

Variability in airborne bacterial and fungal population in the tertiary health care centre

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Abstract An investigation of the air quality and the quantity of airborne microbes was conducted in a private and a government tertiary health care centre of Davanagere in the month of November 2011 to assess the level of air borne pathogens. Using a Merck Microbial Air Sampler MAS-100NT, samples were collected in the morning and in the evening from the different environs of the private and government tertiary health care centre. The media used for the study of fungi was sabouraud dextrose agar. *Aspergillus* spp, *Curvularia* spp, *Alternaria* spp, *Penicillium* spp, *Rhizopus* spp, *Nigrospora* spp, and *Fusarium* spp were found in either of the tertiary health care centre. *Aspergillus* spp was dominant in the Government tertiary health care centre, and *Alternaria* spp and *Curvularia* spp were dominant in the private tertiary health care centre. For the bacteria, quantitative enumeration was done using soyabean casein digest agar and selective media like *Escherichia*

coli and coliform agar and urinary tract infection agar were used in qualitative enumeration. Selected pathogens like *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Enterococcus faecalis* were found in either of the tertiary health care centre. Maximum number of fungi and bacteria were isolated from emergency ward and general ward of government and private health care centre. There was also considerable difference in the morning and in the evening.

Keywords Airborne · Fungi · Bacteria · Indoor · Tertiary health care centre

1 Introduction

The presence of high concentrations of airborne microorganisms within the indoor environments is of increasing concern with respect to many acute diseases, infections, and allergies (Lugauskas 2004). The Indoor environment can potentially place human at greater risk than the outdoor, because enclosed spaces can confine aerosols and build to infection levels (Dutkiewicz and Augustowska 2006).

Indoor environment contains different types of microorganisms, and in hospitals, it is of particular concern (Saad 2003) because patients may serve as a source of pathogenic microbes to other patients, staff,

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and hospital visitors. A diverse range of microbial population is found in the hospital indoor air (Bhatia and Vishwakarma 2010; Ekhaïse et al. 2008). The infrastructure, the surroundings, and closed areas play a pivotal role in the spread of microorganisms. Variation in the operating personnel number and activities leads to the particle concentration fluctuations (Li and Hou 2003).

The sole reason behind hospitalization and medical procedures is to speed up the process of healing. However, this meticulous process unintentionally introduces several pathogenic microorganisms into the body and initiate nosocomial infections (Qudiesat et al. 2009). Hospital infection poses a serious problem of contemporary medicine in the whole world. It is also a true indicator of the quality of the hospital and the level of air pollution (Reanprayoon and Yoonaiwong 2012; Pini et al. 2004; Jurczak et al. 2007). Organisms responsible for such infections may be related to the patients own flora or may be acquired either from health care staff or the environment (Wren et al. 2008).

This study has been aimed at investigating the concentration and type of airborne microbes in the health care centres of Davangere. This goal has been focus to ascertain the nature of the air quality in the hospital environment and to create awareness towards a healthy atmosphere in the health care centre. A second goal has been to compare the microbial contamination in the different sections of the hospitals.

2 Materials and methods

2.1 Sampling sites

To assess the air quality and the quantity of airborne microbes, two prominent tertiary health care centres were selected in Davangere. The private tertiary health care centre, with around 700 beds, is located at the outskirts of the city, surrounded by paddy fields, trees, and a few small scale industries. The government tertiary health care centre is at the heart of the city with 600 beds. Samples were collected in the morning between 9:00 to 11:00 am and in the evening between 4:30 to 6:30 pm. Sampling was conducted in the operating theatres (OT), paediatric ward (Ped), intensive care unit (ICU), emergency ward (Eme), and the general ward (Gen) of the two health care centres.

2.2 Air sampling

The Merck, MAS-100NT microbial air monitoring system is a high performance instrument based on the principle of Anderson air sampler (Andersen 1958). This instrument aspirates air through a perforated plate which results in impaction of particles from an airstream on to the surface of agar medium. It serves to measure the microorganisms in clean rooms and sterile environments. Air is aspirated through a perforated lid and impacted onto the surface of growth media in a standard 90 mm Petri dish. The sampling head is provided with 300 Å— 0.6 mm holes, and aspiration capacity is 100 l/min. The total volume of air that was aspirated onto the agar plate in our sampling was 50 l. The sampler was held at four different corners of the operating theatres near the exit air duct, one foot above the ground level. In the intensive care units, general and emergency wards sampling were done at four different places at a height of 2 to 3 ft above the ground level.

After the incubation period, the number of the fungal and bacterial colony units has been expressed per cubic metre of air: $X = \frac{a \times 1000}{V}$.

where a the number of microbial colonies grown from the indoor air sample; V the volume of the air sample aspirated (litres); and X the number of microbes in one cubic metre of the air expressed in terms of the number of colony forming units in one cubic metre, that is, cfu/m³ (Gniadek and Macura 2007; Javed et al. 2008).

2.3 Isolation and identification of fungi

For the isolation of the fungi, sabouraud dextrose agar (SDA), with 10 mg/l chloram-phenicol, was used. After the sampling, SDA plates were incubated at 25° ± 1 °C during 5 days. Lactophenol blue solution has been used as mounting medium and staining agent for microscopy spore and hypha identification.

2.4 Isolation and identification of bacteria

In case of airborne bacteria isolation, soyabean casein digest agar (SCDA) was used. For selective or differential analysis of certain dominant nosocomial infecting bacteria, HiCrome urinary tract infection (UTI) agar (Collee et al. 1996) and HiCrome

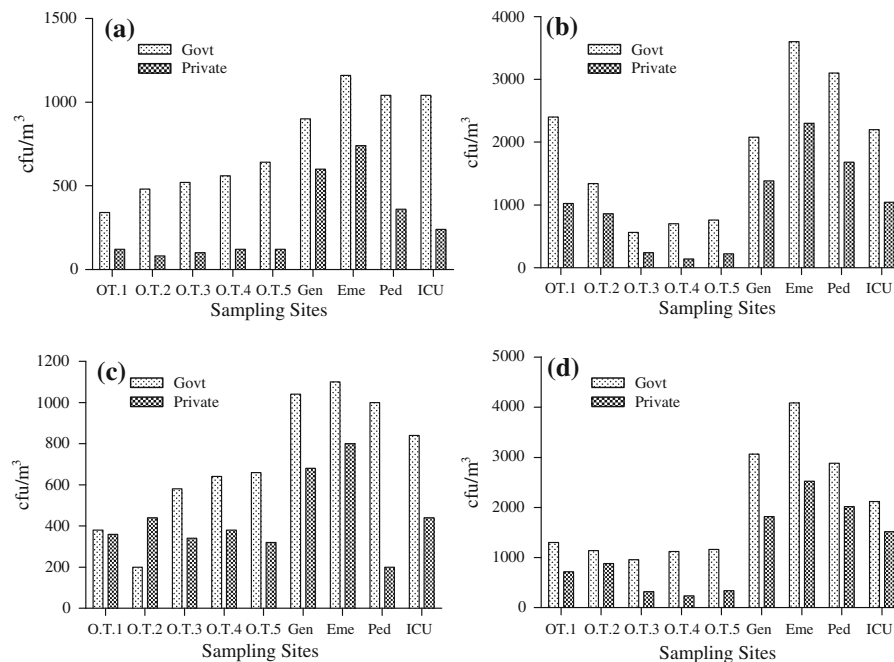


Fig. 1 Fungal concentration in the two tertiary health care centres [Morning] (a), [Evening] (c). Bacterial concentration in the two tertiary health care centres [Morning] (b) [Evening] (d)

Escherichia coli and coliform (ECC) agar media (Delisle and Ley 1989) were used. Sampled plates were incubated at $37 \pm 1^\circ\text{C}$ during 24 to 48 h for the better identification on chromogenic media. Identification was based mainly on formation of chrome by the colony on differential agar, staining, and by biochemical methods.

3 Results

Difference was found in the quantitative and qualitative analysis of fungi and bacteria in the two health care centres (Fig. 1). Considerable differences were recorded in the morning and in the evening. On the other hand, private health care centre counts with lower microbial load than the government health care centre.

In the operating theatres of the government health care centre, fungal colonies ranged from 340 to 640 cfu/m^3 and in the private operating theatres from 80 to 120 cfu/m^3 (Fig. 1a). In the government operating theatres, the bacterial count ranged from 2,400 to 560 cfu/m^3 and in the private health care centre from

1,020 to 140 cfu/m^3 (Fig. 1b). In the evening, the fungal count in the operating theatres of government health care centre ranged from 200 to 660 cfu/m^3 and in the private health care centre from 320 to 440 cfu/m^3 (Fig. 1c). The bacterial count of the government health care centre varied from 960 to 1,300 cfu/m^3 and in the private health care centre from 240 to 880 cfu/m^3 (Fig. 1d).

The percentage of fungi in government tertiary health care centre varied from 6 to 17 % (Fig. 2a) and that of bacteria has been 5 to 22 % (Fig. 2b). A fungal variation of 7 to 23 % has been prevalent in private health care centre (Fig. 2c) and the bacteria varied from 1 to 30 % (Fig. 2d). In the two tertiary health care centres, the highest amount of microbes was isolated from the emergency and general wards, in comparison with the operating theatres and the intensive care units (Fig. 3a, b). In the evening, equal distribution of pathogens has been found in both, the operating theatres of tertiary health care centre (Fig. 3c, d). Fungal concentration in the operating theatres was the upper limit closer to the mean, where as for the bacteria, it was far away from the mean (Fig. 4).

Fig. 2 Percentage of fungi in **a** government **c** private tertiary health care centre. Percentage of bacteria in **b** government **d** private tertiary health care centre

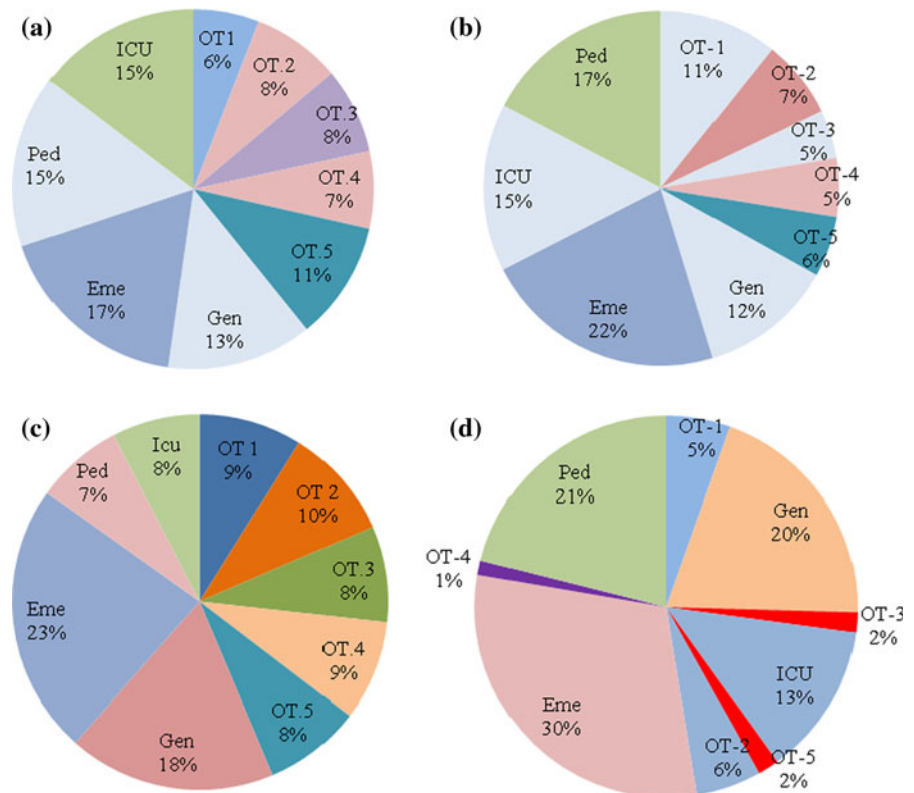
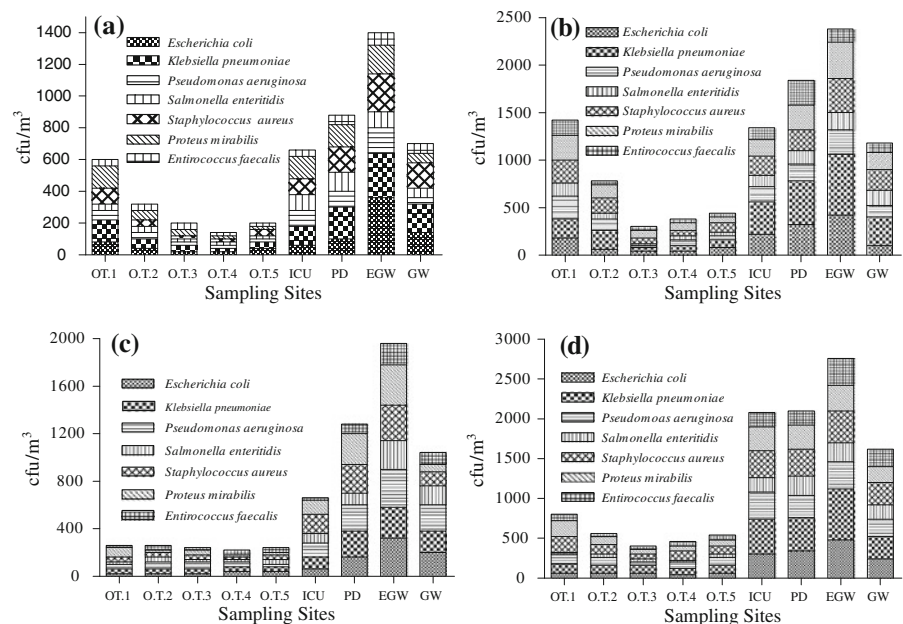


Fig. 3 Pathogen concentration in private tertiary health care centre [Morning] (a), [Evening] (c). Pathogen concentration in government tertiary health care centre [Morning] (b), [Evening] (d)



Aspergillus spp, *Nigrospora* spp, *Rhizopus* spp, *Penicillium* spp, *Curvularia* spp, *Alternaria* spp, and *Fusarium* spp were common in either of the tertiary health care centres; *Aspergillus* spp was

dominant in the Government tertiary care centre; and *Alternaria* spp and *Curvularia* spp were predominant at the private tertiary health care centre.

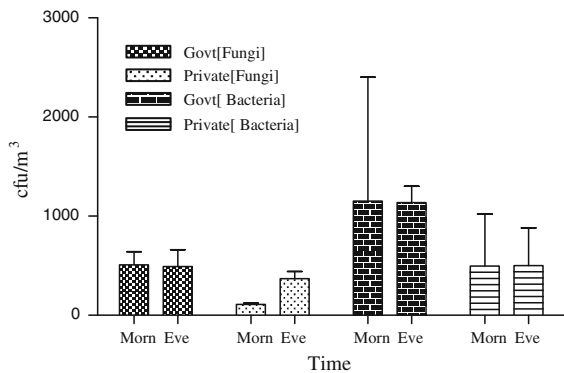


Fig. 4 Variation in the microbial concentration in the operating theatres

4 Discussion

The microflora of any habitat varies with host type, environmental condition, and relation among them (Verma and Khare 2009). Exposure to bioaerosols is hazardous in clinics and hospitals, where they are a major factor in increasing morbidity from respiratory diseases (Sarica et al. 2002). Certain human pathogens also cause infection to healthy individuals (Pastuszka et al. 2005). The indoor air environment can potentially place patients at greater risks than the outside environment because they confine aerosols to infectious levels (Ekhaise et al. 2008).

In both studies, health care centers have been detected with microbial pathogens. Ventilation and human activities in indoor environment are considered important factors affecting the level of these airborne biological contaminants (Balasubramanian et al. 2012).

It has been detected more bacterial and fungal airborne colonies during the evening, probably due to cleaning activities when the doors are open and HVAC (heating ventilation air conditioning) system is not operational. Dust from human activities, such as sweeping, movement, and bed making, increase the amount of microbes (Balasubramanian et al. 2012; Fleischer et al. 2005).

According to the health technical memorandum, HTM 2007, HTM 2025 (Pasquarella et al. 2012), and the European Commission Guide to Good Manufacturing Practice EC GMP (2008), only the OT-4 operating theatres falls under Grade D, with 140 cfu/m³; the other operating theatres cross the limits. Charkowska (2008) considers Class 1 operating theatres

when bacterial concentration is up to 70 cfu/m³ of air, Class 2 when bacteria concentration is up to 300 cfu/m³ of air, and Class 3 when it is up to 700 cfu/m³ of air. In this paper, some operating units of tertiary health care centre were far beyond the counts. Operating theatres 3, 4, and 5 of the private health care centre fall under Class 2 when HVAC system is operational. Awosika et al. (2012) have observed that regular surveillance, restricting the number of medical students, control in the opening and closing of the doors, and minimal movements in the operating theatres may bring the other operating theatres of our study to Class 2 level.

In the morning, fluctuations of the microbes might be related to variations in operating personnel numbers and activities (Li and Hou 2003). Under normal circumstances, the main source of air borne microbial contaminants is microscopic skin fragments given off by staff in the theatre (Hoffman et al. 2002).

In the government tertiary health care centre, the microbial load of fungi and bacteria was high in all the operating theatres probably because of the absence of HVAC system. In this centre, only cleaning procedures, such as fumigation, cleaning of floors, and walls, with disinfectants and the dress code, are practiced. High human activities, especially the theatres staff and medical students, also contribute to the increase of the microbes (Okon et al. 2012; Abt et al. 2000). In cases, the emergency wards and the general wards count with the maximum concentration of microbial pathogens. It could be due to the fact that these wards were flooded with patients.

Aspergillus spp and *Alternaria* spp which were dominant in government and private tertiary health care centre have adverse effects on the human health. *Alternaria* spp, *Aspergillus* spp, and *Penicillium* spp have been identified in the medical literature as dominant species found in the hospitals (Qudiesat et al. 2009). *Aspergillus* spp and *Penicillium* spp are considered as potential threats to the inmates (Krajewska-Kulak et al. 2007; Apetrei et al. 2009). They provoke allergic disease and induce acute intoxication and many adverse effects to human health, such as carcinogenic, mutagenic, teratogenic, hormonal, haemorrhagic, immunotoxic, nephrotoxic, hepatotoxic, dermatotoxic, neurotoxic, antibiotic, hematological changes, releasers of interleukins, and lipid peroxidation (Balasubramanian et al. 2012). Airborne fungi causing respiratory infections and allergic

reactions include *Aspergillus* spp, *Penicillium* spp, *Acremonium* spp, *Paecilomyces* spp, *Mucor* spp, *Alternaria* spp, and *Cladosporium* spp. (Lugauskas 2004).

Gram positive cocci, such as *Staphylococcus aureus*, constituted the main bacteria isolated from air samples in hospital rooms. Yagoub and Agbash (2010) have shown that *S. aureus*, a commensal microorganism, can cause opportunistic infections when host resistance is compromised, especially when a primary infection, such as influenza, is present. Since it exists ubiquitously, mortality rate is high. It is also responsible for many respiratory tract, digestive system, post-operative infections, urinary tract, and skin disorders with multi-antibiotic resistance.

Pseudomonas aeruginosa another gram negative bacterium which was isolated from the tertiary health care centre is responsible for many important nosocomial outbreaks. It has been previously reported that it is present on various surfaces or environments in hospital, including tap water and sink drains (Gilbert et al. 2010). *Klebsiella pneumoniae*, another dominant species in the tertiary health care centre, is found in man's intestines as commensal flora. It becomes an infectious problem when they occur in the upper respiratory tract (Kowalski 2006).

High microbial concentrations in various indoor environments point out a potential health risk such as respiratory diseases. Since the isolated bacteria and fungi could be pathogenic, it is pertinent that their presence should be controlled. Efforts should be made to minimize airborne transmission of opportunistic microorganisms and their potential impact on patients.

5 Conclusion

Taking into account these results, it is important that health care centre be continuously monitored, by controlling the airborne pathogens. It is important for the medical personnel, patients, and also for all those who are in contact with the patient. Air quality control procedures may not negotiate fully the onslaught of the microorganisms but surely will help in their control.

A programmed and periodical cleaning operation and maintenance activities of various indoor environment should be organized as a preventive measure. Increasing the ventilation rate by means of mechanical

or natural systems can also play an important role in improving the indoor air quality.

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