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Isolation And Identification of Indoor Air Microflora in Microbiology Laboratory of Nile University of Nigeria

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ABSTRACT

A study was conducted to isolate and identify air microflora in Microbiology Laboratory of Nile University of Nigeria, Abuja. Nutrient agar and Potato Dextrose agar were prepared and poured into petri dishes and exposed to the air in Microbiology laboratory for 30minutes in the morning before the commencement of class activities and in the afternoon, during class activities. The plates were incubated at 37 ºC and 25 ºC respectively to isolate bacteria and fungi. The isolated bacteria species were *Staphyloccocus aureus, Escherichia coli, Klebsiella* sp. and *Alkaligenes faecalis*, while the fungal species were *Aspergillus niger, Penicillium* sp. *Aspergillus fumigatus, Mucor flavus* and *Fusarium moniliforme*. The microbial counts of the afternoon exposure were much higher than those of the morning hours and the isolated bacterial species demonstrated relatively high sensitivity to antibacterial antibiotics used for the study except for *Klebsiella* sp. which had 20 % resistance and *Alcaligenes faecalis* with 30 % resistance. It is recommended that all environmental conditions that can facilitate the proliferation of these microorganisms in the laboratory should be prevented in order to protect the health of staff and students.

KEYWORDS: Indoor air, Microflora, *Alcaligenes faecalis, Fusarium moniliforme*

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1 | INTRODUCTION

1.1 Background of the Study

Air does not have any indigenous microflora, though a number of microorganisms are present in the air and they represent allochthonous populations transported from aquatic and terrestrial habits into the atmosphere (Aithal, 2009). Air quality of indoor environments is a main factor affecting human health, well-being and productivity. Bacterial air quality (BAQ) is an important problem because people inhale nearly 10 L of air a minute, which amounts to 15,000 L/day (Bragoszewska *et al*., 2020). In spite of this, people hardly pay attention to the effect of the presence of diseases-causing microorganisms in the indoor air (Osuolale *et al*., 2019).

The microorganisms in the air are known as bioaerosols; colloidal suspensions of liquid droplets containing viruses, fungal spores and conidia, bacterial endospores, plant pollen and fragments of plant tissues (Ilusanya *et al*., 2020). These microorganisms usually enter buildings through doors, windows, air conditioners, and individuals coming in from the external environment. The use of air conditioners significantly reduces air exchange in the environments while increasing the concentration of pollutants (irritants, fumes, allergenic pollens, etc.) and infectious agents (viruses, fungi and bacteria) that can add to their pathogenic activity already in itself represented by the particular physical characteristics of an artificially cold and dry air (D'Amato *et al*., 2018).

Some bacteria, particularly Gram-positive bacteria, fungi with spores, and viruses can survive for months in dust particles prevalent in the indoor environment (Sheik *et al*., 2015). Some indoor microbes may be hazardous, secreting poisonous compounds that cause allergies and even fatal disorders (Sekulska *et al.*, 2007). When fungi penetrate from the outdoors and find ideal conditions in the inside environment, they can cause health problems in both immune compromised and healthy persons (Kohler *et al*., 2015). Such health problems include respiratory disorders as well as infections, hypersensitivity, pneumonitis, and toxic responses, among other things, and are contacted mostly through inhalation and deposition in the nasal and bronchial airways.

Because a large number of students attend both theory and practical sessions in the laboratory, there is therefore the need to check and ensure the sanitary conditions of microbiology laboratories, where varieties of microorganisms are handled indoors within an enclosed environment. Moreover, microbial contamination is one of the most significant challenges faced by academics working with microbial cultures around the world. The knowledge of common microbial contaminants in the microbiology laboratory will minimize the false-positive or false-negative results errors that may likely occur in the course of microbiological research conclusions and also prevent loss of valuable laboratory strains. This background knowledge therefore prompted this research activity in Microbiology laboratory of Nile University of Nigeria.

2 | MATERIALS AND METHODS

2.1 Study Area

The study was conducted at the Department of Microbiology in Nile University of Nigeria, which is located in the Federal Capital Territory, Abuja, Nigeria.

2.2 Sample Collection

Samples for the study were collected by sedimentation technique method, which entails opening and displaying of a culture media plate (Sekulska *et al*., 2007). Nutrient agar was used to isolate bacteria while Potato dextrose agar containing 1% erythromycin was used for isolating fungal species. Both media were prepared according to the manufacturer's instructions. At three (3) different locations in the laboratory room, prepared plates of nutrient agar and potato dextrose agar were exposed to the air for 30 minutes, first, at 7:30 am, before the commencement of class activities, and at 2:00 pm, during class activities. The plates were then incubated at 37 °C for 24 hours to isolate bacteria and 25 ^{o}C for 5 days to isolate fungi. Colonies were subcultured onto fresh media respectively to obtain pure cultures.

As control, prepared plates of both nutrient agar and Potato dextrose agar (containing 1% erythromycin) were well sealed with adhesive tapes, placed at the same locations with the exposed plates and were later incubated at temperatures of 37°C and 25°C for 24 hours and 5 days respectively.

2.3 Bacterial Identification

The conventional processes for identifying bacterial isolates were followed, according to Cheesbrough, (2009). Bacterial colonies were identified using morphological features, Gram reactions and biochemical tests. Gram staining and the biochemical tests were carried out according to methods outlined by Baker & Breach, (1980). The identities of the isolates

were confirmed by comparing their characteristics with those of known taxa using Bergey's Manual of Determinative Bacteriology (Bruchanan & Gibbons, 1994).

2.4 Antibiotic Sensitivity Test

The sensitivity of isolates obtained from the laboratory samples on antibiotics was determined by Disc Diffusion Method. Mueller Hinton agar (MHA) plates were prepared and the pure cultures of isolates were streaked on the media in the plates. The antibiotic susceptibility discs, which include Ceftriaxone, Oxacillin, Erythromycin, Ciprofloxacin, Ampicillin/Cloxacillin, Cefuroxime, Doxycycline, Levofloxacin, Tetracycline, Amoxicillin/Clavulanate, were placed in each agar plate and incubated at 37 °C for 24 hours. The results were interpreted based on the Clinical and Laboratory Standard Institute Guide of 2018.

2.5 Identification of Fungal Isolates

The fungi were identified using the cultural characteristics (colour, shape, size and hyphae) and microscopically using lactophenol cotton blue stain as described by Montanari *et al*. (2012). A drop of lactophenol cotton blue stain was made on a grease-free microscope slide with the aid of sterile pipette and using sterilized inoculating needle, a small portion of the mycelium from each fungal culture was sampled and placed on the drop of lactophenol cotton blue stain. The mycelium was spread out on the slide with the aid of two sterile inoculating needles in order to ease viewing. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was mounted on the microscope and observed under x10 and x40 objective lenses The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with Saad, (1992); Klich, (2002) and Samson &Varga, (2007).

3 | RESULTS

3.1 Identification of Microbial Isolates Bacteria

Four bacteria species were isolated and identified in this study and they include, *Klebsiella* sp. *Staphylococcus aureus*, *Escherichia coli* and *Alcaligenes faecalis* (Table 1). *Staphylococcus aureus* was isolated on nutrient agar plates exposed to the indoor air for both morning and afternoon sessions, while *Klebsiella* sp. and *E. coli* were isolated from only the plates exposed in the morning hours (Table 1). *Alcaligenes faecalis* was however isolated only in the afternoon exposure.

Colony counts of bacteria ranged between 30 - 45 CFU/m^3 in the morning and 56 - 167 CFU/m^3 in the afternoon (Table 1). *Staph. aureus* had the highest frequency of occurrence with 35 % while *Klebsiella*. sp. had the lowest frequency of 16 % (Fig. 1). *Staph. aureus* and *E. coli* were sensitive to the ten (10) antibiotics used for susceptibility tests in the study while *Klebs*. sp. and *Alcaligenes faecalis* had 20 % and 30 % resistance rates respectively. The degree of sensitivity is presented in Table 3.

Six fungal species, which include *Aspergillus niger, Penicillium sp., Mucor flavus, Aspergillus flavus, Aspergillus fumigatus, Fusarium moniliforme* were isolated in this study. *A. niger* was isolated in the morning exposure while *A. flavus* and *A. fumigatus* were isolated in the afternoon. *Penicillium* sp. and *Mucor flavus* were also isolated in the morning exposure while *Fusarium moniliforme* only appeared among the afternoon isolates (Table 2). Species of *Aspergillus* occurred most frequently with 36 % occurrence for *A. niger*, 15 % for *A. flavus* and 11 % for *A. fumigatus* (Fig 2). *Penicillium* sp. had the second highest percentage occurrence of 27 % while *Fusarium moniliforme* had the percentage occurrence of 4 %.

No growth was observed in the two control plates, Nutrient agar and PDA after incubation at 37 ºC and 25 ºC for 24 hours and 5 days respectively.

Table 3.1 Cultural, morphological and biochemical characteristics of bacteria isolated from waste dump

Isolates	Colony Characteristics	Colony counts (CFU/m^3)	Cell Shape	Gram's reaction	Motility	Presence of spore	Glucose	Fructose	Lactose	Sucrose	Maltose	Mannitol	MR	VP	Citrate	Catalase	Indole	Oxidase	Starch hydrolysis	Urease	H_2S	Organism
M 1	Spotish white	30	Rod	-	-	-	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	*Klebsiella sp.*
2	Creamy	45	Cocci	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	*Staphylococcus aureus*
3	Grayishwhitish	57	Rod	-	+	-	+	-	+	-	-	+	+	-	-	+	+	-	-	-	-	*Escherichia coli*
A 1	Yellowish	56	Cocci	+	-	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	*Staphylococcus aureus*
2	Chalky white	165	Rod	-	+	+	-	-	-	+	-	-	+	-	-	+	-	+	-	-	-	*Alcaligenes faecalis*
3	Shiny yellow	167	Cocci	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	*Staphylococcus aureus*

MR: Methyl red VP: Voges proskauer H2S: Hydrogen sulphide +: Positive -: Negative

Table 3.2: Cultural and Microscopic characteristics of fungal isolates

Sample no	Colony no (CFU/m³)	Macroscopic characteristics	Microscopic characteristics	Fungal organisms
Morning session 1	10	Black colonies with white edges	Thick septate hyphae with conidia spore in chains	*Aspergillus niger*
2	6	Gray-green	Thin septate hyphae strand with conidia	*Penicillium sp*
3	2	White, heavy, wooly fluffy growth covering entire plate	Thick non septate hyphae with dark sporangiospore	*Mucor flavus*
Afternoon session 1	4	Yellow green	Thick septate hyphae with conidia born in chains	*Aspergillus flavus*
2	3	Blue-green/gray	Thick septate hyphae with conidia in chains from the stigmata	*Aspergillus fumigatus*
3	1	White to purple	Septate hyphae with sickle-shaped	*Fusarium moniliforme*

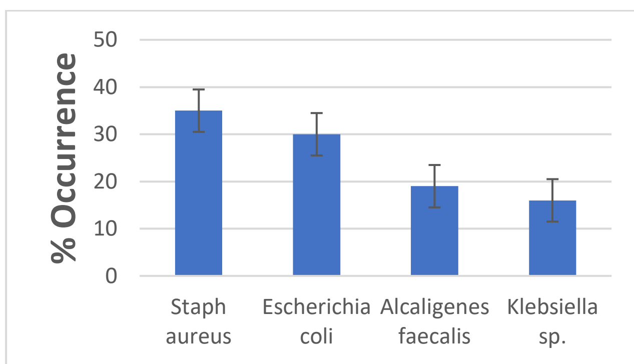

Figure 3.1: Frequency of occurrence of bacterial isolates

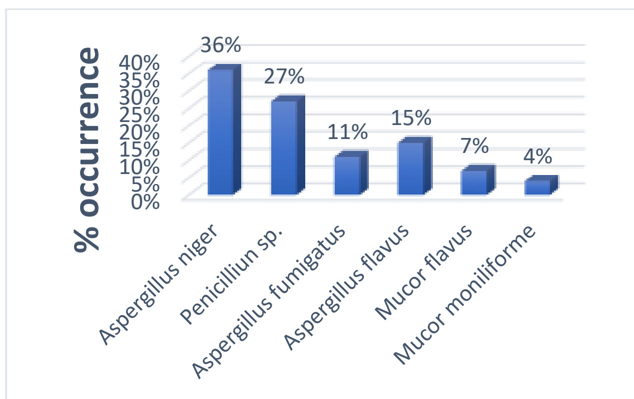

Figure 3.2: Frequency of occurrence of fungal isolate

Table 3.1: Antibiotics Susceptibility Profiles of bacterial isolates

KEY: S = Sensitive, R = Resistance

Antibiotics	*Staphylococcus aureus*	*Klebsiella sp.*	*Alcaligenes faecalis*	*Escherichia coli* S
Ceftriaxone	S	S	S	
Oxacillin	S	S	S	S
Erythromycin	S	S	S	S
Ciprofloxacin	S	S	S	S
Ampicillin/Cloxacillin	S	S	S	S
Cefuroxime	S	S	S	S
Doxycycline	S	S	R	S
Levofloxacin	S	R	R	S
Tetracycline	S	S	S	S
Amoxicillin/Clavulanate **KEY: S = Sensitive,**	S	R	R	S **R = Resistance**

4 | DISCUSSION

This study was conducted in a laboratory, where students enter in batches to carry out practical activities at intervals of time, from morning to afternoon on school days. The prevalence of *Staphylococcus aureus* in the indoor environment, especially in the laboratory environment could be attributed to its easy way of transmission through agents such as throat, skin, cuts, boils, nails, nasopharynx and other activities (Ekhaise *et al.,* 2011). Its' prevalent occurrence in such indoor environments has been reported by Mandal and Brandl (2010), who recommended that, this bacterium be given special attention as it can survive in the air for several days. The survival ability may also be responsible for highest frequency of occurrence among other bacterial species in this study. Osuolale *et al.,* (2019) reported the isolation of *Staphylococcus* and *Klebsiella* species among other microorganisms from an indoor environment. *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* were among the bacteria species isolated from the indoor environment of a hospital by Aladenika and Olaniyan, (2014) as well as Girma and Lamore, (2022). These bacteria are dispersed in air in the droplets of saliva and mucus produced by coughing, sneezing, talking and laughing (WHO, 2009). This could also be the reason for the higher colony counts of these bacteria in the afternoon exposure during practical activities in the laboratory. *E. coli,* which is an important indicator of the presence of faecal material was isolated from indoor and outdoor environments as reported by Rosas *et al.* (1997). The presence of *E. coli* and *Alcaligenes faecalis* in the laboratory indoor air in this study could be an indication of the hygienic conditions of the toilets used by the students as the presence of these bacteria is always associated with fecal matter. *Alcaligenes faecalis* being isolated only in

the afternoon exposure in this study could mean that it was brought into the laboratory as people entered for their practical classes. *A. faecalis* was reported by (Bragoszewska *et al*., 2020) as one of the bacterial species isolated in their indoor air quality study.

The high sensitivity of the bacterial isolates to the antibiotics used for the study could mean that such common antibiotics may not have been abused by the users of the laboratory, from whom the indoor air bacteria could have emanated. The main cause of microbial resistance to antibiotics is misuse and overuse of such antibiotics (WHO, 2020). The resistance of *Klebsiella* sp. and *Alcaligenes faecalis* to some of the antibiotics used could be associated with human exposure to such antibiotics, as Nirwati *et al*. (2019), reported that some of the main contributors in the emergence and spread of high' resistant bacteria for healthcare associated infections (HAI are the intensive and prolonged use of antibiotics in the hospit setting.

The isolation of *Aspergillus* sp., *Penicillium* sp., *and Fusarium* sp. from the indoor environment in this research agrees with report of Reanprayoon and Yoonawong, (2012), who also isolated the same fungi species among other microorganisms in the laboratory environment. *Aspergillus, Penicillium* and *Fusarium* were also among the microorganisms isolated by Osuolale *et al*. (2019). The presence of these fungi in such an environment could be attributed to the enabling room temperature of the laboratory as well as the relative humidity as reported by Gandolfi *et al.* (2015). Aladenika and Olaniyan, (2014) also isolated *Aspergillus, Penicillium* and *Mucor* species in a hospital indoor environment. Such fungal species may have also been discharged into the environment through sneezing, coughing, talking, contact with laboratory materials and the uncontrolled human movement in and out of the laboratory environment.

The high fungal counts in the morning hours before resumption of practical class could serve as a pointer to the cleaning and sanitation status of the laboratory. The reduction in the fungal counts in the afternoon exposure however, could imply that the fungal species in the laboratory air environment could have found new hosts on the staff and students' bodies as well as their properties, which may include their Laboratory coats, practical manuals and other materials. The high concentrations of air microorganisms in Microbiology laboratory as recorded in the present study is of great concern. The indoor environment can potentially place human occupant at higher risk than the outside spaces because enclosed spaces help to confine aerosols, which allows them to buildup to potentially infectious levels (Ekhaise *et al.,* 2011).

5 | CONCLUSION

The concentration of airborne microflora in Microbiology laboratory environment of Nile University of Nigeria Abuja, studied, showed that the laboratory, which is supposed to be a place of high hygienic conditions can also be a source and reservoir of infectious microorganisms. These organisms can cause several infections to the staff and students. In order to improve the quality of indoor air in the laboratory environment, overcrowding should be avoided, good ventilation systems have to be designed and good hygiene practices should be observed.

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Availability of Data and Materials: The data used to support the findings of this study are available from the corresponding author upon request

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