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Isolation and Identification of Air Microflora from Microbiology Laboratory of Adamawa State University Mubi, Nigeria

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***Abstract* - This study investigated the isolation and identification of airborne microflora in the microbiology laboratories of Adamawa State University, Mubi, Nigeria, using the settle plate method. Air samples were exposed on nutrient agar and Sabouraud dextrose agar plates at intervals of 30, 60, and 90 minutes, followed by incubation, colony counting, and microbial characterization through morphological, microscopic, and biochemical techniques. Results showed a progressive increase in both bacterial and fungal loads with longer exposure times, with the highest counts recorded at 90 minutes. Six bacterial species were identified: Streptococcus spp., Bacillus spp., Pseudomonas spp., Escherichia coli, Staphylococcus spp., and Micrococcus spp., of which Bacillus spp. and Staphylococcus spp. were the most prevalent (22.2% each). Five fungal species were also isolated, including *Penicilliumchrysogenum, Aspergillusniger, Fusarium spp., Rhizopus spp.*, and *Aspergillusflavus*, with *Aspergillus* species predominating (53.4%). The findings highlight that microbiology laboratories harbor diverse airborne microorganisms, some of which are opportunistic pathogens that pose risks of contamination and laboratory-acquired infections. This underscores the importance of strict biosafety practices, regular air quality monitoring, and effective environmental control measures to ensure research integrity and safeguard laboratory personnel.**

Keywords: Isolation, Microflora, Identification, Microbiology.

I. INTRODUCTION

Air is a mixture of many gases and tiny dust particles. It is the clear gas in which living things or living organisms live and breathe [1]. It has an indefinite shape and volume. It has a mass and weight, because it is matter. Air is a mixture of Nitrogen, Oxygen, Argon, Carbon dioxide, and moisture. There is an average of about 1% water vapor [2]. Air is however invisible to man's natural eye though a shimmering in hot air can be seen. There are also cells and spores of bacteria, fungi, algae, viruses and protozoa in the air. If air is exposed to sunlight, it has a higher temperature and less moisture. So, if not protected from desiccation most of its microbial forms will die [3]. Air serves as transport or dispersal medium for microorganism, they occur in relatively small number in air when compared with soil or water. Microorganisms are found almost everywhere, and their presence in the air was established by [4]. Microorganisms are found in both indoor and outdoor environments, people spend most of their lives indoors: in houses, industries, offices, colleges, schools, hospitals etc., where they are exposed to many bio aerosols (biological air borne contaminants such as bacteria, viruses, fungi or their by-products). Exposure to these airborne particles can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumon it is and toxic reactions [5].

In addition, longterm contact of people with bio aerosols can influence a person's mental power and learning ability [5]. Despite the critical roles of air types in ensuring safety and efficiency in microbiology laboratories, there is limited documentation on the nature and reliability of airborne microbial contamination in microbiology laboratories in Adamawa State University, Mubi. The lack of data creates a gap in infection control strategies and contamination prevention. This study aims to fill that gap by identifying and isolating air micro flora in microbiology laboratory and assessing the contamination level, identifying potential health hazards, and proper effective control measures. Some bacteria, particularly Gram-positive bacteria, fungi with spores, and viruses can survive for months in dust particles prevalent in the indoor environment [6]. Some indoor microbes may be hazardous, secreting poisonous compounds that cause allergies and even fatal disorders [7]. 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 [8]. 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

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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 [8].

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 [9]. This study focuses on the isolation and identification of airborne microorganisms in microbiology laboratory within Adamawa state University, Mubi. It is limited in scope to samples collected exclusively from this laboratory, using conventional microbiological techniques such as culturing, staining, and microscopic observation for microbial identification. The study does not incorporate molecular or advanced diagnostic methods. Limitations include its restriction to a single location, which may not represent conditions in other environments; the time frame of the study, which may not capture seasonal variations; and the exclusive use of culture-based methods, which may fail to detect non-culturable or fastidious organisms.

II. MATERIALS AND METHODS

2.1 Collection Bacterial Isolation

For bacterial isolation, nine (9) Nutrient Agar (NA) plates were prepared, each containing 20 mL of medium, making a total volume of 180 ml. These plates were exposed at different points in the microbiology laboratory for three durations: three (3) plates for 30 minutes, three (3) plates for 60 minutes, and three (3) plates for 90 minutes, to allow airborne bacteria to settle. According to the manufacturer's formulation (2.8 g of NA powder per 100 mL of water), 5.04 g of NA powder was required to prepare the 180 mL used for the plates.

2.2 Subculturing on Nutrient Agar (NA)

After the initial bacterial growth on Nutrient Agar, colonies were sub-cultured onto nine (9) Nutrient Agar plates. Each plate was prepared with 20 mL of medium, amounting to a total of 180 mL. Using the recommended composition of 5.2 g per 100 mL, 9.36 g of NA powder was measured to obtain the 180 mL required for the experiment [10].

2.3 Fungal Isolation (Sabouraud Dextrose Agar – SDA)

Nine (9) Sabouraud Dextrose Agar (SDA) plates were prepared, each containing 20 mL of medium, resulting in a total volume of 180 mL. These plates were exposed to air in the laboratory at three time intervals: three (3) plates for 30 minutes, three (3) plates for 60 minutes,and three (3) plates for 90 minutes, to capture airborne fungal spores. After exposure, the plates were incubated to support the growth of yeasts and molds. Following the preparation guideline of 6.5 g per 100 mL, 11.7 g of SDA powder was weighed to prepare the 180 mL required [10].

2.4 Settling Plate Method

The settling plate method involved the passive exposure of open Petri dishes containing Nutrient Agar (NA) to the surrounding air, allowing microorganisms to settle by gravity. Plates were positioned at designated locations in the microbiology laboratory and left uncovered for 30 minutes, 60 minutes, and 90 minutes, with three plates assigned to each exposure duration. After the specified time, the plates were immediately covered to prevent further contamination and transferred for incubation. Bacterial plates were incubated at 37 °C for 24–48 hours, while fungal plates were incubated at 25–28 °C for 3–5 days [10].

2.5 Analysis of the Sample

After air sampling, agar plates containing the collected samples were incubated at specific temperatures to encourage microbial growth. Bacterial samples were maintained at 35–37°C for 24–48 hours, while fungal samples were incubated at 25–28°C for 3–5 days. Following incubation, the plates were examined for colony growth, and colony-forming units (CFUs) were counted to estimate the microbial load in the sampled air. To ensure the accuracy of identification, isolated colonies were sub-cultured on fresh agar plates to obtain pure cultures. These purified samples then underwent microscopic, biochemical, and morphological analyses to determine the specific types of microorganisms present.

2.6 Incubation and Colony Counting

Once air sampling was completed, agar plates were placed in incubators under appropriate conditions for microbial growth. Bacterial cultures were incubated at 35–37°C for 24–48 hours, while fungal cultures were maintained at 25–28°C for a period of 3–5 days. After incubation, the growth of microorganisms was assessed by counting CFUs, which helped estimate the concentration of airborne microorganisms in the sampled environment. This quantitative data provided insights into the microbial contamination levels in different locations and conditions [11].

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2.7 Isolation of microorganisms

To obtain pure cultures for further examination, microbial colonies were carefully isolated using the streak plate method. Bacterial isolates were transferred to selective or differential media such as Nutrient Agar to aid in species identification. Fungal isolates were sub-cultured onto Sabouraud Dextrose Agar (SDA), a medium specifically designed to support fungal growth. The isolation process was repeated as necessary to ensure the purity of the microbial cultures before further analysis [11].

2.8 Gram Staining Procedure

A bacterial smear was prepared on a clean glass slide and heat-fixed by gently passing it over a flame or using a fixative. Crystal violet stain was applied to the smear and left for 1–2 minutes, after which the slide was rinsed with water to remove excess stain. Gram's iodine was then applied for 1 minute to fix the primary stain, followed by another rinse with water. A decolorizer, such as ethanol or acetone, was applied for 10–30 seconds to remove the stain from Gram-negative cells, and the slide was rinsed again. A counterstain, usually safranin, was applied for 1–2 minutes before a final rinse. The slide was then observed under a microscope to determine whether the bacteria were Gram-positive (purple) or Gram-negative (pink) [11].

2.9 Catalase Test Procedure

To test for the presence of the catalase enzyme, a bacterial smear was placed on a clean glass slide, and a drop of 3% hydrogen peroxide was added. The formation of bubbles indicated a positive result, demonstrating the breakdown of hydrogen peroxide into water and oxygen by the catalase enzyme. The absence of bubbles indicated a negative result [12].

2.10 Oxidase Test Procedure

For the oxidase test, a smear of the bacterial isolate was made on a clean glass slide or filter paper. A drop of oxidase reagent (such as tetramethyl-p-phenylenediamine) was applied to the smear. The presence of the oxidase enzyme was confirmed by a rapid color change—typically to dark purple or pink—within a few seconds. A positive result was indicated by this color change, while no change suggested a negative result [12].

2.11 Indole Test Procedure

The indole test was performed by inoculating bacterial isolates into tryptone broth and incubating them at 37 °C for 24–48 hours. After incubation, a few drops of Kovac's reagent were added. The appearance of a red or pink layer at the top indicated a positive result, while no color change indicated a negative result [12].

2.12 Citrate Test Procedure

The citrate test involved inoculating isolates onto Simmons citrate agar slants, which were then incubated at 37 °C for 24–48 hours. A positive result was indicated by bacterial growth with a color change of the medium from green to blue, while a negative result showed no growth and no color change [12].

2.13 Triple Sugar Iron (TSI) Reaction

The TSI test was conducted by inoculating isolates into TSI agar by stabbing the butt and streaking the slant, followed by incubation at 37 °C for 24 hours. Acid production was shown by a yellow color in the butt or slant, an alkaline reaction by a red color, gas production by cracks or bubbles in the medium, and hydrogen sulfide (H_2S) production by blackening of the butt [12].

2.14 Fungal Identification

Fungal species were identified using a combination of morphological and staining techniques. Colony morphology on agar, including color, growth rate, and texture, was assessed. Lactophenol Cotton Blue staining was performed to visualize spores and hyphal structures under a microscope. The arrangement and shape of fungal spores further aided in identifying specific fungal species [12].

III. RESULTS AND DISCUSSIONS

Table 1 presents the bacterial colony counts obtained from Microbiology Lab I over three exposure periods (30, 60, and 90 minutes) using the settle plate method. The results indicate a progressive increase in bacterial load with time . At 30 minutes, a total of 210 colonies were recorded across six plates, with a mean ($\bar{x}$) of 105.0 ± 25.5, accounting for 25.15% of the total bacterial count. The count increased to 277 colonies at 60 minutes (138.5 ± 41.7, 33.17%), and peaked at 90 minutes with 348 colonies (174.0 ± 50.9, 41.68%). This trend demonstrates that airborne bacterial deposition in the laboratory environment is time-dependent, with longer exposure allowing more bacterial cells to settle on agar surfaces [13]. The total bacterial load across all time points was 835 colonies, indicating a relatively high microbial presence, likely influenced by human activity, ventilation, and laboratory operations.

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Table 1: Colony Count for Bacteria in Microbiology, using the Settle Plate Method (Lab 1)

Time (min)	No. of Plates	Total Count	x̄ ± SD	% Count
30	6	210	105.0 ± 25.5	25.15
60	6	277	138.5 ± 41.7	33.17
90	6	348	174.0 ± 50.9	41.68
Total	18	835	139.2 ± 41.1	100

Table 2 shows the bacterial colony distribution in Microbiology Lab II also displayed a temporal increase similar to Lab I. At 30 minutes, 193 colonies were observed (96.5 ± 12.0, 21.05%), rising to 316 colonies at 60 minutes (158.0 ± 7.1, 34.46%), and 413 colonies at 90 minutes (206.5 ± 3.5, 45.04%). The total count was 917, slightly higher than that in Lab I, suggesting that Lab II may have a higher level of microbial contamination. The lower standard deviation values at 60 and 90 minutes indicate more consistent deposition patterns across replicate plates. The findings highlight that both laboratories exhibit increased bacterial accumulation with exposure time, emphasizing the importance of routine monitoring and environmental hygiene in microbiology laboratories.

Table 2: Colony Count for Bacteria in Microbiology Lab II, using the Settle Plate Method

Time (min)	No. of Plates	Total Count	x̄ ± SD	% Count
30	6	193	96.5± 12.0	21.05
60	6	316	158.0 ± 7.1	34.46
90	6	413	206.5 ± 3.5	45.04
Total	18	917	206.5 ± 3.5	100

Table 3 shows the fungal colony counts in Lab I also increased over time, reflecting similar deposition dynamics as bacterial colonies. At 30 minutes, 107 fungal colonies were recorded (53.5 ± 2.1, 21.06%), followed by 182 colonies at 60 minutes (91.0 ± 11.3, 35.83%), and 219 colonies at 90 minutes (109.5 ± 26.2, 43.11%). The total fungal count across all exposure periods was 508 colonies. These results indicate that airborne fungi are prevalent in the laboratory environment and that their deposition on agar plates increases proportionally with exposure time [13]. The relatively higher standard deviation at 90 minutes suggests variable fungal dispersal patterns, possibly due to sporadic release from fungal structures or differences in airflow.

Table 3: Colony Count of Fungi in Microbiology Lab I

Time (min)	No. of Plates	Total Count	Ẋ ± SD	% Count
30	6	107	53.5 ± 2.1	21.06
60	6	182	91.0 ± 11.3	35.83
90	6	219	109.5 ± 26.2	43.11
Total	18	508	84.7 ± 28.5	100

Table 4 show in Lab II, fungal counts were consistently higher than Lab I. Initial exposure at 30 minutes yielded 167 colonies (83.5 ± 23.3, 22.66%), which increased to 239 colonies at 60 minutes (119.5 ± 17.7, 32.43%), and 331 colonies at 90 minutes (165.5 ± 33.2, 44.91%). The total fungal count was 737. These observations suggest that Lab II not only supports higher bacterial loads but also exhibits elevated fungal deposition, likely due to factors such as greater airflow, room occupancy, or the presence of nutrient sources that favor fungal survival [13]. The results underscore the importance of environmental control measures to reduce fungal exposure in laboratory settings.

Table 4: Fungal Colony Count in Microbiology Lab II

Time (min)	No. of Plates	Total Count	Total Count	% Count
30	6	167	83.5 ± 23.3	22.66
60	6	239	119.5 ± 17.7	32.43
90	6	331	165.5 ± 33.2	44.91
Total	18	737	122.8 ± 41.8	100

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Table 5 summarizes the morphological, cultural, and biochemical properties of six bacterial isolates recovered from both laboratories. The isolates were identified based on colony morphology, Gram staining, and standard biochemical tests. Notably, Streptococcus spp. displayed small, smooth colonies with cocci in chains, catalase-negative, consistent with its genus characteristics. Bacillus spp. appeared as large, rough colonies with rod-shaped cells and catalase-positive reactions, while Pseudomonas spp. were Gram-negative rods with blue-green pigmentation and oxidase-positive results. Escherichia coliwas identified as smooth Gram-negative rods showing citrate utilization and acid production (A/A). Staphylococcus spp. presented as golden cocci in clusters, catalase-positive, and Micrococcus spp. were yellow-pigmented tetrads with catalase activity. The comprehensive biochemical profiling confirms the presence of both Gram-positive and Gram-negative airborne bacteria, reflecting the microbial diversity in the laboratory environment.

Table 5: Morphological, Cultural and Biochemical Characteristics of Isolated Airborne Bacteria in Adamawa State University, Mubi Microbiology Lab I and II by Settle Plate Method

Isolates	Cultural Characteristics	Cell Shape	Gram Reaction	Catalase	Oxidase	Indole	Citrate	S/B	Possible Bacterial Species
Isolate 1	Small, round, smooth colonies	Cocci in chains and pairs	+	-	-	-	-	**No Change**	*Streptococcus spp.*
Isolate 2	Large, irregular, rough colonies	Rod in chains	+	-	-	-	-	**K/A**	*Bacillus spp.*
Isolate 3	Irregular colonies with blue-green pigmentation	Gram-negative rods	-	+	+	-	+	**K/A**	*Pseudomonas spp.*
Isolate 4	Smooth, round colonies	Gram-negative rods	-	+	-	+	-	**A/A**	*Escherichia coli*
Isolate 5	Small, round, golden colonies	Cocci in clusters	+	+	-	-	-	**A/A**	*Staphylococcus spp.*
Isolate 6	Small, round, smooth colonies, yellow pigmented	Cocci in tetrads and clusters	+	+	+	-	-	**K/K**	*Micrococcus spp.*

Key: S/B = Slant/Butt

The fungal isolates were identified based on colony morphology on Sabouraud Dextrose Agar (SDA) and microscopic features. Penicilliumchrysogenum formed green, powdery colonies with branched conidiophores. Aspergillusniger showed dark brown, concentric colonies with globose vesicles. Fusarium spp. produced pink, cottony colonies with septate hyphae and banana-shaped macroconidia. Rhizopus displayed white cottony colonies with broad aseptate hyphae and sporangiophores, while Aspergillusflavus formed yellow-green colonies with characteristic conidial heads [13]. These results indicate that common airborne fungi in the laboratories belong predominantly to genera known for environmental ubiquity and occasional opportunistic pathogenicity.

Table 6: Morphological and Microscopic Characteristics of Isolated Airborne Fungi in Adamawa State University, Mubi Microbiology Lab I and II

Isolates	Morphology on SDA	Microscopic Characteristics	Suspected Fungi
A	Green colonies Appearance: greenish colonies with powdery texture and white edges	Conidiophores are branched (brush-like), bearing chain of green colonies	*Penicilliumchrysogenum*
B	Dark brown colonies Appearance: concentric rings with powdery texture	Conidiophores terminating in globose vesicle with radiating chains of black conidia	*Aspergillusniger*
C	Pink colonies Appearance: cottony and powdery, pale to bright pink	Septate hyphae, macroconidia that are banana-shaped or sickle-shaped	*Fusarium spp.*
D	White cottony colonies Appearance: fast-growing colonies with cottony and woolly texture	Broad, aseptate hyphae with sporangiophores and sporangia	*Rhizopus*
E	Yellow colonies Appearance: powdery	Conidiophores with radiating conidia,	*Aspergillusflavus*

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	yellow-green colonies	terminating in swollen vesicle	

SDA = Sabouraud Dextrose Agar.

IV. CONCLUSION

This study investigated airborne microbial contaminants in the microbiology laboratories of Adamawa State University, Mubi, using the settle plate method. The results demonstrated a progressive increase in both bacterial and fungal colony counts with longer exposure times (30, 60, and 90 minutes). Statistical analysis revealed significant differences in microbial density across exposure times, with the highest counts recorded at 90 minutes. Six bacterial species were isolated: Streptococcus spp., Bacillus spp., Pseudomonas spp., Escherichia coli, Staphylococcus spp., and Micrococcus spp. Among these, Bacillus spp. and Staphylococcus spp. were the most frequently encountered each representing 22.2% of isolates. Five fungal species were also identified, namely *Penicilliumchrysogenum, Aspergillusniger, Fusarium spp., Rhizopus spp.,* and *Aspergillusflavus.* The genus *Aspergillus* was the most prevalent, accounting for 53.4% of the fungal isolates. The study concludes that the microbiology laboratories of Adamawa State University, Mubi, contain a wide range of airborne microorganisms, including both opportunistic and potentially pathogenic species. The steady increase in microbial load with longer exposure times confirms that extended exposure elevates the risk of contamination. The dominance of *Bacillus spp., Staphylococcus spp.,* and *Aspergillus spp*. highlights their adaptability and persistence in indoor laboratory environments.

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