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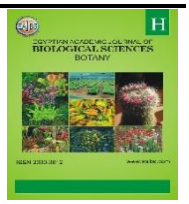

A Checklist of Airborne Fungal Spores from Six Open Markets in Lagos Metropolitan Area, Nigeria.

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ABSTRACT

Fungi are diverse organisms crucial for nutrient cycling and organic matter decomposition. Airborne fungal spores, however, pose potential health risks due to their allergenic properties. This study aimed to isolate, identify, and characterize fungi spores in six major markets in Lagos Metropolitan, Nigeria. Airborne fungal samples were collected from six major markets in Lagos (Ketu, Mile 12, Ikorodu, Oyingbo, Mushin, Ikotun) using open plate method with Potato Dextrose Agar (PDA) as the growth medium. The collection was carried out weekly for twelve consecutive weeks (November, 2023 to January, 2024). Isolated fungi were identified using both morphological (cultural and microscopic) and molecular (DNA extraction, PCR and DNA sequencing) methods. Results from this study revealed the prevalence of eighteen fungi species from the sampled markets. These includes *Aspergillus niger, Aspergillus flavus, Macrophomina sp, Aspergillus wentii, Aspergillus oryzae, Aspergillus sydowii, Aspergillus aculeatus, Aspergillus tubingensis, Aspergillus carbonarius, Curvularia lunata, Aspergillus micronesiensis, Trametes polyzona.* Of all sampled markets, Mushin, Ikorodu, and Mile 12 exhibited the highest prevalence of airborne fungal spores throughout the sampled period. This suggests that regular air quality monitoring and appropriate waste disposal in these open markets are necessary.

INTRODUCTION

Fungi are captivating and diverse life forms characterized by their nucleated, spore-bearing structures, which are crucial for breaking down organic matter and continuous cycling of nutrients (Davis *et al*. 2002). They can impact human and environmental health, contributing to allergies and diseases. Fungal spores are prevalent in the air (Kendrick, 2000) and soil, with varying concentrations influenced by environmental factors. Airborne fungi pose significant health risk, especially in metropolitan settings, and are essential to ecological microbiology. Allergenic fungal spore and allergenic spores can cause severe asthma and seasonal allergic rhinitis (SAR) when inhaled (Woolcock, *et al*. 2006). The concentration and diversity of airborne fungi were shown to be influenced by environmental conditions, including temperature, humidity, wind speed, and air pressure, as well as various urban site functions, such as crowded places with heavy traffic and commercial activity (Nageen *et al.* 2021). The objective of this study is to determine the prevalence and distribution of these fungal species in the selected open markets as well as to isolate, identify, and characterize fungal airsporal in chosen open markets in Lagos Metropolitan, Nigeria.

MATERIALS AND METHODS

Sample Collection:

The samples were collected from six major open markets in Lagos State: Ketu (6.6086 ^{0}N, 3.3956 ^{0}E), Mile 12 (6.6084 ^{0}N, 3.3959 ^{0}E), Ikorodu (6.6206 ^{0}N, 3.5025 ^{0}E), Oyingbo (6.4803 ^{0}N, 3.3836 ^{0}E), Mushin (6.5283 ^{0}N, 3.3539 ^{0}E) and Ikotun (6.5491 ^{0}N, 3.2684 ^{0}E). Airborne fungal spore samples were collected between 11 am – 2 pm from each sampling location using the open plate sedimentation method. By exposing sterile Potato dextrose agar (PDA) plates at 1.5 meters above ground level for 2 to 3 minutes, then covered, sealed and transported to the laboratory for incubation at 25 $\pm$ 2 ^{0}C room temperature for five days. This procedure was repeated weekly for twelve consecutive weeks (Nov. 2023 – Jan. 2024).

Fungi Isolation and Identification:

Mycelial growth from each plate was sub-cultured on freshly prepared Potato dextrose agar (PDA) plates and sub-culturing was repeated until pure plates of isolates were obtained. The fungal isolates were identified using conventional taxonomic techniques (cultural and microscopic) as described by Talbot (1971). The morphology of each fungus was studied and compared with the descriptions of Alexopoulos *et al.* (2007) and Bryce (1992). Genomic DNA of the isolated fungi were extracted from the cultures using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The 16S target region was amplified using One Taq Quick-Load 2X Master Mix (NEB, Catalogue No. M0486) with the primers presented in Table 1. The PCR products were electrophoresed on an agarose gel and clean up enzymatically using the EXOSAP method. The PCR products were sequenced in both forward and reverse directions (Nimagen Brilliant Dye Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit, Catalogue No. D4050). The purified fragments were analysed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample. BioEdit Sequence Alignment Editor version 7.2.5 was used to analyzed the ab1 files generated by the ABI 3500XL Genetic Analyzer and results were obtained by a (NCBI BLAST database search).

Table 1: ITS Primers sequences

Name of Primer	Target	Sequence(5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTACGACTT

RESULTS

Twelve fungal isolates were recovered from the six studied locations: *Aspergillus flavus, Macrophomina species, Aspergillus wentii, Aspergillus oryzae, Aspergillus sydowii, Aspergillus aculeatus, Aspergillus tubingensis, Aspergillus carbonarius, Aspergillus niger, Curvularia lunata, Aspergillus micronesiensis* and *Trametes polyzona*. Extracted genomic DNA from three fungal species that were morphologically unidentifiable displayed intact bands indicating suitability for downstream analysis. The purity of the extracted DNA was within the range of 1.8 - 2.0 ng/ul as expected for pure DNA and the agarose gels of PCR amplicons showed products ranging from 500 base pairs to 600 base pairs as expected for a successful amplification of the Internally Transcribed Spacer (ITS) regions of fungal species. The ITS sequences of the extracted fungi were analyzed to find their match following a preliminary identification. The accession numbers issued by GenBank and the sequence similarity are summarized in Table 2. Notably, *Aspergillus niger* and *Aspergillus flavus* are

the most frequently encountered fungal species in all the sampled markets throughout the sampling period. This was followed by the presence of *Aspergillus sydowii*, while *Aspergillus tubingensis* was the least observed fungal isolate (Tables 3 & 4).

Table 2: Percent Sequence Similarity and GenBank Accession Numbers

Organism	**Percentage Identification**	**GenBank Accession**
Aspergillus micronesiensis	100 %	KP987080.1
Curvularia lunata	99.84 %	KY806118.1
Trametes polyzona	99.84 %	OR100350.1

Table 3: Occurrence of airborne fungi spores from Ikorodu, Mushin and Oyingbo markets.

Fungal isolates	IKORODU												MUSHIN												OYINGBO											
	NOV.				DEC.				JAN.				NOV.				DEC.				JAN.				NOV.				DEC.				JAN.			
	Wk 1	2	3	4	Wk 1	2	3	4	Wk 1	2	3	4	Wk 1	2	3	4	Wk 1	2	3	4	Wk 1	2	3	4	Wk 1	2	3	4	Wk 1	2	3	4	Wk 1	2	3	4
A. niger	√	-	-	-	-	√	-	√	√	-	-	√	-	√	-	√	-	√	√	√	√	-	-	√	-	-	√	√	-	√	√	√	-	-	-	√
A. flavus	-	√	-	√	-	-	-	√	-	-	-	-	-	-	-	-	√	-	-	-	√	-	-	-	-	-	-	√	-	-	-	-	-	-	√	√
A.micronesiensis	-	-	-	-	-	√	-	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. sydowii	√	√	-	-	√	-	-	√	-	√	√	√	√	√	-	√	√	-	-	√	-	√	-	√	-	-	-	-	-	-	-	√	-	√	-
A. oryzae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.carbonarius	-	-	√	-	-	√	-	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. aculeatus	-	-	-	-	--	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. wentii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	√	-	-	√	-	√	-	-
A.tubingensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macrophomna	√	√	-	-	-	-	√	-	√	-	√	√	-	-	√	-	-	-	√	-	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-
C. lunata	-	-	-	√	-	-	√	-	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. polyzona	-	-	-	-	-	-	-	-	-	-	-	-	-	√	√	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\+ indicates present - indicate absent

Table 4: Occurrence of airborne fungi spores from Mile 12, Ketu and Ikotun markets.

Fungal isolates	MILE 12												KETU												IKOTUN											
	NOV.				DEC.				JAN.				NOV.				DEC.				JAN.				NOV.				DEC.				JAN.			
	Wk 1	2	3	4	Wk 1	2	3	4	Wk 1	2	3	4	WK 1	2	3	4	WK 1	2	3	4	WK 1	2	3	4	WK 1	2	3	4	WK 1	2	3	4	WK 1	2	3	4
A. niger	√	-	√	√	√	-	-	√	-	-	√	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
A. flavus	-	√	-	√	-	√	√	-	√	-	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
A.micronesiensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. sydowii	√	-	-	-	√	-	-	-	-	-	-	-	√	√	-	√	-	-	-	√	√	-	-	√	√	√	-	-	√	-	√	-	√	-	-	-
A. oryzae	√	-	√	-	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. carbonarius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. aculeatus	-	-	-	-	√	-	-	-	√	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. wentii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. tubingensis	-	-	-	-	-	-	-	-	-	√	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Macrophomina	-	√	-	√	-	√	-	-	√	√	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C. lunata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T. polyzona	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	√	-	-	-	-	√	-	-	-	-	-	-	-	-	-	-	-	-	-

\+ indicates present -- indicate absent

DISCUSSION

This study explores the prevalence of airborne fungal spores in six major markets in Lagos State, Nigeria. Various fungal taxa were identified with *Aspergillus niger* and *Aspergillus flavus* being the most dominant. These findings are consistent with the findings reported by Odebode, *et al.* (2020) who evaluated airborne fungal composition from five locations in Lagos and reported the abundance of *Aspergillus niger*, *Aspergillus sydowii*, and *Aspergillus flavus*. The study also concurs with findings by Samuel *et al*. (2021) who investigated fungal infections in hospitals located in Lagos and identified *Aspergillus niger, A. flavus, A. fumigatus, A. species, Candida species, Penicillium citrinum* as dominant fungal spores responsible for diseases and infections. Adekunle (2001) reported the presence of *Aspergillus niger, A. flavus, A. fumigatus, A. ochraceus, Trichoderma harzianium, T. viride, Penicillum sp, P.chrysogenum, Fusarium spp, Rhizopus sp, Curvularia spp* in some eating areas at the University of Lagos. The highest occurrence of spore concentrations was found in Mushin, Ikorodu, and Mile 12 markets. It is likely that high levels of human and vehicular activities in these locations contributed to the increased fungal counts observed in Mushin, Ikorodu, and Mile 12. This finding aligns with a study by Fang *et al.* (2007), which

demonstrated that microbial abundance is correlated with population density and the level of human activity. Several authors have proven that exposure to airborne spores of various fungal species that disperse in the atmosphere of these open market places contributes to air pollution, which may have health consequences for human (Nageen *et al.*, 2021; Gao *et al.*, 2022). According to Person *et al*. (2010), the most common fungal species found in this study, *Aspergillus niger*, is known to be a pathogenic fungus and a strong allergen that is linked to lung infections. Invasive aspergillosis, systemic mycosis, cutaneous infections, allergic bronchopulmonary disorders, and in rare instances, pneumonia, can also be brought on by this fungus. *Aspergillus flavus*, the second most isolated fungus in the present research is an opportunistic fungus that can colonize the respiratory tract and cause deadly infections, particularly in patients with impaired immune systems. This group of patients with health impairments may be adversely affected by exposure to this fungus (Nageen *et al.*, 2023). Molecular techniques, including PCR with ITS primers, were used for successful identification of some fungi, highlighting the limitations of traditional morphological methods and the need for advanced techniques to accurately identify "cryptic" species.

CONCLUSION

The findings of this study indicate the significance of airborne fungal spores as important components of indoor and outdoor air quality, with implications for public health and environmental management. The identification of specific fungal taxa and understanding their distribution across different market environments contributes to the body of knowledge aimed at mitigating potential health risks associated with fungal exposure. In order to stop the spread of these pathogenic organisms and improve the general quality of the air in these open spaces, preventive actions such as encouraging appropriate waste management and hygiene habits are crucial as focused interventions going ahead.

Declarations:

Ethical Approval: Since no plant, animal, or human subjects were gathered for the current investigation, ethical issues are not required.

Conflict of interest: The authors declare no conflict of interest.

Authors Contributions: I thus attest that each author listed on the title page has contributed significantly to the idea and planning of the research, has carefully reviewed the manuscript, attested to the veracity and correctness of the data and its interpretation, and has given their approval for submission.

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Availability of Data and Materials: The corresponding author can provide all datasets analyzed and described in this work upon reasonable request.

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