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Microbiological Assessment of Indoor Air Quality in Selected Patient Wards at a Tertiary Hospital in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Patients are at higher risk of nosocomial infections from pathogenic microbes contaminating the indoor air of hospitals. This study evaluated selected patient wards at University of Calabar Teaching Hospital (UCTH), for bacterial and fungal contaminants. The study also determined the microbial contamination level when compared to recommended sanitary standards.
Methodology: To determine microbial load, petri-plate gravitational settling method was used. The set-ups were exposed for one hour (at morning and noon) for passive sampling onto growth media plates, in accordance with the 1/1/1 scheme as stipulated by the index of microbial air contamination standard. Petri-plates were subsequently covered with lids and placed in a cold box. Samples were then transported to the laboratory for further evaluation. Sampling was performed in duplicates within three months, and further analysis were conducted based on standard protocols. Mean counts of bacteria and fungi was recorded and expressed as colony forming units (CFU/m^3).

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Results: Microbial counts in all wards surveyed ranged from 'intermediate' to 'high' when compared to the sanitary standards of the European Commission for non-industrial premises. However, the counts were within the WHO acceptable sanitary standard (<1000 CFU/m^3). Eleven bacterial genera and seven fungal genera were identified. Isolated bacteria were *Staphylococcus aureus* (18.3%), CoNS (6.7%), Bacillus sp (13.3%), Streptococcus spp (6.7%), Corynebacterium sp (1.7%), and Micrococcus sp (1.7%), *Pseudomonas* aeruginosa (15%), *Escherichia coli* (10%), *Klebsiella pneumoniae* (10%), *Acinetobacter baumannii* (8.3%), Salmonella sp (5%) and Enterobacter sp (3.3%). Conversely, isolated fungal contaminants were *A. niger* (25.9%), *A. flavus* (20.4%), Penicillium sp (16.7%), Candida sp (9.3%), Rhizopus sp (3.7%), Mucor sp (7.4%), Cladosporium sp (14.8%), and Fusarium sp (1.9%).
Conclusion: Regular monitoring of indoor air quality in health-care settings is encouraged to effectively guide infection prevention and control strategies and to limit nosocomial infections occurrence.

Keywords: Bioaerosols; hospital wards; indoor air; nosocomial infections; ventilation.

1. INTRODUCTION

Air serves as dispersal or transport medium for microbes and can be categorized as 'indoor air' when found within buildings [1]. The health burden from indoor air pollution has been documented to be associated with over 600,000 deaths in Africa, 99,000 in Europe, 200,000 in the Eastern Mediterranean region, 81,000 in the Americas, and 19,000 deaths in other high-income countries [2]. In Southeast Asia and Western Pacific regions, about 1.69 million and 1.62 million deaths respectively, are linked to indoor air pollution [2]. The Centre for Disease Control and Prevention, estimates that annually, nearly 1.7 million hospitalized patients acquire nosocomial infections while on treatment for other health challenges, thereby resulting to greater than 98,000 deaths [2]. It is also estimated that 11% of deaths in low-income countries are associated with lower respiratory infections caused by indoor air pollution [1,2]. Poor hospital indoor air quality has been associated with a variety of airborne infections, especially with disease-causing bacterial agents [3]. Therefore, hospital indoor air quality represents a major sanitary profile of public importance.

Hospitals and health care settings can be considered as dynamic environments which are influenced by ventilation systems, weather conditions, seasons, and moisture intrusion [4]. Consequently, airborne transmission is one of the routes associated with the spread of several nosocomial infections because, microorganisms are almost always present in air [2,4]. Hospital indoor air contains a diverse range of microorganisms (bacteria, yeasts, fungi, viruses, and parasites), and the transmission of these microbes to humans via air is due to bioaerosols, which can spread to considerable distances, thereby resulting in infection [4].

Bacterial pathogens have been associated with causing most healthcare associated infections (HAIs) because of their ability to survive longer on dry surfaces and their resistance potentials to disinfectants [1,3]. The most common bacterial genera found in indoor air are Bacillus spp, Staphylococcus spp, Clostridium spp, methicillin-resistant Staphylococcus aureus (MRSA), and gentamicin-resistant gram-negative bacteria [1]. Several studies have also reported that nosocomial infections could also be caused by fungal species such as Aspergillus spp and various species of Candida, Penicillium and Cladosporium. Such studies documented that in samples from ventilator systems, air canals, and hospital air instruments, fungal species including Penicillium spp, Cladosporium spp, Aspergillus spp, Chrysosporium, Trichoderma, Rhizopus, and Streptomyces spp have been isolated [4].

Hospital airborne contamination or infection sources play significant roles in the spread of airborne pathogens and could be traced to a variety of factors including patients' activities (e.g., coughing, sneezing, yawning, talking), patients' number per room, patients' normal flora, contaminated fomites, contaminants from staff and visitors as well as air conditioning system [5]. The presence and multiplication of bio-aerosol are aided by deficiency of clean air, occasioned by poorly maintained ventilation systems, increased building insulation, and poorly regulated relative humidity and temperature levels, poor waste management system and presence of unsanitary attached

toilets [4]. The air quality of hospital indoor environment is not always readily controlled and can potentially place patients and other human occupants at high risk [4,5]. In developing countries including Nigeria, design flaws, insufficient ventilation and lack of effective infection prevention and control practices exacerbates poor hospital indoor air quality [5].

To prevent and control the spread of air-borne infections in healthcare environment, it is vital to initiate hospital-wide surveillance through periodic monitoring [4,5]. Routine surveillance uses surface swabs and settle plates and as part of the surveillance protocol, in the sampling of several wards to determine bio-aerosol concentrations [6]. Several hospital wards have been documented for their risk in the acquisition of nosocomial infections due to prolonged admission but, there are limited studies on hospital air quality of major hospital wards in Calabar-Nigeria. Therefore, the present study evaluated the microbial contaminants and contamination level of selected wards at UCTH, Calabar-Nigeria. Findings from this study will effectively aid infection control and surveillance practices.

2. MATERIALS AND METHODS

2.1 Description of Study Center

The University of Calabar Teaching Hospital, Nigeria is about a 610-bed tertiary hospital that came into existence in 1979. The hospital took over the facilities of the then St. Margaret's hospital, Calabar, which was established in 1897 as the first public hospital in Nigeria. The hospital operates 24 hours services for inpatients and outpatients and runs clinical services, out-patient clinics, emergency services, laboratory services, pharmacy services, and specialized investigations. It is located in Calabar, Cross River State at geo-coordinates 4.95485°N 8.35031°E.

2.2 Growth Media Preparation

Growth media utilized for sampling in the present study included MacConkey agar (MCA), 5% Sheep Blood agar (SBA), and Sabouraud Dextrose agar (SDA). For bacterial sampling MCA and SBA were used, while for fungal sampling SDA was used. All growth media were prepared based on the manufacturer's instruction and in line with standard operating procedure, and subsequently dispensed into petri dishes. Sterility tests were performed by incubating a random representative of a prepared growth media batch at 37°C for 24 to 48 hours for bacteria, and at room temperature incubation (25°C) for 3days for fungi. Each sterile petri-dish containing prepared media was properly labelled with vital tags relevant to the experimental set-up.

2.3 Study Design/Sampling Methods

To determine the indoor air quality of selected patient wards at the University of Calabar Teaching Hospital, Calabar-Nigeria, an institutional-based cross sectional study was carried out for three months. The petri-plate gravitational settling sampling method was utilized due to its less complicated methodology and technical feasibility. Samples were collected by exposing prepared petri-plates (9 cm diameter) containing prepared sterile growth media in patient wards (pediatric medical ward, paediatric surgical ward, emergency room, male surgical ward, female surgical ward, gynecology ward, antenatal ward, post-natal ward, male orthopedic ward, female orthopedic ward, male medical ward, and female medical ward) within UCTH. The set-ups were exposed for passive sampling onto prepared media plates in accordance with the 1/1/1 scheme (1 m from the wall, 1 m from the floor, and for a duration of 1 hour) as stipulated by the index of microbial air contamination standard [6]. Samples were obtained twice per day at 9:00 a.m. and 1 p.m. and were quickly covered with their corresponding lids and placed in a cold box before being transported to the Microbiology laboratory for further analysis. The experimental process was performed in duplicates and recorded counts were averaged. After incubation, viable colony counts of airborne bacterial and fungal loads of various wards were recorded and expressed as colony forming units (CFU/m^3).

2.4 Microbiological and Biochemical Identification of Bacterial Isolates

Colonies observed from culture plates containing bacteria were subsequently characterized and identified according to established guidelines as previously described [3,7]. Colonies from MCA, and SBA plates with different morphological characteristics were sub-cultured onto freshly prepared SBA and MCA plates and incubated at 37°C for 24 to 48 hours under aerobic conditions. Bacterial growth

were counted and expressed as colony-forming units (CFU/m^3). The plates were then examined for colony morphology of bacteria, lactose fermentation on MCA, and hemolysis in the SBA medium. Smears were made from unique colonies and gram stained as previously described [3,7]. Hemolysis, catalase, and coagulase tests were used to further identify gram- positive bacteria while gram-negative bacteria were identified through various biochemical tests including indole production, lactose fermentation, MR-VP test, citrate utilization test, triple sugar iron agar test, urease production, motility test. Non-fermenters were identified further using catalase and oxidase tests, growth ability on MCA, and growth at 42°C. All bacterial isolates were identified at genus and species level by conventional methods and further confirmation of isolates identity was conducted using VITEK-2 identification system as detailed in previous studies [8, 9, 10].

2.5 Microbiological Identification of Fungal Isolates

For the identification of fungi, cultures were subsequently inoculated onto Sabouraud Dextrose agar (SDA) slopes and incubated at room temperature (25-27°C) for a period of 3 days in the dark, as per standard procedures [11]. Fungal morphological structures determination was carried out on fungal material mounted in lactophenol cotton blue to differentiate and characterize each fungal type as previously reported [11,12]. Fungal species were subsequently detected to genus and species level using some criteria including rate of growth, surface and reverse colonies coloration grown on SDA, micro- and macro-morphology, pigment production, and slide culture technique as previously described [11,12].

3. RESULTS AND DISCUSSION

Several studies have reported that contamination by airborne microbes in hospital indoor spaces could potentially cause infections, particularly of nosocomial origin [13,14]. Among airborne microorganisms associated with hospital-acquired infections, bacteria and fungi are implicated with causing significant health consequences [3,13,14]. In the present study, all patient wards surveyed within the study period were contaminated with bacteria and fungi, and the microbial counts were higher at noon compared to counts recorded in the morning (Table. 1 & Table. 2). The higher bacterial and fungal counts recorded at noon could be attributed to the busy nature of the hospital during mid-day to evening hours due to the number of patients in-flow, new patient admissions, and the influx of patients' relatives/visitors. This finding corroborates the report that occupants' density is crucial in affecting airborne microbial concentrations, and that occupants' density was time-dependent [5]. Additionally, the highest counts for both bacterial and fungal contaminants were recorded in the emergency room, while the lowest counts were recorded in the paediatric surgical ward (Table 1 & Table 2). This result aligns with findings from a previous study conducted at the University of Benin Teaching Hospital, Nigeria that the accident and emergency ward recorded the highest bacterial and fungal counts from analyzed air samples [5].

Table 1. Mean colony count (CFU/m^3) of bacteria in sampled patient wards

	Mean Colony Count (CFU/m^3)	
Sampling Sites	**Petri-plates exposure at 9 am (1 hour)**	**Petri-plates exposure at 1 pm (1 hour)**
Pediatric Medical Ward	631	652
Pediatric Surgical Ward	385	417
Emergency Room	753	776
Male Surgical Ward	472	492
Female Surgical Ward	459	486
Gynecology Ward	408	435
Antenatal Ward	563	586
Post-natal Ward	529	542
Male Orthopedic Ward	486	518
Female Orthopedic Ward	462	505
Male Medical Ward	612	639
Female Medical Ward	624	641

Table 2. Mean colony count of fungi in sampled patient wards

Sampling Sites	Mean Colony Count (CFU/m^3)	
	Petri-plates exposure at 9 am (1 hour)	Petri-plates exposure at 1 pm (1 hour)
Pediatric Medical Ward	522	541
Pediatric Surgical Ward	211	237
Emergency Room	553	576
Male Surgical Ward	314	338
Female Surgical Ward	326	344
Gynecology Ward	303	336
Antenatal Ward	363	381
Post-natal Ward	385	397
Male Orthopedic Ward	453	484
Female Orthopedic Ward	446	477
Male Medical Ward	538	544
Female Medical Ward	522	539

Although several regulatory institutions have proposed varying limits for microbial load in built environment, there is no universally accepted threshold [3]. Findings from the present study revealed that the microbial load for isolated bacterial and fungal contaminants ranged from 'intermediate' to 'high' (Table 3 & Table 4) when compared to the sanitary standards of the European Commission for non-industrial premises, which considers microbial load < 50 CFU/m^3 as 'very low', 50–100 CFU/m^3 as 'low', 100-500 CFU/m^3 as 'intermediate', 500–2000 CFU/m^3 as 'high' and >2000 CFU/m^3 as 'very high' [1,3]. However, the microbial counts recorded from this study were within the acceptable sanitary standard of the WHO. The WHO sanitary standard places microbial count of <1000 CFU/m^3 as acceptable [3].

Sampled locations which recorded 'high' indoor microbial counts were observed to have had higher patient numbers, frequent opening of doors and total opening of windows compared to wards with 'intermediate' bacterial and fungal counts, which had fewer patient numbers, better sanitary systems, and maintained stringent protocol for entrance and exit. Additionally, in all wards sampled, it was observed that natural ventilation which promotes entry of air contaminants from the external environment was highly relied upon, and there were inadequate mechanical ventilation systems, including poor power supply for steady operation of the few mechanical ventilation systems. Furthermore, dry sweeping which could raise the level of indoor bio-aerosols was observed to be commonly practiced. These observations correlate with findings from previous studies that high patient numbers on admission, poor ventilation and sanitary systems among other factors are cardinal to worsening indoor air quality in health care settings [2, 3]. It has also been reported that temperature range of 26.5–29.5 °C and humidity range of 64.5–85 % provides favorable conditions for bacteria growth and multiplication [2].

In the present study, eleven bacterial genera, and seven fungal genera were identified (Fig. 1 & Fig. 2). The bacterial and fungal species identified were similar to microbial contaminants isolated from a previous study [15]. Among identified species, Staphylococcal species were most isolated. This correlates with report from a previous study that Staphylococcus genus was the most isolated bacterial contaminant of indoor air in four primary health centers in Qatar ranging from 15–80% [13]. Nevertheless, it has been established that locations and seasons could influence variability in the relative contamination level [13,16]. Though, many studies have reported *S. aureus* as pathogenic, recent studies have also shown that coagulase-negative Staphylococci and Micrococci, have potentials of causing several infections in patients who are immuno-compromised [1,11].

Isolated gram-positive bacteria and their percentage occurrence were *Staphylococcus aureus* (18.3%), Coagulase Negative Staphylococci (6.7%), Bacillus sp (13.3%), Streptococcus sp (6.7%), *Corynebacterium* sp (1.7%), and Micrococcus sp (1.7%). Conversely, gram-negative bacteria isolated from various wards were *Pseudomonas aeruginosa* (15%), *Escherichia coli* (10%),

Klebsiella pneumoniae (10%), *Acinetobacter baumannii* (8.3%), Salmonella sp (5%) and Enterobacter sp (3.3%) (Fig. 1). Studies have posited that the presence of gram-negative bacteria could be due to airborne fecal contamination, improper hospital wastes handling and disposal and prevailing climatic conditions [15,16]. These bacterial species isolated have been reported in several research findings as being implicated as causative agents of infections associated with antibiotic resistance [1,3,17]. Generally, bacterial species isolated from the current study aligns with results from similar studies conducted in Qatar, Nigeria, and in a public hospital in Agadir, Morocco [1,3,4,13].

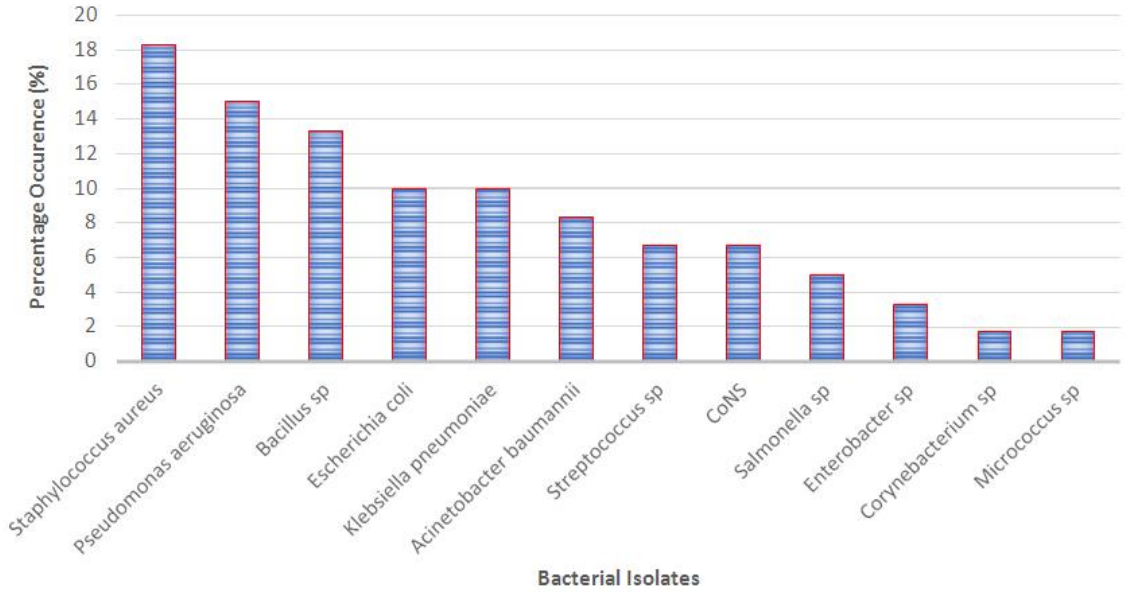

Fig. 1. Percentage frequency of isolated bacterial contaminants recovered from patient wards

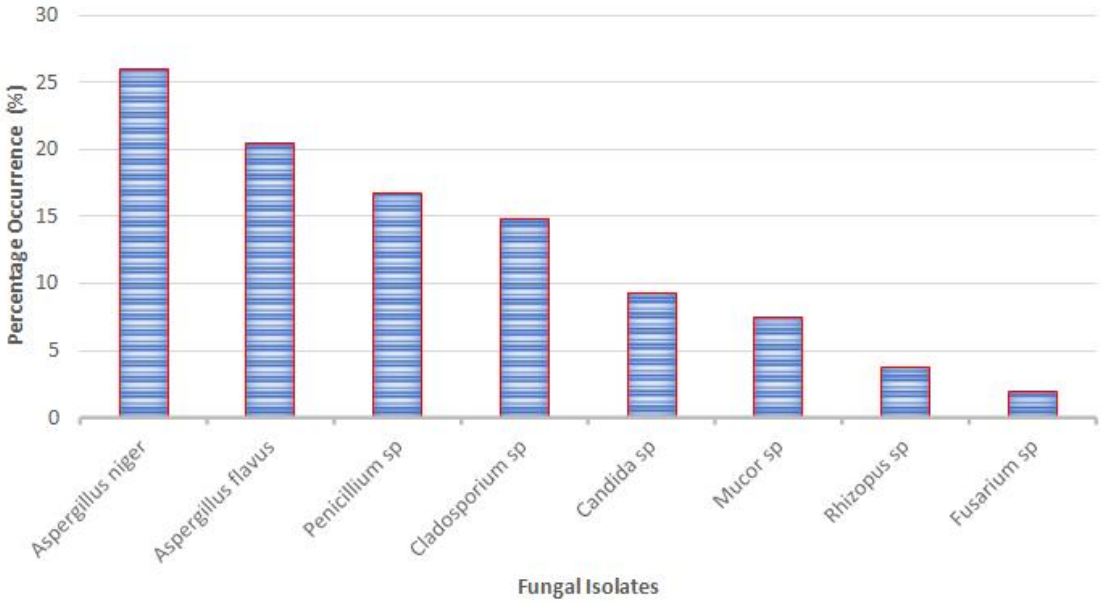

Fig. 2. Percentage frequency of isolated fungal contaminants recovered from patient wards

Table 3. Bacterial contamination level of patient wards based on non-industrial premises sanitary standards of the European Commission

Bacterial Load Range (CFU/m^3)	Contamination Level	Sampling Location and Time																							
		Paediatric Medical Ward		Paediatric Surgical Ward		Emergency Room		Male Surgical Ward		Female Surgical Ward		Gynecology Ward		Antenatal Ward		Post-natal Ward		Male Orthopedic Ward		Female Orthopedic Ward		Male Medical Ward		Female Medical Ward	
		9 am	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*
<50	Very Low																								
50-100	Low																								
100-500	Intermediate			✓	✓			✓	✓	✓	✓	✓	✓					✓		✓					
500-2000	High	✓	✓			✓	✓							✓	✓	✓	✓		✓		✓	✓	✓	✓	✓
>2000	Very High																								

Table 4. Fungal contamination level of patient wards based on non-industrial premises sanitary standards of the European Commission

Fungal Load Range (CFU/m^3)	Contamination Level	Sampling Location and Time																							
		Paediatric Medical Ward		Paediatric Surgical Ward		Emergency Room		Male Surgical Ward		Female Surgical Ward		Gynaecology Ward		Antenatal Ward		Post-natal Ward		Male Orthopedic Ward		Female Orthopedic Ward		Male Medical Ward		Female Medical Ward	
		9 am	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*	*9 am*	*1 pm*
<25	Very Low																								
25-100	Low																								
100-500	Intermediate			✓	✓			✓	✓	✓	✓	✓	✓					✓	✓	✓	✓				
500-2000	High	✓	✓			✓	✓							✓	✓	✓	✓					✓	✓	✓	✓
>2000	Very High																								

In the current study, fungal contaminants with pathogenic potentials were also isolated. Fungal contaminants recovered and their percentage occurrence were *A. niger* (25.9%), *A. flavus* (20.4%), Penicillium sp (16.7%), Candida sp (9.3%), Rhizopus sp (3.7%), Mucor sp (7.4%), Cladosporium sp (14.8%), and Fusarium sp (1.9%) (Fig. 2). These fungal species isolated correlates with those reported in a similar study conducted in Nigeria [3]. Although fungal contaminants were observed to be lower in counts compared to bacteria, their presence in hospital indoor air is of significant concern. For instance, Aspergillus species are reported to be associated with causing allergic reactions, and opportunistic infections notably in immune-deficient patients and children [12,18]. Also, Aspergillus species can cause invasive Aspergillosis as well as produce mycotoxins known to be carcinogens [4]. Furthermore, allergies, respiratory infections, and hypersensitivity reactions may be caused by other fungal spores in healthy individuals as well as immune deficient patients [12]. Overall, the bacterial and fungal pathogens isolated from sampled wards in UCTH were also reported in a similar study conducted at the University of Benin Teaching Hospital, Nigeria [11].

In hospital environment, bioaerosols can compromise sanitary and health standards. Since infectious aerosols are extremely small (<5 μm) they can remain viable as suspended particles in the air stream over a long period, thereby resulting in high risk of airborne infection especially in confined spaces [4,19]. Nosocomial infection is a major and widespread challenge, with one in ten patients likely to be infected during hospital stay [2,20]. While most nosocomial infections are associated with contact, there is increasing evidence of transmission by airborne route [21,22]. It has been estimated that of all endemic nosocomial infections, airborne transmission may account for about 10–20 % [2]. The air quality of hospital indoor spaces where health care workers, patients, and patients' relatives spend significant time, is critical in determining the health status and well-being of such individuals [23,24]. Therefore, the indoor air quality of healthcare facilities requires regular monitoring and hospital-wide surveillance.

4. CONCLUSION

Isolated microbes from indoor air of hospital wards could constitute a diverse microbial reservoir which may potentially increase nosocomial infection risk particularly to patients and staff. Consequently, efforts towards the improvement of hospital sanitary conditions including routine monitoring and evaluation of indoor aeroflora is highly recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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