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Pathogenic Microorganisms Isolated From Indoor Air of Banks And Hospital in Ile-Ife.

[1]Adekunle O.C,[2] Aina A.A, [3]Ojedele R.O
[1,3]Department of Medical Microbiology and Parasitology of Ladoke Akintola University of Technology, Osogbo, Nigeria. [2]Department of Medical Microbiology of Obafemi Awolowo Teaching Hospital, Ile –Ife.
Corresponding Author: Adekunle O.C

Abstract: *Air contains large number of microorganisms including bacteria, fungi and their estimation is important as an index of cleanliness for any particular environment. It becomes imperative to undertake a study of the microbiological assessment of indoor air of our environment. Places where people visit often were selected: hospital and banks. The study sites were General outpatient department of Obafemi Awolowo University Teaching Hospital, First bank, and Guaranty trust bank in Ile-Ife. Different culture media were employed to collect samples and the indoor air samples were assessed using settled plate method for the enumeration of microorganisms. The samples were collected in the morning after cleaning and also after the influx of people. Isolates were identified according to standard methods and sensitivity testing was performed. The mean airborne bacterial load in the hospital and banks ranges from 509.3cfu/min to 740.7cfu/min. While the mean airborne fungal load in GOPD of the hospital and banks ranges from 55.5cfu/min to 231cfu/min respectively. The result revealed the isolation of four bacterial and three fungal isolates. These include Staphylococcus aureus, Klebsiella sp, Escherichia coli, and Proteus sp while the three fungal isolates are Aspergillus sp, Rhizopus sp, and Candida sp. The antibiotic susceptibility pattern of the bacterial isolates varied in their sensitivity and resistance patterns of antibiotic used in the study. The organisms showed high sensitivity to Augmentin, Ofloxacin, Ciprofloxacin and Gentamicin while Erythromycin, Nitrofurantoin, Cefuroxime, Cloxacillin , Ceftrizone , Meropenem and Ceftazidime were highly resistant. The statistical analysis using ANOVA showed that there is no significant difference between the bacterial and fungal population obtained in hospital and banks environment studied.*

Keywords: *pathogenic bacteria, fungal isolates, hospital, banks*

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I. Introduction

Indoor air quality is a term which refers to the air quality within and around building and structures, especially as it relates to the health and comfort of building occupants (1). Indoor air quality can be affected by gases (including carbon monoxide, radon, volatile organic compounds) particulate, microbial contaminants (mold, bacteria), or any mass or energy stressor that can induce adverse health conditions (2). In particular, poor indoor air quality can be harmful to vulnerable groups such as children, young adults, the elderly, or those suffering chronic respiratory and/or cardiovascular diseases (3). Most problems related to indoor air quality result from complex interactions among building occupants, indoor environment (inadequate temperature, excessive humidity), insufficient outdoor air intake, building materials and furnishing, and air contaminants (chemicals, bacteria, molds, vapors) (4). Airborne bacteria are ubiquitous in the earth's atmosphere, and originate from numerous sources, such as lakes, oceans, soils, humans, and animals (5).

Airborne microorganisms such as bacteria, fungi, and viruses are generally found as part of patient's endogenous flora. These airborne microbial particles have negative effects especially on the health of immunocompromised people (6).The infectious aerosols are small and may remain suspended and viable in the air stream over long periods of time. The risks of airborne infection especially in hospitals and other public places e.g. banks, schools, and prisons can be high and may build- up to the infectious levels. Therefore, there is a need to study the quality of indoor air and level of microbial contamination in the places where people visit often such as hospital and banks.

The aim of the study is to compare the microbial concentration of indoor air in General outpatient department of Obafemi Awolowo University Teaching Hospital Ile-Ife and those of banks.

II. Materials And Methods

The study was carried out at Obafemi Awolowo University Teaching Hospital Complex (O.A.U.T.H.C), First bank and Guaranty trust bank at Obafemi Awolowo University Ile-Ife, Osun State, Nigeria. The study was conducted in November, 2018 in Ile-Ife. Air sample were collected in the following area both hospital and banks: General outpatient department, customer area of First bank and Guaranty trust bank.

Sampling procedure

Bacteria and fungi measurement were made by passive air sampling technique. The settle plates method using petri-dishes containing different culture media were used (Crook, 1995). Culture plates containing Sabouraud dextrose agar, MacConkey agar Chocolate agar, Nutrient agar and Desoxycholate -Citrate agar (DCA) were used for sample collection. The sampling was done early in the morning (10:00-11:00am). The culture plates were distributed at different location in the banks and General outpatients department and exposed face upwards to the atmosphere and allowed to stay for 1hour. Thereafter, the plates were covered and transferred immediately to the incubator in the laboratory for incubation.

Microbiological examination

All inoculated plates were incubated at 37^{o}C for 24 hours and Sabouraud dextrose agar plate were incubated room temperature for 5 days as recommended by Kass (1957). The colonial morphology of the colonies formed were examined and characterized by their size, shape, colour, edge, opacity, pigmentation, ability to ferment lactose, haemolysis on chocolate agar. The isolates were also stained to identify if they were gram positive or negative bacteria. Bacteria colonies including those that were identical and non-identical were sub-cultured into MacConkey agar plate and incubated at 37^{o}C for 24hours for proper identification of organisms. The total number of bacterial colony were counted and converted to colony forming units per cubic meters of air using Koch sedimentation method below according to Polish standard (Friberg *et al.,* 1999).

$$\text{CFU/m}^3 = \frac{a \times 10000}{P \times t \times 0.2}$$

Where:
a= the number of colonies on the Petri-plate
p= the space measurement of the Petri-plate (90mm)
t= the time of Petri plate exposure (60mins)
The fungal colonies were enumerated, then examined macroscopically and characterized on the basis of their morphological features such as a distinctive possession of hyphae and spores as described by Betty *et al.,* (2007) when examined under microscope.
Biochemical tests such as gram staining technique procedure, catalase test, coagulase test, indole test, citrate utilization test, urease test and oxidase test were performed.

Antibiotic sensitivity test

The disc diffusion method was used for this study, according to clinical and laboratory standards institute guidelines (CLSI, 2010). All antibiotic disks were purchased from Mast Co. Ltd, UK and used as per manufacturer's instructions. Two colonies were picked from the culture plate and emulsified into 2ml of peptone water broth and then allowed to stay for 5minutes. The bacteria suspension was poured into the sensitivity agar and the excess was discarded. An antibiotic disc containing antibiotic: Augmentin (30µg), Ofloxacin (5µg), Gentamicin (10µg), Ciprofloxacin (5µg), Erythromycin (30µg), Nitrofurantoin (300µg), Meropenem (10µg), Cefuroxime (30µg), Cloxacillin (5µg), Ceftrizone (30µg), Ceftazidime (30µg) was placed on each of the culture and incubated for 24hours at 37^{o}C. The antibiotic diffused into the medium and areas of zone of inhibition around each of the disc were examined. Those organisms that were sensitive to the antibiotics were inhibited at a distance from the disc while those resistant grew round the edge of the disc. The diameters of zones of inhibition of sensitive organism were measured with meter rule.

Statistical analysis

The data that were generated from this study were analysed statistically using ANOVA to find the mean value of the samples in all locations, standard deviation, standard error of mean, and p-value. A p-value less than 0.05 were considered statistically significant.

III. Results

The total viable count for bacteria obtained from each area is shown in table 1, the lowest count was 509cfu/m^3 obtained from the First bank customer's area while the highest count of 740.7cfu/m^3 was obtained from the General outpatient department (GOPD) of the hospital after cleaning.

Table 2 shows the lowest fungi count of 55cfu/m^3 obtained from the First bank customer's area and the highest was obtained from the General outpatient department (GOPD) of O.A.U.T.H.C Ile-Ife (231.5cfu/m^3) in the morning after cleaning. Table 3 shows the minimum range and maximum range of bacteria and fungi obtained from the hospital and banks (509.3cfu/m^3, 740.7cfu/m^3 for bacteria and 55.6cfu/m^3, 231.5cfu/m^3fungi).Table 4 shows that *Staphylococcus aureus* was the most predominant bacterial being isolated in the banks and General out-patient department this was followed by *Klebsiella sp, Proteus sp, Escherichia coli.* Table 5 shows that *Rhizopus sp, Aspergillus sp* was most isolated fungi in the hospital and banks. The airborne fungal isolates include *Aspergillus sp, Candida sp, and Rhizopus sp.* Table 6 and 7 show the summary of statistical analysis of total viable count of bacteria and fungi using ANOVA to find the mean, standard deviation, standard error of mean and P-value. Results showed that there was no significant difference in the bacterial population. (P=0.081) and in the fungi population (P=0.081) of the sampling time in the hospital and indoor air banking. Table 8 shows the antimicrobial susceptibility pattern of the bacterial isolates that varied in their sensitivity and resistance patterns of antibiotic used in the study. The isolates showed high sensitivity to Augmentin (100%), Ofloxacin (100%), Ciprofloxacin (100%) and Gentamicin (100%) while Cefroxime (100%), Cloxacillin (100%), Nitrofurantoin (100%), Erythromycin (100%), Ceftrizone (75%) and Ceftazidime (50%) were highly resistant.

Table 9 shows the antibiotic sensitivity pattern on the isolate obtained from the hospital and banks. Augmentin, Ofloxacin, Ciprofloxacin, Ciprofloxacin have high sensitivity pattern while Cefuroxime, Cloxacillin, Nitrofurantoin, Erythromycin, are highly resistance. Table 10 shows an assessment of air quality in the hospital and banks according to the standard for non-industrial premises. Figure 1 shows correlation between bacteria and fungi concentration at the hospital and banks

Table 1: Number of bacterial colony counts cfu per m^3air at different sampling time of day of exposure

	Sampling time
	10:00am – 11:00am
	Petri – dish exposure time (minutes)
	After cleaning
	60 (minutes)
FB 01	555.6
FB 02	509.3
GTB 01	648.1
GTB 02	611.1
GOPD 01	740.7
GOPD 02	694.4

KEY:
FB:-First bank
GTB:-Guaranty trust bank
GOPD:-General outpatient department

Table 2: Number of fungi colony count cfu per m^3 air at different sampling time of day of exposure

	Sampling time
	10:00am – 11:00am
	Petri – dish exposure time (minutes)
	After cleaning
Sampling sites	60 (minutes)
FB 01	111.1
FB 02	55.6
GTB 01	185.2
GTB 02	138.9
GOPD 01	231.5
GOPD 02	185.2

Table 3: The range of microbe's distribution at Hospital and banks

	Minimum	Maximum
Bacteria(cfu/m^3)	509.3	740.7
Fungi (cfu/m^3)	55.6	231.5

Table 4: Frequency of occurrence of bacterial isolates from the samples

Isolates	FB	GTB	GOPD
Stahylococcus aureus	30	40	57
Klebsiella sp	10	8	42
Proteus sp	0	9	29
Escherichia coli	9	0	28

KEY:
31-60:-Numerous
11-30:-Moderate
1-10:- Scanty
0:-Negative

Table 5: Frequency of occurrence of fungi isolates from the samples

Isolates	FB	GTB	GOPD
Rhizopussp	10	30	40
Aspergillussp	10	30	29
Candida sp	0	0	9

KEY:
31-60:-Numerous
11-30:-Moderate
1-10:- Scanty
0:-Negative

Table 6: Summary of statistical analysis of enumerated bacterial isolates (cfu/m^3 indoor air) in the samples area

Area	Mean	Standard deviation (SD)	Standard error of mean(SEM)
FB	532	32.7	±23.2
GTB	630	26.2	±18.5
GOPD	718	32.8	±23.2

P -value=0.0811

Table 7: Summary of statistical analysis of enumerated fungal isolates (cfu/m^3 indoor air) in the samples area

Area	Mean	Standard deviation	Standard error of mean(SEM)
FB	83.9	39.2	± 27.8
GTB	162.1	32.7	± 23.2
GOPD	208.4	32.7	± 23.2

P- value= 0.0811

Table 8: Antibiotic on the isolated organisms from hospital and banks

	Isolated organisms			
Antibiotic disc	*Stahylococcus aureus*	*Klebsiella sp*	*Proteus sp*	*Escherichia coli*
Augmentin (30µg)	**sensitivity** S	S	S	S
Ofloxacin (5µg)	S	S	S	S
Gentamicin (10µg)	S	S	S	S
Ciprofloxacin (5µg)	S	S	S	S
Erythromycin (30µg)	R	R	R	R
Nitrofurantoin (300µg)	R	R	R	R
Meropenem (10µg)	S	S	R	R
Cefuroxime (30µg)	R	R	R	R
Cloxacillin (5µg)	R	R	R	R
Ceftrizone (30µg)	R	S	R	R
Ceftazidime (30µg)	S	S	R	R

KEY:
S: - Sensitivity
R: - Resistant

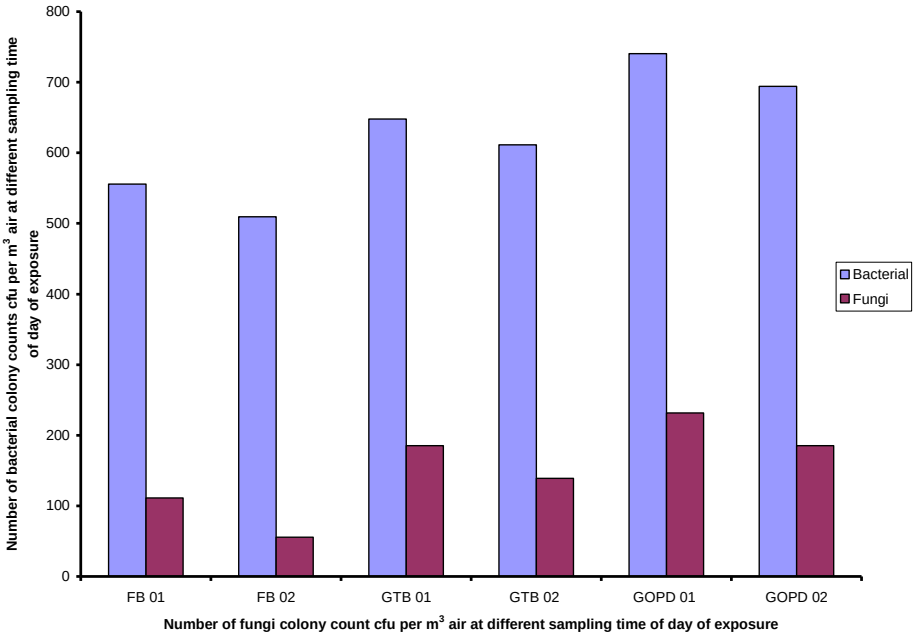

Figure 1: Correlation between bacteria and fungi concentration at the hospital and banks environment.

KEY:
FB:-First bank
GTB:-Guaranty trust bank
GOPD:-General outpatient department

Table 10: An assessment of air quality in the hospital and banks according to the sanitary standard for non-industrial premises

Group of microbes	Range of values(cfu/m³)	Pollution degree	Sampling sites and time					
			FB 01	FB 02	GTB O1	GTB 02	OPD 01	OPD O2
			10:00-11:00a.m	10:00-11:00a.m	10:00-11:00a.m	10:00-11:00a.m	10:00-11:00a.m	10:0-11:00 a.m
Bacteria	<50	Very low						
	50- 100	Low						
	100- 500	Intermediate						
	500-2000	High	✓	✓	✓	✓	✓	✓
	>2000	Very high						
Fungi								
	<25	Very low						
	25-100	Low		✓				
	100-500	Intermediate	✓		✓	✓	✓	✓
	500-2000	High						
	>2000	Very high						

IV. Discussion

The result from this study shows that both GOPD and banks had numerous *Staphylococcus aureus*. *Staphylococcus aureus* causes abscesses, various pyogenenic infections (eg, endocarditis and osteomyelitis), food poisoning, and toxic shock syndrome. *Escherichia coli* were moderate in GOPD and scanty in bank which also supported and lend credence to the previous work of Omoigberale *et al.,* (2013). *Proteus sp* was scanty in bank and moderate in GOPD, this was in accordance to Onipede *et al.,* 2004 (17) from Obafemi Awolowo University Teaching Hospital Ile-ife. *Klebsiella sp* was scanty in banks and numerous in GOPD which supported the earlier claim of Awosika *et al.,* (2012). These microorganisms are known as primary agents of noscomial infections in hospitals. Similar variety of aero-flora was isolated in a hospital in a desert country (15).

It is believed that environment where patients are treated has an important influence on the prospect of such patients receiving or acquiring infection that may complicate their condition (7). The number and type of air borne microorganisms can be used to determine the degree of cleanliness. Air borne contaminants are usually introduced into the air though production of aerosol droplets by human via coughing, sneezing and talking. Many people are actually at risk of inflection while in the hospital or banks. In this study the most frequently isolated bacterial isolates were *Staphylococcus aureus, Klebiella sp, Proteus sp* and *Escherichia coli. Aspergillus sp, Rhizopus sp*, and *Candida sp* were the most frequently isolated fungi isolates in this study. This is similar to that obtained by Omoigberale *et al.*,2013 (6) who isolated six fungal isolates from indoor air of hospital in Ekpoma, Edo State Nigeria. (7) also isolated *Aspergillus sp, Penicillium sp, Micor sp,* and *Candida sp, Verticillium sp* with *Aspergillus sp* and *Penicllium sp* from University of Benin Teaching Hospital, one the study areas. Environmental surveillance of filamentous fungi in three tertiary care hospitals in Greece and indicated that *Aspergillus flavus* and *Aspergillus fumigatus* were the most prevalent species (18), Although, *Aspergillus* may be tolerable for healthy individuals, it may be dangerous for high risk patients. The spores readily invade the airways and could lead to aspergillosis in immuncompromised hosts (13). Pathogenic actinomycetes usually cause chronic exudative inflammatory infection and also cause acute necrotizing pyogenic infection (16). The build up of infectious aerosols exacerbates health care challenges and developing

countries as the role of air borne microorganisms in hospital acquired infections (HAIs) has been recognized (20).

The concentration of bacteria and fungi aerosol in the indoor environment of Obafemi Awolowo University Teaching Hospital Ile-Ife, First bank and Guaranty trust bank of Obafemi Awolowo University estimated with the use of the settle plate method, ranged between 55.6– 740.7cfu/m^3.There is no uniform international standard available on levels and acceptable maximum bio-aerosol load (19). Different countries have different standards. The work conducted by a WHO expert group on assessment of health risks of biological agents in indoor environments suggested that total microbial load should not exceed 1000cfu/m^3. If higher than this, the environment is considered as contaminated (14). Other authors consider that 300cfu/m^3 and 750cfu/m^3 should be the limit for fungi and bacteria respectively (11).

The sensitivity patterns in this study revealed that Augmentin, Ofloxacin, Ciprofloxacin and Gentamicin were highly effective for most of the bacteria isolated with susceptibility rate of 100%, 100%, 100% and 100% respectively. Majority of the isolate showed resistance to Nitrofurantoin (100%), Cefuroxime (100%), Cloxacillin (100%), Ceftrizone (75%), Meropenem (50%) and Ceftazidime (50%). Ciprofloxacin and Gentamicin was sensitive to *Staphylococcus aureus* which is in agreement with Olowe *et al.*, (9). *Klebsiella sp, Proteus sp,* and *Escherichia coli* was sensitive to Ciprofloxacin and Ofloxacin which is in accordance with Olowe *et al.*, (10).

V. Conclusion

The presence of pathogenic microorganisms from hospitals and banks can cause life threatening infection. In conclusion, the results generated in this study clearly suggest that, indoor environment allow aerosols build up which could potentially leads to infection in the hospital and banks. Thus, hospitals and banks should have enhanced practice of good sanitation protocols and infection control measures. The high bacteria and fungi concentrations of air obtained in this study might be potential risk factors spread of infections in the hospital and banks. Immediate interventions is needed to control those environmental factors which favour the growth and multiplication of microbes, also the hospital needs to increase the number of GOPD area which will be sufficient for the outpatients that come from catchment area. Also, the banks need to increase the number of customer's service area to make sufficient atmosphere for the customer's that come to banks regularly.

VI. Recommendation

Overcrowding should be avoided in the hospitals and banks, crowded places increases the chances of contracting an infection and these infections may subsequently spread and antibiotics resistant strains of the pathogens may emerge leading to more severe and untreatable infections. Better and well constructed ventilation system should be provided in hospitals and banks which will go a long way in reducing the microbial load.

Thus, hospitals and banks should have enhanced practice of good sanitation protocols and infection control measures. Through hand washing and use of alcohol rubs by customers' in the bank and medical personnel before and after attending to patient. These could also limit microbial dispersals within the hospitals.

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