AEROBIOLOGICAL ASSESSMENT OF A POLYTECHNIC MEDICAL CENTRE AND ANTIBIOGRAM OF BACTERIA ISOLATED

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Abstract

The aerobiological assessment of a Polytechnic Medical Centre and antibiogram of bacteria isolated was undertaken. The aerobiological assessment was carried out using sedimentation method at the study area for morning and afternoon sessions. The antibiogram of the isolated bacteria were analysed using the disc diffusion method. The results shows that the highest mean bacterial counts for the morning and afternoon sessions are 4.03 x 10^4 cfu/m^3 and 7.95 x 10^4 cfu/m^3 respectively; also, the highest mean fungal counts for the morning and afternoon sessions are 4.00 x 10^4 cfu/m^3 and 2.10 x 10^4 cfu/m^3. The bacteria identified are Bacillus sp., Corynebacterium sp., Enterobacter sp., Klebsiella sp., Pseudomonas sp. and Staphylococcus aureus while the fungi identified are Aspergillus flavus, Debaromyces sp., Fusarium solari, Fusarium sp., Rhizopus sp., Penicillium chrysogenum, Penicillium sp., and Penicillium verrucosum. The antibiogram indicated that all the isolates were susceptible to ciproflox, gentamicin, streptomycin, rifampicin, erythromycin, chloramphenicol, levofloxacin and recorded a multiple antibiotic resistance index (MARI) of 0 except Enterobacter sp. (1) that had 0.1. The study showed that the bioaerosol concentration were high. There are no multiple antibiotic resistances. The study recommends periodic bioaerosol assessment, good sanitary protocol and ventilation system in the Medical Centre.

Keywords: Air Quality, Antibiogram, Bacteria, Fungi, Medical Centre, Polytechnic

Introduction

Indoor air contains a complex mixture of microorganism species, and intermediate products, such as yeast fungi, molds, bacteria, viruses, and volatile microbial organic compounds (Jalili *et al.*2021). The presence of airborne microorganisms is the most important subject in indoor environments, such as residential homes, schools, universities, hospitals, and care centers (Mandal and Brandl**,** 2011).

The complex hospital environment requires special attention so as to ensure healthy indoor air quality (IAQ) to protect patients and healthcare workers from hospital-acquired

(nosocomial) infection and occupational diseases (Leung and Chan, 2006). Airborne microorganisms can originate not only from humans (including patients), but can also be spawned by various indoor hospital characteristics and outdoor environmental sources (Wang *et al*., 2011; Park *et al*., 2013). Hospital buildings may be regarded as dynamic environments affected by season, weather conditions, indoor ventilation system design and operation, intrusion of moisture, outdoor microbial load and the number of occupants, visitors and human activities (Park *et al*., 2013). These factors may be associated with conditions for microbial growth.

The hospital environment is uniquely suited to the spread of infection as houses both susceptible patients and patients with difficult-to-treat infections (Chikere *et al*., 2008). Patients are primarily admitted into hospital wards for proper management of their illnesses, but while on admission some of them acquire other infections than the ones they admitted for. These are called hospital-associated infections (nosocomial infections) which can result from contact with a carrier directly or indirectly through inanimate objects or air (Awosika *et al*., 2004). Bacteria that are often associated with hospital acquired infections are *Staphylococcus aureus, Micrococcus* sp., *Pseudomonas* sp., *Proteus* sp., *Escherichia coli*, *Enterobacter* sp. and *Bacillus cereus* (Ekhaise *et al*., 2008). *Pseudomonas aeruginosa* has been particularly incriminated in nosocomial infection because of its intrinsic resistance to most antibiotics and its ability to survive and multiply at low temperatures and in disinfectant solutions (Ishida *et al*., 2006). Airborne fungi may cause several adverse effects, especially infections, allergenic, and immunotoxic disorders. *Aspergillus*, *Mucor*, and *Rhizopus* are common nosocomial fungi species (Osman *et al.,* 2018).

Many studies have reported on airborne microorganisms in hospitals, and around the world, the studies have shown that the bioaerosol concentration of many units and wards of many hospitals far exceed the WHO (2005) acceptable limit of 1×10^3 cfu/m^3. Also, microbes which are known to spread from numerous sources have been shown to introduce high load of microorganism in to the air. Their presence in the air pose potential hazard as the organisms, particularly the bacteria strains could be pathogenic with bacterial gene pool which could include antibiotic resistance gene. Furthermore, according to Bragoszewska *et al*. (2020), the fast emergence of bacteria which occurs worldwide endangers the potency of antibiotics and may lead to very serious crisis in healthcare. The objectives of this research are to isolate bacteria and fungi from air within the Medical Centre, Captain Elechi Amadi Polytechnic, Rumuola, show variation in bacteria and fungi concentration during the study period and carry out antibiogram analysis on the isolated bacteria.

Based on the above objectives, the following hypotheses were formulated:

i. There are microorganisms (bacteria and fungi) in the air within the Medical Centre;
ii. There will be variation in the microbial concentrations depending on the sampling time;
iii. The opportunistic multiple antibiotic resistant bacteria are found in the air of the investigated medical centre wards;

Materials and Methods

The study was carried out in the Medical Centre, Captain Elechi Amadi Polytechnic Rumuola, Obio Akpor Local Government Area, Rivers State, Nigeria. This is a separate building very close to the major road. It receives out-patients only that includes most staff

and students of the Polytechnic. The sampling points are Female Ward, Male Ward, Open Space, Reception and Laboratory.

For the enumeration of bacteria, Nutrient Agar was used, while Sabouraud Dextrose Agar was used for fungi. The Petri dishes containing appropriate media were exposed to air within the five wards/units of the Medical Centre for fifteen minutes at a height of 1metre. The sample collection was done in two regular intervals of a day. The first set of Petri-dishes containing appropriate medium were exposed at the sampling area as class work commence (9-9:15am) and the same was repeated in the afternoon for the second set (2-2:15pm) (Mostafa *et al*., 2012). After exposing to the indoor air, the Petri dishes containing medium were transferred to the Laboratory and incubated at ambient temperature on the laboratory bench for 24 to 48 hours for bacteria and 3-5days for fungi. The experiment was replicated thrice.

The average of colony forming units (cfu) of both bacteria and fungi was calculated and converted to organisms per cubic metre of air (Stryjakowska-Sekulska *et al*., 2007)

$cfum^3$ = a 10000 /p.t.0.2

Where: a= the number of colonies on the petri dishes; p= surface of the petri dishes; t= the time of Petri dish exposure.

Antibiotic susceptibility of isolates to different agents was determined *in vitro* by employing a disc diffusion test of Kirby-Bauer method. Antibiotics (Optun Laboratories Nigeria Limited) used in the study were ciproflox (10µg), norfloxacin (10µg), gentamycin (10µg), amoxil (20µg), streptomycin (30µg), rifampicin (30µg), erythromycin (30µg), chloramphenicol (30µg), ampiclox (20µg) and levofloxacin (20µg). Inoculum of each isolate was prepared from an 18 hour broth culture adjusted to obtained turbidity comparable to 0.5 McFarland Standard. Sterile cotton tipped swab was then dipped into the standardised bacterial solution and used to streak the entire dried surface of the medium. The inoculated plates were incubated for 5 minutes to remove excess moisture to dry. Thereafter, the antibiotic discs were then pressed firmly onto the agar with the sterile forceps to ensure complete contact with the agar. The plates were incubated aerobically at 37^oC for 18-24 hours. The diameter of zones of inhibition surrounding the antibiotic discs were measured in mm. Isolates were classified as resistant, intermediate and sensitive (CSLI, 2006).

The multiple antibiotic resistant index (MARI) was carried out as described by Matyar *et al*. (2007) with slight modification. MARI = resistant antibiotics ÷ total antibiotics tested.

All the data obtained during the study period was analyse using Mean and presented in Tables.

Results

The results shows that the highest mean bacterial counts for the morning and afternoon sessions are 4.03×10^4 cfu/m^3 and 7.95×10^4 cfu/m^3 respectively; also, the highest mean fungal counts for the morning and afternoon sessions are 4.00×10^4 cfu/m^3 and 2.10×10^4 cfu/m^3.

Table 1: Mean Bacterial and Fungal Counts obtained from the Air Samples within the Medical Centre, Captain Elechi Amadi Polytechnic

Microorganism	Sampling Point	Counts Obtained (cfu/m^3) Morning	Afternoon
Bacteria	Female Ward	4.13×10^3	4.02×10^3
	Male Ward	4.03×10^4	7.95×10^4
	Open Space	1.05×10^4	4.79×10^3
	Reception	1.15×10^4	2.55×10^3
	Laboratory	1.17×10^4	5.52×10^3
Fungi	Female Ward	1.30×10^4	1.04×10^4
	Male Ward	9.30×10^3	7.60×10^3
	Open Space	2.00×10^4	2.10×10^4
	Reception	4.00×10^4	1.39×10^4
	Laboratory	1.50×10^4	1.70×10^4
WHO (2005) Acceptable Limit		**1.0×10^3 cfu/m^3**	

The characterisation and identification of bacteria isolated from the air samples within the Medical Centre is presented in Table 4.2. The isolated, characterised and identified bacteria are *Bacillus* sp., *Corynebacterium* sp., *Enterobacter* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Staphylococcus aureus*. The fungi identified are *Aspergillus flavus*, *Debaromyces* sp., *Fusarium* solari, *Fusarium* sp., *Rhizopus* sp., *Penicillium chrysogenum*, *Penicillium* sp., and *Penicillium verrucosum*.

The antibiotic susceptibility patterns and multiple antibiotic resistant index (MARI) of Bacteria isolated from air within Captain Elechi Amadi Polytechnic Medical Centre is presented in Table 2. The results show that all the isolates were susceptible to ciproflox, gentamicin, streptomycin, rifampicin, erythromycin, chloramphenicol and levofloxacin, while higher susceptibility of 90% was observed for norfloxacin, amoxil and ampiclox. This result also shows that all the test bacteria recorded a multiple antibiotic resistance index (MARI) of 0 except *Enterobacter* sp. (1) that recorded a MARI of 0.1 indicating high susceptibility level.

Table 2 Antibiotic Susceptibility Patterns and Multiple Antibiotic Resistant Index (MARI) of the Bacteria isolated from air within Captain Elechi Amadi Polytechnic Medical Centre

	Antibiotic/Zone of Inhibition (mm)										
Isolate	CPX	NR	GN	AML	S	RD	E	CH	APX		
	LEV	R	I	S		MARI					
Corynebacterium sp.	30S	18S	28S	20S	26S	26S	30S	30S	22S	32S	
	0(0%)	0(0%)	10(100%)		0						
Pseudomonas sp.(1)	26S	18S	22S	18S	26S	22S	26S	26S	22S	32S	
	0(0%)	0(0%)	10(100%)		0						
Pseudomonas sp.(2)	32S	22S	26S	22S	26S	22S	28S	28S	18S	26S	
	0(0%)	0(0%)	10(100%)		0						
Klebsiella sp.	26S	16S	20S	22S	26S	22S	28S	22S	20S	26S	
	0(0%)	0(0%)	10(100%)		0						
Enterobacter sp.(1)	26S	0R	16S	30S	30S	22S	22S	22S	12I	30S	
	1(0%)	1(10%)	9(90%)		0.1						
Enterobacter sp.(2)	28S	22S	28S	10I	30S	22S	28S	30S	22S	28S	
	0(0%)	1(10%)	9(90%)		0						
Enterobacter sp.(3)	28S	18S	26S	18S	28S	18S	28S	22S	16S	28S	
	0(0%)	0(0%)	10(100%)		0						
Staphylococcus aureus	26S	22S	28S	22S	28S	22S	24S	28S	30S	28S	
	0(0%)	0(0%)	10(100%)		0						
Bacillus sp. (1)	30S	24S	26S	22S	28S	26S	30S	26S	13I	30S	
	0(0%)	1(10%)	9(90%)		0						
Bacillus sp. (2)	26S	18S	22S	18S	26S	22S	26S	26S	22S	32S	
	0(0%)	0(0%)	10(100%)		0						

KEY

CPX Ciprofloxcin **NR** Norfloxacin **GN** Gentamicin **AML** Amoxil **S** Streptomycin

RD Rifampicin **E** Erythromycin **CH** Chloramphenical **APX** Ampiclox **LEV** Levofloxacin

sp. species **R** Resistant **I** Intermediate **S** sensitive (susceptibility)

MARI Multiple Antibiotic Resistant Index

Discussion

Indoor air microbial communities play significant roles on the increase in hospitals acquired infections globally (Mohammed and Haruna, 2019). In view of the importance of indoor air bacterial quality in the transmission of nosocomial infections, this study was conducted to assess the indoor air bacteria load from the Medical Centre, Captain Elechi Amadi Polytechnic, Rumuola.

The results from the study reveals that the highest mean bacterial counts for the morning and afternoon sessions are 4.03 x 10^4 cfu/m^3 and 7.95 x 10^4 cfu/m^3 respectively; also, the highest mean fungal counts for the morning and afternoon sessions are 4.00 x 10^4 cfu/m^3 and 2.10 x 10^4 cfu/m^3. The observed high counts could be attributed to large turnout of patients observed during sampling. Zhang *et al*. (2007) reported that density of people greatly affect the population of airborne bacteria. Generally more counts were recorded in the morning than in the afternoon hours. Since it is for outpatients especially students, there are

increase activities in the morning hours. Also, the counts in this study were above the WHO (2005) acceptable limit of 1.0 x 10^3 cfu/m^3. Generally, in the Medical Centre under study, there are non-sterile devices such as personal belongings of patients and visitors, as well as overcrowding of patients, especially at peak points and may be the reason why the study area may have a high diversity and density of bioaerosols (Hosseinzadeh *et al*., 2012). As suggested by Sautour *et al*. (2009), the Medical Centre's indoor air quality maybe affected by the outdoor air quality due its location and activities around it. This is true because there were construction activities going on within the study period. Various factors such as the sampling season, impact of outdoor on hospital indoor air, type of admitted patient, type of ventilation system and its effectiveness, and efficiency of disinfection can affect frequency and diversity of isolated fungi as well as bacteria from indoor hospital air (Hoseinzadeh *et al*. 2013)

The bacteria identified are *Bacillus* sp., *Corynebacterium* sp., *Enterobacter* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Staphylococcus aureus* while the fungi identified are *Aspergillus flavus*, *Debaromyces* sp., *Fusarium* solari, *Fusarium* sp., *Rhizopus* sp., *Penicillium chrysogenum*, *Penicillium* sp., and *Penicillium verrucosum*. Bacteria such as Bacillus sp., *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* have been reported by Iroha *et al*. (2020) in a Hospital environment. Iroha *et al*. (2020) stated that the observed trend in prevalence/incidence of these pathogens in hospital infections further highlights them as important public health challenge with increasing economic and human impact. *Pseudomonas aeruginosa* has been particularly incriminated in nosocomial infection because of its intrinsic resistance to most antibiotics and its ability to survive and multiply at low temperatures and in disinfectant solutions (Ohsaki *et al*., 2007). The presence of airborne *Staphylococcus* spp. indicates the possible presence of pathogenic microorganisms, in which antibiotic resistance has been observed with increasing frequency over the past several decades (Małecka-Adamowicz *et al*., 2020). Mohammed and Haruna (2019) opined that since the isolated bacteria could be pathogenic if contact is established with patients, it is pertinent that their presence should be controlled. Most fungi are known to be associated with asthma in both children and adults (Jalili *et al*., 2021), Jaakkola *et al*. (2010) also noted that fungi inhalation could lead pulmonary aspergillosis.

Antimicrobial resistance (AMR) is a worldwide public health concern that has drawn attention in the recent time (Wanja *et al*., 2020). The antibiotic susceptibility pattern of airborne bacterial isolates revealed that ciproflox, gentamicin, streptomycin, rifampicin, erythromycin, chloramphenicol and levofloxacin recorded 100% susceptibility while higher susceptibility of 90% was observed for norfloxacin, amoxil and ampiclox for both Gram-positive and Gram-negative bacteria from all the units/wards sampled during this study. This high susceptibility is in agreement with the findings of Iroha *et al*. (2020). High susceptibility of isolates to gentamicin and chloramphenicol also agrees with Wanja *et al*. (2020) and Makut *et al*. (2014) respectively.

Multiple antibiotic resistance indexing has been an effective and efficient tool in tracing bacteria exposure (Osundiya *et al*., 2013). This result also shows that all the test bacteria recorded a multiple antibiotic resistance index (MARI) of 0 except *Enterobacter* sp. (1) that recorded a MARI of 0.1 indicating very low resistance. This is a good result and could be as a result of hygienic level, low traffic of patients due to the service specifications as well as the location of the Medical Centre. Subashkumar *et al*. (2006) stated that isolates that exhibit multi antibiotics resistance index (MARI) value greater than 0.2 depicts high

level of antibiotics resistance due to either indiscriminate use of antibiotic or horizontal gene transfer. The rapid emergence of resistant bacteria, which occurs worldwide, endangers the efficacy of antibiotics and may lead to a serious crisis in healthcare. Therefore, a need for better understanding of antibiotic-resistant bacteria populations is obvious (Bragoszewska *et al*., 2020).

Conclusion

The present study revealed the presence and high concentration of bacteria and fungi in the Medical Centre, Captain Elechi Amadi Polytechnic, Rumuola. The bacteria identified are *Bacillus* sp., *Corynebacterium* sp., *Enterobacter* sp., *Klebsiella* sp., *Pseudomonas* sp. and *Staphylococcus aureus* while the fungi identified are *Aspergillus flavus*, *Debaromyces* sp., *Fusarium* solari, *Fusarium* sp., *Rhizopus* sp., *Penicillium chrysogenum*, *Penicillium* sp., and *Penicillium verrucosum*. The study also revealed that there is variation in the microbial counts during the sampling period, as more counts were recorded in the morning than in the afternoon hours. The antibiogram indicated that all the isolates were susceptible to ciproflox, gentamicin, streptomycin, rifampicin, erythromycin, chloramphenicol, levofloxacin and recorded a multiple antibiotic resistance index (MARI) of 0 except *Enterobacter* sp. (1) that had 0.1. All the antibiotics used for the study were good and there are no multiple antibiotic resistances. There should be periodic evaluation of indoor air within the Polytechnic Medical Centre; also, sustained good ventilation and hygiene practices including disinfection/sterilisation are very critical for effective control of airborne bacterial concentrations in the wards/units and around the Medical Centre environment.

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