Original Research Article

Isolation and Identification of Air Microflora in Clifford University Medical Center, Ihie Campus, Owerrinta, Abia State, Nigeria.

Abstract

The settling plate method was used to examine the air microflora in 6 units at the Clifford University Medical Centre, Ihie campus, Owerrinta, Abia state, Nigeria. The male ward, male restroom, female ward, female restroom, theater room, and environment were among the 6 units. At each of the sample locations, the culture plates containing potato dextrose agar and nutrient agar media were exposed to the atmosphere for about 15 minutes. A total count of 11 CFU and 295 CFU fungal and bacterial isolates respectively were identified from the medical center but 5 fungal isolates and 29 bacterial isolates were obtained as pure cultures. These fungi were *Aspergillus flavus, Aspergillus niger*,*Trichophyton* sp, *Penicillium*sp, and white mold. The bacterial genera were *Staphylococcus* sp, *Streptococcus* sp, *Micrococcus* sp, and *Bacillus* sp. *Staphylococcus*sp and *Aspergillus* sp. were the two most prevalent microorganisms in this investigation. It can be said that the Clifford University Medical Centre employed for this study featured numerous types of Gram-positive bacteria, a high prevalence of pathogenic bacteria, and a high prevalence of pathogenic fungi in both the indoor and outdoor air. The majority of the fungal isolates are molds, whereas certain bacterial species are commensals that live on human skin, the fungal isolates are of environmental origin. The bacterial and fungal isolates contained a few non-pathogenic species. The study was carried out to determine if air microfloral affects the infection rate in the University Medical Centre.

Keywords: Air Microflora, University Medical Centre, Fungal and Bacterial Isolates, Pathogenic and Non-Pathogenic Organisms.

1. Introduction

The study of living microorganisms (bioaerosols) and toxins that are suspended in the atmosphere is known as Aeromicrobiology [1]. Although the quantity of microorganisms in the air is far lower than in the soil and water, they are nevertheless numerous enough to have an impact on the atmosphere. These aerosols have ecological significance because they are linked to illnesses in people, animals, and plants. [2,3]. The majority of the particles that end up suspended in the air come from terrestrial and aquatic environments, and air turbulence. In addition to being largely transported by wind, bioaerosols can also be deposited by a number of different mechanisms, such as gravity, contact with surfaces, or mixing with rain to bring the dust back down to the earth's surface [4,5]. Bioaerosols can spread infections, endotoxins, and allergens that sensitive people are allergic to. The types of bacteria or toxins, the types of particles they are connected with, such as dust or mist, and the gases in which the bioaerosols are suspended, are just a few of the variables that affect the content and size of bioaerosols. Fungi, bacteria, viruses, and pollen are examples of bioaerosols. Bioaerosol survival rates are influenced by a variety of biotic and abiotic parameters, such as weather patterns, ultraviolet (UV) light, temperature, and humidity, as well as the availability of resources in clouds or dust [6]. Bacteria make up the majority of bioaerosols found in marine areas, whilst those found in terrestrial ecosystems are abundant in bacteria, fungi, and pollen [7].

Healthcare facilities present an extra, particular issue in addition to the infectious threats faced by all office and commercial buildings, a high density of potentially infectious and immune-compromised individuals. Ventilation is necessary in hospitals and other healthcare facilities because they are complicated environments that must manage hazardous emissions while providing comfort for patients [8]. Furthermore, patients may spread dangerous microbes to staff, hospital visitors, and other patients, making the biological quality of the air in hospital environments a special issue [9]. Despite the fact that hospitalization and medical operations are intended to treat illnesses, they can occasionally unintentionally introduce dangerous bacteria into the body and start a Nosocomial Infection (NI), the infected patient is the main source of airborne germs inside the hospital [10]. Airborne transmission occurs when pathogenic microorganisms are transmitted from an infected person to a vulnerable person through the air. The primary process that makes infections airborne, is the production of aerosol droplets from coughing or sneezing, along with the subsequent loss of water that permits them to float in the air for long periods of time and over great distances [11]. Biological aerosols include spores of yeast, mold, bacteria, viruses, and other microorganisms, skin lesions may potentially be a source of airborne particles in specific clinical situations [10]. Some microorganisms, especially Gram-positive ones like *Staphylococcus aureus* and *Streptococcus pneumoniae*, can persist for several months on dust particles. Viruses and fungus spores can both endure for longer lengths of time. Since many modern buildings are enclosed and feature self-contained circulating air systems for temperature management, the prevalence of airborne infections has increased [12].

Bioaerosol properties are complex and diverse, and have a direct impact on the environment, climate, and human health, to accurately obtain the atmospheric chemical characteristics of bioaerosols, their effective identification in the atmosphere is very significant [13]. Airborne bacteria and fungi may cause several adverse effects, especially infectious, allergenic, and immunotoxic disorders [13]. Microbiological air quality is an important criterion that must be taken into consideration when indoor workplaces are being designed to provide a safe environment. [14]More people are dying every year from hospital infections. Although, many pathogens can cause hospital infection those that are able to survive in the hospital environment for long periods and resist also disinfections are particularly important in this respect [15]. This study was designed to identify both pathogenic and non-pathogenic air microflora and determine if they affect the infection rate in the hospital environment, bacterial and fungal aerosols were collected in this study using the settling plate technique and isolated in the microbiology laboratory.

2. Materials and Methods

2.1. Study site

Clifford University Medical Centre was used as a case study for this research project. Air samples were collected at the following units of the hospital: Male ward, Female ward, Male toilet, Female toilet, Theatre room, and External environment and investigated by settling plate technique.

2.2 Sample Collection and Isolation of Organisms

2.2.1 Collection of Sample

The 12 culture plates containing the Nutrient agar media and the 12 culture plates containing Potato dextrose agar were exposed to the medical centres' atmosphere for a period of 15 minutes where 2 culture plates were exposed at each of the sampling units. After which, the culture plates were carefully covered back and wrapped in Santana nylons and placed in a cool, dry, and clean carton, and transported back to the microbiology laboratory for isolation of organisms.

2.2.2 Isolation of Organisms

After 24 hours of incubation at 37 ˚C, 29 bacterial pure cultures were isolated in the microbiology laboratory while after 3-5 days of incubation at 25˚C, 5 fungal pure cultures were isolated.

2.3 Identification of Organisms

Various biochemical tests were carried out in this study after the isolation for the identification of the bacteria isolated from the medical centre while fungal staining and microscopy were carried out for the identification of fungi isolated from the medical centre. The biochemical tests are catalase, coagulase, citrate utilization, oxidase, hydrogen sulphide production, sugar fermentation, and indole tests

3. Results

The bacterial counts and fungal counts of each unit of the medical centre ranged from 19 CFU to 115 CFU and 1 CFU to 4 CFU respectively. The microbial counts (CFU) are presented in Table 1, the biochemical characteristics and identification of bacterial isolates are presented in Table 2 while Table 3 is the cultural characteristics of fungal isolates.

Table 1: Counts of Indoor and Outdoor Air Microflora of Clifford University Medical Centre**.**

SAMPLE SOURCE	TOTAL BACTERIAL COUNTS PER UNIT(CFU)	TOTAL FUNGAL COUNTS PER UNIT (CFU)
Male Toilets	25	2
Male Wards	115	2
Female Toilets	37	1
Female Wards	19	4
External Environment	28	1
Theatre room	71	1
Total Microbial Counts	**295**	**11**

The bacterial isolates identified in the various units of Clifford University Medical Centre were *Micrococcus* sp., *Staphylococcus* sp., *Streptococcus* sp., and *Bacillus* sp. The occurrence of the bacterial isolates is presented in Table 2.

Table 2: Biochemical Characteristics and Identification of Bacterial Isolates from Different Units of Clifford University Medical Centre.

Sample source	Cultural characteristics	Shape	Gram reaction	Catalase	Coagulase	Oxidase	Indole	Citrate	SFT				Possible Bacterial Species (sp)
									S	B	G	H_2S	
MT1A	Orange, flat, punctiform, smooth.	Cocci	Positive	+	-	-	-	-	+	+	-	-	*Staphylococcus* sp.
MT1B	Milky, flat, punctiform, smooth.	Cocci	Positive	+	-	+	-	+	+	+	-	-	*Staphylococcus* sp.
MT2	Milky, flat, smooth, punctiform.	Cocci	Positive	+	-	+	-	+	+	+	-	-	*Staphylococcus* sp.
MW1A	Milky, flat, smooth, punctiform.	Cocci	Positive	+	-	+	-	+	-	+	-	-	*Micrococcus* sp.
MW1B	Milky, round, smooth, flat.	Cocci	Positive	+	-	+	-	+	+	+	-	-	*Staphylococcus* sp.
MW2A	Milky, round, flat, smooth.	Short rod	Positive	+	-	+	-	+	-	+	+	+	*Bacillus* sp.
MW2B	Creamy, flat, round, smooth.	Long rod	Positive	+	-	+	-	+	-	+	-	-	*Bacillus* sp.
FT1A	Milky, flat, round, smooth.	Cocci	Positive	+	-	+	-	+	-	-	+	-	*Micrococcus* sp.
FT1B	Milky, flat, punctiform, smooth.	Cocci	Positive	+	-	+	-	-	+	+	-	-	*Micrococcus* sp.
FT2A	Milky, flat, punctiform, smooth.	Cocci	Positive	+	-	+	-	+	+	+	+	-	*Staphylococcus* sp.

FT2B	Milky, opaque, round, smooth.	Cocci in chain	Positive	+	-	+	-	-	-	-	-	-	*Micrococcus* sp.
FW1A	Milky, rhizoid, hilly, rough.	Short rod	Positive	+	-	+	-	-	+	+	+	-	*Bacillus* sp.
FW1B	Milky, opaque, round, smooth.	Cocci in chain	Positive	+	-	+	-	-	+	+	-	-	*Staphylococcus* sp.
FW1C	Milky, smooth, punctiform.	Short rod	Positive	+	-	-	-	+	-	-	-	-	*Bacillus* sp.
FW2A	Milky, smooth, dark-centered, round.	Cocci	Positive	+	-	+	-	+	+	+	-	-	*Staphylococcus* sp.
FW2B	Milky, smooth, round.	Cocci	Positive	+	-	+	-	+	+	+	-	-	*Staphylococcus* sp.
FW2C	Milky, smooth, punctiform.	Cocci	Positive	+	-	+	-	+	+	+	+	-	*Staphylococcus* sp.
EE1A	White, rough, dark-centered, round.	Cocci	Positive	+	-	-	-	+	+	+	+	-	*Staphylococcus* sp.
EE1B	Creamy, rough, rough, rhizoid.	Cocci	Positive	+	-	-	-	+	+	+	-	-	*Staphylococcus* sp.
EE1C	Orange, round, hilly, smooth.	Cocci	Positive	+	-	-	-	-	+	+	+	-	*Staphylococcus* sp.
EE1D	Milky, round, flat, smooth.	Cocci	Positive	+	-	-	-	-	+	+	-	-	*Staphylococcus* sp.

EE2A	White, rough, dark-centered, filamentous, rhizoid.	Cocci in chain	Positive	+	-	-	-	-	+	+	-	-	*Staphylococcus* sp.
EE2B	Milky, round, smooth, flat.	Cocci in chain	Positive	-	-	-	-	-	-	+	-	-	*Streptococcus* sp.
EE2C	Milky, smooth, punctiform, flat.	Long rod	Positive	+	+	-	-	-	+	+	+	-	*Bacillus* sp.
T1	Milky, rhizoid, rough.	Cocci	Positive	-	-	+	-	-	-	+	-	-	*Streptococcus* sp.
T2	Orange, round, dark-centered, moist, flat	Cocci	Positive	+	-	-	-	+	+	+	-	-	*Staphylococcus* sp.
T3	White, rough, dry, umbolate.	Short rod	Positive	-	-	+	-	-	+	-	-	-	*Bacillus* sp.
T4	Milky, flat, smooth, punctiform.	Cocci	Positive	+	-	+	-	-	+	+	-	-	*Staphylococcus* sp.
T5	Milky, round, smooth, moist.	Short rod	Positive	+	-	+	-	-	-	+	+	-	*Bacillus* sp.

KEY: MT- Male Toilet, MW- Male Ward, FT- Female Toilet, FW- Female Ward, EE- External Environment, T- Theatre, SFT- Sugar Fermentation Test, S- Slant, G- Gas, B- Butt, H_2S- Hydrogen sulphide, + (positive), - (negative)

Table 3: Cultural Characteristics of Fungal Isolates from Different Units of Clifford University Medical Centre

SAMPLE SOURCE	ORGANISM	CULTURAL CHARACTERISTICS
THEATRE	White mold	White and filamentous
ENVIRONMENT	*Trichophyton* sp.	Orange and non-filamentous
MALE WARD	*Aspergillus* sp.	Non-filamentous, velvet, circular and leave-green with white circumference.
	Penicillium sp.	Non-filamentous, velvet, circular, greenish-blue with white circumference.
MALE TOILET	*Aspergillus* sp.	Non-filamentous, velvet, circular, dark-brown with white edges.
	Trichophyton sp.	Orange and non-filamentous
FEMALE TOILET	White mold	White and filamentous
FEMALE WARD	*Aspergillus* sp.	Non-filamentous, velvet, circular, dark-brown with white edges.
	Aspergillus sp.	Non-filamentous, velvet, circular and leave-green with white circumference.
	Penicillium sp.	Non-filamentous, velvet, circular, greenish-blue with white circumference.
	White mold	White and filamentous

Discussion of Results

The bacterial isolates from the different units of Clifford University Medical Centre: male ward, male toilet, female ward, female toilet, theatre, and the environment, were subcultured for 24 hours at 37 ˚C. Afterward, 29 pure cultures were isolated and grouped based on their different sources of the sample.

The results of the current study showed that the air at Clifford University Medical Centre included a great diversity of bacteria. *Micrococcus*, *Staphylococcus*, *Streptococcus*, and *Bacillus* were the bacterial genera isolated. *Staphylococcus* predominated in the environment. In a related study, *Staphylococcus*, *Proteus*, *Streptococcus*, and *Micrococcus* were isolated from indoor air of laboratories, while *Micrococcus*, *Paracoccus*, *Staphylococcus*, and *Enhydrobacter* were isolated in the study of indoor and outdoor environments of child care facilities [16, 17]. Also, Sanaa & Amani [15] isolated *S. aureus*, *E. coli*, *Klebsiella* sp, *P. aeruginosa*, and *Bacillus*sp from hospital delivery and nursing rooms in Khartoum district Sudan. All of the bacteria found in this study were Gram-positive rods and cocci, just like the ones found in hospital lobbies' air [18]. Since Gram-positive bacteria have a thicker covering of peptidoglycan in their cell walls than Gram-negative bacteria, this is not surprising. As a result, they dominated the study's bacterial flora.

The organisms recovered in this study can be categorized as either commensals or pathogens depending on how they interact with their human hosts. Either humans or the soil environment is the source of these bacteria. The commensals are part of the typical human body flora and are present on the skin, nose, and mouth. They do not infect their host normally, with the exception of when the immune system is weak [19]. *Bacillus*, *Paenibacillus*, and *Chaetomium* were among the bacterial genera reported by Yan *et al* [3]. The *Staphylococcus* genus, which was discovered in this study, is found on human and other animals' skin and mucous membranes.

They are a part of the microbial flora of soil and are present all over the planet. *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* are the three most significant species of human pathogens [19], with *S. aureus* being the most pathogenic of all and responsible for serious infections in healthcare facilities like Pneumonia; which most frequently affects people with underlying lung disease, Osteomyelitis; a bone infection that can be brought on by staphylococcal bacteria entering the bloodstream or being directly introduced, as in the case of intravenous (IV) drug abuse, vaginal infections, etc. endocarditis, and many other conditions [20].

Staphylococcusaureus wasone of the dominant isolated organisms, this is a common pathogenic bacterium that is associated with various diseases such as respiratory tract, digestive system, and post-operative infections, also urinary tract, and skin disorders, it is resistant to antibiotics, its presence might be due to post-sterilization, or the environment contamination [15]. Anyone can become infected with *Staphylococcus*, but some people are more susceptible than others, such as those who have diabetes, cancer, etc. The risk is also higher in hospitals where there are patients with compromised immune systems, those who have had specific surgeries, those in intensive care units (ICUs), and those who have had medical devices implanted in their bodies [20]. Although the organism can be found in many places, including on the surface of the skin, in dust, water, and soil, in this investigation, *Micrococcus* sp. was not particularly prevalent in the medical center. In this investigation, the genus *Bacillus* was also isolated. These saprophytic organisms are common in the air and several other ecosystems. Medically significant pathogenic species include *Bacillus cereus*, traditionally considered foodborne pathogens that establish occasional opportunistic infections, and have naturally evolved to cause fatal anthrax-like disease. This serves as a reminder that the field of medical microbiology is constantly changing, posing new challenges that require ongoing vigilance and research [21] and *Bacillus anthracis*, which produces anthrax. Bacilli endospores are more resistant to heat, drying, and disinfectants than vegetative cells are. They may survive better in the air and can be found in dirt, dust, and plant products [22]. In this study, the gram-positive, and catalase-negative streptococci were the final bacterial genera discovered. These organisms belong to the natural flora but turn pathogenic when they are exposed to an immune system that is weak. *Streptococcus pyogenes* (group A *Streptococcus*) is one of the most important bacterial causes of skin and soft tissue infections (SSTIs) worldwide. There is no other pathogen that causes as many diverse clinical entities as *S. pyogenes*. [23].Specifically, this organism causes infections in the superficial keratin layer (impetigo), the superficial epidermis (erysipelas), the subcutaneous tissue (cellulitis), the fascia (necrotizing fasciitis), or muscle (myositis and myonecrosis) and it is also the etiologic agent of scarlet fever and Streptococcal Toxic Shock Syndrome (StrepTSS). Impetigo is a non-life-threatening infection, but can result in post-streptococcal acute glomerulonephritis (AGN). Cellulitis and erysipelas can be mild or moderately severe, while necrotizing fasciitis, myonecrosis, and StrepTSS are life-threatening [23].

The fungal isolates from the different units of Clifford University Medical Centre: male ward, male toilet, female ward, female toilet, theatre, and the environment, were subcultured for 3-5 days at 25˚C. Afterward, 5 pure cultures were isolated and grouped based on their different sources of the sample.

This study found that the air at Clifford University Medical Centre contained a great diversity of fungi. White Mold, *Trichophyton terrestre*, *Aspergillus flavus*, *Aspergillus niger*, and *Penicillium* were among the fungi that were isolated; *Aspergillus* species predominated in the medical center, with the female ward having the highest fungal count (4 CFU). In a related study, *Saccharomyces cerevisiae*, *Aspergillus* sp., *Rhizopus stolonifera*, and *Alternaria* sp. were isolated in the study of Indoor Air Microflora of some Daycare Centres in Illorin south local government area [24]. Due to their ability to sporulate and reputation for pathogenicity, the *Aspergillus spp*. isolated in this investigation dominated the genera of fungus isolated. These are *Aspergillusniger*, which can cause aspergillosis and generates oxalic acid, malformin, and other chemicals, and *Aspergillus flavus*, which is known to create mycotoxins. *Aspergillus flavus*, one of the two isolated species, is known to produce aflatoxin [25]. In several offices at the University of Ilorin in Ilorin, Nigeria, previous research also noted the presence of *Aspergillus niger, Aspergillus flavus, Aspergillus glaucus, Aspergillus versicolor*, *Alternaria alternata*, *Geotrichumcandidum*, and *Rhizopus* [26].

Penicillium spp. are among the most prevalent fungi in the environment and are typically thought to not be harmful to people. This result is in agreement with Jalili [13] who reported the isolation of species of yeast, *Aspergillus*, and *Penicillium*in Shahrekord Hospitals in Iran, these organisms are highly pathogenic or opportunistic pathogens/medically important microorganisms. Most fungi are known to be associated with asthma in both children and adults [13] These fungi lead to pulmonary aspergillosis when inhaled [27].Cases of contamination occur mostly in people with underlying illnesses and low immunity levels [28]. Approximately 49.1% of *Aspergillus* sp. outbreaks within hospitals can be attributed to the construction work in or around hospitals [29]. In a study conducted in Benin City, Nigeria, *Penicillium*, *Mucor*, *Aspergillus*, and *Fusarium* were among the fungi species isolated from the hospital indoor air [30]. Airborne fungi and their spores have the potential to be blown into buildings with natural ventilation. They can pose a threat to the life of immunocompromised patients when are blown in through the windows of the relevant wards [31]. A study conducted in hospitals in Sari, Iran isolated predominantly pathogens which are *Mucorales* sp., *Candida* sp., *Fusarium* sp., *and Aspergillus* sp. [32].

CONCLUSION AND RECOMMENDATION

Conclusion

The results of this study showed that the Clifford University Medical Center's indoor and outdoor air harbour a variety of genera of Gram-positive cocci and rod bacteria, *Staphylococcus* sp predominated in the hospital environment and the male ward having the highest bacterial count (115 CFU). Of the fungi isolated, *Aspergillus* sp has the greatest fungal count (4 CFU). A few pathogenic species were isolated among bacteria and fungi such as *Staphylococcus*, *Bacillus*, *Streptococcus*, *Aspergillus,* and *Trichophyton* spp respectively. Therefore, the air microflora in this medical centre may have possibly contributed to the infection rate in the Medical Centre.

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