

Pathogenic potentials and DNA sequencing of airborne *Aspergillus* species from Indoor environments in Nigeria

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Abstract

Background: Air pollution by fungal spores poses a potential threat to the health and wellbeing of people. Airborne *Aspergillus* species play an important role in causing allergenic and invasive infections in susceptible individuals.

Objectives: We conducted this study to determine the prevalence and pathogenic potentials of airborne *Aspergillus* species in homes, offices and hospitals in Northern part of Anambra State.

Methodology: A total of 420, 70 and 59 air samples were collected from homes, offices and hospitals respectively using A6 single stage air sampler. Nasal swabs of occupants of same indoor environments were examined.

Results: The pathogenic potentials of some isolates in immunocompromised and immunocompetent Balb C mice were investigated using pharyngeal aspiration technique. Some of the fungal isolates were subjected to molecular studies to establish the similarity between air and nasal isolates. Twelve species of *Aspergillus* were recovered from homes and hospitals while 11 species were recovered from offices. *Aspergillus niger* was the predominant fungus (76.6%, 61.4%, 62.7%) followed by *A. fumigatus* (51.4%, 34.2%, 38.9%). Consequently *A. niger* was the dominant fungus in the nasal swabs of occupants of these environments (homes 66%, offices 19% and hospitals 14%). Immunosuppressed Balb C mice inoculated with spores of *A. fumigatus* died 51, 54 and 58 hours after inoculation while mice inoculated with *A. niger* developed lesions on the lungs.

Conclusion: DNA sequence of airborne *A. niger* and *A. niger* from nasal swab of occupant of same environment showed genetic relationship. Considering the presence of fungi with pathogenic potentials, environmental monitoring is necessary to prevent possible infection.

Keywords: Airborne fungi; DNA Sequencing; Pathogenic potentials; Indoor environment; *Aspergillus*

1. Introduction

Fungi are saprophytic usually multicellular eukaryotic organisms that are heterotrophs and have important roles in nutrient cycling in an ecosystem. Fungi are found in diverse types of environment, in soil, decaying organic matter, seeds and grains where they exist as saprophytes. Under conducive environment they grow in their vegetative form while in

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adverse environment, they exist in their resistant structures as spores. Fungi grow in living and non-living particles including furniture, paper or books, leather bags, shoes, clothes and air which serve as a means of dispersal [1].

Out of the 200 known species of *Aspergillus*, only a small number of them are pathogenic to humans [2] causing infections and allergies in man and producing toxins that affect agricultural produce. Members of the *Aspergillus* and *Fusarium* species are among the pathogenic and allergenic fungi that cause fatal diseases in humans and animals. *Aspergillus fumigatus* is the most common aetiologic agent of invasive infections in humans, followed by *A. flavus*, *A. terreus* and *A. niger* [3]. These fungi can cause infection in healthy individual and increasingly in immuno-compromised patients. They are abundant in nature and produce large amounts of asexual, haploid conidia which are highly hydrophobic making them easily airborne. The small diameters of the conidia make them to be easily inhaled into the lungs where they penetrate the alveoli and other parts of the lung. In immune-compromised individual, the inhaled conidia can germinate and produce hyphae that invade the surrounding lung tissue leading to the development of invasive pulmonary aspergillosis, which is the major cause of death in these individuals resulting to about >90% mortality rate, aspergilloma and different forms of hypersensitivity pneumonitis, allergic asthma and allergic broncho-pulmonary aspergillosis [4].

Aspergillus species especially *A. fumigatus*, *A. flavus*, and *A. terreus* can also cause infections in immune-competent individual who may or may not suffer from trauma. These include chronic sphenoid sinusitis, intracranial invasive aspergillosis, corneal infections, onychomycosis and otomycosis [5].

Aspergillus species are ubiquitous mould in homes, offices and hospital environments. Exposure to these moulds in indoor environment is not always considered a specific risk factor in the aetiology of fungal diseases unless some special conditions are present, which are essential for specific infections, as seen in hospitalized immuno-compromised patients and immuno-compromised patients in homes. Several studies have previously revealed that exposure to indoor airborne fungi can result in adverse health effects.

2. Methodology

The study was approved by the Ethical Committee Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus. ERC/FHST/NAU/2013/046. All procedure performed in this study, collection of nasal swabs from human participants, were in accordance with the ethical standards of this institution and with the 1964 Helsinki Declaration and its later amendments.

The investigation was carried out using A6 single stage microbial air sampler with malt extract agar supplemented with chloramphenicol 0.05mg/ml. About 420, 70 and 59 air samples were collected from randomly selected 84 homes, 28 offices, and 7 hospitals respectively, giving a total of 549 air samples. The temperature and relative humidity of indoor environments were taken. Two hundred and twenty six (226) nasal swabs were also collected from occupants of homes, offices and care givers in hospitals sampled. The microbial air sampler is operated at an air flow rate of 28 LPM and was set up at a height representative of the normal human breathing zone i.e 1.5m above floor level and 1m above floor level in offices [6]. The sampling time was 5-7 minutes according to the environmental situation of the measurement condition to avoid drying of the agar surface and overloading of the plate. Samples were collected in the morning hours immediately after morning cleaning. Inoculated plates were sealed with masking tape to prevent contamination and incubated at room temperature for 4-14 days and observed daily for yeast and mould growth [8]. The nasal swabs were streaked on Sabouraud dextrose Agar, supplemented with chloramphenicol at 0.05mg/ml and incubated at room temperature for 2-7 days.

When growths were adequate, the mature fungal growth were examined macroscopically and microscopically. The procedures used were those employed in most mycological examinations involving a pathogenic and nonpathogenic fungi [7].

Identification of fungal isolates was based on gross colonial morphology, microscopic image observed which were matched against those contained in Atlases of Pathogenic Fungi by De Hoog [10].

2.1. Pathogenicity Evaluation

Fungal isolates were tested for their pathogenic potentials, using immune-competent and immuno-compromised BALB/C albino mice by the pharyngeal aspiration technique. Spore suspensions for inoculation was prepared by growing the organisms on Potato dextrose agar at 22°C until profuse sporulation had occurred, usually in 4 to 6 days. These spores were harvested, by addition of sterile normal saline with 0.1% of Tween 80 and shaking with glass beads.

Tween 80 was added to spore suspension to avoid their clumping. Large particles were allowed to sediment under gravity and the supernatant spore suspension was decanted. After counting in a hemacytometer chamber, 5×10^6 number of spores were used for inoculation [9].

2.2. Animal Inoculation

2.2.1. The Pharyngeal Aspiration Technique

Each mouse was anaesthetized by placing it in a jar with a wire-mesh floor over gauze moistened with isoflurane and observed for signs of decreased mobility and unsteady gait for about 20s. When fully anaesthetized, the mouse was taken out of the jar and gently placed on a slant board. The animal was positioned so that its back was against the board while its neck and the lower cranium rested on a metal wire. Then the mouth was gently opened and the mouse was suspended from the upper thin rubber band by its incisor teeth. The tongue was gently pulled aside from the oral cavity and maintained in full extension by small forceps. A 5×10^6 number of spores were pipetted at the base of the tongue and the tongue restraint was continued until at least 2 deep breaths were completed but for not longer than 15seconds. Following release of the tongue, the mouse was gently lifted off the board, placed on its left side on a flat surface, and observed for anesthetic recovery [11].

A total of sixteen (16) BALB/C albino mice of both sexes, weighing between approximately 30-35g were used for this study. Treated mice received 5 mg/mouse of hydrocortisone, intra-peritoneally using syringe and 26 Gauge needle for 4 days before the spores were injected and once per week after infection [12]. The control animals were inoculated with heat killed spores of the fungi, inactivated by autoclaving at 121°C for 15 minutes. Their non-viability was confirmed by failure to grow on potato dextrose agar medium after four days of incubation.

The animals were allowed for a period of two months and observed daily for any visible sign or death. At the end of the 8th week, the mice were sacrificed and the lungs of the mice were harvested. Samples were collected from the lesions observed on the organs, cultured and observed for recovery of these organisms. The harvested organs were stored in 10% formalin for tissue processing.

2.3. DNA Sequencing of Fungal Isolates

Four species of *Aspergillus* isolated from air and nasal swabs of occupants of the different environments sampled, were subjected to molecular typing. Three to four days old cultures of the isolates, grown on potato dextrose agar supplemented with chloramphenicol (0.05mg/ml) were used. rDNA of the test fungi was extracted and the molecular analysis employing ITS was undertaken in Inqaba Biotech Laboratory, South Africa

3. Results

A total of 54, 46, 52 species of fungi were isolated from homes, offices and hospitals respectively, out of which 12 species were *Aspergillus*. The predominant fungi in these environments were *A. niger* 322(76.6%) and *A. fumigatus* 216(51.4%) in homes, *A. niger* 43(61.4%) and *A. fumigatus* 24(34.2%) in offices and *A. niger* 37(62.7%), and *A. fumigatus* 28(38.9%) in hospitals. *Aspergillus niger* and *A. fumigatus* were the predominant fungal isolates from the nasal swabs of occupants of the various environments. Three of the immunosuppressed mice inoculated with spores of *A. fumigatus* died 51, 54 and 58 hours respectively after inoculation. Death also occurred in one of the untreated or non- immunosuppressed mice infected with spores of *A. fumigatus* 7 days after infection. The inoculated fungus, *A. fumigatus*, was recovered from the samples collected from lesions/abscesses found on the lungs of dead mice after culturing on Sabouraud dextrose agar.

Three immunosuppressed mice inoculated with spores of *A. niger*, and one immune-competent mouse inoculated with spores of *A. fumigatus* developed lesions on their organs. There was also a reduction in weight in these 4 mice by 4 - 5 g per mouse. The immunocompetent control mice in all the groups developed no lesion on their organs, and had no weight loss while the immunosuppressed control mice had reduction in weight between 1 - 2 g per mouse.

The phylogenetic relatedness was constructed to ascertain the relationship between the fungi isolated from the nasal of an occupant in an environment and the fungi isolated from the air of the same environment. Isolate 003A is an airborne isolate of *A. niger* from a particular home while 003N is *A. niger* isolated from the nasal of an occupant of same home. The phylogenetic tree (Fig 2) showed that the two fungal isolates designated 003A and 003N are closely related.

Table 1 Prevalence of *Aspergillus* species in Homes, Offices and Hospitals located in Northern Part of Anambra State

S/no	Isolates	Homes (%)	Offices (%)	Hospitals (%)
1	<i>Aspergillus fumigatus</i>	216(51.4)	24(34.2)	28(38.9)
2	<i>A. tamari-kita</i>	150(35.7)	16(22.8)	8(13.5)
3	<i>A. terreus</i>	124(29.5)	10(14.2)	13(22.0)
4	<i>A. versicolor</i>	4(0.95)	0.0	2(3.3)
5	<i>A. niger</i>	322(76.6)	43(61.4)	37(62.7)
6	<i>A. flavus</i>	182(43.3)	8(11.4)	13(22.0)
7	<i>A. clavatus</i>	102(24.2)	2(2.9)	5(8.4)
8	<i>A. alliaceus</i>	104(24.7)	14(20.0)	12(20.3)
9	<i>A. candidus</i>	53(12.6)	5(7.1)	9(15.2)
10	<i>A. aculeatus</i>	156(37.1)	17(24.3)	20(33.8)
11	<i>A. penicillioides</i>	62(14.7)	1(1.4)	13(22.0)
12	<i>A. ochraceus</i>	146(34.7)	17(24.3)	13(22.0)

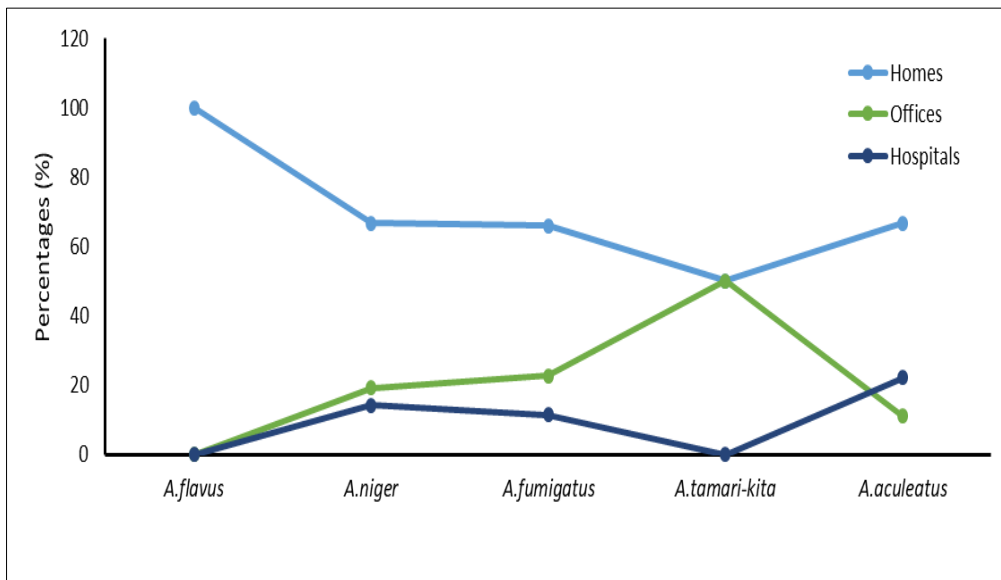


Figure 1 Frequency of fungal isolates for the nasal swabs of sampled individuals in Northern part of Anambra State

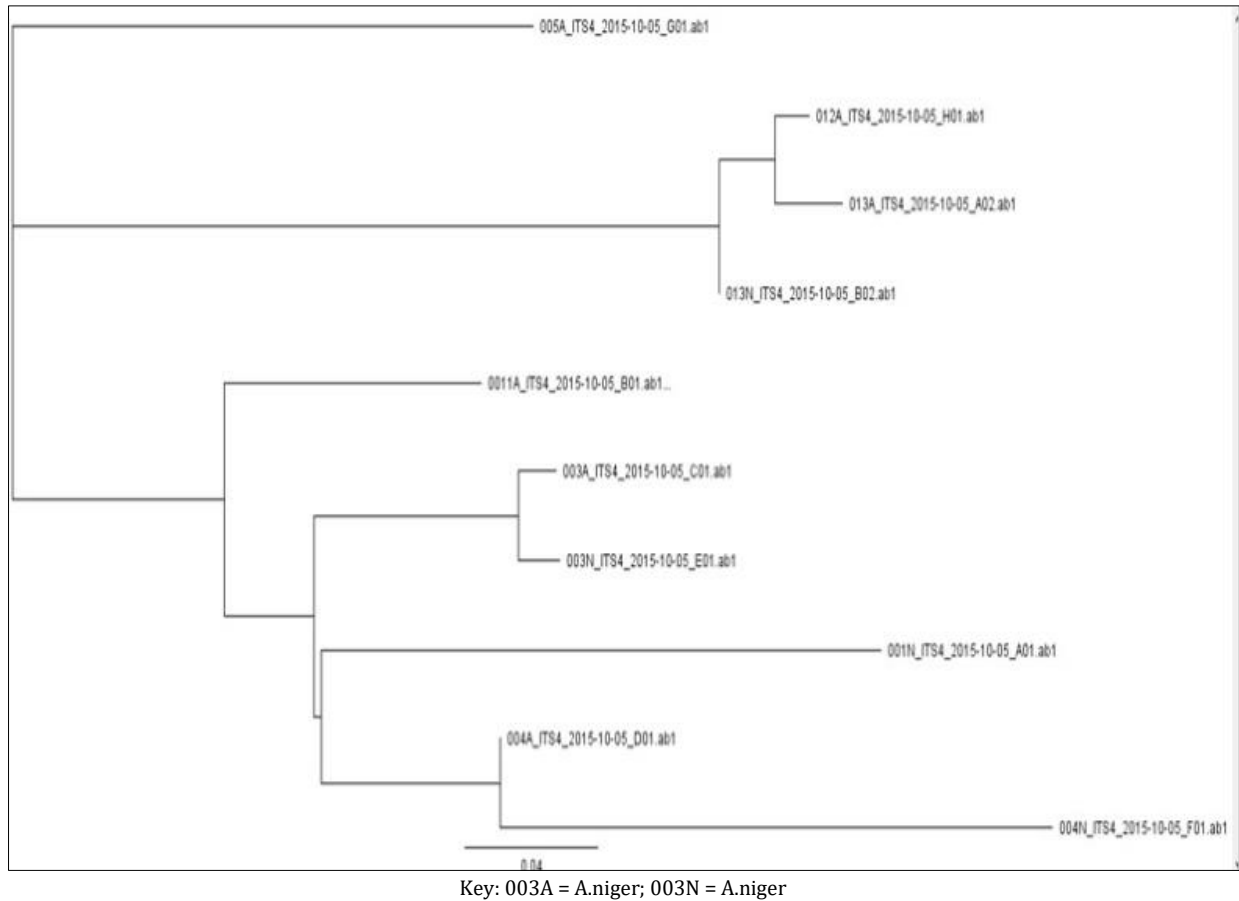


Figure 2 The phylogenetic tree of airborne and nasal fungal isolates showing relatedness between air and nasal isolates

4. Discussion

One of the basic rights of humanity is healthy indoor air. The air we breathe is essential for life yet, it is a potential source of contaminants associated with diseases. Most people spend up to 90% of their time each day indoors (in homes, offices, schools, hospitals, day care centres, retirement homes and other private and public buildings). Exposure of these individuals to bio aerosols like fungi, bacteria, dust mites, microbial volatile organic compounds, toxins and allergens have already been associated with a wide range of health effects such as allergies, infectious diseases or toxic effect especially in immune compromised individuals.

Previous studies by [13], carried out in North, South and Central America showed that species of *Cladosporium*, *Penicillium*, *Aspergillus* and *Alternaria* were the most prevalent indoor airborne fungi. Studies by [14] and [15] on indoor environment also reported similar fungal isolates. *Aspergillus niger* and *A. fumigatus*, are known allergenic and pathogenic fungi that causes rhinitis, sinusitis, aspergillosis and hypersensitivity pneumonitis in immune-compromised individuals.

The dominant fungal species from nasal swabs of occupants of various environments were consistently the dominant airborne fungi in the corresponding indoor environment. This result is similar to the study by [16] who demonstrated similar fungi from nasal cavity of healthy people. One of the nasal isolates *A. fumigatus* is the main agent of aspergillosis and allergic bronchopulmonary aspergillosis in immune-compromised patients and a lot of other diseases [17].

The result of the phylogenetic analysis support the fact that spore inhalation is the usual route of fungal infection especially *Aspergillus* infections, suggesting a determining role of environmental contamination by spores in the epidemiology of airborne fungal infection.

Aspergillus species and some saprophytic fungi are known opportunistic pathogens that cause infections in immunosuppressed individual, hence the use of cortisone immunosuppressed mice in this study. *Aspergillus fumigatus*

is a pathogenic toxigenic and allergenic mould causing invasive aspergillosis, allergic asthma, hypersensitivity pneumonitis, allergic broncho-pulmonary aspergillosis, aspergillus sinusitis etc in immunosuppressed patients. The result of this study is similar to the outcome of the study by [9], on pathogenic potential of *Aspergillus* species using cortisone suppressed mice. Similar to this study, death occurred in mice pre-treated with hydrocortisone and untreated mouse.

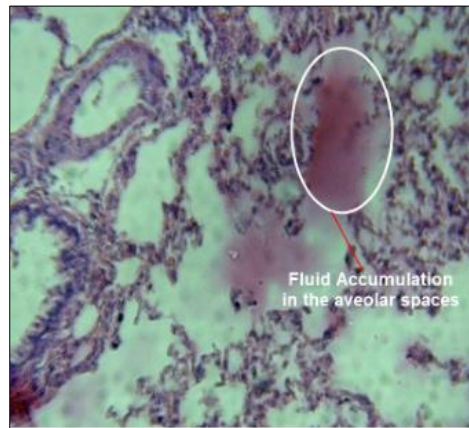


Figure 3 Photomicrograph of H&E stained lung tissues of one immunosuppressed mouse inoculated with spores of *A.fumigatus*

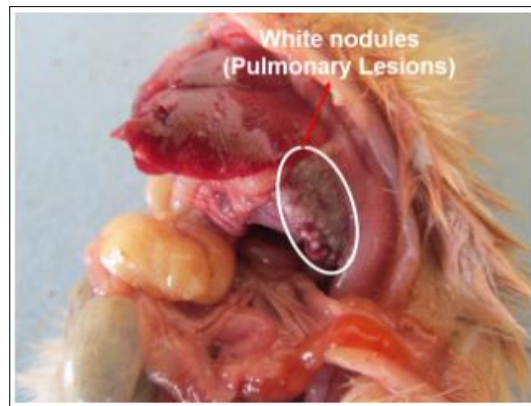


Figure 4 Aspergillosis in mouse infected with Spores of *A.fumigatus*. Pulmonary lesions characterized by white plaques and nodules



Figure 5 Control mouse without pulmonary lesions

5. Conclusion

Characterization of fungal isolates found in indoor environments, indicates the type of fungi individuals are exposed to. It is evident from the research, that there is presence of potentially pathogenic fungi in our indoor environments. There is need for enlightenment of the occupants of various residences on the likely risks posed by these fungi. Findings underline the importance of environmental surveillance and strict application of preventive measures to reduce the concentration of these fungi in our indoor environments.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

The study was approved by the Ethical Committee Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus.

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