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Evaluation of the Diversity and Antifungal Sensitivity Profile of Aeroterrestrial Fungi around a Health Care Facility

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ABSTRACT

Air and soil surrounding healthcare facilities are potential reservoirs of diverse fungi, some of which can have the potential to cause various kinds of mycoses. Some may harbor antifungal resistance genes (ARGs) which can pose therapeutic difficulty. This study evaluated the diversity of aerroterrestrial fungi around a health care facility in Port Harcourt and their antifungal resistance. Seventy-two (72) air and soil samples were collected over a period of twelve calendar months from July 2021 to June 2022. The isolated and identified fungal species were *Acremonium radiatum, Aphanoascus flavascens, Chrysosporium tropicum, Colletotrichum fructicola, Fusarium solani, Microsporium canic, Penicillium aurantiogriseum, Peniciliium vanluykii, Phaeacremonium aleophilum, Scedosporium aurantiacum, Syncephalastrum racemosum, and Trichosporon asahii.* The most abundant genus was *Penicillium*. Diversity indices revealed a rich and varied fungal community associated with the health facility, all of which demonstrated resistance to one or more antifungal drugs used for the study. *Trihosporon asahii* demonstrated multidrug resistance to all tested antifungal drugs, namely Ketoconazole, Nystatin, Fluconazole and Griseofulvin. All other fungi isolates were susceptible to Ketoconazole and Fluconazole, with intermediate susceptibility to Nystatin. Thus, the drugs of choice recommended for treatment are Ketoconazole and Fluconazole. The observation of several antifungal resistant fungi suggests the potential of the isolates harboring antifungal resistance genes in the environment. These findings highlight the importance and potential impact of aeroterrestrial fungi on human health, and a guide for future public health strategies for infection control, antifungal therapy, and the development of targeted interventions to mitigate the risk of mycoses in health care facilities.

Keywords: Healthcare Facilities, Air, Soil, *Penicillium*, Fungi, Antifungal Resistance, *Trichosporon asahii.*

Introduction

Air and soil surrounding healthcare facilities are potential reservoirs of diverse fungi, some of which can have the potential to cause various kinds of fungal diseases. Some may harbor antifungal resistance genes (ARGs) which can pose extreme therapeutic difficulties (Garvey *et al*., 2022). The diversity of aeroterrestrial fungi encompasses a wide range of multifunctional roles, allowing these fungi to adapt to different environmental conditions and participate in various ecological processes, including pathogenicity, industrial applications, national development, and public health (Disegha *et al*., 2024).

Antifungal resistance has emerged as a significant public health concern, complicating the treatment of fungal infections and leading to increased morbidity and mortality rates (Vitiello *et al*., 2023).

We aim to provide insights that could inform treatment protocols and guide future research in the field of mycology.

Fungi are eukaryotic, non-photosynthetic micro-organisms, with a wide range of members, including yeasts, rusts, smuts, mildews, molds, and mushrooms (Moore, 2019; Alexopoulos, 2023). Fungi are ubiquitous and can be found in various ecological settings. Some fungi are beneficial, while others pose significant threats to public health in both indoor and outdoor environments (Khan and Karuppayil, 2012; Dellagi *et al*., 2020).

Soil serves as a complex and nutrient-rich environment that supports the growth and proliferation of fungi and various other organisms (Jacoby *et al*., 2017).

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Fungi demonstrate significant phenotypic plasticity, which allows them to dynamically modify their morphological and physiological traits in response to external stressors or adverse environmental conditions (Alster *et al*., 2021). This pronounced adaptability facilitates their survival across a wide variety of soil environments. Fungi synthesize a diverse range of extracellular enzymes that break down complex organic matter in the soil, releasing essential nutrients such as carbon for metabolism. This decomposition is crucial for regulating nutrient balance and cycling within the soil ecosystem (Kües, 2015).

The atmosphere contains dust, spores, moisture, and various inorganic particles, with fungal spores being a significant component. Airborne fungi are the primary cause of fungal infections in humans, plants, and animals (Khan and Karuppayil, 2012). Outdoor air significantly influences the presence, prevalence, and rapid distribution of fungal spores in indoor environments, including educational institutions, offices, laboratories, auditoriums, hospitals, conference halls, greenhouses, mushroom farms, and post-harvest storage facilities. Consequently, outdoor air serves as a major source of fungal contamination and infections in these settings (Masoomeh *et al*., 2014; Knogge, 1996).

In health center environments, a wide range of activities occurs, which, when coupled with inadequate maintenance, poor building designs, and vehicular traffic, can lead to adverse health outcomes, including Sick Building Syndrome (SBS) (Khan and Karuppayil, 2012). SBS is characterized by a collection of non-specific health and comfort symptoms experienced by occupants, particularly in healthcare settings where air quality is critical. The etiology of SBS in health centers involves several factors, including fungal infestations on damp walls, fabrics, and flooring, which contribute to degraded indoor air quality.

Furthermore, psychological factors such as stress, along with physiological factors related to individual susceptibility, may intensify these symptoms. Common presentations include headaches, fatigue, and difficulty concentrating, with symptoms typically improving upon leaving the affected space.

Effective diagnosis and management of SBS in health centers require a multifaceted approach that addresses air quality issues, implements antifungal treatments for affected surfaces, considers psychological impacts, and attends to the unique needs of individuals in these environments (Nag, 2018).

Fungi contribute to biogeochemical recycling, biotransformation, bioremediation, and the removal of pollutants, which supports sustainable development (Obire *et al*., 2008; Ataikiru *et al*., 2018; Ajar *et al*., 2022). However, in health center environments, fungi pose several dangers. They can cause opportunistic infections, particularly in immunocompromised patients, leading to serious health complications. Fungal spores may trigger allergic reactions and respiratory issues in sensitive individuals, impacting both patients and healthcare workers (Low and Rotstein, 2011). Additionally, fungi can contaminate medical equipment, surfaces, and air quality, jeopardizing infection control and overall hygiene. The rise of antifungal-resistant strains further complicates the treatment of fungal infections, presenting significant challenges in patient care and management (Kohler *et al*., 2015; Konopka *et al*., 2019).

Reports concerning the populations, physiology, diversity, and diseases associated with aeroterrestrial fungi have raised significant public health concerns, particularly in health center environments (Köhler *et al*., 2015; Disegha and Nrior, 2021). Although some studies have been reported, specific issues related to the mycological profile of the health centre remain inadequately explored. This research aims to address this knowledge gap and contribute to epidemiological investigations pertinent to public health. It will focus on the dynamics, ecological relationships, and geographical distribution of aeroterrestrial fungi in relation to their environmental contexts around health centers (Disegha and Nrior, 2021).

An extensive study of fungi within health center environments, particularly at Rivers State University, has not been conducted, especially regarding their georeferenced spatial locations. This study aims to fill this gap by mapping the fungal profile present in the air and soil around health care facility.

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The study investigates the physiological and nutritional diversity of air and soil fungi in health care facility environment, which is vital for understanding health implications. It covers various fungal categories, including pathogenic fungi that pose health risks. The research will also examine entomopathogenic, mycoparasitic, saprophytic, toxigenic, edible, and poisonous fungi, as well as antibiotic-producing fungi like dermatophytes.

By characterizing these fungal groups and their distributions, the study aims to provide insights into public health risks and inform management strategies for fungal exposure. It will serve as a baseline survey, offering quantitative data on how anthropogenic activities affect the presence of pathogenic, opportunistic, and toxigenic fungi in air and soil.

The study aimed to assess the diversity of fungal species of aeroterrestrial environment around health facility and analyze the relationship between fungal counts. In addition, antifungal susceptibility testing was conducted using selected synthetic antifungal agents to evaluate the effectiveness of these treatments and identify antifungal resistance aeroterrestrial fungi and characterizing their profile by tabulation. Finally, statistical analysis will be performed on relevant subsections of the objectives to enhance interpretation.

Materials and Methods

Study Area

The Health Care Facility of Rivers State University in Port Harcourt was the study area. The inner surroundings of the University are best described as sandy soil, thinly covered in grass, and consistently groomed. The Health Care Facility GPS coordinates are. (4.7930224, 6.9816542. The Health Care Facility at Rivers State University plays a crucial role in the university's commitment to health education and community service, located on its campus in Port Harcourt, Nigeria. It provides healthcare services to students, staff, and the local community, focusing on preventive and therapeutic care. Services include outpatient consultations in general medicine, pediatrics, and women's health, as well as emergency care. The facility features an on-site pharmacy and comprehensive laboratory services, along with health education programs promoting healthy lifestyles. It also serves as a training ground for students in nursing, medicine, and public health, engaging in research that benefits both the academic and local communities. Outreach programs and partnerships with health organizations further enhance service delivery and community ties, aiming to improve health standards and foster health awareness.

Sample Collection

Soil samples were collected from six (6) designated substation in the campus Health Care Facility environment at Monthly intervals for a period of twelve (12) calendar months from July 2021 to June 2022, covering both the wet and dry seasons. The soil samples were collected with the aid of a sterile hand held auger from a depth of 10 to 15 cm. The samples were collected into sterile plastic zip-lock bags and transported to the laboratory for analysis. Samples from the 6 substations were mixed together to form a composite soil sample. A total of 12 composite soil samples (each weighing about 50 grams) were analyzed during the study.

Air samples were collected using sedimentation plated method using potato dextrose total agar (PDA), Sabauroud Dextrose agar (SDA) and Malt extract agar (MEA) agar. Sedimentation (Settle plates) using sterile Petri dishes were prepared and labeled appropriately. A total of 72 settle plates were used for monthly sampling. Six plates were used for each substation and each plate was labeled indicating media type, sample type, date, time, sampling location and plate number. All media and distilled water used were sterilized in an autoclave for 15 minutes. Pipettes and other glassware were sterilized in a hot-air oven at 160^{o}C. About 85% alcohol was used to sterilize laboratory benches (Brown *et al*., 2016).

Mycological analysis was performed at the Microbiology Laboratory, Rivers State University, Port Harcourt. The soil-dilution/spread-plate method was used to cultivate, enumerate, and isolate fungi from soil samples. A 10-gram soil sample was mixed with 90 ml of sterile distilled water in a 250 ml Erlenmeyer flask, creating a 1:10 dilution. Two additional ten-fold serial dilutions were prepared from this stock. A 0.1 ml aliquot of the 10^{-3} dilution was aseptically spread on duplicate plates, with ampicillin added to prevent bacterial growth.

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Air samples were collected using sedimentation plates, which were placed face up on a flat rack 1.5 meters high and 1 meter away from obstructions for 30 minutes. After exposure, the plates were covered and incubated inverted at 30°C for 2 to 5 days. Fungal colonies from both soil and air were enumerated, isolated into pure cultures, and identified based on morphological features and growth characteristics. Fungal counts were expressed in colony-forming units per gram (CFU/g) for soil and per minute per square meter (CFU/min/m²) for air (Al-Shaarani *et al.*, 2023).

Preparation of fungal isolates of fungal colonies from aeroterrestrial sources were isolated into pure cultures, and identified by observing their colonial characteristics by macroscopy and microscopy. Examination by macroscopy included observing morphological features such as surface topography, surface texture, pigmentation, and type of mycelium, medium of growth and pace of growth. Microscopy included observing distinctive microscopic structures after staining with lactophenol blue and viewing under light microscope using 40x objective magnification (Alsohaili and Bani-Hasan, 2018). The isolates were identified using. Koneman's colour atlas and textbook of diagnotistic microbiology (6th Ed.) (Koneman *et al.*, 1997) and were verified by using reverse image identification schemes (https://imagesift.com; https://yandex.com/images.

Determination of Physiological diversity of Fungi around the Health Facility

Physiological diversities of fungi were assessed by categorizing the fungal isolates into their functional groups based on already existing reports (Disegha *et al*., 2024).

Determination of soil quality was carried out by estimating physicochemical parameters such as temperature, pH, moisture content, water holding capacity, electrical conductivity, total organic carbon, soil organic matter, available nitrogen, available phosphorus, available potassium, magnesium, calcium, sulfate, and zinc were measured. Soil samples were air-dried, ground to fine particles, sieved through a 2mm mesh sieve, and subjected to physical and chemical analysis using standardized methods (Kekane *et al.*, 2015).

Antifungal Susceptibility Testing (AFST) and Identification of Resistant Fungi

Antifungal susceptibility testing was conducted using the modified method of Obire *et al.* (2020) with Ketoconazole (K), Nystatin (N), Fluconazole (F), and Griseofulvin (G). The disk diffusion assay was performed to assess fungal resistance on culture plates. Disks contained Nystatin (100 units), Fluconazole (10 µg/ml), Griseofulvin (10 µg/ml), and Ketoconazole (10 µg/ml). Inocula were prepared from five distinct spores (1 mm each) from 24-hour cultures grown on potato dextrose agar, incubated at 35°C. Spores were suspended in 5 ml of sterile 0.85% saline, vortexed, and adjusted to 1 x 10^6 to 5 x 10^6 cells/ml (0.5 McFarland standard).

A sterile cotton swab moistened with the suspension was used to inoculate mycological plates. After drying for 10 minutes, impregnated disks were placed on the media. Plates were incubated for 72 hours at 37°C, with slowly growing isolates incubated for an additional 96 hours. Zones of inhibition were measured in millimeters, and results were interpreted according to NCCLS (2020) criteria for Resistant (R), Intermediate (I), and Susceptible (S). Fungal species resistant to antifungal drugs were identified based on these results.

The drugs used for antifungal susceptibility testing included Ketoconazole 200 mg USP, Ketoconazole USP 200 mg; Nystatin 16 ; Fluconazole in 200 mg tablets; and Griseofulvin (G) as Griseofulvin 500 mg. The standard recommended zone diameters for antifungal susceptibility tests are given according to CLSI (2012) standard: a zone diameter of 14 mm or less indicates resistance (R), 15 to 18 mm indicates intermediate susceptibility (I), and 19 mm or greater indicates susceptibility (S).

Statistical Analysis of Field and Experimental Data

All data obtained from laboratory and field studies will be analyzed using GraphPad Prism Version 8. Data entry, structuring and transformations will be performed using MS Excel for Windows Version 2010. Analysis of Variance will be performed on quarterly populations.

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Results

Figure 1 shows the mean monthly fungal populations of air and soil fungi at the Health Centre environment. In the air, the mean fungal population ranged from 2.5 to 2.8 Log_{10} CFU/min.M^2. There was steady count values recorded throughout the sampling period from July 2021 to June 2022, with smooth fluctuations throughout the sampling period. The lowest and highest aerial fungal populations were observed in August 2021 and January 2022 respectively.

In the soil samples, the mean fungal population ranged from 5.0 in June to 6.3 Log_{10} CFU/g in March 2022. There was slight continuous decrease in soil fungal population from 5.6 Log_{10} CFU/g in July 2021 to 5.4 Log_{10} CFU/g in October 2021, which then increased to 6.5 in January 2022, with a sudden decline in population to 5.7 recorded in February 2022, and then a continuous fluctuation for the rest of the months of sampling period.

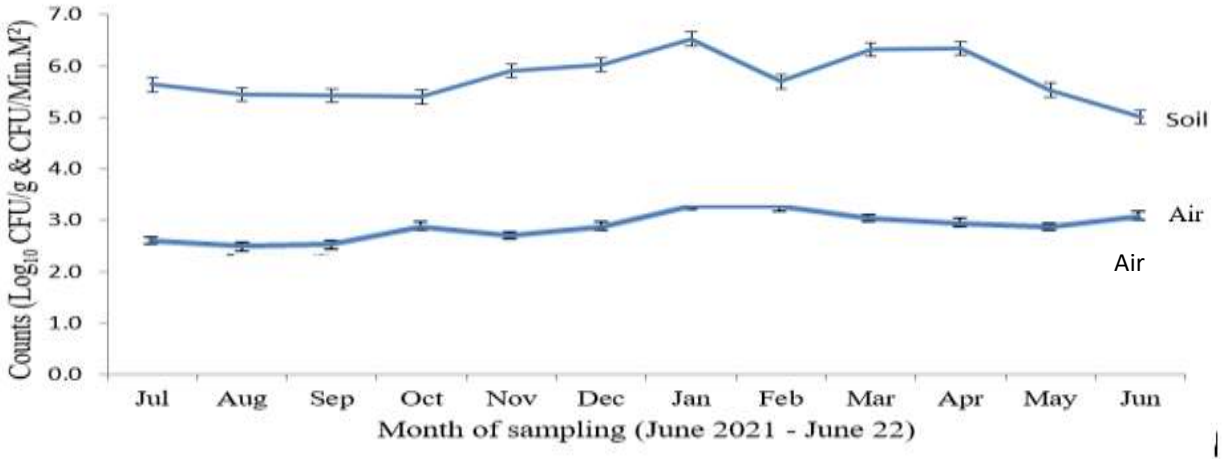

Figure 1: Monthly Populations of Soil and Air and Soil Fungi at Health Care Facility

Figure 2 shows mean seasonal counts of Air (Log_{10} CFU/min.M^2) and Soil (Log_{10} CFU/g) fungi at the Health Care Facility for Dry and Wet seasons during the sampling period.

In the air sampling, actual mean air fungal populations for Dry and Wet seasons were 2.75 (Log10 CFU/min.M^2 and 2.63 (Log10 CFU/min.M^2) respectively. Highest and lowest Air fungal populations were 2.9 (Log10 CFU/min.M^2) and 2.60 (Log_{10} CFU/min.M^2) respectively for Dry season while in Wet Season the highest population was 2.8 (Log10 CFU/min.M^2) and 2.4 (Log10 CFU/min.M^2) respectively. Fungal population in Dry season was signigicantly higher than that of the Wet season at $p < 0.05$.

In the soil samples, mean seasonal populations at the Health Care Facility during the sampling period, showed 5.14 Log10 CFU/g and 4.89 Log10 CFU/g) for dry and wet seasons respectively. Highest and lowest Soil fungal populations for Dry Season were 5.529 (Log10 CFU/g) and 4.69 Log_{10} CFU/g) respectively while in Wet Season the highest population was 5.63 (Log10 CFU/g) and 4.42 (Log10 CFU/g) respectively. There was no significant difference in soil fungal populations for Dry and Wet seasons at $p < 0.05$. There was very high signficance difference in fungal populations recorded for air and soil samples for both dry and wet seasns, which values were inconsequential due to different methods use for estimation.

In Figure 3, the highest air fungal population (2.82 Log_{10} CFU/min.M^2) was recorded in the first quarter (Q1) (January - March), followed by population in the second quarter (Q2) (2.71 Log_{10} CFU/min.M^2) (April – May) and the lowest fungal population was recorded in the fourth quarter (Q4)(2.56 Log_{10} CFU/min.M^2)(October-December). Results of mean separation of air fungal counts in Post Hoc analysis using Turkey test for quarterly means showed that there was no significant difference amongst the quarters of sampling at $P < 0.05$.

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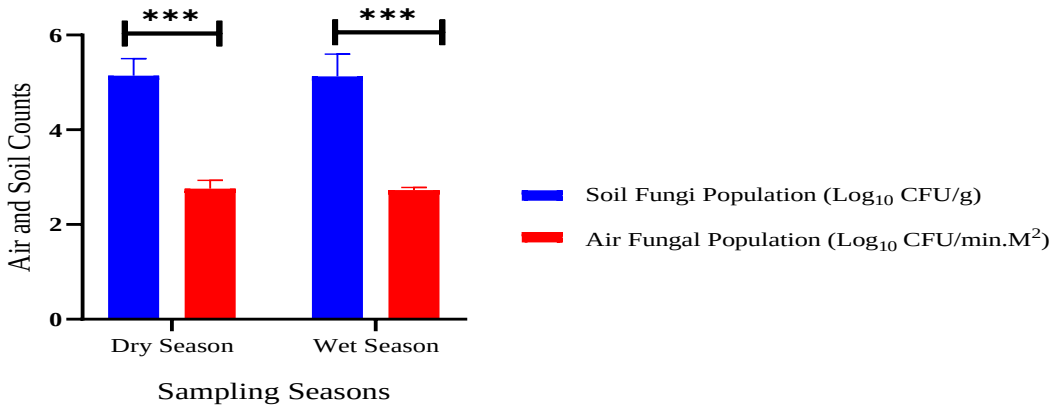

Figure 2: Mean of Seasonal Populations of Air (Log_{10} CFU/min.M^2) and Soil (Log_{10} CFU/g) Fungi

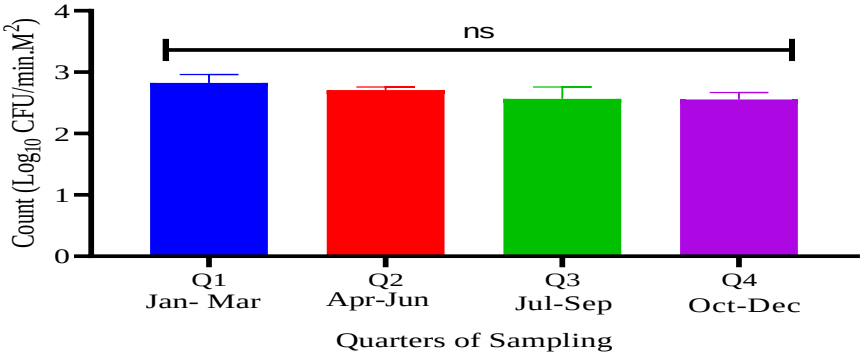

Figure 3: Quarterly Populations of Air Fungi (Log_{10} CFU/min.M^2) during the Sampling Period

In Figure 4, the highest soil fungal population (5.18 Log_{10} CFU/g) was recorded in the first quarter (Q1) (January - March), followed by population in the second quarter (Q2) (4.96 Log_{10} CFU/g) (April – May) and the lowest fungal population was recorded in the fourth quarter (Q4)(4.78 Log_{10} CFU/g)(October-December). Results of mean separation of soil fungal counts in Post Hoc analysis using Turkey test for quarterly means showed that there was no significant difference amongst the quarters of sampling at $P < 0.05$.

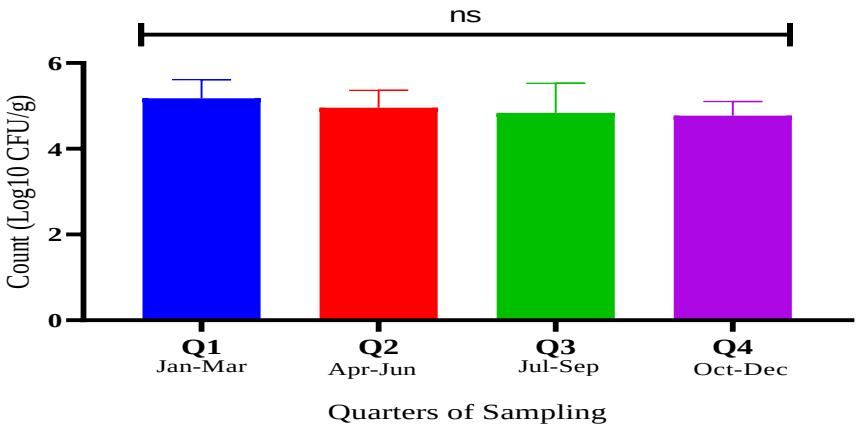

Figure 4: Multiple Comparison of Quarterly Populations of Soil Fungi during the Sampling Period

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Table 3 presents results obtained from mycological analyses of soil samples within the Health Care Facility at the Rivers State University Main Campus. Each result presentation is made with respect to contextual criteria as specified in the sections. Some aeroterrestrial fungi detected from the health care facility were observed culturally, morphologically and microscopically. These characteristics were used as bases for their phenotypic identification for the fungal isolates.

Table 3: Cultural and Morphological Characteristics of Fungi Isolated from around the Health Facility

SN	Macroscopy	Microscopy	Identity
1	A usually initially compact at first and wet, typically slow-growing, and have fine, hyaline hyphae that mostly produce simple phialides. Colonies appear less wooly or granular, may be white or various shades of light pasted green and yellow	Conidia are often aggregated in heads with a slimy tip, one-celled, globose to cylindrical, or elongated, hyaline or pigmented particles. Conidia mimic an eastern letter or "dipththeroidal" pattern by being lengthy and loosely clustered in a criss-cross pattern. A long, thin, delicate conidiophore that culminates in a blunt point gives birth to each cluster at its tip. Macroconida don't exist.	*Acremonium radiatum*
2	Range in color from white to cream, depending on the quantity of brown ascomata that are present on the surface, with aflat topology.	Have numerous, large club-shaped conidia with older colonies containing fruiting bodies, or ascomata, at their centres. These fruiting bodies are rather substantial.	*Aphanoascus flavascens*
3	Colonies have a flat, light- to medium-fast growth rate, white to tan to beige color, and frequently a powdery or granular surface texture. In age, reverse pigment may be absent or become pale brownish-yellow.	Conidia are visible under a microscope and have distinct basal scars and are abundant, hyaline, single-celled, clavate to pyriform, smooth, and slightly thick-walled. The tips of the hyphae, on short or long lateral branches, or sessile along the hyphae are where the conidia are generated (intercalary). No hyphal spirals or macroconidia are visible.	*Chrysosporium tropicum*
4	Dark pigmented Colonies with white aerial mycelium, consisting of numerous black sclerotia and light brown-coloured conidial masses, reverse is dark brown	Sclerotia are usually abundant, setose, spherical and are often confluent. Conidia are straight, fusiform, attenuated at the ends, Appressoria are common, clavate, brown, variable in shape.	*Colletotrichum fructicola*
5	The descriptions below are based on growth on potato flakes agar at 25°C with on/off fluorescent light cycles that last roughly 12 hours each. Macroscopic morphology may differ greatly on various medium. Rapid expansion. Colonies have a cream reverse, a cream to white Air mycelium, and a woolly to cottony appearance. Sporodochia, which are typically wet and cream-colored, are clusters of conidiogenous cells or conidia that can be seen as elevated regions with the naked eye. Sporodochia is seldom orange, but rarely it might be blue-green or blue.	They have hyaline and septate hyphae. Conidiophores are monophialides that are simple (non-branched) or branched (phialides with a single opening); 4-6 × up to 65 m long, moderately curved, robust, thick-walled, often 3-5 septate, macroconidia are borne on short conidiophores that quickly develop into sporodochia. Microconidia are one to three cells long, 2-5 x 8-16 m, and only found in false heads. They are produced by long monophialides (in clusters of conidia at the tip of the phialide). Chlamydoconidia are prevalent (sometimes profuse) and can be seen both alone and in pairs.	*Fusarium solani*

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6	**Table 3 Continued** Characterized by colonies that initially appear white to cream-colored, developing yellow or brownish pigmentation as they mature. The surface is fluffy or powdery, creating a hairy appearance due to mycelium growth. These colonies grow rapidly, often reaching several centimeters in diameter within a week, and typically have a circular shape with irregular edges.	Displays distinct microscopic features. The macroconidia are large, spindle-shaped, thick-walled, and often rough, typically found in chains and containing multiple septa. Smaller microconidia are generally globose or oval, produced singly along the hyphae, and smooth-walled, though less abundant. The mycelium consists of septate hyphae that are thick-walled, may appear branched, and can be either smooth or slightly rough in texture.	*Microsporum canis*
7	Its colonies typically display distinctive colors ranging from orange to grayish-green, depending on growth conditions and media. The surface is often velvety or powdery, with a dense, fluffy appearance due to abundant conidia. Colonies grow rapidly, often reaching several centimeters in diameter within a few days and usually exhibit a circular, filamentous growth pattern.	exhibits distinct microscopic features. Its conidiophores are upright, branched structures that may be septate and commonly bear conidia in a brush-like arrangement at their tips. The conidia themselves are typically smooth, varying in shape from globose to ellipsoidal, and often appear in chains. The mycelium consists of septate hyphae that are usually hyaline (transparent) and can be either smooth or slightly rough.	*Penicillium aurantiogriseum*
8	The colonies typically exhibit a green to bluish-green coloration, which may vary slightly based on environmental conditions. The surface is often velvety or powdery, creating a dense, fluffy appearance due to conidia production. Colonies grow rapidly, often reaching several centimeters in diameter within a few days, and generally have a circular shape with a filamentous growth form.	Exhibits distinct microscopic features. Its conidiophores are typically tall and branched, often septate, and bear conidia at their tips in a brush-like arrangement. The conidia themselves are usually smooth, varying in shape from globose to ellipsoidal, and often form chains during production. The mycelium consists of septate hyphae, which are generally hyaline (transparent) and may have either a smooth or slightly rough texture.	*Penicillium vanluykii*
9	characterized by colonies that appear dark brown to black, often with an olive or grayish hue. The surface is velvety or downy, reflecting dense mycelial growth. Colonies grow moderately slowly, typically reaching a few centimeters in diameter over several weeks, and exhibit a circular shape with irregular edges.	displays distinct microscopic features, including conidia that are hyaline to dark brown, oval to cylindrical, and may have smooth or slightly rough walls. The mycelium comprises septate hyphae that are darkly pigmented, thick-walled, and occasionally branched. Ascomatal structures may also be present, varying in shape and often embedded in the substrate.	*Phaeacremonium aleophilum*
10	Characterized by colonies that typically appear orange to yellowish-brown, often featuring a distinct bright orange center. The surface is generally velvety or fluffy, indicating a dense aerial mycelium. Colonies grow rapidly, often reaching several centimeters in diameter within a week, and usually exhibit a circular shape with a smooth to slightly irregular edge.	Exhibits distinct microscopic features, including oval to cylindrical conidia that are darkly pigmented and may have smooth or slightly rough surfaces, often produced in chains. The mycelium comprises septate hyphae that are typically hyaline to light brown, thin-walled, and may exhibit branching. Ascomatal structures can also be present, varying in shape and often embedded in the substrate.	*Scedosporium aurantiacum*

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Table 3 Continued

11	Characterized by colonies that typically appear white to grayish or cream-colored. The surface is fluffy or cottony with a velvety texture. Colonies grow rapidly, often reaching several centimeters in diameter, and exhibit a spreading growth pattern that may form a dense, mat-like structure. Additionally, branched sporangiophores are present, giving the colonies a distinctive clustered appearance.	Displays distinct microscopic features, including septate hyphae that are broad and irregular in width. The organism has branched sporangiophores that arise from the hyphae, often arranged in a racemose pattern. The sporangia are spherical to oval, typically large, and contain numerous spores, which are borne at the tips of the sporangiophores. The spores themselves are generally smooth-walled and can vary in size, usually appearing as small, round structures.	*Syncephalastrum racemosum*
12	Characterized by colonies that typically appear white to cream or pale yellow. The surface is smooth to slightly wrinkled, with a moist or creamy appearance. Colonies grow rapidly, often reaching several centimeters in diameter within a few days, and have a circular shape with a well-defined edge; they may also develop a powdery or granular texture over time.	Characterized by round to oval yeast cells measuring 3 to 10 micrometers in diameter. It may also display elongated pseudohyphae with constricted septa and form arthroconidia, which are rectangular cells resulting from hyphal fragmentation. Additionally, budding is common, with yeast cells showing a distinctive budding pattern.	*Trichosporon asahii*

Table 4 presents the physiological diversity of aeroterrestrial fungi identified at Health Facility location in the Rivers State University, Port Harcourt, focusing on 12 species across 11 genera. The fungi are categorized based on their physiological roles. Notably, *Acremonium radiatum* and *Fusarium solani* are classified as both pathogenic and saprophytic, indicating their ability to thrive on dead organic matter while also causing disease. Other species, such as *Colletotrichum fructicola*, are recognized as phytopathogenic, affecting plants directly. Dermatophytes, including *Chrysosporium tropicum* and *Trichosporon asahii*, are linked to skin infections. Additionally, *Penicillium aurantiogriseum* and *Penicillium vanluykii* are highlighted for their antibiotic-producing capabilities, emphasizing their industrial relevance. Overall, the Table illustrates a rich diversity of fungi with varied ecological and practical implications (Disegha *et al*., 2024).

Table 4: Physiological categories of Aeroterrestrial Fungi detected around the Health Facility

Fungal species	Physiological categories
Acremonium radiatum	Pathogenic, Saprophytic fungi
Aphanoascus flavascens	Saprophytic
Chrysosporium tropicum,	Dermatophyte
Colletotrichum fructicola	Phytopathogenic
Fusarium solani	Phytopathogenic, Saprophytic
Microsporium canis,	Pathogenic, Dermatophyte
Penicillium aurantiogriseum	Pathogenic, Antibiotic Producing
Penicillium vanluykii	Antibiotic Producing, Industrially important
Phaeacremonium aleophilum	Pathogenic
Scedosporium aurantiacum	Saprophytic
Syncephalastrum racemosum	Pathogenic, Dermatophyte
Trichosporon asahii,	Pathogenic, Dermatophyte

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Antifungal sensitivity profiles of fungal isolates from synthetic antifungal are shown in Table 6. The table summarizes the responses of various fungi to different antifungal agents, highlighting their effectiveness. In general, fungi such as *Aphanoascus flavascens*, *Chrysosporium tropicum*, *Colletotrichum fructicola*, *Fusarium solani*, *Microsporium canis*, *Penicillium aurantiogriseum*, *Penicillium vanluykii*, *Phaeacremonium aleophilum*, *Scedosporium aurantiacum*, and *Syncephalastrum racemosum* were Susceptible (S) to Ketoconazole and Nystatin; but showed Intermediate (I) sensitivity to Fluconazole, and Resistant (R) to Griseofulvin. In contrast, *Trichosporon asahii* is resistant to all tested agents, indicating broader effectiveness. In conclusion, most fungi are susceptible to Ketoconazole and Nystatin, show intermediate sensitivity to Fluconazole, and are resistant to Griseofulvin. *Trichosporon asahii* is uniquely susceptible to all agents, making it easier to treat. This information is vital for guiding clinical treatment decisions.

Table 6: Antifungal Susceptibility Values of Fungi Treated with Antifungal Agents

S/N	**Fungi**	**Ketoconazole (10 µg/ml)**	**Nystatin (100 units)**	**Fluconazole (25 µg/ml)**	**Griseofulvin (10 µg/ml)**
1.	*Aphanoascus flavascens*	S	I	S	R
2.	*Chrysosporium tropicum*	S	I	S	R
3.	*Colletotrichum fructicola*	S	I	S	R
4.	*Fusarium solani*	S	I	S	R
5.	*Microsporium canis*	S	I	S	R
6.	*Penicillium aurantiogriseum*	S	I	S	R
7.	*Penicillium vanluykii*	S	I	S	R
8.	*Phaeacremonium aleophilum*	S	I	S	R
9.	*Scedosporium aurantiacum*	S	I	S	R
10.	*Syncephalastrum racemosum*	S	I	S	R
11.	*Trichosporon asahii*	R	R	R	R

Key: S – Susceptible; I – Intermediate; R – Resistant.

Discussion

The analysis of air fungal populations at the Health Centre, as observed in the study, reveals a mean monthly fungal population ranging from 2.5 to 2.8 Log_{10} CFU/min.M² throughout the sampling period from July 2021 to June 2022. This consistent range indicates a stable fungal presence in the air of the healthcare environment, which is critical for assessing potential health risks, particularly for immunocompromised patients (Tukaszuk. *et al*. (2011; Low and Rotstein, 2011). The steady fungal counts throughout the year, with fluctuations primarily between the minimum and maximum values, suggest that the Health Centre maintains a relatively constant airborne fungal load. Notably, the lowest fungal population was recorded in August 2021, while the peak was observed in January 2022. These variations may be attributed to seasonal changes, such as temperature and humidity, which are known to influence fungal dispersal and viability (Tukaszuk. *et al*. (2011).

The observed air fungal counts are consistent with findings from other healthcare environments. For instance, a study conducted by (Samuel *et al*., 2021). reported similar concentrations of airborne fungi in hospital settings, emphasizing the need for ongoing monitoring in such environments. Additionally, previous research has indicated that airborne fungi tend to proliferate during specific seasonal conditions, correlating with our findings of peaks in January and troughs in August (Samuel *et al*., 2021).

The analysis of mean monthly soil fungal populations at the Health Care facility, as observed in the study, indicates notable fluctuations in fungal abundance over the sampling period. The mean population ranged from 5.0 Log_{10} CFU/g in June to a peak of 6.3 Log_{10} CFU/g in March 2022.

The study revealed a slight continuous decrease in soil fungal populations from 5.6 Log_{10} CFU/g in July 2021 to 5.4 Log_{10} CFU/g in October 2021. This decline may reflect seasonal changes that affect fungal activity, such as temperature and moisture levels.

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A significant increase to 6.5 Log_{10} CFU/g was recorded in January 2022, indicating favorable conditions for fungal growth in the soil during the wet months, likely due to increased soil moisture and organic matter availability. Following the peak in January, a sudden decline to 5.7 Log_{10} CFU/g was observed in February 2022, followed by continuous fluctuations for the remainder of the sampling period. This pattern suggests a dynamic soil environment influenced by various biotic and abiotic factors (Yang and Yang, 2021).

The observed fluctuations in fungal populations highlight the complex interactions within the soil ecosystem within the health care facility. The initial decline from July to October may indicate a period of reduced fungal activity, potentially linked to environmental stressors. The subsequent increase in January aligns with findings from other studies that indicate winter conditions can enhance fungal growth due to higher soil moisture levels. The sudden decline in February could be attributed to rapid environmental changes or competition among microbial communities (Gil-Martínez, 2021).).

These findings are consistent with previous research emphasizing the influence of seasonal variations on soil fungal dynamics. For example, studies have shown that soil fungi often exhibit increased activity during cooler, wetter months, which aligns with the observed peak in March Additionally, fluctuations noted in this study are similar to those reported in other healthcare settings, where soil microbial populations are affected by environmental conditions and human activities (Baldrian *et al*., 2022)

The analysis of mean seasonal counts of air fungi at the Health Care Facility, as observed in the study, reveals important insights into the dynamics of airborne fungal populations during dry and wet seasons. The actual mean air fungal populations were 2.75 Log_{10} CFU/min.M² for the dry season and 2.63 Log_{10} CFU/min.M² for the wet season, indicating a statistically significant difference ($p < 0.05$) between the two seasons (Odebode *et al*., 2020).

The highest recorded air fungal population during the dry season was 2.9 Log_{10} CFU/min.m², while the lowest was 2.60 Log_{10} CFU/min.m². This range suggests a relatively stable fungal presence, with slight fluctuations. Wet Season: In contrast, the wet season exhibited a highest population of 2.8 Log_{10} CFU/min.m² and a lowest of 2.4 Log_{10} CFU/min.m². The overall mean was lower than that of the dry season, reinforcing the observed trend (Tabatabaei *et al*., 202).

The significant difference in fungal populations between the dry and wet seasons highlights the impact of environmental conditions on airborne fungi. The higher mean in the dry season may be attributed to lower humidity levels, which can facilitate the dispersal and viability of fungal spores. Conversely, the wet season's increased moisture may lead to conditions that suppress airborne fungal populations due to the settling of spores and increased competition among microbial communities (Nageen *et al.*, 2023)

These findings are consistent with previous studies that have reported seasonal variations in airborne fungal populations. For instance, research has shown that dry conditions often correlate with higher concentrations of airborne fungi, while wet conditions can lead to reduced fungal viability due to increased precipitation and humidity (Oppliger and Duquenne, 2015). This aligns with the results observed in this study, where the dry season consistently showed higher fungal counts compared to the wet season.

To effectively manage the risks associated with airborne fungi in healthcare settings, we recommend implementing continuous monitoring of airborne fungal populations throughout the year. This ongoing assessment will help in understanding seasonal dynamics and potential health impacts. Additionally, it is crucial to improve air quality management, particularly during the wet season, by enhancing ventilation and utilizing dehumidifiers to minimize fungal growth and exposure (Al-Shaarani, *et al*., 2-23).

The analysis of mean seasonal counts of soil fungi at the Health Care Facility provides valuable insights into the dynamics of soil fungal populations during dry and wet seasons. The actual mean soil fungal populations were recorded at 5.14 Log10 CFU/g for the dry season and 4.89 Log10 CFU/g for the wet season. Notably, there was no significant difference in soil fungal populations between the two seasons at $p < 0.05$.

In the Dry Season, the highest soil fungal population recorded during the dry season was 5.529 Log_{10} CFU/g, while the lowest was 4.69 Log_{10} CFU/g. This indicates a relatively stable range of fungal populations, with a peak suggesting favorable conditions for fungal growth.

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Whereas, in the Wet Season, the highest population reached 5.63 Log_{10} CFU/g, and the lowest was 4.42 Log_{10} CFU/g. Despite the higher peak in the wet season, the overall mean was lower than that of the dry season.

The lack of significant difference in soil fungal populations between the dry and wet seasons suggests that environmental factors influencing soil fungi may not be as pronounced as those affecting airborne fungi. While the dry season exhibited a higher mean, the wet season's peak indicates that moisture levels can also support substantial fungal growth. This stability across seasons may reflect the resilience of soil fungal communities to varying moisture conditions, as they can thrive in both dry and wet environments.

These findings align with previous studies that have reported similar patterns in soil fungal dynamics. Research has shown that soil fungi can maintain populations across different moisture conditions, although specific species may respond differently to seasonal changes. The observed peaks in both seasons suggest that soil fungi are well-adapted to fluctuating environmental conditions, which is consistent with findings from other ecological studies.

The mycological analysis of soil samples from the Health Facility of the Rivers State University revealed various fungal isolates, characterized by distinct cultural and morphological traits. The findings outlined in the study provide a comprehensive overview of the identified fungi, facilitating their phenotypic identification.

The fungal isolates exhibited a diverse range of cultural characteristics, with colors varying from white to dark brown and textures ranging from fluffy to powdery. Notable isolates included *Acremonium radiatum*, *Aphanoascus flavascens*, and *Fusarium solani*, each displaying distinct macroscopic and microscopic features. Microscopic analysis revealed variability in the shapes, sizes, and arrangements of conidia. Many isolates showed either hyaline or pigmented hyphae, aiding in their identification. For instance, *Microsporum canis* presented spindle-shaped macroconidia, while *Penicillium aurantiogriseum* displayed smooth, globose conidia.

The results from the mycological analysis highlight the diversity of fungal populations present in the soil of the Health Facility at the Rivers State University Main Campus.

The characterized isolates underscore the ecological significance of fungi in soil environments. Continued research and management efforts are vital for leveraging these organisms for agricultural and environmental benefits.

The study reveals a vibrant community of aeroterrestrial fungi, showcasing twelve species from eleven genera, each with distinct physiological roles. This diversity speaks to the intricate ecological makeup of the sampled environment and the fungi's potential impact. Notably, the fungi exhibit a range of lifestyles, including pathogenic, saprophytic, and dermatophytic, with some species, like *Acremonium radiatum* and *Microsporum canis*, demonstrating a flexible nature by functioning in multiple categories. The presence of two *Penicillium* species, known for their industrial and antibiotic potential, suggests the location could be a source of valuable biological compounds. When compared to other ecological surveys, the identified genera, such as *Fusarium* and *Colletotrichum*, are commonly found, confirming their widespread adaptability. The discovery of various pathogenic species, including *Colletotrichum fructicola* and *Phaeacremonium aleophilum*, reinforces the known risks these fungi pose to both plant health and human well-being. Ultimately, the study emphasizes the rich and multifaceted aeroterrestrial fungal community, highlighting its ecological importance and the need for further investigation to unlock its potential in areas like biotechnology and medicine.

The antifungal susceptibility testing results presented in Tables 6 and 7 provide critical insights into the effectiveness of various antifungal agents against a range of fungal isolates. The analysis includes twelve different fungal species tested against four antifungal drugs: Ketoconazole (K), Nystatin (N), Fluconazole (F), and Griseofulvin (G).

Susceptibility Profiles of most fungal isolates, including *Aphanoascus flavascens*, *Chrysosporium tropicum*, *Colletotrichum fructicola*, *Fusarium solani*, *Microsporium canis*, *Penicillium aurantiogriseum*, *Penicillium vanluykii*, *Phaeacremonium aleophilum*, *Scedosporium aurantiacum*, and *Syncephalastrum racemosum*, exhibited susceptibility (S) to Ketoconazole and Nystatin. They showed intermediate (I) sensitivity to Fluconazole and resistance (R) to Griseofulvin.

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Unique Resistance was observed with respect to *Trichosporon asahii* which demonstrated resistance to all tested antifungal agents, indicating a significant challenge in treatment options for infections caused by this species. The majority of the fungal isolates displayed a consistent pattern of susceptibility to Ketoconazole and Nystatin, which aligns with previous findings that suggest these agents are effective against a broad spectrum of fungi (McKeny *et al.*, 2023). The intermediate sensitivity to Fluconazole indicates a potential for resistance development, which warrants further monitoring.

The resistance patterns exhibited in the study highlights *Trichosporon* as resistant to all tested agents is of concern and underscores the need for alternative treatment strategies for infections caused by this organism. The resistance patterns observed in studies indicate that *Trichosporon* species exhibit resistance to all tested antifungal agents, which raises significant concerns regarding treatment options. This resistance underscores the urgent need for alternative treatment strategies for infections caused by this organism. Current first-line therapies, such as azoles (voriconazole and posaconazole), have limited effectiveness due to the high rates of resistance, particularly in immunocompromised patients. (Colombia *et al*., 2011).

The susceptibility profiles observed in this study are consistent with earlier reports that identified shown Penicillium and Fusarium species as generally susceptible to Ketoconazole and Nystatin, while showing varying responses to Fluconazole and Griseofulvin. The resistance of *Trichosporon asahii* corroborates findings from other studies that have documented its challenging treatment profile due to multi-drug resistance (Rodriguez-Tudela *et al*., 2005).

The susceptibility data should guide clinicians in selecting appropriate antifungal therapies, particularly favoring Ketoconazole and Nystatin for the majority of fungal infections. Monitoring Continuous surveillance of antifungal susceptibility patterns is essential to detect emerging resistance, especially for Fluconazole and Griseofulvin. (CLSI, 2012).

In essence, the antifungal susceptibility testing results indicate that most fungal isolates are susceptible to Ketoconazole and Nystatin, exhibit intermediate sensitivity to Fluconazole, and are resistant to Griseofulvin.

The unique resistance of *Trichosporon asahii* underscores the need for careful treatment planning. These findings are crucial for informing clinical decisions and improving patient outcomes in fungal infections. Most fungal isolates, including *Aphanoascus flavascens*, *Chrysosporium tropicum*, *Colletotrichum fructicola*, *Fusarium solani*, *Microsporium canis*, *Penicillium aurantiogriseum*, *Penicillium vanluykii*, *Phaeacremonium aleophilum*, *Scedosporium aurantiacum*, and *Syncephalastrum racemosum*, exhibited susceptibility (S) to Ketoconazole and Nystatin. These isolates demonstrated intermediate (I) sensitivity to Fluconazole and resistance (R) to Griseofulvin. Notably, *Trichosporon asahii* showed resistance to all tested antifungal agents, presenting a significant treatment challenge (Zomorodian *et al*., 2011).

A consistent susceptibility pattern to Ketoconazole and Nystatin aligns with previous studies indicating their broad-spectrum efficacy against fungi. The intermediate sensitivity observed for Fluconazole raises concerns about potential resistance development, necessitating ongoing surveillance. The complete resistance of *T. asahii* underscores the need for alternative treatment strategies for infections caused by this species (Sanguinetti and Posteraro, 2018).

The susceptibility profiles in this study corroborate earlier findings that identified *Penicillium* and *Fusarium* species as generally susceptible to Ketoconazole and Nystatin, while responses to Fluconazole and Griseofulvin varied. The resistance of *T. asahii* is consistent with existing literature documenting its multi-drug resistance, emphasizing the need for effective management protocols for infections associated with this organism (Tuft *et al*., 2024).

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