

Original Article

Microbial assessment of indoor air of the applied microbiology laboratory, Nnamdi Azikiwe University, Awka, Nigeria

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Abstract

Air is made up of enormous number of microorganisms mainly fungi and bacteria spores. Their estimation is important as an indication of cleanliness of any particular environment. The present study was carried out to assess microorganisms in indoor air of the Applied Microbiology laboratory of Nnamdi Azikiwe University, Awka. Prepared plates of Sabouraud Dextrose agar (SDA), Nutrient agar (NA), and Blood agar were exposed for ten minutes for culturing of microorganisms. The NA and Blood agar plates were incubated at 37°C for 24h while the SDA plate was incubated at room temperature for 48h. A total of ten (10) microorganisms were isolated from the samples. These include six (6) bacteria and four (fungal) species. The bacterial isolates include; *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus* sp., and *Proteus* sp. The fungal isolates include *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and *Rhizopus* sp. The most frequently isolated bacteria were *Staphylococcus aureus* and *Bacillus* sp. with 25% and 35% occurrence. *Proteus* sp., *Streptococcus* sp., *E. coli*, and *P. aeruginosa* had 20%, 10%, 5%, and 5% percentage occurrences respectively. *Aspergillus* and *Penicillium* sp. were the most frequently isolated fungal isolates with 40% and 30% occurrence respectively. *Fusarium* sp. and *Rhizopus* sp. both had 20% and 10% occurrence respectively. With this result, attention must be given to control those environmental factors which favor the growth and multiplication of microbes in the indoor air of the laboratory and air sterilization should be carried out to safeguard the health of users and workers.

Keywords: Microbial assessment, airborne microbial, indoor air assessment, biological air pollutants

Introduction

The majority of tasks are completed indoors, especially in laboratories. Issues with indoor air quality have been identified by the World Health Organization (WHO) as significant risk factors for human health in low-, middle-, and high-income nations. People spend a significant amount of time at home and at work, where open-air habitats have been replaced by airtight, energy-efficient ecosystems as a result of global lifestyle changes (Molhave, 2011). These situations frequently lead to "Sick Building Syndrome" (SBS), a condition in which people report negative health effects that seem to be connected to living in a building, as a result of inadequate maintenance, bad building design, or people activities (Zain, 2011). A variety of factors influence indoor air quality. Many of these are associated with the building's structure and decoration, as well as its ventilation, internal temperature, and humidity. Additional factors include outside pollution and, unavoidably, contamination by microorganisms, particularly fungi.

Airflow, which includes indoor air, is a fundamental factor influencing human body function. The European Environment Agency (EEA) and the World Health Organization (WHO) both assert that environmental dangers like air or water pollution have a substantial effect on people's health. As well as natural compounds

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in much larger concentrations, all elements present in the Earth's atmosphere that are not natural components are regarded as air pollutants. Gases and vaporized chemical compounds, airborne ashes, dust, acid rain, trace metals, and biological pollutants were all present in the atmosphere (European Environment Agency (EEA), 2018).

Pollen, fungi, bacteria, and viruses are examples of biological air pollutants, also referred to as bioaerosols. In addition, mycotoxins, enzymes, and pieces of plant and animal tissues are produced by bacteria. Virtually every habitat has microorganisms, and intriguingly, bacteria and fungi have been found at different atmospheric levels. The bioaerosol's dispersed phase particles have sizes ranging from 1 to 200 μm . For instance, a single bacterial cell can be anywhere between 0.5 and 2.0 μm and several fungal and mould spores can be anywhere between 3.0 and 17 μm in size. Bioaerosols with diameters between 1.0 and 5.0 μm usually float in the atmosphere, while those with diameters greater than this prefer to settle on surfaces. Respirable bioaerosol, which is defined as a fraction smaller than 7, poses the greatest risk to human health (Douglas et al., 2018).

Airborne microorganisms and bioaerosols can spread in all ecosystems due to their ability to travel long distances through wind and precipitation. Some may be pathogens or allergens carriers, putting the public's health at risk (Griffin, 2004). As a result, the presence of microorganisms, particularly those that cause infectious diseases, in both ambient air and indoor environments can be extremely harmful. Research has shown that microorganisms in the air are most often responsible for immune system irregularities such as infections and allergies. Exposure to mould and other dampness-related microbial agents, according to WHO, carries the potential for hypersensitivity pneumonitis, allergic alveolitis, or chronic rhinosinusitis, as evidenced in studies both in vivo and in vitro (Heseltine & Rosen, 2009).

There are still many unsolved concerns and little awareness of the existence of microorganisms in the air. According to the number of scientific publications, research on microorganisms in the air is still understudied, and little is known about the composition of the microbial community in the atmosphere. The European Environment Agency (EEA) just released a study (2018) that discusses the causes, types and potential effects of air pollution on human health and ecosystems, but it omits any mention of biological agents in the atmosphere. This work, therefore, is aimed at assessing microorganisms in the indoor air of the Applied Microbiology laboratory of Nnamdi Azikiwe University, Awka.

Methods

Sample collection

This study employed the plate exposure approach by means of opening plates containing particular culture media for a predetermined amount of time. (Ekhaie et al., 2010). This method allows bacteria or fungi in the air to settle on the respective culture media. Prepared plates of Sabouraud Dextrose agar (SDA), Nutrient agar, and Blood agar were exposed for ten minutes for culturing of microorganisms. The plates were kept on the floor (Sample A), the workbench (Sample B), and breathing level (Sample C) and exposed from 10:00 AM to 10:10 AM. After sampling, the plates were incubated in the laboratory.

Isolation of Microorganisms

The plates were incubated for 24 hours at 37°C and for 48 hours at 28°C (visible fungi colony could take more than 24 hours). In order to isolate these distinct colonies, bacteria were isolated using the streaking method, and fungi were isolated via stab

inoculation. For identification purposes, the distinct colonies were re-inoculated into the proper media slants and stored at 4°C. (Cheesbrough, 2005).

Identification of isolates

Standard microbiological techniques were used for bacterium identification. Gram staining, motility, catalase, coagulase, oxidase, and indole tests were a few examples of microscopic and biochemical tests carried out using standard techniques. Fungal isolates underwent microscopic and macroscopic investigations, including staining for morphological traits, in order to be identified as fungus. Utilizing cultural and morphological traits such as colony development pattern, conidial morphology, and pigmentation, the fungal isolates were identified. When employing cotton blue in lactophenol cotton blue stain to identify the isolated fungi, Cheesbrough's (2010) method was also used. Using a mounting needle and a little piece of the aerial mycelia from the representative fungal cultures, the stain was applied to a clean slide to make the identification. This was followed by adding a drop of lactophenol. On the slide with the needle, the mycelium was evenly distributed. To remove air bubbles, a cover slip was put delicately and lightly. After mounting, the slide was examined using a light microscope equipped with 10- and 40-objective lenses. Using the fungal atlas to compare these traits, identification was carried out to determine the isolates to genus level.

Results

The types of bacteria and fungi isolated from the Microbiology Laboratory in Nnamdi Azikiwe University, Awka are provided in Table 1 and Table 2. They were characterized based on shape, elevation, color, margin, surface, and transparency. The isolates are all opaque and possess smooth surfaces. Colour varied as some are white and some are creamy, some colonies are flat and some are also raised. The shape was also observed as most were circular and some were irregular. The margin also revealed that most of the isolates were entire and some were undulated.

Table 1. Plate count of the bacterial and fungal isolates from the indoor air samples

Samples	Bacteria (10 ⁴ cfu/m ³)	Fungi (10 ² cfu/m ³)
A	45	23
B	55	29
C	37	19

Table 2. Morphological characteristics of the bacterial isolates from the indoor air Samples

Isolate	Shape	Color	Elevation	Margin	Surface	Transparency
1	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
2	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
3	Circular	White	Raised	Entire	Rough	Opaque
4	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
5	Irregular	Creamy	Flat	Undulated	Smooth	Opaque
6	Irregular	Creamy	Flat	Flat	Undulated	Smooth

Six species of bacteria were isolated from the laboratories: *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus* sp., and *Proteus* sp. In this study, the most frequently isolated bacteria were *Staphylococcus aureus* and *Bacillus* sp (Figure 1A).

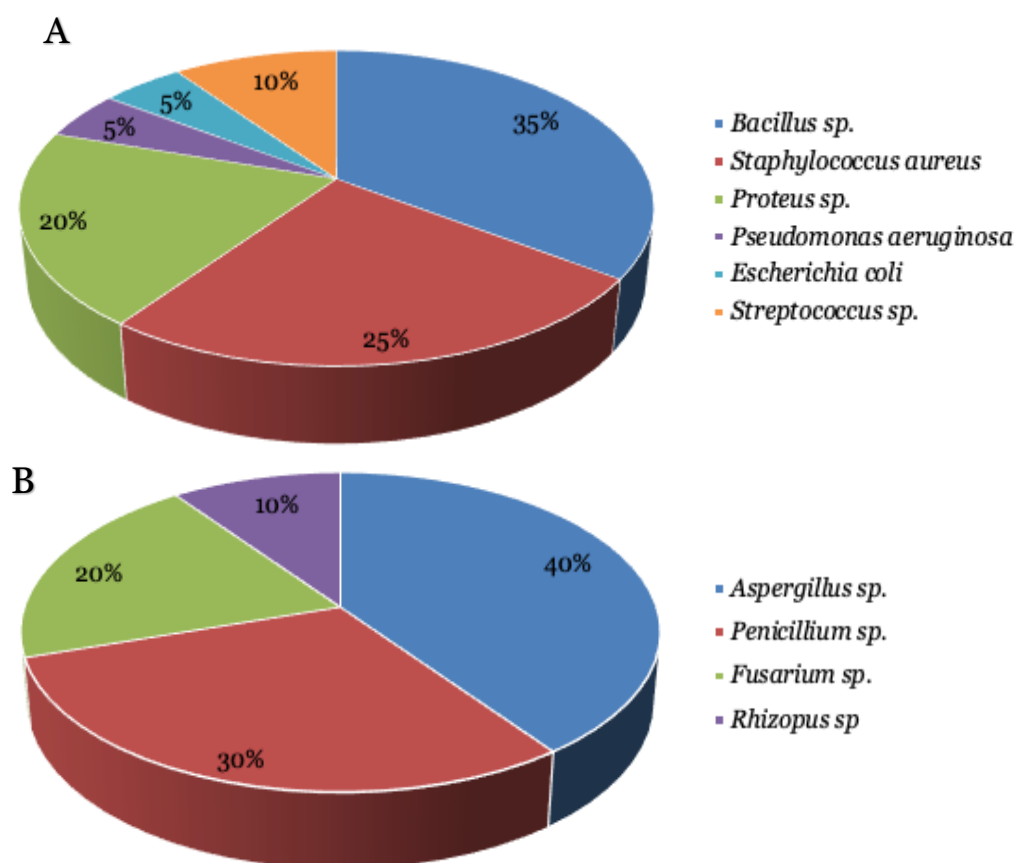


Figure 1. Percentage of type of isolated bacteria (A) and fungi (B)

The isolates displayed different reactions to Gram staining (Table 3). The reactions to different biochemical tests were also presented in Table 3. The probable isolated were *Staphylococcus aureus*, *Bacillus sp.*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus sp.*, and *Proteus sp.* Some of the Bacteria have the ability to ferment sugar to produce acid and gas (Table 4).

Table 3. Microscopic and Biochemical tests for the identification of the bacterial isolates

Isolates	Characters							Probable organism
	Gram stain	Shape	M	Ca	U	I	Ci	
1	-	Rods	+	+	-	-	-	<i>Pseudomonas aeruginosa</i>
2	-	Rods	+	+	+	-	+	<i>Proteus sp.</i>
3	+	Rods	+	+	-	-	+	<i>Bacillus sp.</i>
4	-	Rods	+	+	-	+	-	<i>Escherichia coli</i>
5	+	Cocci	-	+	+	-	+	<i>Staphylococcus aureus</i>
6	+	Cocci	-	-	-	-	-	<i>Streptococcus sp.</i>

M: motility, Ca: catalase, U: urease, I: indole, Ci: citrate

Table 4. Sugar Fermentation Test of the Bacterial Isolates

Isolates	Fru	Suc	Gal	Mal	Lac	Glu	Probable organism
1	AG	AG	AG	A	A	AG	<i>Pseudomonas aeruginosa</i>
2	A	A	A	A	A	AG	<i>Proteus</i> sp.
3	A	A	A	A	A	AG	<i>Bacillus</i> sp.
4	-	AG	-	-	AG	AG	<i>Escherichia coli</i>
5	AG	AG	AG	A	A	AG	<i>Staphylococcus aureus</i>
6	AG	A	A	A	A	AG	<i>Streptococcus</i> sp.

Fru: fructose, Suc: sucrose, Gal: galactose, Mal: maltose, Lac: lactose, Glu: glucose, A: acid, G: gas, -: negative

The fungi isolated from the laboratories include *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., and *Rhizopus* sp. The most frequent isolated fungi were *Aspergillus* and *Penicillium* sp (Figure 1B). Table 5 shows the microscopic and biochemical tests for the identification of the fungal isolates and the probable fungal organisms. The results showed the probable fungal isolates were *Fusarium* sp., *Aspergillus* sp., *Rhizopus* sp., and *Penicillium* sp.

Table 5. Morphology and biochemical test of the fungal isolates

Isolates	Color	Appearance	Color Underside	Microscopic Appearance	Probable Organisms
1	Bluish/green	Powdery	White	Septate, hyaline, conidia-singled-celled and round with smooth walls in chains.	<i>Penicillium</i> sp.
2	Brown	Powdery	White	Broad hyphae, scarcely septate, round sp.	<i>Rhizopus</i> sp.
3	Green brown	Dry	White and brown	Conidiophores end with a sac-like structure. Phialides are attached to this sac-like structures and conidia are attached to phialides in chains.	<i>Aspergillus</i> sp.
4	Orange	Wooly	Orange	Septate, hyaline. Phialides are long, cylindrical and branched. Microconidia; single-celled macroconidia-curved foot cell at the base	<i>Fusarium</i> sp.

Discussion

In this study, the most frequently isolated bacteria were *Staphylococcus aureus* and *Bacillus* sp. (Figure 1A). These airborne micro floras were obtained using similar methods to those used by Ekhaie et al. (2010), who reported the isolation of bacterial isolates, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Bacillus* sp., *Proteus mirabilis*, and *Streptococcus* sp., with *Staphylococcus aureus* being the most common bacterial (Ekhaie et al., 2010).

Skin, deeper tissue, and organ infections have been linked to *Staphylococcus aureus*. Numerous causes may have contributed to the atmospheric presence of these isolated microorganisms. These include the typical student vegetation, staff, and student

clothing, guests, and supplies. Activities by staff and students, such as coughing, talking, yawning, etc. (Ekhaïse et al., 2008).

The soil is the principal habitat for the *Bacillus* species, which are widely scattered throughout the environment. This bacteria causes systemic infections, deep-seated soft tissue infections, localized infections associated with trauma, and food poisoning (e.g. meningitis, endocarditis, etc.) (Barrie et al., 1994). *Staphylococcus aureus* causes numerous infections at various sites of the body, some of which include: skin infections, infections of surgical and trauma wounds, food poisoning, and gastrointestinal tract infections that may be caused by consuming food contaminated with *Staphylococcus aureus*. Infections can also affect intravascular devices such as artificial heart valves and shunts, but they also frequently affect prosthetic joints, catheters, and major wounds.

Aspergillus species, *Penicillium* species, *Fusarium* species, and *Rhizopus* species are among the fungi that have been isolated from the labs. The two most frequently isolated fungal isolates in this study were *Aspergillus* and *Penicillium* sp (Figure 1B). For healthy people, *Aspergillus* might be tolerated, but for people who are at high risk, it might be harmful. The spores easily enter the airways and may cause Aspergillosis in humans with weakened immune systems (Gangneux, 2004). The morphological and biochemical procedures for identifying fungi isolates and likely fungi are shown in Table 5. This table displays their diverse responses to the reagents. Additionally, the table listed the likely fungi isolates, which included *Fusarium* species, *Aspergillus* species, *Rhizopus* species, and *Penicillium* species.

It is well known that temperature and relative humidity play crucial roles in the production, release, and dispersal of fungal spores. This is especially true in indoor environments, where a dry atmosphere and high temperatures during dry seasons affect the movement of airborne microbial particles and provide evidence for the presence of a variety of fungal species during the time period. With the exception of *Penicillium* species, all isolated bacteria and fungi are potential pathogens that might spread disease (Adediran et al., 2003). Moulds are present everywhere in the biosphere, and their spores are frequently found in the dust in homes and workplaces. When mould spore concentrations are extremely high, they can pose very dangerous health concerns to people, including allergic reactions or mycotoxin poisoning (Dallongeville et al., 2015) or causing fungal infection (mycosis). *Aspergillus niger* infects a person's lungs and grows, eventually forming a fungus ball in the lungs. Hearing issues and even hearing loss are side effects of *Aspergillus niger*.

From the current study, indoor air contains pathogenic and non-pathogenic microorganisms. Therefore, good hygiene should be maintained in the laboratory to reduce the prevalence of these microorganisms (Rosiello et al., 2021).

Conclusion

In conclusion, the findings of this study strongly imply that, regardless of the season, indoor environments permit the build-up of aerosols that may result in illnesses, contaminate testing equipment, and produce erroneous results. Population of students in a laboratory at a time may also increase the proliferation of airborne contaminants. Proliferation of airborne contaminants in the laboratories could also be influenced by poor and deficient hygiene conditions, also a low degree of cleanliness, and minimal disinfection procedures used against airborne contaminants. Improper activities of the staff and students such as working without taking precautions could also contribute to the air contaminants. The use of air filters should be encouraged to promote less contaminated air by sterilization. It is advisable that strict measures should be put in place to check the increasing microbial

loads in the laboratories and all activities in the laboratory must be performed with precautions and according to standards.

Authors' contributions

EAS, NCM, UIO, ECC, and OO conceived the idea, EAS, NCM, UIO, ECC, and OO retrieved the data, wrote and reviewed the manuscript. All authors approved the final version of the document.

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Conflict of interest

All authors declare no conflict of interest.

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