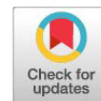


Research Article



Identification and Antifungal Susceptibility Profile of *Aspergillus niger* Isolates from Chicken Blood in the Federal Capital Territory, Nigeria

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Abstract | Increasing incidence of invasive aspergillosis necessitated this study which aimed to isolate and identify *Aspergillus niger* in chicken blood from local markets within F.C.T, Nigeria. Three hundred and sixty (360) chicken blood samples were collected from six Area Councils (AC) in FCT. Isolation and preliminary identification were done using Potato Dextrose Agar (PDA) and Microscopy. Antifungal Susceptibility Testing (AFST) was done with liofilchem® antifungal disc using Disc Diffusion method by Kirby bauer and Ten (10) antifungal agents were tested. Fifty-four (54) isolates were confirmed positive for *Aspergillus niger* with prevalence of 16 (29.6%). The distribution of *A. niger* from the different Area councils were 8 (2.2%) from Abaji, AMAC 5 (1.4%), Bwari 6 (1.7%), Gwagwalada, 21 (5.8%), Kuje 10 (2.8%) and Kwali A4 (1.1%) bringing the overall prevalence to 15%. Isolates showed 100% susceptibility to Posaconazole, Variconazole, Nystatin, Ketoconazole and Itraconazole while multiple drug resistance was observed in routinely used antifungal agents like Metronidazole, Fluconazole, Capsogin, Amphotericin B, and Griseofulvin with a MARI of greater than 0.2 from all antifungal agents tested which was significant indicating that isolates were all multi drug resistance organisms. This study discovered the presence of *A. niger* from chicken blood in the Federal Capital Territory which is a first-time report in Nigeria and hence there is need for isolation and invivo AFST studies with regards to other animal species in Nigeria, as this will guide clinicians in antifungal agents' selection towards achieving effective therapy as a control strategy for eradicating the disease.

Keywords | *Aspergillus niger*, MARI, Zoonotic, Antifungal agents, Potato dextrose agar, *Aspergillosis*

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INTRODUCTION

Infectious diseases rank second globally in terms of cause of death, after cardiovascular disorders (Lopez and

Mathers, 2006) and even though viruses or bacteria are the primary cause of many diseases, opportunistic fungal infections in humans and animals are becoming more commonplace globally (Seyedmousavi *et al.*, 2015), with a

rising incidence of resistant / recurrent mycoses, exist as subcutaneous, systemic, and superficial mycoses which is the most prevalent form (Gnat *et al.*, 2021).

Around 300,000 persons are afflicted with serious fungal diseases that can be fatal, accounting for 161 000 fatalities (53.7%) (Bongomin *et al.*, 2017). About 11.5 million people have fungal asthma, which may be the cause of 46 000 asthma fatalities annually. These revised estimates point to an annual occurrence of 38.5 million deaths and 65.5 million invasive infections caused by fungi, of which 25.5 million (68%; range 35-90) occurred directly related to fungal infections (Denning, 2024).

The fungal hyphae of *Aspergillus* are reported to be septate with the spores (conidia) having a black rough margin, giving a black mass microscopically (Dibia *et al.*, 2007). *Aspergillus niger* is commonly found in cereals, grapes, coffee, processed food/drinks like red wine and have been reported to produce mycotoxins (Susca *et al.*, 2007). They are worldwide in distribution and are important food contaminants which may be present at distinct stages of processing such as during the pre-harvesting stage, dehydration stage, depository stage and transfer stage (Taniwaki and Pitt, 2013). Mycotoxins are secondary metabolites made by a large variety of filamentous fungus that harm humans, animals, and crops, causing disease and monetary losses. Mycotoxins are thought to infect 25% of the world's food-grade grains (Eskola *et al.*, 2020), particularly those used as components of commercial chicken feed (Slizewska *et al.*, 2016). *Aspergillus niger* is reported to be responsible for 75% of the deaths that occurs in animals and human globally due to Aspergillosis (Fianchi *et al.*, 2004; Seyedmousavi *et al.*, 2015).

Aspergillus niger is a filamentous fungus, which appears macroscopically as initially white but changes to black after a few days due to conidial spore production. Colonies of *Aspergillus* on Potato dextrose agar (PDA) have a downy to powdery texture on medium with an uncolored or light-yellow backside (The reverse colour), which can range from cream in *Aspergillus niger* with the edges of the colonies appearing pale yellow producing radial fissures, orange to purple in *Aspergillus versicolor* and from purple to olive in some strains of *Aspergillus nidulans*. *A. flavus* colonies start out looking yellow-green but eventually turn a darker green. The texture is occasionally grainy and feels like wool or cotton (Marta *et al.*, 2022).

Clinical signs that have been reported in animals include: reduced daily weight gain, decreased feed efficiency, polyuria, polydipsia, poor sperm quality, fetal death, fetal resorption, and abortion (Manafi *et al.*, 2015). Rapid weight loss, lethargy, hudding, decreased appetite, decreased thirst, weakness, reduced egg production, poor hatchability, thin

shelled eggs, production of egg yolk with blood or meat spots, decreased body temperature, excessive urates, and yellow diarrhea in chickens (Gianni *et al.*, 2010).

Due to *Aspergillus*'s emergence of resistance, antifungal medications used to treat various kinds of aspergillosis encounter difficulties (Perlin *et al.*, 2017). In addition, one of the main causes of developed drug resistance is continued usage of commonly available antifungal agents for medication. Along with these factors, the location, type of *Aspergillus* species, and antifungal medications all play a role (Goncalves *et al.*, 2016) in the prevention and control of the disease. Even though information on drug-resistant genes and genome mutations is accessible, there are few therapeutic options, which make it challenging to control invasive secondary fungal infections like invasive aspergillosis (Perlin *et al.*, 2015).

The paucity of information on cultural isolation of *Aspergillus niger* from chicken blood in Nigeria, necessitated this study. Invasive aspergillosis is a zoonotic disease of great public health concern worldwide, with an increasing report (About 4.8 million) of emerging cases of aspergilloma (fungus ball in the lung cavity) in immunocompromised patients and animals, hence there is need for determination of antifungal agents' susceptibility profile, which will aid effective and successful therapy of aspergillosis in Nigeria.

MATERIALS AND METHODS

STUDY AREA

The federal capital territory is located north of the confluence of the Rivers Niger and Benue. It is bordered by Niger state to the West and North, Kaduna state to the northeast, Nasarawa state to the east and south and Kogi state to the southwest. Lying between latitude 8.25° and 9.20° north of the equator and longitude 6.45° and 7.39° east of Greenwich Meridian, from a geographic perspective, the Federal Capital Territory is situated in the middle of the nation (Suleiman and Bako, 2021).

The federal capital territory has a landmass of approximately 7,315 km², and it is situated within the Savannah region with moderate climatic conditions. The territory is currently made up of six Area councils, namely: Abaji, Abuja Municipal, Gwagwalada, Kuje, Bwari and Kwali (Abubakar, 2014).

STUDY DESIGN

This is a cross sectional study which is a type of observational study conducted to determine the prevalence of *Aspergillus niger* from chicken blood in the Federal Capital Territory, Nigeria. Sixty (60) blood samples were collected from each Area Council weekly making a total of 360 blood samples.

The samples were collected based on availability of chickens sold at the major local markets using convenient sampling method due to limited access to the entire target population and reluctance of some of the marketers to allow sample collection from their chickens, hence sampling was done based on their availability, willingness of marketers and ease of access to the chickens as described by [Castello et al. \(2009\)](#). This sampling method is cost effective and easy to apply but may lack varieties in samples collected as sampling is usually based on availability and ease of access to samples.

The major local markets of the six (6) Area Councils of the Federal Capital Territory were each sampled weekly on their various market days.

Blood samples were taken from 360 chickens in the FCT (AMAC, Gwagwalada, Kwali, Abaji, Bwari and Kuje Area councils), the sampling was carried out from February, 2022 to April, 2022, during the dry season.

SAMPLE SIZE DETERMINATION AND SAMPLING TECHNIQUES

The sample size was determined using the formula recommended by [Thrusfield \(2007\)](#): Using 50.0% prevalence. In this study, 360 blood samples were collected so as to increase precision. Convenient sampling method was used to collect blood from chickens for this research, from the local markets in the Federal Capital Territory, Nigeria.

SAMPLE COLLECTION

COLLECTION OF BLOOD SAMPLES AND SAMPLE PROCESSING

Two (2) ml of blood was collected aseptically using a 2ml syringe and 21-gauge needle and were transferred into clean test tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) for culture ([Adah et al., 2018](#)), and test tubes were labeled appropriately. All samples were transported to the Veterinary Microbiology Laboratory of the Faculty of Veterinary Medicine Gwagwalada, in a leak proof container packed with ice ([Bertu et al., 2010](#)). The samples were stored in a refrigerator until required for processing.

LABORATORY CULTURE AND IDENTIFICATION

MEDIA PREPARATION

Potato Dextrose Agar (HIMEDIA, USA) was made in accordance with the manufacturer's instructions, weighing 39g of the media and dissolving in 1 litre of distilled water, then followed with the addition of 250 mg of Chloramphenicol to every 500 ml of agar prepared. The media was then thoroughly mixed and divided into 20 ml and 10 ml portions, respectively, for each petri dish and universal bottle. The universal bottles were placed slantwise at a 45-degree angle so as to enable the media to solidify

([Dynowska et al., 2011](#)).

CULTURE OF BLOOD SAMPLES

Using a sterile pasteur loop, the samples were inoculated into sterile labeled plates and universal bottles containing the prepared sterile media infused with antibiotics ([Ezekiel and Sombie, 2014](#)). All of these were then incubated aerobically at room temperature (25 °C) for three to five days and disposed of after two to three weeks ([Thomas and Thomas, 2001](#)).

MICROSCOPIC EXAMINATION

The resultant colonies were stained using Lactophenol Cotton Blue Stain (LPCB, HIMEDIA, Kelton, USA) following standard procedure as described by the manufacturers and viewed under the microscope at low power (x4 and x10) and oil immersion (x100) respectively for observation of fungal hyphae and conidia as described by [Knoll et al. \(2023\)](#).

ANTIFUNGAL SUSCEPTIBILITY TEST

Antifungal sensitivity testing was done using the Liofilchem® antifungal disc, from Italy. A subculture was made from the growth on the petri dishes and antifungal susceptibility disc (Liofilchem® Italy) was placed on the surface of the inoculated media (PDA) using the disc diffusion method ([CLSI, 2010](#)) and this was left at room temperature for 1 to 3 days.

The antifungal agents with the concentration used for antifungal susceptibility testing in this study include; Metronidazole (MTZ, 50µg), Griseofulvin (AGF 10µg), Fluconazole (FLU, 100µg), Ketoconazole (KCA, 15µg), Nystatin (NY, 100 iu), Voriconazole (VC, 1µg), Posaconazole (POS, 5µg), Itraconazole (ITC, 50µg), Amphotericin B (AMB, 20µg), and Caspofungin (CAS, 5µg) ([Patterson et al., 2016](#); [Ullmann et al., 2018](#)). At the end of the incubation period, the inhibition halos or zones were measured using a metric ruler and compared with the diameters of the standard Clinical Laboratory Standard Institute ([CLSI, 2010](#)) and recorded as sensitive, intermediate or resistant.

RESULTS AND DISCUSSION

CULTURAL ISOLATION

Out of the 360 samples collected and processed, 54 (15.0%) were positive for *Aspergillus* species while 306 (85.0%) samples were negative. Colonial morphologies were observed on Potato Dextrose Agar (PDA) at 25°C after 7 days, which signifies the occurrence of *Aspergillus niger* in the blood of chickens in this study as shown in [Table 1](#).

Table 1: Distribution of positive isolates based on Local Government Area.

Local Govt. area	No. of samples collected	No. of positive samples (%)	No. of negative samples (%)
Abaji	60	8(2.2)	52(14.4)
AMAC	60	5(1.4)	55(15.3)
Bwari	60	6(1.7)	54(15.0)
Gwagwalada	60	21(5.8)	39(10.8)
Kuje	60	10(2.8)	50(13.9)
Kwali	60	4(1.1)	56(15.6)
Total	360	54(15)	306(85)

The colonial morphology observed were initially white colonies which turn dark cinnamon brown and quickly became black powdery colonies indicating conidial production with a reverse of white to pale yellow in colour with radial fissures as seen in Figure 4, 5, respectively, this is a classical feature that describes *Aspergillus niger* isolates (CLSI, 2010).

The fungal hyphae were observed to be septate with the spores (conidia) having a black rough margin giving a black mass microscopically at x 40 magnification and this was observed using Leica DM 300 Binocular compound microscope after staining with Lactophenol Cotton Blue stain (LPCB HIMEDIA, Kelton, USA) as shown in Figure 2.

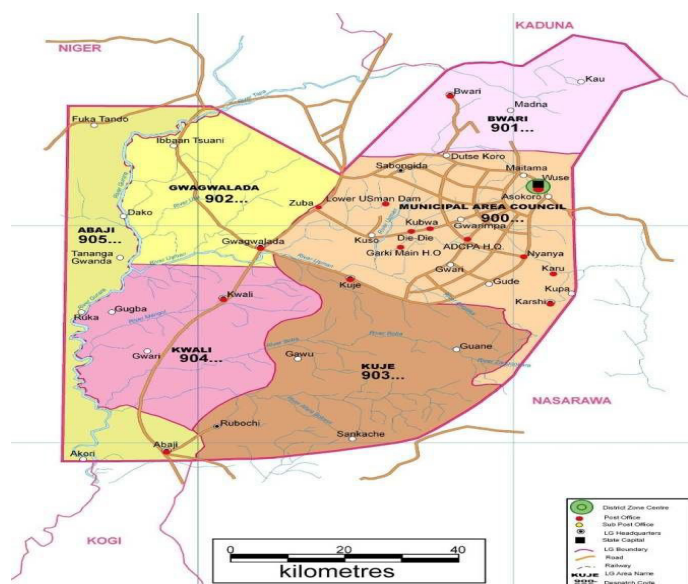


Figure 1: Map of the federal capital territory showing the six area councils sampled.

Source: Ozioma *et al.* (2017).

The distribution of *Aspergillus niger* isolated from Abaji Area Council was 3.3% (2), While from AMAC was 3.3% (2) and from Bwari LGA was 3.3% (2), While from Gwagwalada LGA was 10.0% (6), From Kuje LGA was *Aspergillus niger* 5.0% (3), *Aspergillus fumigatus* 6.7% (4)

and from Kwali LGA its 1.7% (1), as shown in Figure 1 and Table 3.

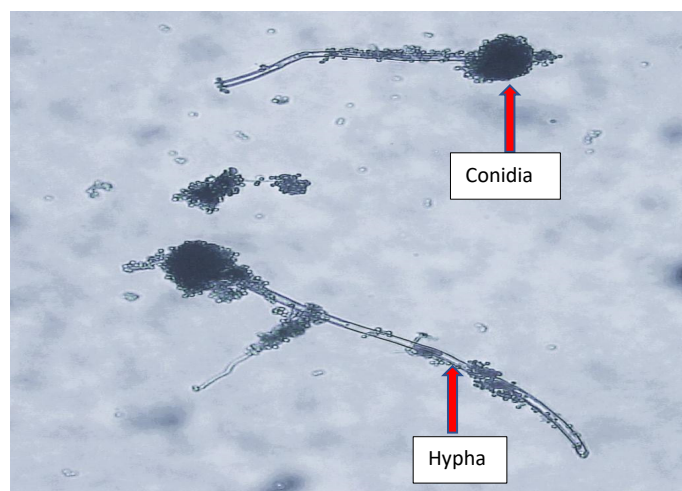


Figure 2: Showing the microscopic appearance of *Aspergillus niger* using Lacto Phenol Cotton Blue stain on Leica DM 300 binocular compound microscope at x40 Magnification.

Table 2: Prevalence of the various isolates based on LGA.

Aspergillus specie	LGA	No of isolates	Percent-age (%)
Aspergillus niger	ABAJI	2	3.3
Aspergillus niger	AMAC	2	3.3
Aspergillus niger	BWARI	2	3.3
Aspergillus niger	GWAGWALADA	6	10.0
Aspergillus niger	KUJE	3	5.0
Aspergillus niger	KWALI	1	0.7

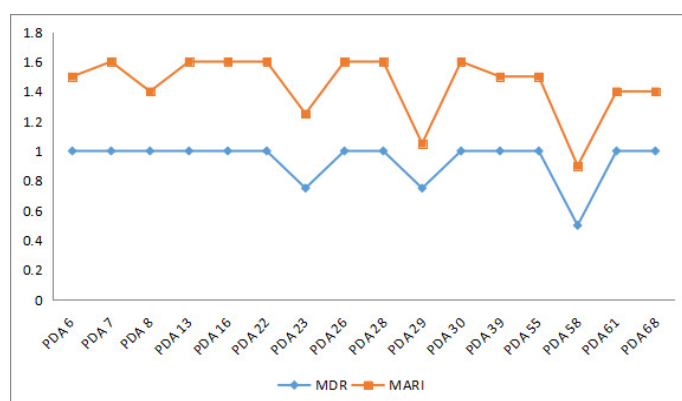


Figure 3: Line graph showing the multiple drug resistance (MDR) and multiple antifungal resistance index (MARI) of the isolates.

The distribution of positive isolates and their percentage positive and negative samples based on Local Government Area as shown in Table 1 and Figure 1 as follows: Gwagwalada area council had the highest percentage positive samples of 21.0% (5.8), Kuje area council had a percentage positive samples of 10.0 % (2.8), Abaji area

council had a percentage positive samples of 8.0% (2.2), Bwari Area Council had a percentage positive samples of 6.0% (1.7), AMAC had a percentage positive samples of 5.0 % (1.4), and Kwali Area Council had the lowest percentage positive samples of 4.0% (1.1).

Table 3: Antifungal Susceptibility profile of *Aspergillus niger* to various antifungal agents.

Antifungal agents	Concentration (µg/iu)	Sensitivity (%)	Intermediate (%)	Resistance (%)
Fluconazole	100 µg	6(37.5)	0(0)	10(62.5)
Voriconazole	1 µg	16(100)	0(0)	0(0)
Posaconazole	5 µg	16(100)	0(0)	0(0)
Itraconazole	50 µg	7(43.7)	8(50)	1(6.25)
Ketoconazole	15 µg	10(62.5)	6(37.5)	0(0)
Metronidazole	50 µg	0(0)	0(0)	16(100)
Caspofungin	5 µg	0(0)	0(0)	16(100)
Amphotericin B	20 µg	0(0)	1(6.25)	15(93.7)
Nystatin	100iu	15(93.7)	1(6.25)	0(0)
Griseofulvin	10 µg	1(6.25)	0(0)	15(93.7)

Metronidazole 50µg, Posaconazole 5µg, Fluconazole 100µg, Ketoconazole 15µg, Itraconazole 50µg, Voriconazole 1µg, Caspofungin 5µg, Nystatin 100iu, Amphotericin B 20µg, Griseofulvin 10µg.

Kwali Area Council had the highest percentage negative samples of 56.0% (15.6), AMAC had a percentage negative sample of 55.0% (15.3), Bwari Area Council had a percentage negative sample of 54.0% (15.0), Abaji Area Council had a percentage negative sample of 52.0% (14.4), Kuje Area Council had a percentage negative sample of 50.0% (13.9), and Gwagwalada Area Council had the lowest percentage negative samples of 39.0% (10.8) as

displayed in Table 2.

Table 3 displays the antifungal susceptibility profile of the isolates to various antifungal drugs. All the isolates showed high percentage susceptibility of 100.0% to Voriconazole (1µg) and Posaconazole (5µg), followed by 93.7% to Nystatin (100iu), 62.5% to Ketoconazole (15µg), 43.7% to Itraconazole (50µg), 37.5% to Fluconazole (100µg), and 6.25% to Griseofulvin (10µg). All the isolates showed high percentage resistance of 100% to Metronidazole (50µg) and Caspofungin (5µg), followed by 93.7% to Amphotericin B (20µg) and Griseofulvin (10µg), 62.5% to Fluconazole (100µg) and 6.25% to Itraconazole (50µg).

All the isolates obtained showed multiple drug resistance (MDR) to 2 or more classes (Azoles, Echinocandins, Polyenes and Allylamines) of antifungal drugs used in this study and 10 antifungal agents were used in this study belonging to 4 categories of antifungal agents, which are as follows:

Seven (7) isolates which are on PDA 7, PDA 13, PDA 16, PDA 22, PDA 26, PDA 28 and PDA 30 were all resistant to six (6) antifungal agents belonging to (4) classes of antifungal with a multiple drug resistance index (MARI) of 0.6, respectively.

Aspergillus species especially *A. niger* have been reported to be the major cause of hepatocellular carcinoma and liver failure in animals/humans especially in chemotherapy, transplant, HIV/AIDS and other debilitating/terminal disease patients, and as such the impact and devastating effects of infection with *Aspergillus* species and its isolation

Table 4: Antifungal resistant pattern of *Aspergillus niger* isolates to various antifungal agents.

S. N	Isolates	Resistance pattern	Number of resistant antifungal agent
1	PDA 6	MTZ, FLU, CAS, AMB, AGF	5
2	PDA 7	MTZ, FLU, KCA, CAS, AMB, AGF	6
3	PDA 8	MTZ, CAS, AMB, AGF	4
4	PDA 13	MTZ, FLU, KCA, CAS, AMB, AGF	6
5	PDA 16	MTZ, FLU, KCA, CAS, AMB, AGF	6
6	PDA 22	MTZ, KCA, ITC, CAS, AMB, AGF	6
7	PDA 23	MTZ, FLU, KCA, CAS, AGF	5
8	PDA 26	MTZ, FLU, KCA, CAS, AMB, AGF	6
9	PDA 28	MTZ, FLU, KCA, CAS, AMB, AGF	6
10	PDA 29	MTZ, CAS, AMB	3
11	PDA 30	MTZ, FLU, KCA, CAS, AMB, AGF	6
12	PDA 39	MTZ, KCA, CAS, AMB, AGF	5
13	PDA 55	MTZ, FLU, CAS, AMB, AGF	5
14	PDA 58	MTZ, FLU, KCA, CAS	4
15	PDA 61	MTZ, CAS, AMB, AGF	4
16	PDA 68	MTZ, CAS, AMB, AGF	4

Table 5: Multi drug resistance (MDR) pattern and multiple antifungal resistance index (MARI) of *Aspergillus niger* isolates.

S. N	Isolates	Resistance pattern	No of antifungal agents resistant (n = 10)	Multiple antifungal resistance index (MARI)	No of antifungal categories resisted (n = 4)	Multiple drug resistance (MDR)
1	PDA 6	MTZ, FLU, CAS, AMB, AGF	5	0.5	4	1
2	PDA 7	MTZ, FLU, KCA, CAS, AMB, AGF	6	0.6	4	1
3	PDA 8	MTZ, CAS, AMB, AGF	4	0.4	4	1
4	PDA 13	MTZ, FLU, KCA, CAS, AMB, AGF	6	0.6	4	1
5	PDA 16	MTZ, FLU, KCA, CAS, AMB, AGF	6	0.6	4	1
6	PDA 22	MTZ, KCA, ITC, CAS, AMB, AGF	6	0.6	4	1
7	PDA 23	MTZ, FLU, KCA, CAS, AGF	5	0.5	3	0.75
8	PDA 26	MTZ, FLU, KCA, CAS, AMB, AGF	6	0.6	4	1
9	PDA 28	MTZ, FLU, KCA, CAS, AMB, AGF	6	0.6	4	1
10	PDA 29	MTZ, CAS, AMB	3	0.3	3	0.75
11	PDA 30	MTZ, FLU, KCA, CAS, AMB, AGF	6	0.6	4	1
12	PDA 39	MTZ, KCA, CAS, AMB, AGF	5	0.5	4	1
13	PDA 55	MTZ, KCA, CAS, AMB, AGF	5	0.5	4	1
14	PDA 58	MTZ, FLU, KCA, CAS	4	0.4	2	0.5
15	PDA 61	MTZ, CAS, AMB, AGF	4	0.4	4	1
16	PDA 68	MTZ, CAS, AMB, AGF	4	0.4	4	1

from blood as recorded in this study cannot be underestimated (Berkow *et al.*, 2020).

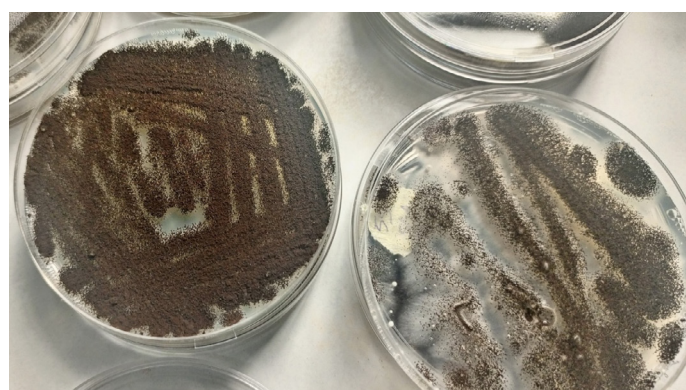


Figure 4: Powdery black colonies of *Aspergillus niger* on PDA.

The macroscopic appearance of powdery black colonies with a pale-yellow reverse on Potato Dextrose Agar exhibiting radial fissures is significant for *Aspergillus niger* as described by the atlas of medical mycology, 2018 and also as reported by Teh and Latiffah (2018). This finding indicates that Potato Dextrose Agar is a selective media which is conducive for the characteristic growth of *Aspergillus niger* as reported by De-Farias *et al.* (2010) and Woo and Wook (2005).

The microscopic appearance of septate hyphae with spores (conidia) having a black rough margin giving a black mass observed in this study indicates that the isolates obtained are

Aspergillus niger as shown in Figure 2, and this microscopic observation serves as a preliminary identification which suggests *Aspergillus* specie as previously reported by Teh and Latiffah (2018).

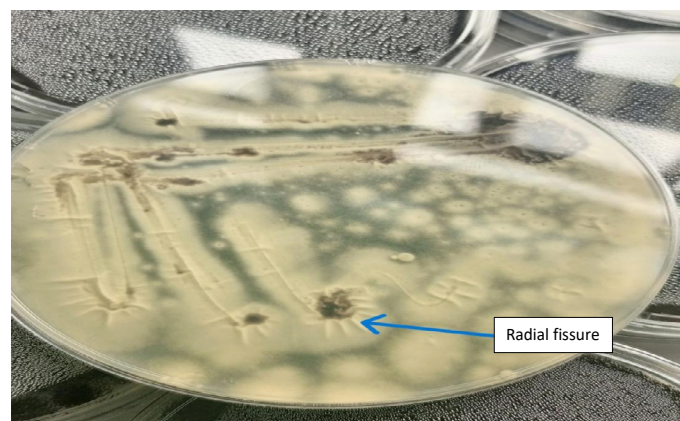


Figure 5: Reverse white cultural morphology of *Aspergillus niger* on PDA after 7 days of culture at 25°C with arrow showing the radial fissure.

This study isolated *A. niger* from blood which is a first time report and this shows the occurrence of *Aspergillus niger* in chicken blood (fungemia) in the Federal Capital Territory Nigeria, the finding of *Aspergillus* specie in the blood of chicken is of great significance because this fungi specie have been reported to be the causative agents of invasive aspergillosis in immunocompromised patients with detrimental multiple organ effects in both animals and humans with great public health significance worldwide

since it can be transmitted from animals to humans and vice versa (Pu *et al.*, 2018).



Figure 6: Clear zones of inhibition from antifungal susceptibility testing of *Aspergillus niger* on PDA using the Disc diffusion method after 20 hours of incubation on PDA.

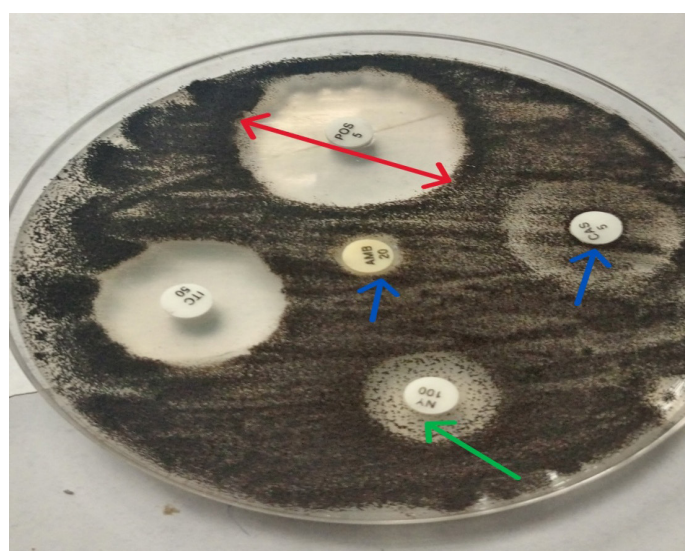


Figure 7: Antifungal resistance of *Aspergillus niger* to various antifungal agents on PDA with blue arrows indicating resistant zones while red arrow indicates clear zone of inhibition suggestive of isolate susceptibility to the antifungal agent.

The prevalence rate of 29.6% obtained in this study is lower than the 75.1% reported by Habib *et al.* (2015), and this might be attributed to the difference in sample location, sample type and type of media utilized since the previous study collected poultry feed samples in Kaduna state, while this study collected chicken blood in the Federal Capital Territory (FCT) and also, the isolation media utilized for sample processing in this study was Potato dextrose agar (PDA) while the previous study used Rose Bengal Chloramphenicol Agar.

Fungi species especially *Aspergillus* species have been reported as common on cereals, which are the major components of poultry feed and these poultry feed easily becomes contaminated through small quantities of fungal spores contaminating the grains used for poultry feed formulations during pre-harvesting, harvesting, transportation or storage stage of grain processing (Amadi and Adeniyi, 2009), which might also be a reason for the high prevalence obtained in the previous studies from cereals.

The prevalence rate (29.6%) reported in this study is higher than the prevalence rate of 27% reported by Muhammad *et al.* (2021) and these differences might be as a result of difference in sample type, since the previous study reported its prevalence from sun dried okro from only one Area Council which is Gwagwalada while the present study sampled chicken blood from six (6) Area Councils of the Federal Capital Territory. The lower result obtained in the previous study could have been due to inactivation or inhibition of *Aspergillus* species on the sun dried okro through sun drying thereby reducing the fungal concentration as Ultra Violet rays from sunlight have been reported to inactivate both *Aspergillus* species and their spores (Tsado, 2015).

The percentage distribution of 6 (10%) for *Aspergillus niger* isolated from chicken blood from Gwagwalada Area Council in this study is higher than that obtained from the other Area Councils, which might indicate that there are poor biosafety measures leading to higher level of contamination in the Gwagwalada area council markets where samples were collected, as evident during sample collection at the Gwagwalada area council market.

The percentage distribution of 10% for *A. niger* obtained in this study is lower, when compared to that reported in Mailafia *et al.* (2017) and hence disagrees with the previous findings, which reported a prevalence of 38% for *A. niger* from fruits (pineapple, watermelon, orange, pawpaw, and tomatoes) in Gwagwalada. This difference in sample type could be the reason for the difference in prevalence obtained in this present study and this finding implies that there is possibly high level of zoonosis due to close contacts between the chickens and human observed during sampling, which can lead to possible increase in the endemicity of the disease and also a possibility of a reverse zoonosis.

The overall prevalence for *Aspergillus niger* (35%) obtained in this study in Gwagwalada is lower than the 70% reported in Gwagwalada by Mailafia *et al.* (2017) and this variance in results in this present study was attributed to the fact that the sample type and sample size collected were different, as this study sampled sixty (60) chicken blood

while the previous study sampled hundred (100) fruits from Gwagwalada Area council. *Aspergillus niger* has been reported to thrive more readily in fruits and vegetables than poultry blood.

Gwagwalada had the overall highest prevalence of *Aspergillus niger* isolated as shown in Figure 1 and this indicates a high level of infection in chickens compared to other Area Council in the FCT, which might be attributed to the high population density observed at the poultry market where chickens are sold for slaughter, which can also lead to high level of contamination due to poor hygienic practices observed as most chickens for slaughter are kept for sales together in a crowded cage and some kept on the moist floor tied together as restraint. These kind of management practices leads to high level of contamination as *Aspergillus niger* are associated with thriving on wet environment and transmission is mostly via inhalation of spores and direct contact with contaminated animals and formites (Yang *et al.*, 2023). The high infection observed in chicken blood from Gwagwalada might also indicates direct inhalation of spores from contaminated soil and cage floor suggesting that *Aspergillus* species exist as normal flora of the soil especially in spore forms and are easily inhaled with any little disruption of the soil which is similar to previous reports of Berkow *et al.* (2020).

The percentage distribution of 2(3.3%) isolated from chicken blood from the local markets in Abaji Area Council in this study is in line with the findings of Akueche *et al.* (2012) who reported the occurrence of different fungal species, including *Aspergillus* (*A. niger* and *A. flavus*) from sesame seeds obtained from the local markets in Abaji Area Council. According to Bankole and Mabekoje (2004), *Aspergillus* species. were among the most common fungal contamination of Nigerian food commodities (grains, nuts, beverages, herbs, spices, milk, meat, poultry products) which also have been extensively reported by Atehnkeng *et al.* (2008). According to Jamime-Garcia and Cotty (2004), *Aspergillus* have a very high occurrence when compared to other fungal species because they are easily able to survive in soil, plant debris, and insects which act as a reservoir that can infect grains and animals in the field.

The percentage distribution of *Aspergillus* species isolated from chicken blood from the local markets in Abuja Municipal Area Council in this study is 2 (3.3%) which is lower than the prevalence of 12.2% reported by Aboh and Oladosun (2014) from Kunun-zaki in AMAC. This low prevalence might be as a result of *Aspergillus* species being more readily common and isolated from sputum and lung biopsy samples and rarely isolated from blood samples and more so, *Aspergillus* organism being reported as a fermentative organism, it will most likely proliferate

more easily on fermentative products like kunun-zaki, also poor hygienic practices which might lead to contamination from utensils, water for processing and packages (Nylon bags/plastic bottles) used for its distribution might also lead to increase prevalence of the organism in Kunun-zaki compared to its isolation from chicken blood. Nevertheless, the importance of isolating the organism from chicken blood cannot be underestimated because *Aspergillus* is a zoonotic organism that can cause a variety of health issues, such as lung infections, organ infections, and allergic reactions in humans and animals (Gnat *et al.*, 2021).

The percentage distribution of *Aspergillus* species isolated from chicken blood from the local markets in Bwari Area Council in this study showed isolation rate for *Aspergillus niger* 2(3.3%) which is lower than that reported by Mairami *et al.* (2018), who reported a prevalence of 58% from fruits in Bwari Area Council. This differences in prevalence were attributed to the difference in sample type and sample size, as the previous study had a larger sample size of seventy (70) fruits compared to this present study which sampled sixty (60) blood samples from chickens in Bwari Area Council. Given that *Aspergillosis* is a zoonotic disease with a high morbidity and mortality rate and that chickens are a major source of infection for both humans and animals, the isolation of *Aspergillus* species from chicken blood is extremely important for public health since Aryse *et al.* (2018) established that aspergillosis which is caused by *Aspergillus* species kills more than 1 million people each year and impacts the health of over 15 million people.

The percentage distribution of *Aspergillus* species isolated from chicken blood from the local markets in kuje Area Council in this study showed isolation rate for *Aspergillus niger* as 3(5.0%) which is in line with the reports of previous findings reported by Yusuf *et al.* (2021), which reported similar incidence rate for *Aspergillus* specie from maize seeds from the local markets in Kuje which signifies that *Aspergillus* species thrive more in cereals especially in the tropics where the environmental condition is humid hence favours the proliferation of *Aspergillus* species.

The percentage distribution of *Aspergillus* species isolated from chicken blood from the local markets in Kwali Area Council in this study showed isolation rate of 1(1.7%) which is in line with the findings of Yusuf *et al.* (2021) which reported a similar incidence for *Aspergillus* species from maize (Yellow corn) in Kwali Area Council.

This study obtained different percentage prevalence for all six Local Government Area Councils sampled and this might be attributed to the difference in temperature, chicken housing or cage system and management practices encountered in the various Local Government Area Councils. The extremely high chi-square value (328.98)

compared to critical value (11.07) displayed a very small p-value ($p < 0.0001$) which indicates that the differences in prevalence observed from the six Area Councils are not random and also further reveals that the prevalence between the Area Councils is statistically significant. Strong evidence of real disparities between area councils might have resulted from their hygiene and risk of uneven health outcomes across councils.

This finding of *Aspergillus niger* from chicken blood is of great concern as this organism have been reported to secrete mycotoxins like ochratoxins, patulin and fumonisins which is capable of causing nephrotoxicity, renal tumors, immunotoxicity, hepatotoxicity and teratotoxicity in poultry birds and also in other animal species, which makes it a potential hazard to human health through their consumption.

Aspergillus niger causes severe reduction in production yield in poultry farms and industries as these organisms can penetrate the eggshell and contaminate eggs in the hatchery and causes aspergillosis in poultry birds leading to high morbidity and mortality thereby causing great economic losses to poultry farmers globally.

Antifungal susceptibility testing in this study reveals increased susceptibility to members of the Azole class of antifungal drugs (Itraconazole, Ketoconazole, Posaconazole and Voriconazole) and polyene class of antifungal drugs (Nystatin), with isolates showing hundred percent (100.0%) susceptibility to Posaconazole and Voriconazole and 93.7% susceptibility to Nystatin antifungal agent suggesting that the use of these antifungal drugs in aspergillosis therapy will be effective (Revie *et al.*, 2018) and also assist in improving a patient's health state, hence acting as a potential disease control measure as previously reported by Van Daele *et al.*, (2019). In addition, the isolates were highly resistant to members of the Azole (Metronidazole and Fluconazole), Polyenes, (Amphotericin B), Allyamines (Griseofulvin) and Echinocardins (Capsogugin) class of antifungal agents, with isolates showing 100.0% resistance to Metronidazole (Azole class) and Capsogugin (Echinocardin class), with 93.7% resistance to Amphotericin B (Polyene class) and Griseofulvin (Allyamines) antifungal agents. However, this multidrug resistant (MDR) observed in this study as shown in Table 4, was attributed to indiscriminate use of these five (5) Antifungal drugs by quacks and poultry owners/ farmers as these agents are cheaper and readily available (Vanreppelen *et al.*, 2023) in our environment of study when compared to Posaconazole, Itraconazole, Voriconazole and Nystatin antifungal agents, which are more frequently used in developed countries where resistance have not been widely established (Berkow *et al.*, 2020), resulting to invasive aspergillosis infection

characterized by drug resistance and therapeutic failures in patients (Arendrup *et al.*, 2012). Also, this multi drug resistance observed in this study as shown in Table 5 and Figure 3, is of great public health concern globally as antifungal resistance increases the risk of topical and systemic fungal infections, which can affect the mucous membrane, hair, nails, lungs, integument, and other body parts leading to great morbidity and mortality in humans and animals. Furthermore, antifungal resistance contributes to major crop losses experienced by farmers and this challenges crop production and its quality thereby leading to some devastating economic losses in agricultural industries from damage to food crops and other plants by antifungal resistant strain of fungi usually difficult to manage as these fungi organisms have developed ability to defeat the drugs routinely designed to eliminate them.

There is an increase in clinically available triazoles especially in developing countries like Nigeria and hence increase routine utilization in triazole antifungal therapy as the major drug of choice based on availability (Hope *et al.*, 2013) and their indiscriminate use has led to the development of emerging acquired resistance reported recently, in fungal species such as *Candida* and *Aspergillus* species (Berkow *et al.*, 2020). This probably might also be the major reasons for the high resistance to the triazole class of antifungal agents observed in this study, which gives rise to limited treatment alternatives as only four classes of antifungal agents are available (azoles, polyenes, flucytosine and the echinocandins) which might lead to problems in aspergillosis management and control due to triazole antifungal therapy failures, hence increasing the endemicity of aspergillosis in Nigeria, due to the possibility of a fungus developing resistance to a particular class of drug not responding to any readily available treatments hence serves as a serious public health crisis.

The significance of carrying out antifungal susceptibility testing (AFST) in this study was to determine the susceptibility of the isolates as shown in figure 6 and resistance of the isolates to antifungal agents as shown in Figure 7, is to serve as a guide for veterinary practitioners within the area of study towards making informed decisions as to the right choice of antifungal agent required for effective therapy when handling cases of aspergillosis especially when the patient is suddenly not responding to therapy or when the fungal infection is invasive and have developed drug resistance which might lead to therapy failure in patients (Arendrup *et al.*, 2016). The 100.0 % and 93.7 % susceptibility observed in this study to Posaconazole, voriconazole and nystatin respectively indicates that treatment with these antifungal agents in clinical cases of aspergillosis will lead to effective management and control of the disease here in Nigeria

possibly decreasing the endemicity and prevalence of the disease in both animals and humans in Nigeria.

CONCLUSIONS AND RECOMMENDATIONS

The prevalence of *Aspergillus niger* from poultry blood was established and the results obtained will also serve as a baseline data for subsequent studies on *Aspergillus species* in Abuja and Nigeria at large. This study obtained an overall prevalence of 29.6% for *Aspergillus niger* from chicken blood. According to this study, *A. niger* isolates showed 100.0% multi drug resistance (MDR) to Fluconazole, and Metronidazole, while it showed 93.7% resistance to Amphotericin B, Caspofungin and Griseofulvin, while in terms of susceptibility, the *A. niger* isolates were 100.0% susceptible to Voriconazole, and posaconazole, while 93.7% susceptible to Itraconazole, Nystatin and Ketoconazole. It also obtained a Multiple Antifungal Resistance Index (MARI) of greater than 0.2 which is significant.

Major stakeholders like veterinarians and poultry farmers need to ensure constant use of strict standard operating procedures (SOPs) in the clinics, field and in the farms respectively to aid reduction in the rise of antifungal resistance and this can be achieved by ensuring clinicians always complete the full dosage regime of all antifungal medications, practice regular hand washing, improving stewardship of all existing chemicals in the clinics and farms, promoting and supporting new antifungal drugs detection and development and also capitalizing on emerging technologies for substitute solutions which will help decrease or completely eliminate antifungal drug resistance globally.

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NOVELTY STATEMENT

This research demonstrated the isolation and antifungal susceptibility testing of *Aspergillus niger* from chicken blood which has never been reported in any of the six Area Councils of the Federal Capital Territory and Nigeria at large.

AUTHOR'S CONTRIBUTION

Bridget Maria Jessica ADAH: Carried out the actual conceptualization, formal analysis, investigations, resources,

writing of the original draft, study design, methodology, and data curation of the manuscript.

Samuel MAILAFIA, H.O.K OLABODE, Monday ONAKPA, and James Agbo AMEH: Were involved in the project administration and supervision of this manuscript. Martha Echioda OGBOLE, Enid GODWIN, and Chinwe Elizabeth OKOLI: Were involved in the visualization and funding acquisition.

Nnabuike Nicodemus Mkpuma: carried out the software and validations required.

Ebenezer Odey ODEY and Rabi Rebecca MAIRABO: Were involved in the visualization and formal analysis (Statistical Analysis) required.

Oluwa Adikpe AGBONU, Ifeanyi Cajetan Cashmir and Hakeem Onigbanjo: All contributed to the project administration.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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