3)}\) Cabo Verde et al,\(^{(27)}\) Gizaw et al\(^{(1)}\), Ashuro et al,\(^{(7)}\) and Mwambete et al.\(^{(19)}\)

Open doors and windows contribute to higher fungal loads in indoor air. Thus, majority of the findings of current study are concordant with those of other studies. However, the trivial number of discordant findings could possibly be explained by the variations among the settings and methodologies.

**CONCLUSIONS**

Hospitals are considered as dynamic environments favouring microbial transmission among the inhabitants unless the infectious patients are isolated and barrier precautions are followed. This study determined the quantity and diversity of culturable, aerobic, mesophilic micro organisms in the hospital indoor air samples utilizing settle plate method which is simple and cost effective. The baseline data generated will serve as a guide to emphasize, formulate and implement more stringent infection prevention & control policies and guidelines in all the OPDs, wards and OTs of this health care setting.

This teaching hospital has an inpatient bed strength of 2000 and provides optimal and quintessential health care services for people of 2 districts. Ensuring healthy indoor air quality with effective multidisciplinary team approach, can protect patients, students and health care workers from HAI's. This would create remarkable positive impact on the physical and mental health of the occupants.
Summary
➢ Paediatric, antenatal, post natal and postoperative wards had low microbial load.
➢ Labour and septic labour wards had high microbial load.
➢ All places sampled in the study had bacterial and fungal load within permissible range, namely 105 - 2000 CFU/m$^3$ and 26 -105 CFU/m$^3$ respectively.
➢ All of the sampled sites had hand washing stations, good biomedical waste management and adequate & appropriate cleaning and disinfection frequencies.
➢ Though the activities of personnel regarding clean rooms are satisfactory, periodic training regarding adherence to environmental safety guidelines is crucial.

Limitations
➢ In this study, the microbial contamination of outdoor air was not assessed.
➢ Conducted at single centre with low sample size (60).
➢ Surface sampling with swabs for aerobic bacteria in association with settle plate sampling, could yield better, comprehensive results of microbial loads in hospital environment.
➢ Viable but non culturable forms (VBNC) of bacteria which may be in low concentration but can cause infections, had not been determined. The study of them necessitates sensitive, expensive, labour intensive and sophisticated Polymerase Chain Reaction (PCR) techniques.

Acknowledgement: The authors thank and feel grateful to Indian Council of Medical Research for sponsorship for this study.

Legends:
IMA Index of Microbial Air contamination
S.D Standard deviation
ICU Intensive Care Unit
NICU Neonatal Intensive Care Unit
PICU Paediatric Intensive Care Unit
OT Operation Theatre
TAT Trans Abdominal Tubectomy
OG Obstetrics and Gynaecology
CFU Colony Forming Units

REFERENCES


22. Patel Pritikumari, Shalini Malhotra , Saloni Garg, Renu Yadav, NK Bhatia, Nandini Duggal, Air Bacteriology of Operation Theatres and Intensive Care Units from a Tertiary Care Hospital in New Delhi International Journal of Innovative Research in Medical Science (IJIRMS) 2019; 04(11). 627-29
27. Cabo Verde S, et al., Microbiological assessment of indoor air quality at different hospital sites, Research in Microbiology 2015;03(004)
31. Valentina Y. and Umadevi S. Phenotypic Detection and Quality Assessment of Indoor Air-Borne Microorganisms Using Passive Air Sampling Technique (Settle Plate) at A Tertiary Care Teaching Hospital in Puducherry, J Pure Appl Microbiol,2019; 13(1), 241-245

DECLARATIONS
1.Ethics approval and consent to participate: Ethics committee approval was obtained from the Institutional ethics committee, Tirunelveli Medical College, Tirunelveli. Consent to participate in the study– voluntary and written informed consent obtained from all the staff of OTs and wards.
Consent for publication: yes
Availability of data and materials: all are available with the corresponding author
Competing interest: None
Funding: Indian Council of Medical Research, New Delhi, India
Acknowledgement: The authors are thankful to the Indian Council of Medical Research, for funding and financial support.