EVALUATION OF AIRBORNE FUNGAL ISOLATES FROM SELECTED PRIMARY HEALTHCARE CENTRES IN EGOR LOCAL GOVERNMENT AREA IN EDO STATE

BY

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BENIN CITY.

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN MICROBIOLOGY, UNIVERSITY OF BENIN, BENIN CITY.

OCTOBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **Ayevbosa AJAYI-EBOHON** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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This project work was carried out by **AJAYI-EBOHON AYEVBOSA** in partial fulfilment of the award of a Bachelor of Science, B.Sc (Hons) degree in the Department of Microbiology, University of Benin, Benin City.

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PROF. E.O. IGBINOSA **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty, for bringing me this far in life. I am truly grateful.

ACKNOWLEDGEMENT

I wish to begin by expressing my profound gratitude to God Almighty, whose endless love, grace, and mercy have been my constant source of strength and guidance throughout this academic journey. His divine favor has been instrumental in the successful completion of this project.

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ABSTRACT

Indoor air quality in healthcare environments is an important determinant of patient safety and occupational health. Airborne fungi are among the major biological contaminants in hospitals and primary health centers, where they can cause allergic reactions, opportunistic infections, and surface deterioration. This study was designed to evaluate the airborne fungal load and diversity in selected Primary Health Centers (PHCs) within Egor Local Government Area, Edo State, Nigeria. Air samples were collected weekly for three weeks from Uselu, Evbotubu, and Evhuogida PHCs using the settle plate technique. Fungal colonies were enumerated as colony-forming units per cubic meter (CFU/m^3), and isolates were identified based on cultural and morphological characteristics. Antifungal susceptibility testing was conducted using standard disc diffusion methods. Results revealed that fungal load ranged from 2.4 $\times 10^2$ to 3.5 $\times 10^2$ CFU/m^3, with Uselu PHC recording the highest mean load (3.33 $\times 10^2$ CFU/m^3) and Evbotubu PHC the lowest (2.45 $\times 10^2$ CFU/m^3). Four fungal genera *Aspergillus sp.*, *Penicillium sp.*, *Cladosporium sp.* and *Fusarium sp.* were identified, with *Aspergillus sp (38%)* being the most predominant. *Alternaria* sp. was the least frequent. Antifungal screening showed that *Aspergillus* sp. was most sensitive to ketoconazole and amphotericin B, while *Penicillium* sp. showed resistance to fluconazole and ketoconazole. The findings highlight that primary health centers in Egor LGA harbor airborne fungal contaminants, with variations linked to environmental and infrastructural factors. Regular environmental monitoring, adequate ventilation, and periodic sanitation are recommended to reduce potential exposure to pathogenic fungi within healthcare environments.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Airborne fungi are ubiquitous in both indoor and outdoor environments, contributing significantly to the microbial load of the air we breathe. These microorganisms, primarily spores, hyphal fragments, and other fungal propagules, are dispersed through air currents and can settle in various surfaces or be inhaled by individuals, potentially causing health risks. In healthcare facilities, such as primary health centers (PHCs), the presence of airborne fungi is of particular concern due to the vulnerability of patients, many of whom may have compromised immune systems or underlying health conditions (Araújo*et al*., 2008). Fungi such as *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* are commonly isolated from indoor air in hospitals and health centers, and their presence can lead to nosocomial infections, allergic reactions, and respiratory ailments (Atya*et al*., 2019; Kiasat*et al*., 2017).

Primary Health Centers (PHCs) serve as the first point of healthcare delivery in Nigeria, particularly in Edo State, providing essential medical services to a large proportion of the population. These facilities are often located in both urban and semi-urban areas, where environmental factors such as high humidity, inadequate ventilation, and overcrowding can exacerbate the proliferation of fungal growth and airborne fungal spores (OkonkwoandOnwujekwe, 2016). Edo State, located in Nigeria's southern region, experiences a

tropical climate with high rainfall and humidity, creating an ideal environment for fungal growth and dissemination. The indoor air quality of PHCs in this region is a critical public health issue, as these facilities cater to diverse patient groups, including immunocompromised individuals, pregnant women, and children, who are particularly susceptible to fungal infections (Suleyman and Alangaden, 2021)

Studies worldwide have shown that airborne fungi in healthcare settings are associated with significant health risks. For instance, a study conducted in Iranianhospitals found that *Cladosporium*, *Aspergillus*, and *Penicillium* were the predominant fungal genera in indoor air, with concentrations ranging from 0 to 280 CFU/m³ in various wards (Kiasat*et al*., 2017). Similarly, a study in Sri Lanka reported high levels of airborne fungi in hospital environments, linking their presence to poor ventilation systems and high human activity (Sivagnanasundaram*et al*., 2019). These findings underscore the need for regular monitoring and evaluation of airborne fungal isolates to assess the microbial air quality in healthcare facilities and mitigate associated health risks.

In Nigeria, limited research has been conducted on airborne fungal contamination in PHCs, particularly in primary healthcare settings. Most studies focus on tertiary hospitals in urban centers, leaving a gap in understanding the microbial air quality in rural and semi-urban PHCs (Atya*et al*., 2019). Given the critical role of PHCs in Edo State's healthcare delivery, evaluating the prevalence, diversity, and concentration of airborne fungal isolates in these facilities is essential for informing infection control measures and protecting public health.

The indoor air quality of PHCs in Edo State is a largely unexplored area of research, despite the potential for fungal contamination in these settings. Poor ventilation, high humidity, and limited resources for environmental sanitation in many PHCs create conditions conducive to fungal growth and dissemination of airborne fungal spores. These fungi can act as opportunistic

pathogens, causing infections such as invasive aspergillosis or candidiasis, particularly in immunocompromised patients (Van den Nest *et al*., 2021). Additionally, prolonged exposure to fungal spores can exacerbate respiratory conditions like asthma and allergic bronchopulmonaryaspergillosis (ABPA) in both patients and healthcare workers (Oliveira *et al*., 2023).

The lack of standardized guidelines for monitoring airborne fungi in Nigerian PHCs further complicates efforts to address this issue. Without empirical data on the prevalence and types of fungal isolates in these facilities, it is challenging to implement effective infection prevention and control strategies. Moreover, the tropical climate of Edo State, characterized by high humidity and temperatures, increases the risk of fungal proliferation, making it imperative to investigate the extent of airborne fungal contamination in PHCs. This study seeks to fill this knowledge gap by evaluating the diversity, concentration, and potential health risks of airborne fungal isolates in selected PHCs across Local Government Areas (LGAs) in Edo State.

1.3 Aim and Objectives of the Study

1.3.1 Aim

The aim of this study is to evaluate the airborne fungal isolates from primary health centers in selected Local Government Areas in Edo State, Nigeria, to assess their diversity, concentration, and potential health implications.

1.3.2 Specific Objectives

1. isolate and identify airborne fungal species present in the indoor environments of selected PHCs in Edo State.

2. determine the concentration of airborne fungal spores in different areas of the PHCs, such as waiting areas, consultation rooms, and wards.
3. determine the abtifungal susceptibility pattern of the fungi isolates

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Airborne Fungi

Airborne fungi constitute a critical subset of bioaerosols, which are microscopic biological particles, including bacteria, viruses, pollen, and fungal elements, suspended in the atmosphere. These fungal microorganisms, encompassing spores, hyphal fragments, and other propagules, are pivotal in environmental microbiology and public health due to their potential to trigger infections, allergic reactions, and respiratory ailments (Haleem Khan and Mohan Karuppayil, 2012). In indoor settings, particularly primary health centers (PHCs) in tropical regions like Edo State, Nigeria, airborne fungi present a significant challenge. The region's high humidity, warm temperatures, and often inadequate ventilation systems create optimal conditions for fungal proliferation, posing risks to patients, healthcare workers, and visitors (OkonkwoandOnwujekwe, 2016). This section provides an in-depth exploration of the definition and characteristics of airborne fungi, common fungal genera, sources in indoor environments, and factors influencing their dispersal, with a focus on their relevance to PHCs in Edo State.

2.2 Definition and Characteristics of Airborne Fungi

Airborne fungi are defined as fungal particles, primarily spores, hyphal fragments, and other propagules, that are aerosolized and transported through air currents. Fungal spores are specialized reproductive or survival structures, typically ranging from 1 to 100 micrometers in diameter, designed for dispersal across diverse environments (Madigan *et al*., 2015). These spores are lightweight, often possessing thick, protective cell walls that confer resistance to environmental stressors such as desiccation, extreme temperatures, and ultraviolet (UV) radiation (Araújo*et al*., 2008). Hyphal fragments, which are fragmented segments of fungal mycelium, can also become airborne, contributing to the bioaerosol load. Other propagules, such as conidia (asexual spores), yeast cells, or chlamydospores, vary by fungal species and environmental conditions (Haleem Khan and Mohan Karuppayil, 2012).

The physical and biological characteristics of airborne fungi enhance their ability to persist and disperse in indoor environments. Their small size and aerodynamic properties allow them to remain suspended in the air for hours or even days, depending on air turbulence and settling rates (Hinds, 1999). For instance, spores of *Aspergillusfumigatus*, measuring 2–5 micrometers, are small enough to penetrate deep into the alveolar regions of the lungs, posing a risk of invasive infections in immunocompromised individuals (Latgé, 1999). Additionally, fungal spores often exhibit hydrophobicity, enabling them to adhere to dust particles or surfaces, which can act as secondary reservoirs for resuspension. In healthcare settings like PHCs, this persistence is particularly concerning, as fungal particles can contaminate medical equipment, linens, or air-handling systems, increasing the risk of nosocomial infections.

The viability of airborne fungi is another critical characteristic. Many fungal spores can remain viable for weeks to months under favorable conditions, such as high humidity and moderate temperatures, which are prevalent in Edo State's tropical climate (Ekhaise*et al*., 2008). This durability allows fungi to colonize new substrates upon deposition, perpetuating their presence in indoor environments. Furthermore, some fungi produce secondary metabolites, such as mycotoxins or volatile organic compounds (VOCs), which can exacerbate health risks by causing irritation or toxicity upon inhalation (Fischer andDott, 2003). These characteristics underscore the need for vigilant monitoring of airborne fungi in PHCs, where vulnerable populations, including immunocompromised patients, pregnant women, and children, are frequently exposed.

2.3 Common Genera of Airborne Fungi

Airborne fungi are a critical component of indoor bioaerosols in primary health centers (PHCs), particularly in tropical regions like Edo State, Nigeria, where environmental conditions such as high humidity, warm temperatures, and often inadequate infrastructure foster their proliferation.

The most prevalent genera in these settings include *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Fusarium*, and *Candida*, each characterized by unique morphological, physiological, and ecological traits that enable them to thrive in healthcare environments. These fungi pose significant public health risks, causing nosocomial infections, allergic reactions, and respiratory diseases, especially in vulnerable populations such as immunocompromised patients, children, pregnant women, and healthcare workers in PHCs (Atya

et al., 2019; Kiasat*et al*., 2017). In Edo State, where PHCs often face challenges like poor ventilation, overcrowding, and limited sanitation resources, these genera are particularly concerning. This section provides a comprehensive, detailed analysis of each genus, emphasizing their prevalence, health implications, ecological adaptations, and specific relevance to PHCs in Edo State, supported by global and local studies.

2.3.1. *Aspergillus*sp.

The *Aspergillus* genus, encompassing over 300 species, is one of the most prevalent and clinically significant airborne fungi in healthcare settings due to its small, hydrophobic conidia (2–5 micrometers) that are easily aerosolized and capable of deep respiratory penetration (Latgé, 1999). Key species include *Aspergillusfumigatus*, *Aspergillusniger*, *Aspergillusflavus*, *Aspergillusterreus*, and *Aspergillusnidulans*, with *A. fumigatus* being the most pathogenic. Its thermotolerance (optimal growth at 37–50°C) and ability to produce abundant conidia make it a leading cause of invasive aspergillosis, a life-threatening infection with mortality rates of 30–90% in immunocompromised patients, such as those with leukemia, HIV/AIDS, or organ transplants.*A. flavus* produces aflatoxins, potent carcinogenic mycotoxins that pose additional risks through inhalation, potentially leading to liver toxicity or hepatocellular carcinoma (Bennett andKlich, 2003). *A. terreus*, noted in microbial vitamin production studies, is also a concern in

healthcare settings due to its resistance to amphotericin B, complicating treatment (Steinbach *et al*., 2004).

In PHCs, *Aspergillus* spores are introduced through outdoor air, dust, or contaminated surfaces like medical equipment, linens, or water-damaged walls. A study in Iranian hospitals reported *Aspergillus* concentrations up to 280 colony-forming units per cubic meter (CFU/m^3) in intensive care units (ICUs), where poor air filtration increases risks (Kiasat*et al*., 2017). In Edo State, the tropical climate (25–35°C, humidity >70%) creates ideal conditions for *Aspergillus* growth, particularly during the rainy season (April–October), when damp building materials foster mold proliferation (Ekhaise*et al*., 2016). PHCs with open windows, common in resource-limited settings, allow outdoor spores from soil or vegetation to infiltrate, while construction activities near facilities can resuspend dust-laden spores, as observed in a Brazilian hospital study (Araújo*et al*., 2008). *Aspergillus*'s ability to form biofilms on surfaces and resist environmental stressors like desiccation and UV radiation complicates control efforts in PHCs, where maintenance is often substandard. High-efficiency particulate air (HEPA) filtration and regular disinfection are critical but rarely implemented, highlighting the need for targeted interventions to protect vulnerable patients in Edo State.

2.3.2.*Penicillium*sp.

Penicillium, with over 400 species, is a ubiquitous genus known for its prolific production of small conidia (2–3 micrometers) that contribute significantly to indoor bioaerosols. Thriving in high-humidity environments with organic substrates like damp walls, dust, or decaying food, *Penicillium* is a common contaminant in PHCs (Haleem Khan and Mohan Karuppayil, 2012). Notable species include *Penicilliumchrysogenum*, used industrially for penicillin production, and *Penicilliummarneffei*, an opportunistic pathogen causing penicilliosis in immunocompromised individuals, though less common in Nigeria (Pitt, 2000). *Penicillium* spores are potent allergens,

triggering immunoglobulin E (IgE)-mediated hypersensitivity reactions, leading to allergic rhinitis, sinusitis, and asthma exacerbation, particularly in patients with pre-existing respiratory conditions (Verhoeff and Burge; 1997). Some species produce mycotoxins, such as ochratoxin A and citrinin, which can cause nephrotoxicity, hepatotoxicity, or immunosuppression upon chronic inhalation (Pitt, 2000).

In Edo State's PHCs, *Penicillium* is frequently isolated from poorly ventilated areas, such as wards or storage rooms, where dust and moisture accumulate. A Nigerian hospital study reported *Penicillium* concentrations exceeding 150 CFU/m³ in wards with inadequate air exchange, linking its prevalence to high humidity and organic debris (Ekhaise*et al*., 2008). The tropical climate of Edo State, with humidity levels often above 70% during the rainy season, promotes *Penicillium* growth on damp surfaces like ceilings, furniture, or medical supplies (OkonkwoandOnwujekwe, 2016). In resource-constrained PHCs, inconsistent cleaning protocols exacerbate *Penicillium* contamination, as the genus can colonize low-nutrient surfaces and form resistant conidia. Its spores are easily resuspended by human activities, such as sweeping or patient movement, increasing exposure risks in crowded PHCs. Humidity control, improved ventilation, and regular sanitation are essential to mitigate *Penicillium*'s impact, but these measures are often limited in Edo State's healthcare facilities.

2.3.3.*Cladosporium* sp.

Cladosporium, with over 700 species, is one of the most abundant fungal genera in indoor and outdoor air, characterized by darkly pigmented conidia (2–10 micrometers) that are highly resistant to UV radiation, desiccation, and temperature fluctuations (Bensch*et al*., 2012). Common species, including *Cladosporiumcladosporioides*, *Cladosporiumherbarum*, *Cladosporiumsphaerospermum*, and *Cladosporiummacrocarpum*, are frequently isolated in healthcare settings. These spores are potent allergens, associated with allergic rhinitis, asthma,

and hypersensitivity pneumonitis, particularly in atopic individuals or those with chronic respiratory conditions like chronic obstructive pulmonary disease (COPD) (Sivagnanasundaram*et al*., 2019). *Cladosporium*'s small, aerodynamic spores enable prolonged suspension in air, increasing inhalation risks in crowded PHC areas like waiting rooms.

A Sri Lankan study reported *Cladosporium* concentrations up to 400 CFU/m^3 in hospital waiting areas, attributing its dominance to poor ventilation and high human traffic, which resuspends settled spores. In Edo State, *Cladosporium* is likely introduced through open windows, as outdoor sources like soil, vegetation, and agricultural fields are rich in its spores. The genus's ability to grow across a wide temperature range (5–35°C) and tolerate low moisture levels makes it a persistent concern year-round, even during the dry season (November–March) when dust resuspension is prevalent (Ekhaise*et al*., 2016). In PHCs, *Cladosporium* may colonize damp surfaces or dust, particularly in poorly maintained facilities with water leaks or inadequate cleaning. While rarely invasive, its allergenic potential poses significant risks to asthma-prone patients and children, who are frequent PHC attendees. Enhanced ventilation systems and regular surface disinfection are crucial to reducing *Cladosporium* loads in Edo State's PHCs.

2.3.4.*Alternaria*sp.

Alternaria, comprising over 300 species, is a potent allergenic genus characterized by large, multicellular conidia (20–200 micrometers) that are less easily aerosolized but highly immunogenic. *Alternariaalternata*, the most prevalent species, thrives in environments with decaying organic matter, such as damp building materials, soil, or plant debris, making it common in tropical climates (Denning *et al*., 2006). *Alternaria* is strongly associated with allergic bronchopulmonaryaspergillosis (ABPA), a hypersensitivity lung disease, and severe asthma, particularly in individuals with fungal sensitization. Its spores trigger IgE-mediated allergic responses, causing symptoms like wheezing, dyspnea, and nasal congestion, which can

be severe in tropical settings with high spore concentrations (Verhoeff and Burge, 1997). *Alternaria* also produces mycotoxins, such as alternariol, tenuazonic acid, and altertoxins, which may cause cytotoxicity, genotoxicity, or immunosuppression upon chronic exposure (Pitt, 2000).

In PHCs, *Alternaria* is often detected in areas with water damage or high outdoor infiltration. A tropical hospital study reported *Alternaria* concentrations ranging from 50 to 200 CFU/m³, with peaks during the rainy season when humidity promotes fungal growth (Srinivasan*et al*., 2019). In Edo State, where PHCs frequently suffer from poor maintenance, *Alternaria* may colonize damp walls, ceilings, or floors, releasing spores that are resuspended by activities like sweeping or patient movement (Haleem Khan and Mohan Karuppayil, 2012). The large size of *Alternaria* conidia causes rapid settling, but their resuspension in crowded PHCs increases exposure risks, particularly for asthma-prone patients. The rainy season in Edo State exacerbates dampness, amplifying *Alternaria* proliferation and necessitating improved building maintenance and air filtration to reduce allergenic risks.

2.3.5. *Fusarium*sp.

Fusarium, with over 300 species, is less prevalent in indoor air than *Aspergillus* or *Penicillium* but is a significant opportunistic pathogen, particularly in immunocompromised patients. It produces macroconidia and microconidia that can become airborne, especially in environments with contaminated soil, water, or plant material (Leslie andSummerell, 2006). Key species, such as *Fusariumsolani*, *Fusariumoxysporum*, *Fusariumverticillioides*, and *Fusariumproliferatum*, are associated with cutaneous infections, keratitis, onychomycosis, and systemic fusariosis, which can have mortality rates exceeding 50% in patients with hematological malignancies or bone marrow transplants (Van den Nest *et al*., 2021). *Fusarium*'s resistance to multiple antifungal agents, including azoles, echinocandins, and amphotericin B, complicates treatment, making its presence in PHCs a critical concern (NucciandAnaissie, 2007).

In healthcare settings, *Fusarium* is often introduced through outdoor air or contaminated water systems, such as hospital plumbing, humidifiers, or sinks. A Brazilian hospital study reported *Fusarium* concentrations up to 50 CFU/m³ in areas near open windows, highlighting environmental infiltration (Araújo*et al*., 2008). In Edo State, where many PHCs are located in semi-urban or rural areas surrounded by agricultural fields or vegetation, *Fusarium* spores may enter through natural ventilation, particularly during the rainy season when soil moisture promotes fungal growth (Ekhaise*et al*., 2016). *Fusarium* produces mycotoxins, such as fumonisins, trichothecenes, and zearalenone, which can cause toxicity, immunosuppression, or carcinogenic effects upon chronic exposure (Leslie andSummerell, 2006). In PHCs with limited resources for water treatment or air filtration, *Fusarium* poses a significant risk to vulnerable patients, requiring rigorous environmental monitoring and maintenance of water systems.

2.3.6.*Candida*sp.

Candida, a genus of yeast-like fungi with over 200 species, is less commonly airborne than filamentous fungi but can become aerosolized in healthcare settings, particularly in areas with high patient density or contaminated surfaces. Key species, including *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and the emerging multidrug-resistant *Candida auris*, are leading causes of nosocomial bloodstream infections (candidemia), mucosal infections, and, rarely, pulmonary candidiasis, with candidemia mortality rates ranging from 20% to 40% (PfallerandDiekema, 2007). *Candida* cells or fragments can become airborne during activities like wound dressing, linen handling, or catheter manipulation, contributing to indoor bioaerosols (Van den Nest *et al*., 2021). *C. auris* is particularly concerning due to its persistence on surfaces for weeks and resistance to multiple antifungals, including fluconazole and amphotericin B (Lockhart *et al*., 2017).

In PHCs, *Candida* is often associated with contaminated medical equipment, catheters, or healthcare workers' hands, especially in settings with suboptimal sterilization practices. An Indian hospital study identified *Candida* on ICU surfaces, suggesting that poor sanitation facilitates its aerosolization (Atya*et al*., 2019). In Edo State, where resource constraints may limit autoclaving, disinfectant availability, or hand hygiene compliance, *Candida* poses a significant risk in crowded wards or consultation rooms. The tropical climate, with high humidity and temperatures, further promotes *Candida* survival on surfaces, increasing the likelihood of aerosolization during patient care activities (OkonkwoandOnwujekwe, 2016). The emergence of *C. auris* in global healthcare settings underscores the need for enhanced infection control measures, such as rigorous surface disinfection and hand hygiene protocols, to reduce *Candida*'s spread in Edo State's PHCs.

The genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Fusarium*, and *Candida* are pivotal contributors to airborne fungal contamination in PHCs, each with distinct morphological, ecological, and pathogenic characteristics that exacerbate risks in Edo State's tropical climate. Their ability to cause severe infections, allergic reactions, and respiratory diseases, combined with their prevalence in resource-constrained settings with poor ventilation and sanitation, poses significant challenges to public health. In Edo State, where PHCs serve vulnerable populations, these genera necessitate species-specific identification and targeted interventions, including improved ventilation, regular disinfection, and infrastructure maintenance, to mitigate their impact and ensure safe healthcare environments.

2.4. Sources of Airborne Fungi in Indoor Environments

Airborne fungi in primary health centers (PHCs) in Edo State, Nigeria, originate from a multifaceted combination of internal and external sources, which interact with the region's tropical climate—characterized by high humidity (>70%), warm temperatures (25–35°C), and

distinct wet and dry seasons—to elevate indoor fungal loads. These fungi, including genera such as *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Fusarium*, and *Candida*, pose significant public health risks by contributing to nosocomial infections, allergic reactions, and respiratory diseases among vulnerable populations, including immunocompromised patients, children, pregnant women, and healthcare workers (Atya*et al*., 2019; Kiasat*et al*., 2016). In resource-constrained PHCs, where poor ventilation, overcrowding, and limited sanitation exacerbate contamination, understanding these sources is critical for developing effective infection control strategies. This section provides a comprehensive analysis of the primary sources of airborne fungi in indoor environments, with a focus on their ecological roles, health implications, and relevance to Edo State's PHCs.

2.4.1.Dust and Organic Matter

Dust particles are a primary reservoir for fungal spores and hyphal fragments, serving as carriers that facilitate their dispersal and resuspension in indoor air. Composed of organic and inorganic materials, dust provides a stable environment for fungal propagules, which can remain viable for weeks to months under favorable conditions, such as high humidity and moderate temperatures (Haleem Khan and Mohan Karuppayil, 2012). Organic matter, including food residues, decaying wood, damp carpets, paper materials, or biological debris (e.g., skin cells or hair), acts as a nutrient source for fungal growth, enabling species like *Aspergillus* and *Penicillium* to colonize surfaces and release spores. In PHCs, dust accumulation on medical equipment, furniture, floors, windowsills, and shelves is a significant concern, particularly in facilities with limited cleaning resources. A Nigerian hospital study identified dust as a primary source of fungal contamination in outpatient departments, reporting concentrations of *Aspergillus* and *Penicillium* exceeding 150 colony-forming units per cubic meter (CFU/m^3) in areas with poor sanitation practices (Ekhaise*et al*., 2008).

In Edo State's PHCs, overcrowding and infrequent cleaning exacerbate dust accumulation, especially in high-traffic areas like waiting rooms, consultation rooms, and wards. Human activities, such as sweeping, walking, or moving furniture, resuspend dust-bound spores, increasing airborne fungal loads and inhalation risks. For example, *Cladosporium* spores, which are small (2–10 micrometers) and lightweight, are easily aerosolized from dust, contributing to allergic rhinitis and asthma exacerbation in susceptible patients (Alam*et al*., 2022). The tropical climate of Edo State, with humidity levels often exceeding 70% during the rainy season (April–October), promotes fungal growth on organic debris, such as food particles or plant material brought into PHCs by patients or staff. Dust can also harbor mycotoxins, such as aflatoxins from *Aspergillusflavus*, which pose additional health risks through inhalation (Bennett andKlich, 2003). Control measures, such as wet mopping instead of dry sweeping, regular surface disinfection with antifungal agents, and dust control protocols, are essential but often limited by resource constraints in Edo State's PHCs. Implementing low-cost strategies, like ensuring proper waste disposal and minimizing clutter, could reduce dust-related fungal contamination.

Alam, S., Nisar, M., Bano, S. A., and Ahmad, T. (2022). Impact of aerial fungal spores on human health.In *Hazardous Environmental Micro-pollutants, Health Impacts and Allied Treatment Technologies* (pp. 219-240). Cham: Springer International Publishing.

2.4.2. Human Activity

High human traffic in PHCs significantly contributes to fungal dispersal by resuspending settled spores into the air, particularly in areas like waiting rooms, consultation rooms, and wards. Activities such as walking, coughing, sneezing, handling linens, or moving medical equipment disturb dust and organic matter, releasing fungal propagules into the indoor environment. In tropical settings like Edo State, where PHCs are often overcrowded due to high patient demand and limited healthcare facilities, human activity amplifies fungal loads, increasing exposure risks

for patients and healthcare workers (Sivagnanasundaram*et al*., 2019). A Sri Lankan study reported fungal concentrations up to 400 CFU/m³ in hospital areas with high patient turnover, attributing this to frequent disturbance of settled spores by foot traffic and linen handling (Srinivasan*et al*., 2019).

In Edo State's PHCs, overcrowding is a pervasive issue, particularly in rural and semi-urban facilities serving large populations. Waiting areas often accommodate dozens of patients simultaneously, leading to continuous air turbulence that resuspends spores of *Cladosporium*, *Alternaria*, and *Aspergillus* (Ekhaise*et al*., 2016). Coughing or sneezing by patients with respiratory infections can aerosolize fungal particles, particularly *Candida* species, which may originate from mucosal surfaces or contaminated hands (PfallerandDiekema, 2007). Healthcare workers' activities, such as changing bed linens, adjusting medical equipment, or performing procedures like wound dressing, further contribute to spore dispersal, especially in poorly ventilated wards. For instance, *Candida auris*, an emerging multidrug-resistant pathogen, can be aerosolized during patient care activities, increasing the risk of nosocomial infections (Lockhart *et al*., 2017). The lack of standardized cleaning protocols and limited staff training in Edo State's PHCs exacerbate this issue, as settled spores are not effectively removed. Control measures, such as limiting patient numbers in waiting areas, using face masks to reduce respiratory emissions, and implementing air purifiers, could mitigate human activity-related dispersal, but these are often infeasible due to resource constraints.

2.4.3. External Sources

Outdoor air is a major contributor to indoor fungal contamination in PHCs, particularly in facilities relying on natural ventilation through open windows or doors, a common practice in Edo State due to limited mechanical ventilation systems. Outdoor sources, including soil, vegetation, decaying organic matter, agricultural fields, and construction sites, release large

quantities of fungal spores, such as *Aspergillus*, *Cladosporium*, *Fusarium*, and *Alternaria*, which infiltrate indoor spaces (OkonkwoandOnwujekwe, 2016). In Edo State's tropical climate, seasonal factors amplify outdoor fungal loads, with the rainy season (April–October) promoting fungal growth in soil and vegetation due to increased humidity and moisture. A Nigerian hospital study found that outdoor air contributed up to 60% of indoor fungal spores in facilities with open windows, with concentrations peaking at 200 CFU/m³ during wet months (Ekhaise*et al*., 2008).

In rural and semi-urban PHCs in Edo State, located near agricultural fields or forested areas, outdoor spores are readily introduced through natural ventilation, especially during the rainy season when humidity exceeds 70%. For example, *Fusarium* spores, prevalent in soil, are carried by wind into PHCs, posing risks to immunocompromised patients (Leslie andSummerell, 2006). Construction activities near PHCs, such as road repairs or building expansions, further exacerbate outdoor fungal inputs by disturbing soil and releasing dust-bound spores, as observed in a Brazilian hospital study where *Aspergillus* concentrations increased during nearby construction (Araújo*et al*., 2008). The lack of high-efficiency particulate air (HEPA) filtration in most PHCs allows unimpeded entry of outdoor spores, compounding indoor contamination. Control strategies, such as installing window screens, sealing building gaps, or using basic air filters, could reduce outdoor fungal infiltration, but these measures are rarely implemented due to cost and logistical challenges in Edo State.

2.4.4. Building Materials and Water Damage

Poorly maintained infrastructure, a common issue in resource-constrained PHCs, creates microenvironments conducive to fungal growth. Damp walls, leaking roofs, cracked ceilings, and water-damaged floors provide ideal substrates for molds like *Aspergillus*, *Penicillium*, and *Alternaria*. Water damage, often resulting from heavy rainfall or plumbing failures, promotes fungal colonization by maintaining high moisture levels (>60%) on porous surfaces like plaster,

wood, or drywall (Haleem Khan and Mohan Karuppayil, 2012). A Brazilian hospital study found that water-damaged areas were significant sources of fungal spores, with concentrations exceeding 100 CFU/m³ in affected wards, particularly for *Aspergillus* and *Penicillium* (Araújo*et al*., 2008).

In Edo State, the rainy season exacerbates water damage in PHCs, many of which have aging infrastructure with inadequate roofing or drainage systems. Leaking roofs and poor plumbing allow water to seep into walls and floors, creating damp niches where fungi thrive (OkonkwoandOnwujekwe, 2016). For example, *Penicillium* can colonize damp plaster, releasing spores that contribute to indoor air contamination, while *Alternaria* grows on water-soaked wood or textiles, increasing allergenic risks. The lack of regular maintenance and funding for repairs in Edo State's PHCs perpetuates these conditions, making water-damaged areas persistent sources of fungal spores. Health risks include inhalation of mycotoxins, such as ochratoxin A from *Penicillium*, which can cause nephrotoxicity (Pitt, 2000). Control measures, such as prompt repair of leaks, use of dehumidifiers, and application of antifungal paints, are critical but often unfeasible due to limited resources. Low-cost alternatives, like improving drainage around PHC buildings, could help mitigate water damage-related fungal growth.

2.4.5. Medical Equipment and Textiles

Contaminated medical equipment, linens, and textiles serve as secondary sources of airborne fungal contamination in PHCs. Fungal spores can adhere to surfaces like stethoscopes, blood pressure cuffs, catheters, or surgical instruments, becoming aerosolized during handling or cleaning. Textiles, such as curtains, bed linens, and patient gowns, can harbor spores of *Aspergillus* or *Candida*, particularly if stored in damp or poorly ventilated areas. Improper sterilization or inadequate storage practices exacerbate this issue in resource-limited settings. An

Indian hospital study identified contaminated curtains as a reservoir for *Aspergillus* spores, contributing to indoor air pollution with concentrations up to 50 CFU/m³ (Atya*et al*., 2019).

In Edo State's PHCs, limited access to autoclaves, disinfectants, or proper storage facilities increases the risk of fungal contamination on equipment and textiles. For example, *Candida auris*, a multidrug-resistant yeast, can persist on surfaces for weeks, becoming airborne during linen handling or patient care activities (Lockhart *et al*., 2017). Crowded wards and consultation rooms, combined with infrequent washing of linens, amplify the risk of spore dispersal. Healthcare workers' hands, often inadequately sanitized due to limited supplies, can transfer fungal spores to equipment, further contributing to contamination (PfallerandDiekema, 2007). Health risks include nosocomial infections, such as candidemia, which has a mortality rate of 20–40% (PfallerandDiekema, 2007). Control measures, such as regular sterilization of equipment, frequent washing of textiles with antifungal detergents, and strict hand hygiene protocols, are essential but challenging to implement in Edo State's PHCs due to resource and training limitations.

2.4.6. Air-Handling Systems

In PHCs with mechanical ventilation, poorly maintained air-conditioning units, fans, or ductwork can become breeding grounds for fungi, dispersing spores into indoor air. Fungal spores can colonize moist filters, cooling coils, or duct surfaces, particularly in humid environments where condensation occurs. Species like *Aspergillus* and *Penicillium* thrive in these conditions, releasing spores during system operation (Haleem Khan and Mohan Karuppayil, 2012). A study in a tropical hospital reported fungal concentrations up to 80 CFU/m³ in areas with unmaintained air-conditioning units, highlighting the role of air-handling systems in contamination (Srinivasan*et al*., 2019).

In urban PHCs in Edo State, where air-conditioning may be used intermittently due to power outages or cost constraints, irregular maintenance exacerbates fungal growth in air-handling systems. For example, *Aspergillus* spores can colonize damp filters, becoming aerosolized when units are turned on, increasing exposure risks in wards or consultation rooms (Araújo*et al*., 2008). In rural PHCs, reliance on fans without proper cleaning further contributes to spore dispersal. The absence of HEPA filtration, which can remove 99.97% of particles ≥0.3 micrometers, is a significant limitation in Edo State's PHCs, allowing fungal spores to circulate freely (Hinds, 1999). Health risks include inhalation of allergenic or pathogenic spores, particularly for immunocompromised patients. Regular cleaning and replacement of filters, along with installation of basic air purifiers, could reduce contamination, but these measures are often cost-prohibitive in resource-limited settings.

2.5. Factors Influencing Fungal Dispersal

The dispersal and concentration of airborne fungi in PHCs are governed by a complex interplay of environmental, physical, and anthropogenic factors, which are particularly pronounced in Edo State's tropical climate. These factors influence the growth, release, and spread of fungal spores, impacting indoor air quality and posing health risks to patients and healthcare workers. Understanding these factors is essential for designing effective control measures in resource-constrained PHCs.

2.5.1. Air Currents

Air currents, whether natural (e.g., wind through open windows) or mechanical (e.g., fans or air conditioners), are primary drivers of fungal dispersal in PHCs. Natural air currents introduce outdoor spores, while mechanical systems or human movement create turbulence that resuspends settled spores. In poorly ventilated PHCs, stagnant air leads to spore accumulation, while

turbulent currents from fans or foot traffic increase airborne concentrations. A Sri Lankan study found fungal concentrations up to 500 CFU/m³ in areas with low air exchange rates, highlighting the role of air currents in spore dispersal (Sivagnanasundaram*et al*., 2019).

In Edo State's PHCs, reliance on natural ventilation through open windows introduces outdoor spores, particularly during the rainy season when wind carries *Fusarium* or *Cladosporium* from soil or vegetation (Ekhaise*et al*., 2016). Fans, commonly used in urban PHCs, exacerbate resuspension of dust-bound spores, especially in crowded waiting areas. For example, *Aspergillus* spores, which are small and lightweight, can remain airborne for hours under turbulent conditions, increasing inhalation risks (Latgé, 1999). Control measures, such as installing window screens or using low-cost air purifiers, could reduce spore dispersal, but these are rarely available in Edo State's PHCs, necessitating alternative strategies like optimizing air exchange through strategic window placement.

2.5.2. Temperature

Fungal growth and spore release are highly temperature-dependent, with most fungi thriving between 20°C and 30°C, conditions typical of Edo State's tropical climate. Higher temperatures accelerate fungal metabolism, increasing spore production and release. For instance, *Aspergillus* species exhibit optimal growth at 25–30°C, contributing to their prevalence in tropical healthcare settings (Madigan *et al*., 2015). A Nigerian study reported that indoor temperatures in hospitals, often exceeding 28°C due to poor cooling systems, correlated with elevated fungal loads, particularly for *Penicillium* and *Aspergillus* (Ekhaise*et al*., 2008).

In Edo State's PHCs, the lack of consistent air-conditioning or cooling systems results in indoor temperatures mirroring outdoor conditions, promoting fungal proliferation on surfaces like walls or linens. During the dry season (November–March), higher temperatures may enhance spore

release from desiccated fungi, increasing airborne concentrations. Health risks include increased exposure to pathogenic spores, such as *A. fumigatus*, which can cause invasive aspergillosis (Latgé, 1999). Temperature control through improved ventilation or cooling systems could mitigate fungal growth, but power outages and cost constraints limit these options, requiring low-cost solutions like shading or passive cooling designs.

2.5.3. Humidity

Relative humidity is a critical determinant of fungal proliferation, with levels above 60% promoting mold growth on surfaces and subsequent spore release. In Edo State, where humidity often exceeds 70% during the rainy season (April–October), PHCs are highly susceptible to fungal contamination. A Nigerian hospital study reported a positive correlation between humidity and fungal loads, with concentrations of *Aspergillus* and *Penicillium* peaking at 200 CFU/m³ during wet months (Ekhaise*et al*., 2008). High humidity facilitates fungal colonization of damp surfaces, such as walls, ceilings, or textiles, releasing spores into the air.

In PHCs, inadequate roofing and plumbing exacerbate humidity-related fungal growth, particularly in poorly maintained facilities. For example, *Alternaria* thrives in damp environments, contributing to allergenic spore loads that exacerbate asthma (Barnes, 2019) .Mycotoxins, such as ochratoxin A from *Penicillium*, are also produced under high humidity, posing toxicity risks (Pitt, 2000). Dehumidifiers and improved ventilation could reduce humidity, but these are rarely feasible in Edo State's PHCs. Alternative strategies, such as sealing leaks and using moisture-absorbing materials, could help control humidity-related fungal proliferation.

2.5.4. Seasonal Variations

Tropical climates like Edo State's exhibit distinct wet and dry seasons, significantly influencing fungal populations. The rainy season (April–October) creates ideal conditions for fungal growth due to high humidity and frequent rainfall, promoting spore production in outdoor sources (e.g., soil, vegetation) and indoor substrates (e.g., damp walls). A Nigerian study found fungal concentrations in hospitals increased by 30–50% during the rainy season, with *Cladosporium* and *Fusarium* dominating (Ekhaise*et al*., 2016). Conversely, the dry season (November–March) reduces outdoor fungal loads but increases dust resuspension, elevating indoor concentrations of *Aspergillus* and *Alternaria* (OkonkwoandOnwujekwe, 2016).

In Edo State's PHCs, seasonal variations exacerbate contamination risks, particularly in facilities with open windows or water-damaged infrastructure. For example, *Fusarium* spores from agricultural fields are more prevalent during the rainy season, infiltrating PHCs and posing risks to immunocompromised patients (Leslie andSummerell, 2006). Seasonal monitoring and adaptive cleaning protocols, such as increased sanitation during the rainy season, could mitigate these risks, but limited resources hinder implementation.

2.5.5. Ventilation Systems

Inadequate ventilation exacerbates fungal contamination by trapping spores indoors or introducing outdoor spores without filtration. In Edo State's PHCs, reliance on natural ventilation through open windows allows unimpeded entry of *Cladosporium* and *Fusarium* spores, while poorly maintained mechanical systems, such as fans or air conditioners, disperse spores from colonized filters or ducts. A Brazilian study reported fungal concentrations up to 80 CFU/m³ in areas with unmaintained air-conditioning units (Araújo*et al*., 2008). HEPA filtration, which removes 99.97% of particles ≥0.3 micrometers, is effective but rarely implemented in resource-limited settings (Hinds, 1999).

In urban PHCs, intermittent use of air conditioners due to power outages promotes fungal growth in moist ducts, while rural PHCs rely on fans that resuspend spores. Health risks include increased exposure to pathogenic spores, such as *A. fumigatus*, in poorly ventilated wards (Latgé, 1999). Low-cost ventilation improvements, such as window screens or exhaust fans, could enhance air exchange, but funding and maintenance challenges limit adoption in Edo State.

2.5.6. Human and Structural Factors

Overcrowding, a common issue in PHCs, increases air turbulence and spore resuspension, particularly in waiting areas and wards. Structural issues, such as cracked walls, leaking roofs, or poor sanitation, create niches for fungal growth. A Nigerian teaching hospital study reported fungal concentrations exceeding 200 CFU/m³ in areas with high patient density and substandard infrastructure, with *Aspergillus* and *Penicillium* dominating (Ekhaise*et al*., 2008). In Edo State, PHCs often lack proper waste disposal systems, allowing organic debris to accumulate and support fungal growth.

Human activities, such as improper waste handling or inadequate hand hygiene, further exacerbate contamination. For example, *Candida* spores can be transferred via healthcare workers' hands, increasing nosocomial infection risks (PfallerandDiekema, 2007).

2.7. Specific Diseases Linked to Airborne Fungi

Airborne fungi are directly linked to several specific diseases, ranging from invasive infections to allergic and respiratory conditions, each with significant implications for PHC attendees in Edo State.

2.7.1. Invasive Aspergillosis

Invasive aspergillosis, primarily caused by *Aspergillusfumigatus*, is a life-threatening fungal infection affecting immunocompromised patients, such as those with HIV/AIDS, leukemia, organ transplants, or prolonged corticosteroid use. The small size (2–5 micrometers) and hydrophobicity of *Aspergillus* spores enable them to penetrate deep into the alveolar regions of the lungs, where they can germinate and invade tissues, leading to pulmonary or disseminated infections (Latgé, 1999). Mortality rates for invasive aspergillosis range from 30% to 90%, depending on the patient's immune status and treatment access (Van den Nest *et al*., 2021). A study in Iranian hospitals reported *Aspergillus* concentrations up to 280 CFU/m³ in intensive care units (ICUs), highlighting the risk in healthcare settings (Kiasat*et al*., 2019).

In Edo State's PHCs, where immunocompromised patients, particularly those with HIV/AIDS (prevalent in Nigeria), frequently seek care, invasive aspergillosis is a significant concern. The lack of advanced diagnostic tools, such as galactomannan assays, and limited access to antifungal drugs like voriconazole in resource-constrained PHCs delays diagnosis and treatment, increasing mortality risks (Ekhaise*et al*., 2016). Poor ventilation and dust accumulation in PHCs, coupled with outdoor spore infiltration through open windows, elevate *Aspergillus* exposure, particularly during construction activities near facilities, as observed in a Brazilian hospital study (Araújo*et al*., 2008). Preventive measures, such as HEPA filtration and antifungal prophylaxis for high-risk patients, are critical but rarely feasible in Edo State, necessitating alternative strategies like improved sanitation and dust control.

2.7.2. Allergic BronchopulmonaryAspergillosis (ABPA)

Allergic bronchopulmonaryaspergillosis (ABPA) is a hypersensitivity lung disease caused by immune responses to *Aspergillus* antigens, primarily affecting patients with asthma or cystic

fibrosis. Characterized by wheezing, pulmonary infiltrates, and bronchiectasis, ABPA results from IgE-mediated allergic reactions to *Aspergillus* spores, leading to chronic lung damage if untreated (Denning *et al*., 2006). *Alternaria* and *Cladosporium* spores can also contribute to similar hypersensitivity reactions. A global study estimated that ABPA affects 1–2% of asthma patients, with higher prevalence in tropical climates due to elevated fungal loads (Abel-Fernández*et al*., 2023).

In Edo State, where asthma is a common respiratory condition, ABPA poses a significant risk in PHCs, particularly during the rainy season when *Aspergillus* and *Alternaria* spore concentrations peak due to high humidity (Ekhaise*et al*., 2016). Crowded waiting areas and poorly ventilated wards increase exposure to fungal allergens, exacerbating symptoms in asthma patients. The lack of diagnostic tools, such as specific IgE testing, and limited access to corticosteroids or antifungal therapy in PHCs complicates ABPA management, leading to recurrent exacerbations and reduced quality of life. Control measures, such as reducing indoor humidity and improving ventilation, could mitigate ABPA risks, but resource constraints in Edo State's PHCs hinder implementation.

2.7.3. Asthma and Other Respiratory Conditions

Airborne fungi, particularly *Alternaria*, *Cladosporium*, and *Penicillium*, are potent allergens that exacerbate asthma and other respiratory conditions, including allergic rhinitis, sinusitis, and hypersensitivity pneumonitis. *Alternaria* spores, large (20–200 micrometers) and immunogenic, are strongly associated with severe asthma, triggering IgE-mediated responses that cause airway inflammation and bronchoconstriction (Denning *et al*., 2006). *Cladosporium* spores, small and abundant, contribute to allergic rhinitis and asthma, with concentrations up to 400 CFU/m³ reported in hospital waiting areas in a Sri Lankan study (Sivagnanasundaram*et al*., 2019; Hughes *et al*., 2022). Chronic exposure to fungal spores can also lead to hypersensitivity pneumonitis, a

lung inflammation caused by immune responses to fungal antigens, particularly in patients with prolonged exposure in poorly ventilated environments (Haleem Khan and Mohan Karuppayil, 2012).

In Edo State's PHCs, asthma patients, including children and adults, are at high risk due to elevated fungal loads in crowded, humid environments. A Nigerian study reported that 30% of respiratory complaints in hospital outpatients were linked to fungal exposure, with *Cladosporium* and *Alternaria* dominating (Ekhaise*et al*., 2008). The tropical climate, with humidity exceeding 70% during the rainy season, promotes fungal growth on damp surfaces, releasing allergenic spores into indoor air. The lack of air purifiers and inadequate cleaning in PHCs exacerbates exposure, particularly for patients with pre-existing respiratory conditions. Management strategies, such as allergen avoidance and inhaled corticosteroids, are limited by cost and availability, highlighting the need for environmental controls like dehumidification and regular sanitation to reduce asthma triggers in PHCs.

2.8. Prevalence and Distribution of Airborne Fungi

The prevalence of airborne fungi in Nigerian healthcare facilities varies across geopolitical zones due to differences in climate, urbanization, and environmental factors. In the South-South region, including Edo State, a study at the University of Benin Teaching Hospital (UBTH) reported fungal concentrations ranging from 50 to 200 colony-forming units per cubic meter (CFU/m^3) in wards and outpatient departments, with *Aspergillusniger*, *Aspergillusfumigatus*, *Cladosporiumcladosporioides*, and *Penicilliumchrysogenum* as dominant species (Ekhaise*et al*., 2008). The high humidity (>70%) and proximity to agricultural fields in Edo State contribute to elevated *Aspergillus* and *Cladosporium* loads, particularly in rural PHCs relying on natural ventilation.

In the North-East, a study in Wukari, Taraba State, documented *Aspergillusflavus* and *Penicillium* species at concentrations up to 250 CFU/m³, with peaks in the morning due to dry, hot conditions (30–40°C) facilitating spore dispersal (Atya*et al*., 2019). The semi-arid climate of this region, with intense dry seasons, promotes dust resuspension, increasing *Aspergillus* prevalence. In the North-Central region, a Jos hospital's special care baby unit reported a 20.2% fungal contamination rate, with *Aspergillus* species comprising 29.6% of isolates, highlighting risks to neonates in cooler, high-altitude climates (15–25°C) (Okolo*et al*., 2020). In the South-West, a Lagos tertiary hospital recorded high fungal concentrations in crowded outpatient areas, driven by urban pollution and high human traffic (Durugbo*et al*., 2023).

These regional differences reflect climatic influences, with southern regions (e.g., Edo, Lagos) showing higher fungal diversity due to humidity, while northern regions (e.g., Taraba, Jos) exhibit elevated concentrations of xerophilic species like *Aspergillus* during dry seasons. In Edo State's PHCs, the tropical rainforest climate amplifies fungal prevalence, necessitating region-specific monitoring and control strategies.

Fungal prevalence varies significantly between facility types, with tertiary hospitals, secondary hospitals, and PHCs showing distinct contamination profiles. Tertiary hospitals, such as UBTH in Edo State, manage complex patient populations and have higher fungal loads in critical areas like intensive care units (ICUs), operating theaters, and hematology wards. A Nigerian study reported *Aspergillus* concentrations up to 280 CFU/m³ in ICUs, linked to construction activities and poor air filtration (Okokon*et al*., 2018). These facilities often have mechanical ventilation systems, but irregular maintenance allows fungal colonization of ducts, dispersing *Penicillium* spores.

Secondary hospitals, serving as referral centers, show moderate fungal loads (100–200 CFU/m³), with *Cladosporium* and *Alternaria* dominating in outpatient departments due to high patient

turnover (Ekhaise*et al*., 2016). PHCs, prevalent in rural and semi-urban Edo State, exhibit lower but significant concentrations (50–150 CFU/m³), attributed to simpler infrastructure, reliance on open windows, and limited cleaning resources. A Benin City PHC study identified *Aspergillusniger* and *Penicillium* in 60% of air samples, with concentrations peaking in waiting areas and consultation rooms (Ekhaise*et al*., 2010). The lack of high-efficiency particulate air (HEPA) filtration in PHCs allows unimpeded entry of outdoor spores, particularly *Fusarium* from nearby farmlands (Odebode, 2017).

Facility-specific patterns also reflect patient care activities. For example, *Candida* species, including *Candida auris*, are more prevalent in wards with invasive procedures (e.g., catheterization), as aerosolization occurs during patient handling .

Seasonal variations significantly influence fungal prevalence in Nigerian healthcare facilities, driven by humidity, temperature, and wind patterns. During the rainy season (April–October), high humidity (>70%) and frequent rainfall promote fungal growth in soil, vegetation, and damp indoor surfaces, increasing indoor spore concentrations. A Benin City study reported a 40–50% increase in *Aspergillus* and *Penicillium* loads in hospital wards during wet months, with concentrations reaching 200 CFU/m³ in poorly ventilated areas (Ekhaise*et al*., 2010). *Alternaria* and *Fusarium* also peak during this period, as moist conditions favor their proliferation (Odebode, 2017).

In contrast, the dry season (November–March) is characterized by dust resuspension and lower humidity (40–60%), elevating concentrations of xerophilic species like *Cladosporium* and *Aspergillusflavus*. A Wukari study noted *Aspergillus* concentrations up to 250 CFU/m³ in morning hours during the dry season, linked to dry winds and human activity. The dominant fungal species in Nigerian healthcare settings reflect their ecological adaptability to tropical conditions and healthcare environments. *Aspergillus* species, particularly *A. niger*, *A. fumigatus*,

and *A. flavus*, are ubiquitous, detected in 50–70% of air samples across facilities. *A. fumigatus*, with small spores (2–5 micrometers), is prevalent in ICUs and wards, posing risks of invasive aspergillosis (Latgé, 1999). *A. flavus*, common in northern regions like Taraba, produces aflatoxins, increasing toxicity risks (Shabeer*et al*., 2022). *Penicillium* species, including *P. chrysogenum*, thrive in damp areas, contributing to allergic reactions and mycotoxin production (e.g., ochratoxin A) (Bennett andKlich, 2003).

Cladosporiumcladosporioides, with lightweight spores (2–10 micrometers), is abundant in outpatient areas and PHCs, causing allergic rhinitis and asthma exacerbation (Denning *et al*., 2006). *Alternariaalternata*, prevalent in humid southern regions like Edo State, is a potent allergen associated with severe asthma, detected in 30% of PHC air samples (Ekhaise*et al*., 2016). *Fusarium* species, linked to soil and agricultural fields, infiltrate rural PHCs, posing risks of cutaneous infections in immunocompromised patients (NucciandAnaissie, 2007). *Candida* species, particularly *Candida auris*, are emerging threats in tertiary hospitals, aerosolized during patient care and contributing to candidemia (Sabino*et al*., 2020).

2.9. Infection Control and Air Quality Management in Healthcare Facilities

Environmental controls aim to reduce fungal growth and spore dispersal by managing indoor conditions, such as humidity, temperature, and surface cleanliness, which are critical in PHCs where fungal loads are elevated due to tropical climates and infrastructural limitations.

2.9.1. Humidity and Moisture Control

Relative humidity above 60% promotes fungal growth on surfaces, leading to increased spore release into indoor air. In Edo State, where humidity often exceeds 70% during the rainy season (April–October), PHCs are highly susceptible to fungal contamination, particularly by *Aspergillus* and *Penicillium* (Haleem Khan and Mohan Karuppayil, 2012). A Nigerian hospital

study reported a positive correlation between humidity and fungal concentrations, with *Aspergillus* loads reaching 200 colony-forming units per cubic meter (CFU/m³) in humid wards (Ekhaise*et al*., 2008). Dehumidifiers can reduce indoor humidity to below 50%, inhibiting fungal growth, but their high cost and reliance on stable electricity make them impractical for most PHCs in Edo State.

Low-cost alternatives include improving building drainage to prevent water accumulation, sealing leaks in roofs and walls, and using moisture-absorbing materials like silica gel in storage areas. For example, repairing leaking roofs, a common issue in Edo State's PHCs, can significantly reduce dampness that fosters *Alternaria* growth (OkonkwoandOnwujekwe, 2016). Applying antifungal paints or coatings on walls, which contain agents like quaternary ammonium compounds, can further inhibit fungal colonization, offering a cost-effective solution for resource-limited settings (Srinivasan*et al*., 2019). Regular monitoring of indoor humidity using affordable hygrometers can guide maintenance efforts, ensuring conditions remain unfavorable for fungal proliferation.

2.9.2. Temperature Regulation

Fungal growth is temperature-dependent, with most species, such as *Aspergillus* and *Penicillium*, thriving between 20°C and 30°C, conditions typical of Edo State's tropical climate (Madigan *et al*., 2015). Indoor temperatures in PHCs often mirror outdoor conditions due to limited air-conditioning, promoting fungal spore production. A study in a tropical hospital found that temperatures above 28°C correlated with elevated *Penicillium* concentrations, exacerbating respiratory risks for patients (Ekhaise*et al*., 2016).

Air-conditioning units can maintain temperatures below 25°C, reducing fungal growth, but their intermittent use in Edo State's PHCs, due to power outages and high costs, limits effectiveness.

Passive cooling strategies, such as installing reflective roofing materials, shading windows with awnings, or enhancing cross-ventilation through strategic window placement, offer feasible alternatives to regulate indoor temperatures. For instance, shading PHC buildings with trees or canopies, a low-cost approach, can reduce indoor temperatures by 2–5°C, inhibiting fungal metabolism (Araújo*et al*., 2008). Regular maintenance of fans, commonly used in PHCs, is also essential to prevent spore dispersal from dust accumulation on blades.

2.9.3. Surface Cleaning and Disinfection

Dust and organic matter on surfaces, such as medical equipment, furniture, and floors, serve as reservoirs for fungal spores, which can become airborne during human activities. Regular cleaning and disinfection are critical to reducing fungal contamination in PHCs. A Nigerian hospital study identified dust as a primary source of *Aspergillus* and *Cladosporium* spores, with concentrations exceeding 150 CFU/m³ in poorly cleaned areas (Ekhaise*et al*., 2008). Wet mopping with antifungal disinfectants, such as sodium hypochlorite (bleach) or hydrogen peroxide, is more effective than dry sweeping, which resuspends spores (Haleem Khan and Mohan Karuppayil, 2012).

Limited cleaning resources and staff training hinder effective sanitation. For example, *Candida auris*, a multidrug-resistant yeast, can persist on surfaces for weeks, contributing to nosocomial infections if not properly disinfected (Lockhart *et al*., 2017). Low-cost cleaning protocols, such as using diluted bleach solutions (1:10) and reusable microfiber cloths, can effectively remove fungal spores from surfaces. Scheduling cleaning during low-traffic periods minimizes spore resuspension, while ensuring proper waste disposal prevents organic debris accumulation that supports fungal growth (OkonkwoandOnwujekwe, 2016). Training healthcare workers on cleaning techniques and infection control is essential to maximize the impact of limited resources.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling Area

The study was conducted in the Egor Local Government Area of Edo State, Nigeria, a peri-urban region within Benin City, characterized by a tropical rainforest climate with high humidity (>70%), warm temperatures (25–35°C), and distinct wet (April–October) and dry (November–March) seasons. Three PHCs were selected as sampling sites: Uselu PHC, Okohokugbo PHC and Evhuogida PHC. These facilities were chosen due to their high patient traffic, poor ventilation, and potential for airborne fungal contamination, exacerbated by overcrowding and inadequate sanitation infrastructure. The PHCs serve diverse populations, including immunocompromised patients, children, and the elderly, who are particularly vulnerable to fungal-related health risks such as allergies, asthma, and nosocomial infections (Ekhaise*et al*., 2008).

3.2 Sample Collection

Air samples were collected from the three selected PHCs in Egor LGA to assess airborne fungal contamination. Sampling was performed in triplicate at each PHC to ensure representativeness and account for variability in fungal distribution. At each PHC, three sampling points were identified: the waiting area, consultation room, and ward, chosen for their high human activity and potential for fungal contamination due to patient turnover, poor ventilation, and dust accumulation.

Air sampling was conducted using the settle plate method, (Omeliansky, 1940; WHO, 2009). Sterile Petri dishes (90 mm, Borosil) containing Potato Dextrose Agar (PDA) supplemented with 0.1 g/L chloramphenicol to inhibit bacterial growth were exposed to air for 30 minutes at a height of 1 meter above the floor, simulating human breathing zones. Three plates were exposed simultaneously at each sampling point, yielding nine samples per PHC (3 points × 3 replicates) and a total of 27 samples across the three PHCs. Plates were covered immediately after exposure, sealed with Parafilm, and labeled with the PHC name, sampling point, date, and replicate number. Samples were transported in a cooled insulated box (4–8°C) to the Microbiology Laboratory at the University of Benin within 4 hours of collection to preserve fungal viability. Upon arrival, plates were incubated at 28°C for 5–7 days, with daily inspections for fungal growth, and stored at 4°C if further processing was delayed.

3.3 Sterilization of Materials

To prevent contamination and ensure reliable results, all materials used in air sampling and fungal analysis were rigorously sterilized. Glassware, including Petri dishes, pipettes, conical flasks, test tubes, McCartney bottles, stirring rods, and measuring cylinders, was washed with detergent, rinsed with distilled water, and air-dried. Items were wrapped in aluminum foil to

maintain sterility and sterilized in a hot-air oven at 160°C for 1 hour, following standard protocols (Madigan *et al*., 2015). Metal tools, such as inoculation loops and forceps, were flame-sterilized using a Bunsen burner before and during use.

Sterility was validated by incubating randomly selected sterilized items on PDA plates at 28°C for 48 hours to confirm the absence of microbial growth. The laboratory work surface was disinfected with 70% ethanol before and after each procedure, and a Bunsen burner flame was maintained during sample handling to create a sterile zone, minimizing airborne contamination.

3.4 Preparation and Sterilization of Media

All media and reagents were prepared under aseptic conditions to support fungal growth and isolation. Glassware used for media preparation, including test tubes, beakers, conical flasks, Petri dishes, McCartney bottles, stirring rods, and measuring cylinders, was sterilized at 160°C for 1 hour in a hot-air oven, as described in Section 3.3. Culture media, primarily Potato Dextrose Agar (PDA), were sterilized in an autoclave at 121°C and 15 psi for 15 minutes to eliminate contaminants (Hinds, 1999). Media were prepared fresh on a need-basis to maintain potency and stored at 3–4°C in a refrigerator for up to one week. Sterility was confirmed by incubating uninoculated PDA plates at 28°C for 48 hours.

3.4.1 Preparation of Potato Dextrose Agar (PDA)

39 g of PDA powder was dissolved in 1 liter of distilled water in a 2-liter conical flask. The flask was sealed with cotton wool and aluminum foil to prevent contamination. The mixture was stirred for homogeneity and autoclaved at 121°C for 15 minutes. After cooling to 45–50°C in a water bath, 0.1 g/L of chloramphenicol was aseptically added to inhibit bacterial growth. The medium was poured into sterile Petri dishes, with each plate receiving approximately 20 mL of

medium. Plates were allowed to solidify, inverted to prevent condensation, and incubated at 25°C for 72 hours to confirm sterility before use.

3.5 Enumeration and Isolation of Fungi

Air samples on PDA plates were incubated at 28°C for 3–5 days in an incubator (Model INCU-Line IL 53, VWR), Plates were inspected daily for fungal colony formation, with colony counts recorded as colony-forming units per cubic meter (CFU/m³). Distinct colonies were selected based on morphological characteristics, such as size, shape, texture, and color, to capture species diversity. Selected colonies were subcultured onto fresh PDA plates using a sterile inoculation loop to obtain pure cultures. A total of 50–100 isolates were targeted across all samples, depending on colony diversity, to ensure comprehensive representation of fungal populations. Purified isolates were preserved on PDA slants in McCartney bottles at 4°C for subsequent identification and antifungal susceptibility testing. The colonies were calculated using the formula:

$$\text{CFU/m}^3 = \text{CFU/ml} = \frac{number\ of\ colonies\ x \times 1000}{\text{Plate area in cm}^2\ x\ \text{exposure time in minutes x air sampling rate}}$$

(Srinivasan *et al*., 2019).

3.5.1 Preparation of Pure Culture

To isolate pure fungal strains, colonies with unique morphological features were subcultured onto fresh PDA plates. A small portion of each colony was aseptically transferred using a sterile inoculation loop to a new PDA plate under. Plates were incubated at 28°C for 5–7 days to allow growth of pure cultures, which were inspected for uniformity to confirm the absence of contaminants. Pure cultures were transferred to PDA slants in sterile McCartney bottles, sealed

with Parafilm, and stored at 4°C. Each slant was labeled with the isolate's code, PHC name, sampling point, and date to maintain traceability.

3.6 Identification of Fungal Isolates

Fungal isolates were identified based on cultural, morphological, and microscopic characteristics, following standard mycological protocols (Pitt & Hocking, 2009). Identification was conducted in the Microbiology Laboratory at the University of Benin, using macroscopic and microscopic techniques for accuracy.

3.6.1 Cultural Characteristics

Each colony morphology e.g., size, texture, color, reverse color was determined by physical examination.

3.6.2 Preparation of Pure Cultures

To confirm purity, a single colony with distinct morphology was selected and re-streaked as a primary inoculum on a fresh PDA plate using a sterile inoculation loop. The plate was incubated at 25°C for 72 hours to verify purity. The pure culture was then streaked onto a PDA slant in a McCartney bottle, incubated at 25°C for 72 hours, and stored at 4°C for further analysis. Each slant was labeled to ensure traceability.

3.6.3 Lactophenol Cotton Blue Staining

Microscopic identification was performed using lactophenol cotton blue staining to visualize fungal structures. A drop oflactophenol cotton blue stain was placed on a clean microscope slide. A small tuft of fungal mycelium was aseptically transferred using a sterile wire loop and smeared into the stain. A cover slip was gently placed over the preparation, and the slide was examined

under a microscope at 400× magnification. The stain's components—phenol (fungicide), lactic acid (clearing agent), cotton blue (stains cytoplasm), and glycerin (preserves preparation) enabled clear visualization of hyphae, spores, and reproductive structures (Pitt & Hocking, 2009). Morphological features, such as conidiophores, spore shape, and septation, were compared with standard references to identify species.

3.7 Antifungal Susceptibility Testing

Antifungal susceptibility testing was conducted to evaluate the resistance profiles of isolated fungal strains against common antifungal agents: amphotericin B, fluconazole, ketoconazole, and griseofulvin. The disk diffusion method, adapted from the Clinical and Laboratory Standards Institute (CLSI) M44-A2 guidelines, was used due to its cost-effectiveness and applicability in resource-limited settings (CLSI, 2009). Fungal isolates were grown on PDA plates at 28°C for 5–7 days. A fungal suspension was prepared by transferring colonies to sterile saline (0.85% NaCl) and adjusting turbidity to the 0.5 McFarland standard (approximately $1–5 \times 10^6$ CFU/mL) using a spectrophotometer (Model UV-1800, Shimadzu).

The suspension was spread evenly on PDA plates using a sterile cotton swab. Sterile filter paper discs (6 mm, Whatman) impregnated with antifungal agents amphotericin B (25 µg), fluconazole (25 µg), ketoconazole (30 µg), and griseofulvin (50 µg)—were placed on the inoculated plates using sterile forceps. Plates were incubated at 28°C for 24–48 hours, allowing antifungal diffusion and inhibition of fungal growth. The diameter of inhibition zones was measured in millimeters using a ruler, with larger zones indicating susceptibility and smaller or absent zones indicating resistance. Results were interpreted based on CLSI breakpoints, where available, or published studies for non-standardized fungi: zones >20 mm indicated susceptibility, 10–20 mm indicated partial susceptibility, and <10 mm or no zone indicated resistance (Pfaller andDiekema, 2007). Each test was performed in triplicate to ensure reproducibility.

3.8 Statistical Analysis

Data on fungal counts (CFU/m^3), species prevalence, and antifungal susceptibility (inhibition zone diameters) were collected and analyzed using Microsoft Excel (Version 16.0, Microsoft) and Statistical Package for the Social Sciences (SPSS, Version 22.0, IBM Corp., Chicago, IL, USA). Normally distributed data were expressed as mean ± standard deviation (Ogbeibu, 2014).

CHAPTER FOUR

RESULTS

Table 4.1 presents the mean fungal load of air samples from the selected primary health centers (PHCs) in Egor LGA (Okohokugbo PHC, Evhuogida PHC, and Uselu PHC) over a three-week period. The lowest fungal load was recorded at Okohokugbo PHC in Week 1 ($2.4 \pm 0.10 \times 10^2$ CFU/m³), while the highest was observed at Uselu PHC in Week 2 ($3.5 \pm 0.16 \times 10^2$ CFU/m³).

Figure 4.1 illustrates the total mean fungal load (expressed in CFU/m³) recorded in air samples collected from the three primary health centers (PHCs) in Egor Local Government Area over a three-week period. The results indicate that Uselu PHC recorded the highest mean fungal load at 3.33×10^2 CFU/m³, followed by Evhuogida PHC with 2.77×10^2 CFU/m³, and Okohokugbo PHC with the lowest load of 2.45×10^2 CFU/m³.

Table 4.2 presents the cultural and morphological characteristics of fungal isolates. Four distinct fungal genera were identified: *Fusarium* sp., *Cladosporium* sp., *Penicillium* sp., and *Aspergillus* sp. Identification was based on colony morphology, texture, elevation, margin, hyphal characteristics, and spore type.

Table 4.3 and Figure 4.2 show the distribution of airborne fungal isolates across the three weeks. *Fusarium* sp. was the least frequently isolated fungus, accounting for 11.5% of total isolates, followed by *Penicillium* sp**.** (23.0%), *Cladosporium* sp**.** (30.8%), and *Aspergillus* sp. being the most prevalent at 38.5%. The highest number of isolates was obtained in Week 2 (10 isolates), while Week 1 recorded the lowest (8 isolates).

Table 4.4 presents the antifungal sensitivity pattern of the fungal isolates to five tested antifungal agents (fluconazole, ketoconazole, amphotericin B, itraconazole, and voriconazole). The highest resistance was observed in *Penicillium* sp., which was resistant to fluconazole and ketoconazole.

In contrast, *Aspergillus* sp. was the most sensitive isolate, showing full sensitivity to ketoconazole and amphotericin B. Overall, amphotericin B and voriconazole were the most effective antifungal agents against the isolated fungi, while fluconazole and itraconazole exhibited variable activity.

Table 4.1: Mean Fungal Load (×10² CFU/m³) of Air Samples from Selected PHCs in Egor LGA Over Three Weeks

Week	Uselu PHC	Evhuogida PHC	Okohokugbo PHC
Week 1	$3.2 \pm 0.15 \times 10^2$	$2.7 \pm 0.12 \times 10^2$	$2.4 \pm 0.10 \times 10^2$
Week 2	$3.5 \pm 0.16 \times 10^2$	$2.8 \pm 0.13 \times 10^2$	$2.5 \pm 0.11 \times 10^2$
Week 3	$3.3 \pm 0.15 \times 10^2$	$2.8 \pm 0.12 \times 10^2$	$2.45 \pm 0.10 \times 10^2$

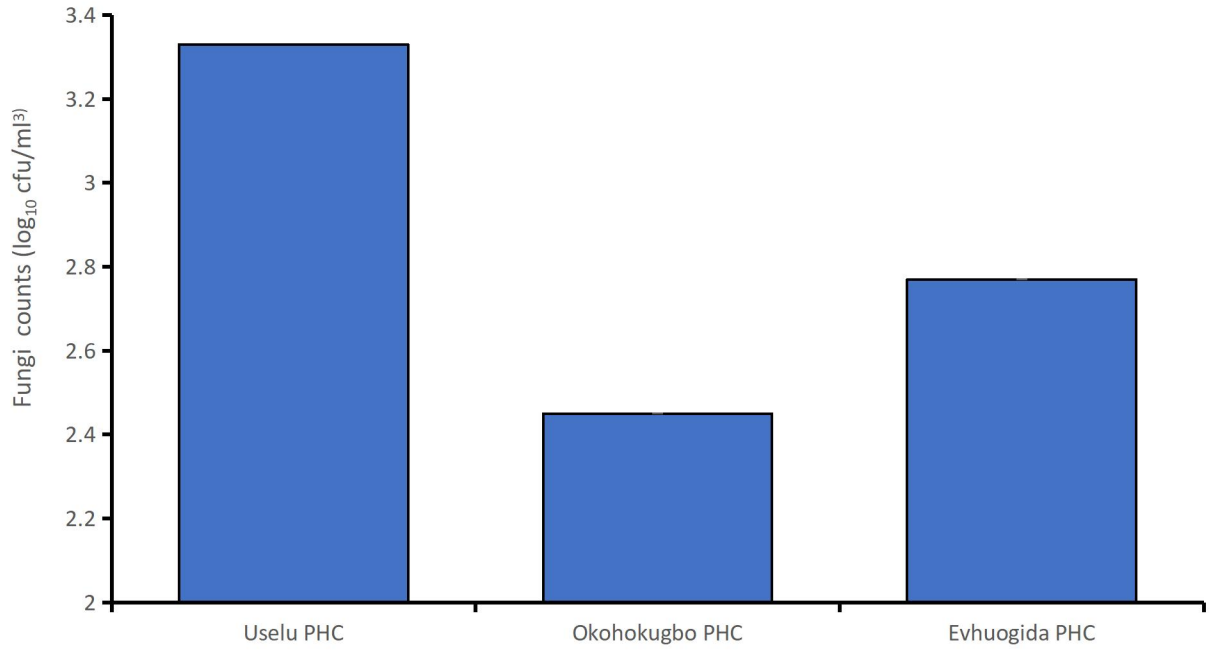

Figure 4.1. Total Mean Fungal Load ($\times 10^2$ CFU/m³) of Air Samples from Selected PHCs in Egor LGA Over Three Weeks

Table 4.2: Cultural and Morphological Characteristics of Fungal Isolates from Selected PHCs in Egor LGA

Characteristics	F1	F2	F3	F4
Cultural				
Nature of Colony	Greenish dome-shaped colonies with velvety texture and pale reverse side	Blue-green colonies, powdery texture, flat elevation, irregular margin	Olive-green colonies, velvety texture, raised elevation, entire margin	Pinkish/white colonies with cottony texture, raised elevation, entire margin
Morphological				
Nature of Hyphae	Septate	Septate	Septate	Septate
Spore Type	Conidiospore	Conidiospore	Conidiospore	Macroconidia
Organism	*Aspergillus* sp.	*Penicillium* sp.	*Cladosporium* sp.	*Fusarium* sp.

Table 4.3: Distribution of Airborne Fungal Isolates from Selected PHCs in Egor LGA Over Three Weeks

Fungal Isolates	Week 1 (n, %)	Week 2 (n, %)	Week 3 (n, %)	Total (n, %)
Aspergillus sp.	3 (37.5%)	4 (40.0%)	3 (37.5%)	10 (38.5%)
Penicillium sp.	2 (25.0%)	2 (20.0%)	2 (25.0%)	6 (23.0%)
Cladosporium sp.	2 (25.0%)	3 (30.0%)	3 (37.5%)	8 (30.8%)
Fusarium sp.	1 (12.5%)	1 (10.0%)	1 (12.5%)	3 (11.5%)
Total Isolates	8 (100%)	10 (100%)	9 (100%)	27 (100%)

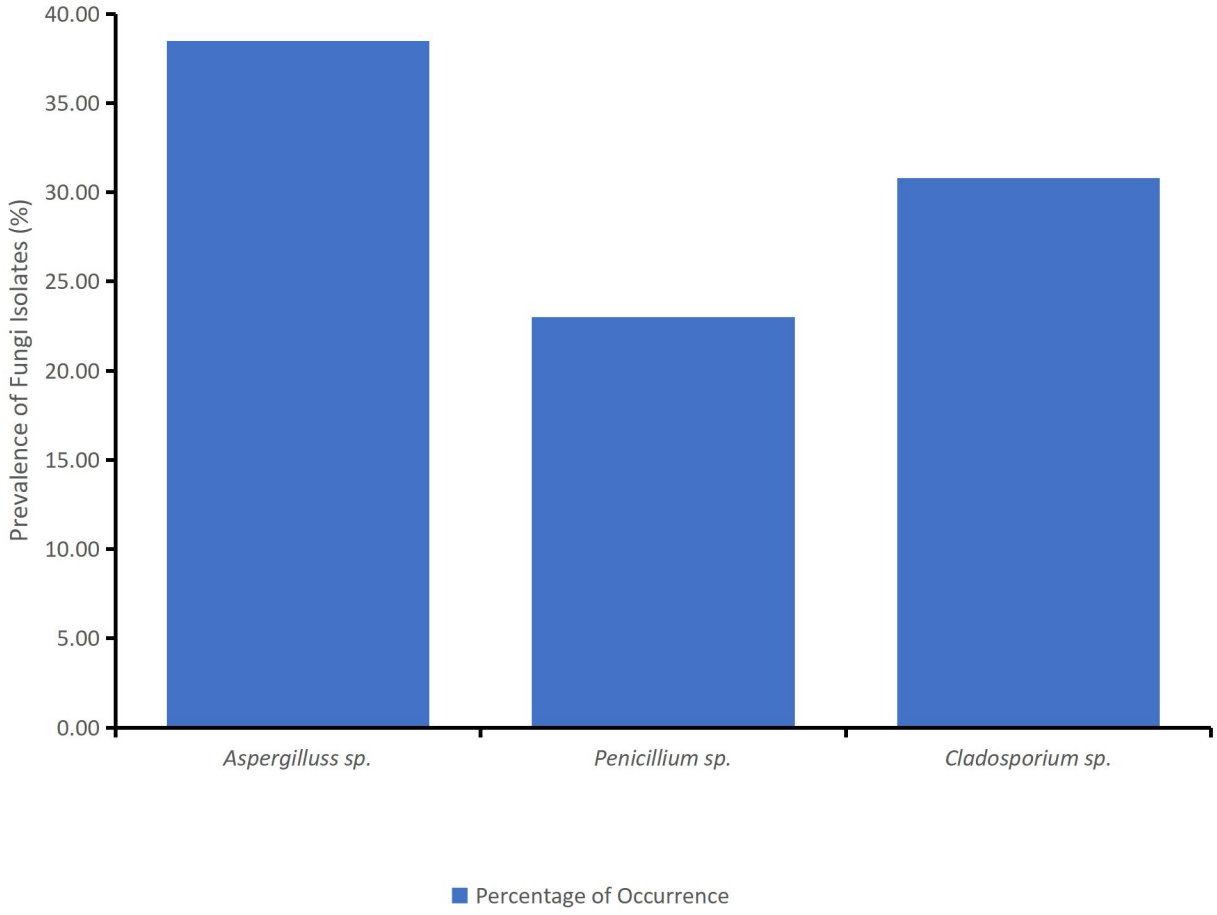

Figure 4.2. Percentage Frequency of Occurrence of Fungi isolates

Table 4.4: Fungal Sensitivity Pattern to Tested Antifungal Agents

Isolates	FLC	KET	AMB	ITC	VRC
Aspergillus sp	12 (I)	20 (S)	18 (S)	12 (I)	14 (I)
Penicillium sp	9 (R)	10 (R)	12 (I)	16 (S)	22 (S)
Cladosporium sp	11 (I)	15 (I)	17 (S)	13 (I)	16 (S)
Fusarium sp	14 (I)	18 (S)	20 (S)	10 (R)	16 (S)

Key:

R (Resistant): ≤ 10 mm

I (Intermediate): 11–15 mm

S (Sensitive): ≥ 16 mm

Fluconazole (FLC), Ketoconazole (KET), Amphotericin B (AMB), Itraconazole (ITC), Voriconazole (VRC)

CHAPTER FIVE

DISCUSSION

Airborne fungi are important environmental contaminants, particularly in healthcare settings, due to their potential to cause opportunistic infections and respiratory complications (Reponen *et al*., 2011; Pasanen, 2001). Hospitals and primary health centers (PHCs) are high-risk environments where immunocompromised individuals are exposed to fungal spores through inhalation or contact with contaminated surfaces (Burge, 2002; Golah *et al.,* 2017). The present study evaluated the presence, distribution, and antifungal susceptibility of airborne fungal isolates from selected PHCs in Egor Local Government Area, Edo State, Nigeria

The study revealed that airborne fungal loads varied across the three PHCs over the three-week sampling period. Uselu PHC recorded the highest mean fungal load (3.33×10^2 CFU/m³), followed by Evhuogida PHC (2.77×10^2 CFU/m³), with Okohokugbo PHC showing the lowest load (2.45×10^2 CFU/m³). The lowest fungal load was recorded at Okohokugbo PHC in Week 1 ($2.4 \pm 0.10 \times 10^2$ CFU/m³), while the highest was observed at Uselu PHC in Week 2 ($3.5 \pm 0.16 \times 10^2$ CFU/m³).

These results are consistent with findings by Awad *et al*. (2014), who reported that fungal concentrations in indoor healthcare environments are influenced by factors such as human activity, ventilation, humidity, and the presence of patients. The observed higher fungal load at Uselu PHC may reflect higher patient traffic, poor ventilation, or inadequate cleaning practices, which create a conducive environment for fungal proliferation. Conversely, the lower fungal load at Okohokugbo PHC may be attributed to better environmental control measures, fewer occupants, or effective cleaning practices. The variation in fungal load across locations underscores the need for regular air quality monitoring in healthcare facilities to minimize exposure risks, especially for vulnerable populations such as children, the elderly, and immunocompromised patients (Beggs, 2003; Sivagnanasundaram *et al.,* 2019).

Four fungal genera were identified: *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp. and *Fusarium* sp. *Aspergillus* sp. formed greenish, dome-shaped colonies with a velvety texture; *Penicillium* sp. exhibited blue-green colonies with powdery texture; *Cladosporium* sp. produced olive-green colonies; and *Fusarium* sp. had pinkish-white cottony colonies. All isolates were septate fungi, with *Aspergillus, Penicillium*, and *Cladosporium* producing conidiospores, while *Fusarium* produced macroconidia.

The predominance of *Aspergillus* sp. (38.5% of total isolates) aligns with numerous studies indicating that *Aspergillus* is the most common airborne fungal genus in healthcare facilities globally (Burge, 2002; Aihara *et al*., 2013; Viegas *et al.,* 2019). *Aspergillus* species are opportunistic pathogens capable of causing respiratory infections, allergic reactions, and systemic mycoses, particularly in immunocompromised patients (Latgé, 1999; Raj *et al*., 2025). The presence of *Penicillium* and *Cladosporium* further highlights the potential for allergic and respiratory disorders, as these fungi are known sources of allergenic spores (Eduardo *et al*., 2012; Levetin et al., 2016). *Fusarium*, although least prevalent (11.5%), is clinically significant due to its association with keratitis, onychomycosis, and invasive infections in immunocompromised individuals (Guarro *et al*., 2000; Nucci and Anaissie, 2023).

The diversity of fungal genera observed suggests that PHCs in Egor LGA harbor a complex airborne mycobiota, likely influenced by environmental conditions such as temperature, humidity, ventilation, and human activity (Pasanen, 2001). These findings emphasize the importance of routine environmental surveillance and implementation of effective infection control measures, including improved ventilation, air filtration, and regular disinfection.

The weekly distribution of fungal isolates showed that Week 2 had the highest number of isolates (10 isolates), while Week 1 had the lowest (8 isolates). *Aspergillus* sp. was the most frequent isolate, followed by *Cladosporium* sp., *Penicillium* sp. and *Fusarium* sp.

This distribution pattern is consistent with seasonal and temporal variations in airborne fungal loads reported by Eduard *et al*. (2012), who noted that fungal concentrations often peak during periods of increased humidity and human activity. The slightly higher isolation in Week 2 may reflect environmental conditions that favor sporulation, such as increased rainfall or higher indoor humidity levels. Monitoring temporal changes in airborne fungal populations is critical for assessing exposure risk and implementing timely mitigation strategies in healthcare facilities.

The antifungal susceptibility testing revealed significant differences among the fungal isolates. *Penicillium* sp. exhibited the highest resistance, particularly to fluconazole and ketoconazole, while *Aspergillus* sp. was the most sensitive, showing full susceptibility to ketoconazole and amphotericin B. Overall, amphotericin B and voriconazole were the most effective antifungal agents against the isolates, whereas fluconazole and itraconazole showed variable activity.

These findings are in line with previous reports indicating that resistance to azoles is common among *Penicillium* and *Fusarium* species (Morrison *et al*., 2013; Pfaller and Diekema, 2007). Amphotericin B and voriconazole remain highly effective against a broad spectrum of filamentous fungi, supporting their continued use in clinical management of fungal infections (Denning, 2002). The observed resistance patterns highlight the need for targeted antifungal therapy based on susceptibility testing rather than empirical treatment, which may contribute to treatment failure and the development of multidrug-resistant fungi.

The presence of airborne fungi, particularly *Aspergillus* and *Fusarium*, in primary health centers (PHCs) presents a significant public health concern, especially for immunocompromised patients and healthcare staff (Singh *et al*., 2011; Spagnolo, 2025). Chronic exposure to fungal spores in indoor healthcare environments can lead to a range of adverse health outcomes, including respiratory allergies, asthma exacerbation, and opportunistic infections (Eduardo *et al*., 2012;

Baxi *et al*., 2016; Oliveira *et al.,* 2023). The detection of resistant strains, such as *Penicillium* sp., underscores potential challenges in clinical management and highlights the importance of implementing effective environmental control measures within healthcare facilities (Garvey and Rowan, 2023).

5.2 CONCLUSION

The findings from this study underscore the role of primary health centers (PHCs) in Egor Local Government Area as reservoirs of diverse airborne fungal populations, including *Aspergillus* sp., *Cladosporium* sp., *Penicillium* sp. and *Fusarium* sp. The consistent isolation of *Aspergillus* sp., which was the most prevalent fungus, indicates that environmental conditions and human activity within these healthcare facilities facilitate fungal proliferation. The presence of *Penicillium* sp. and *Fusarium* sp., including strains exhibiting resistance to common antifungal agents, is particularly concerning as it suggests potential health risks to patients, especially immunocompromised individuals, and to healthcare workers exposed to contaminated air. Moreover, antifungal susceptibility testing revealed that while amphotericin B and voriconazole were effective against most isolates, variable sensitivity to fluconazole and itraconazole highlights the possibility of treatment challenges in clinical settings. These findings call for proactive public health interventions, including routine monitoring of indoor air quality, proper ventilation, surface disinfection, and staff education on infection control measures. Implementing such strategies is crucial for reducing fungal exposure, preventing opportunistic infections, and mitigating the risk of antifungal resistance.

5.3 RECOMMENDATIONS

Based on the findings of this study, several recommendations are proposed to mitigate the risks associated with airborne fungal contamination in primary health centers (PHCs) in Egor LGA:

1. **Routine Monitoring of Indoor Air Quality:**

Regular surveillance of airborne fungal loads should be instituted in PHCs to track changes in fungal diversity and concentration. This will enable timely identification of elevated fungal levels and facilitate appropriate interventions.

2. **Environmental Control Measures:**

   Healthcare facilities should implement strategies to minimize fungal proliferation, including proper ventilation, installation of high-efficiency particulate air (HEPA) filters in critical areas, moisture control, and prevention of dust accumulation. These measures are crucial in reducing environmental conditions favorable for fungal growth.

3. **Surface Cleaning and Disinfection:**

   Frequent cleaning and disinfection of surfaces, particularly in high-traffic and critical areas, should be enforced. This will help reduce the risk of fungal spores settling on surfaces and being aerosolized into indoor air.

4. **Antifungal Susceptibility Testing:**

   Routine antifungal susceptibility testing of clinical fungal isolates should be conducted to guide effective treatment and reduce the risk of therapeutic failure. This is particularly important given the detection of resistant strains such as *Penicillium* sp. in this study.

5. **Staff Training and Public Awareness:**

   Healthcare workers should receive continuous training on infection prevention and control, including proper hygiene practices and awareness of fungal exposure risks. Patients should also be educated on measures to reduce fungal exposure within healthcare facilities.

6. **Further Research:**

   Future studies should investigate seasonal variations in fungal populations, molecular identification of isolates for precise species-level detection, and the impact of environmental interventions on reducing fungal load. Such research will provide

comprehensive data to improve infection control strategies and clinical management of fungal infections in PHCs.

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