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Indoor Microbiological Air Quality in Some Wards of a Tertiary Health Institution in Port Harcourt, Nigeria.

Douglas, Salome Ibietela* and Robinson, V. K.
Department of Microbiology, Faculty of Science,
Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.
Corresponding Author: Douglas

Abstract: *Indoor air within a hospital building may act as a source of disease causing organisms, which may be transmitted by the patient and could cause airborne or hospital acquired infections. The indoor air of a tertiary health institution in Port Harcourt, Rivers State was investigated to know the level of microbial contamination. The Koch sedimentation technique was used. In this technique, Nutrient agar (NA), Mannitol salt agar(MSA) and Sabouraud Dextrose agar(SDA) plates were exposed to the ambient air of the study sites in duplicates so as to enumerate the total heterotrophic bacterial, Staphylococcal and fungal populations. The mean total heterotrophic bacterial, Staphylococcal and fungal populations of the Hospital wards ranged from 2.54 to 3.40log$_{10}$Cfu/m^3, 2.12 to 2.99 log$_{10}$Cfu/m^3 and 1.78 to 2.51 log$_{10}$Sfu/m^3 respectively. The bacterial loads recorded in this study were high and exceeds the suggested standard of bacterial load in air within a building while the fungal counts recorded were under admissible limits. The bacterial isolates identified in this study includes: Bacillus species, Chryseobacterium sp, Escherichia coli, Staphylococcus aureus, and Staphylococcus epidermidis. While Aspergillus flavus, Aspergillus niger, Candida sp, Mucor sp, and Penicillium sp, were the fungi isolated. High microbial populations observed may be due to high human activities within the wards, lack of proper cleaning and disinfection and poor ventilation among others. The bacterial and fungal isolates identified in this study could be opportunistic pathogens and could cause hospital acquired infections. Attention must be given to control those environmental factors which favour the growth and multiplication of microbes in indoor environment. There is also need for regular monitoring of the indoor air quality of the hospital, in order to ascertain the actual source of these contaminants and to further prevent them.*

Keywords: *bacteria, Staphylococcal, fungi, indoor air, tertiary institution*

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I. Introduction

The quality of air within a building in relation to the health of the people inhabiting that building is referred to as the indoor air quality of that building, and it is very important in health care settings which accommodates large number of individuals (Agbagwa and Onyemaechi, 2014). The contamination of the indoor air by microorganisms especially in the health institutions is of public health concern as current studies have suggested that the presence of microorganisms in the indoor air could cause airborne infections as well as hospital acquired infections (Wemedo and Robinson, 2018; Latika and Ritu, 2011). An understanding of microbial air contamination is a crucial criterion for assessing the hygienic conditions of the environment. Globally, people stay longer in an indoor environment especially at places of work, relaxation centres, homes and schools and this is as a result of the changes in the way of life (Chao *et al*., 2003). Also, in a study by Yang *et al* (2011), it was reported that about eighty percent of the life of a human is spent in an indoor environment. According to Botkin and Keller (2007), environment that is in a good condition is strongly connected with the human health especially those within the building (staff, patients, including visitors). One of the ways through which diseases especially those that are associated with hospital acquired infections are transmitted is via the air (Claudete *et al*., 2006). Previous studies have referred to infections which were acquired by patients only in the hospital environment as Nosocomial infection or hospital acquired infection (Beggs, 2003; Omoigberale *et al*., 2014). The indoor air of health institutions could be contaminated by pathogens or normal flora of patients or healthy carriers from the nasal/pharynx, oral cavity, bronchial secretions, skin and from the digestive tracts. These contaminants can remain in the air under favourable conditions as their survival depends on their level of resistance to the outer environment, type of the object and the conditions of the environment which favor the survival of microorganisms are especially represented by the absence of light, particularly of direct solar radiations, high humidity and low temperature (Cernei *et al.,* 2013). Wemedo and Robinson (2018) in a recent study has reported that these organisms could be discharged into the atmosphere through various activities such as sneezing, coughing, laughing, and sweeping including other activities and that when these aerosols are

present in the atmosphere, transmission could either be by inhalation as mentioned earlier or by contact with non-critical surfaces after these particles must have settled. The major microbial contaminants are bacteria, viruses, fungi, mites and pollen (Khan and Karuppayil, 2011). The general physical condition of humans could be drastically affected via the contact with microorganisms present in the air. Thus, contact with these pollutants could impair respiration and functions of the heart which may lead to high rate of mortality and morbidity of the population (Baccarelli *et al*, 2008; Kunzli *et al*, 2000, 2004). The proceeds of an organization which is the output by the workers could be affected by the quality of the indoor air. This implies that an environment that is not properly ventilated could lead to the inhalation of toxins, microbes and other substances which could affect the health of the workers leading to their absence from duty (Wong *et al*., 2007). This study was aimed at assessing the indoor microbiological air quality of some wards in a tertiary health institution in Port Harcourt.

II. Materials and Methods

Description of Area under study

This study was carried out in the Kelsey Harrison Hospital which was formerly called New Niger Hospital (up until 2009) Port Harcourt and it is located along Emenike Street, of the Diobu axis, in Port-Harcourt City Local Government Area, Rivers State, Nigeria. This facility became functional in January, 2013 after revamping. This hospital has 150 bed space capacities, equipped with State-of-the art facility, serving as a referral hospital, that receive cases from the Health Centres across the state. It is a two storey building consisting of more than eleven hospital wards. It is one of the major specialist hospitals in the State and is owned by the Rivers State Government. The exact areas were the samples were collected includes; Outpatient ward, Female ward, Injection room, Accident and Emergency ward and the Maternity wards.

Microbiological Analysis of Air Samples

The total heterotrophic bacteria, *Staphylococcal* and fungal populations were determined using Koch sedimentation method. Despite reports by previous studies that the result provided by the sedimentation technique when compared with results obtained from air sampler are high, (Fleischer, 2006), it is believed that the sedimentation techniques enables us to know the varieties of microbes available in the air as well as estimating the populations of bacteria and fungi in the air (Silviu *et al.*, 2015). In this method, Petri dishes containing freshly prepared growth media (Nutrient agar, Sabouraud dextrose agar and Mannitol salt agar) in duplicates were exposed one metre(1m) above the floor (Wemedo and Robinson, 2018; Douglas and Robinson, 2018) to the ambient air of the various study sites for fifteen (15) minutes. Sampled plates were closed at the end of sampling and transported to the Microbiology Laboratory of the Department of Microbiology, Rivers State University, where they were incubated at 37ºC for 24 hours. Sampling was carried out at the peak of work activity (at noon) in each of the study sites. The study was for a period of three months (January- March, 2018).

Enumeration of Colony forming Units

The number of microorganisms after 24 and 72 hours of incubation (for bacteria and fungi respectively) expressed as CFU/ m^3 for bacterial population and Sfu/m^3 for fungi were estimated according to the formula adopted by Douglas and Robinson (2018). The formula also known as Koch's sedimentation formula is presented below;

$$A = \frac{a \times 10000}{0.2 \times \pi r^2 \times t}$$

Where

- A= Cfu/M^3 or Sfu/m^3
- a= average number of colonies
- πr^2 = area of the Petri dish
- t = time of exposure of the plate

Purification and Characterization of bacterial and fungal Isolates

The bacterial isolates after incubation were sub-cultured using the streak plate method on fresh sterile Nutrient, Sabouraud Dextrose and Mannitol salt agar plates (for *Staphylococcal* isolates). This was done until pure bacterial isolates were obtained. The pure isolates were then preserved in 10% (v/v) glycerol and were kept frozen in the fridge (Haler thermocool) for further identification studies. The bacterial isolates were characterized using morphological and biochemical tests as described by Cheesbrough (2005). The tests employed were sugar fermentation tests, citrate utilization, coagulase test, haemolysis test, catalase test, motility test, gram's reaction. After the various test, the bacterial isolates were identified using Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). The fungal isolates were identified based on their macroscopic (i.e. colour, shape of spores and growth pattern) and microscopic appearance (by staining spores using lactophenol blue before viewing under the microscope using the X10 and X40 objective lens). Further confirmation of fungal identity was done using Fungal Identification Manual (Barnett *et al.,* 1983).

III. Results

Microbial load

The total heterotrophic bacterial load of the indoor air for the respective wards is presented in Fig 1. The mean total heterotrophic bacterial load ranged from 2.5×10^2 to 2.2×10^3 Cfu/M^3 for the month of January. While the months of February and March ranged from 3.3×10^2 to 3.1×10^3 and 5.2×10^2 to 2.8×10^3 Cfu/M^3 respectively. The highest microbial load was observed in the female ward in the month of January and March while the outpatient ward had the highest bacterial load in the month of February. Thus, the results revealed that the bacterial load was not even as fluctuations within the wards as well as between the months were observed. The *Staphylococcal* load of the various wards is illustrated in Fig. 2, which revealed that the female ward had the highest load of Staphylococci of 1.3×10^3 Cfu/M^3. Similar observations as those of the total heterotrophic bacterial load were observed in the Staphylococcal counts. The staphylococcal load as revealed in Fig 2 increased between the months (i.e. there was a continuous increase from the month of January to March, 2018).

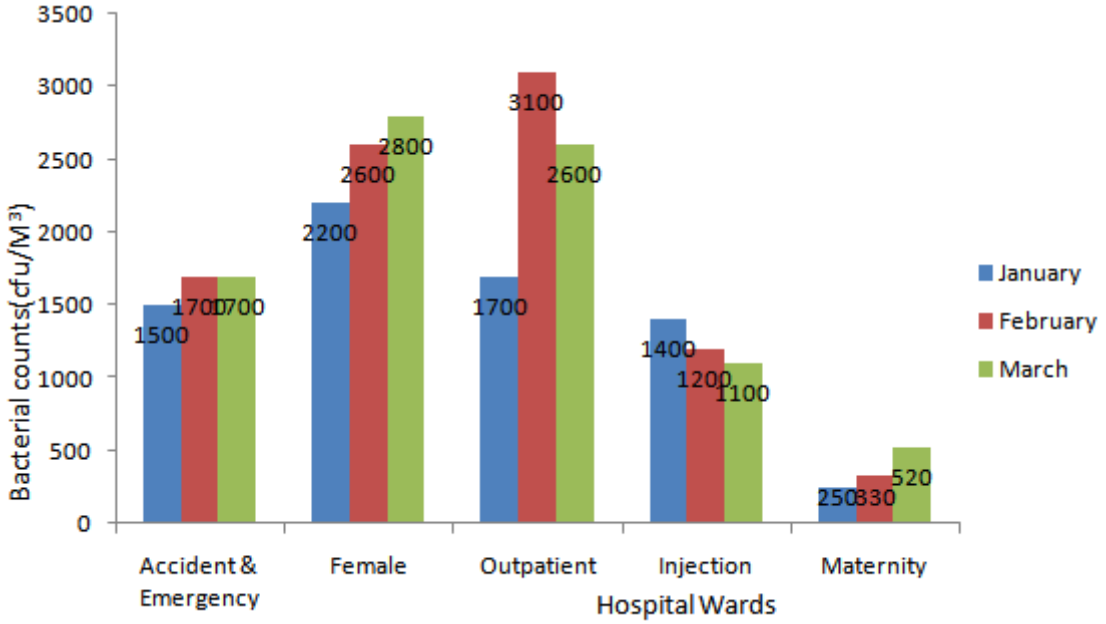
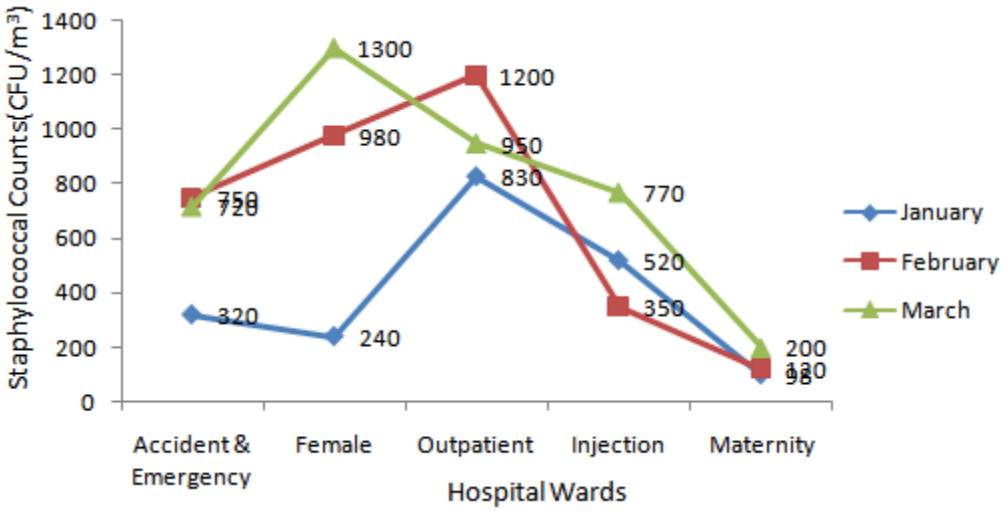


Fig. 1: Total heterotrophic Bacterial load of the Kelsey Harrison Hospital from January to March



Fig, 2: Trend in the total Staphylococcal load of the Kelsey Harrison Hospital from January to March (Cfu/m^3)

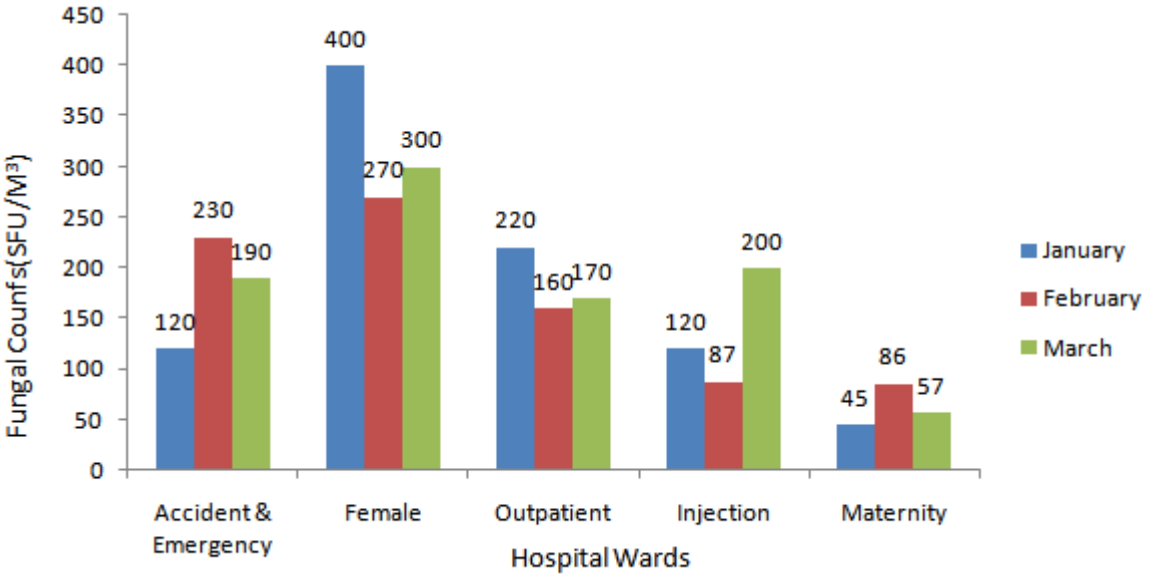


Fig. 3: Total Fungal load of the Kelsey Harrison Hospital from January to March (Sfu/m^3)

The total heterotrophic fungal loads of the wards for the month of January ranged from 4.5×10 to 4.0×10^{2}Sfu/m^3 while the fungal load for the month of February and March ranged from 8.6×10 to 2.7×10^{2}Sfu/m^3 and 5.7×10 to 3.0×10^{2}Sfu/m^3 respectively. Generally, the highest fungal population was recorded in the female ward in the three months sampled. The mean microbial populations in $\log_{10}$Cfu/m^3 of the Harrison Hospital revealed that the highest bacterial and fungal counts were observed in the female ward while the highest Staphylococcal counts were recorded in the outpatient ward (Table 1). Also, the Two-way ANOVA analysis revealed a significant difference in the total heterotrophic bacterial load of the various wards at $P>0.05$, while a significant difference in the total Staphylococcal load was observed in the maternity ward. Furthermore, there was also a significant difference in the fungal population across the wards at $P>0.05$.

Table 1: The Mean Microbial Populations ($\log_{10}$Cfu/m^3) of the Various Wards in Hospital.

Wards	Total heterotrophic bacteria	Total Staphylococci	Total fungi
Accident & Emergency	3.21±0.03^{bc}	2.74±0.21^{b}	2.23±0.15^{b}
Female	3.40±0.06^{d}	2.83±0.38^{b}	2.51±0.08^{c}
Injection	3.09±0.0^{b}	2.71±0.17^{b}	2.10±0.18^{b}
Maternity	2.54±0.16^{a}	2.12±0.16^{a}	1.78±0.14^{a}
Outpatient	3.38±0.13^{cd}	2.99±0.09^{b}	2.25±0.07^{b}

Means with the same alphabet across columns shows no significant difference ($p>0.05$)

Bacteria and Fungi isolated

The bacteria and fungi isolated from the various hospital wards are presented in Tables 2 and 3 respectively. *Escherichia coli* were not isolated from the outpatient and injection wards but were isolated in the female, accident & emergency and the maternity wards. *Bacillus and Chryseobacterium* species were isolated from all the wards while *Staphylococcus* species were isolated from all the wards except in the maternity ward. Also, *Aspergillus niger* was isolated in all the respective wards while *Aspergillus flavus* were only isolated in the accident & emergency and the female wards. *Candida* species were also isolated from all the wards except from the outpatient ward, while *Mucor and Penicillium* species were isolated from all the wards except the maternity ward. The absence of these microbes in some wards may not necessarily mean that they are not there but rather at the time of sampling, they were not detected.

Table 2: Bacterial Isolates Identified in the various Wards of the Hospital

Isolates	Accident & Emergency	Female	Injection	Maternity	Outpatient
Bacillus species	+	+	+	+	+
Chryseobacterium species	+	+	+	+	+
Escherichia coli	+	+	-	+	-
Staphylococcus species	+	+	+	-	+

Positive (+): present, negative (-): absent.

Table 3: Fungal Isolates Identified from the various Wards of the Hospital

Isolates	Accident & Emergency	Female	Injection	Maternity	Outpatient
Aspergillus niger	+	+	+	+	+
Aspergillus flavus	+	+	-	-	-
Candida species	+	+	+	+	-
Mucor species	+	+	+	-	+
Penicillium species	+	+	+	-	+

Positive (+): present, negative (-): absent.

IV. Discussion

The total heterotrophic bacterial loads of the indoor air in this study ranged from 2500 to 3100Cfu/m^3, which was high. This range is higher than the 480 and 1468 cfu/m^3 which was reported by Gizaw *et al.* (2016) of Gondar University teaching hospital. Also, the bacterial load in this current study is less than the 2123 and 9733 cfu/m^3 that was reported by Fekadu and Getachewu (2015) of Jima University specialized hospital. Furthermore, the mean bacterial load in this study was less than those reported by Wemedo and Robinson (2018). Also, the fungal loads in this current study were less than those reported by Douglas and Robinson (2018), and the 365.8 and 578.5 Sfu/m^3 of indoor fungi of Golestan Hospital in Ahvaz, Iran reported by Goudarzi *et al.* (2016). In a study by Gizaw *et al.* (2016), it was reported that there are no uniform International Standard available on levels and acceptable maximum bacterial loads in indoor air. A group of the World Health Organization having assessed the risks posed by biological particles on health opined that the number of microorganisms of an indoor air should not exceed 10^3 cfu/m^3 (Nevalainen and Morawaska, 2009). However, other researchers deliberated that 7.5×10^2 cfu/m^3 should be the limit for bacteria (Francisco and Luiz, 2000; Cappitelli *et al.,* 2009). Microorganisms in the air within the range of 4.50×10^3 to 10.0×10^4 cfu/m^3 have also been proposed as the upper limit for ubiquitous bacterial aerosols (Nevalainen, 1989). The sanitary standards of European Commission for non-industrial premises consider less than 50 CFU/m^3 as 'very low' bacterial load, 50–100 CFU/m^3 as 'low', 100–500 CFU/m^3 as 'intermediate', 500–2000 CFU/m^3 as 'high' and above 2000 CFU/m^3 as 'very high' load (Commission of European Communities, 2016). With reference to these standards, the bacterial load in this current study is considered to be high. Also, Silviu *et al* (2015) reported that the admissible level for air fungi load is 550 CFU/m^3. Thus, according to this standard, the fungal load in this study is under the admissible limit.

Four bacterial genera belonging to *Chryseobacterium, Escherichia, Bacillus and Staphylococcus* (both coagulase negative and positive *Staphylococcus*) species were identified in this study. Except for *Chryseobacterium* species which is present in this study, the bacterial isolates from this study agreed with those reported by previous studies to be associated with nosocomial infections (Agbagwa and Onyemaechi, 2014; Emuren and Ordinioha, 2016; Prescott *et al*., 2011; Calderon *et al*., 2011). They are mostly transmitted either through water, food or by person to person or through contact with noncritical surfaces. Also, *Chrysobacterium* species are commonly found in the soil, fresh and marine water including chlorinated water. As an environmental contaminant, it can colonize sinks and taps and also serves as reservoirs for infections within health care environments. Infections through contaminated medical equipment such as respirators, mist tents, and humidifiers for new babies and adults with compromised immune system have been reported (Jeffrey *et al.,* 2004; Calderón *et al.,* 2011). Thus, there prevalence in the sampling sites of the study locations could be attributed to the flow of air current from the outside environment or through the use of contaminated water to clean floor surfaces and others.

Four fungal genera belonging to *Aspergillus*, *Candida*, *Mucor* and *Penicillium* were identified in this study. Results revealed that *Penicillium species, Mucor species* and *A. flavus* were not identified in the maternity ward while *Candida* and *A. flavus* were not identified in the outpatient ward. *Candida* species had the highest frequency in the female ward amongst other study sites (wards). This is understandable as *Candida* species are normal flora of the female vagina (Prescott *et al.,* 2011) and its dominance could also imply that there is a yeast infection as weakened immune system could cause the proliferation of *Candida*. This is in conformity with Prescott *et al* (2011). Furthermore, the fungal isolates in this study have been reported by previous studies to be able to cause infections including hospital acquired infections (Douglas and Robinson, 2018; Latika and Ritu, 2011; Qudiesat *et al.,* 2009). The presence of fungi in the inhaled air is not new and many persons could inhale

it without falling ill due to their immune strength. But in patients or persons with compromised immune system, infections could occur. Hospital mortality caused by invasive aspergillosis (acquired by inhalation of dust particles contaminated with fungi spores) has been reported (Rainer *et al.,* 2000).

V. Conclusion

The bacterial load in this study is quite high whereas the fungal load is below the admissible limit. The microbes identified in this study could contain pathogenic strains which may lead to delayed convalescence discharge. The quality of the indoor air should be a priority for not just the health workers alone but also to the hospital management. Thus, the indoor environment should be properly ventilated and always clean. There is also need for regular monitoring of the indoor air quality of the hospital, in order to ascertain the actual source of these contaminants and to further prevent them.

Competing interests

Authors have declared that no competing interests exist.

Consent and Ethical Approval

Ethical approval to undertake the study was sort and obtained from the Rivers State Health Management board and the informed consent form was obtained from the medical officer of Port Harcourt City Local Government Area.

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