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*Case Report*

# Laboratory diagnosis of *Aspergillosis* in poultry and humans and detection of fungal contaminants in poultry vaccines in Jos, Plateau, Nigeria

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**ABSTRACT:** Fungal mycoses pose a significant threat to ecosystem health. It is a major impediment of the poultry industry and an important source of respiratory problems in humans. This case report described the laboratory diagnosis of *Aspergillosis* in poultry and humans and the presence of fungal contaminants in poultry vaccines in Jos, Plateau State, Nigeria. The cases were diagnosed based on clinical signs, necropsy and mycological evaluation of tissue, sputum and vaccine samples. The fungal isolates were identified using both macroscopic morphology and microscopically using lactophenol cotton blue stain. Antifungal susceptibility profiles of the fungal isolates were determined using disc diffusion methods. The major isolates identified were *Aspergillus flavus, A. fumigatus, A. niger*, *Penicillium* species and *Fusarium* species. Susceptibility of *A. niger* to antifungal agents showed the following profiles, resistance to nystatin, fluconazole and Anidulafungin, and susceptible to amphotericin B, voriconazole itraconazole, and econazole. This case report described the importance of accurate laboratory diagnosis in achieving efficient treatment and reducing losses associated with increased cost of treatment. Also, it emphasized the importance of One-Health as a good model for achieving improved treatment options.

**Keywords**: Fungal contamination, fungal genera, vaccine.

## INTRODUCTION

*Aspergillosis* is the most predominant fungal disease associated with early and high mortality in chicks. It is a non-contagious systemic fungal disease caused by *Aspergillus* species including Aspergillus *niger, A. fumigatus, A. flavus* and *A. nidulans* (Oladele *et al*., 2022; Bitrus *et al*., 2024). In poultry, the disease affects different domestic and wild birds, especially those in captivity, and can lead to a major economic loss due to high mortality, morbidity and compromised growth and feed conversion efficiency in affected birds (Ameji *et al*., 2020; Bitrus *et al.,*

2024). The disease exists in two forms; acute and chronic. The acute form is associated with high morbidity and mortality from inhaling many spores, while the chronic form is characterized by low mortality, and mainly affecting immunocompromised birds. Mortality rates in spontaneous infections range from 4.5 to 90%, with affected birds aged between 3 days and 20 weeks (Arne *et al*., 2011).

In the twentieth century, several vaccine products were unintentionally contaminated with unwanted viruses during their production (Merten, 2002; Sawyer *et al*., 1944). This included the contamination of the poliovirus vaccine with simian virus 40 (SV40) (Shah and Nathanson, 1976), for which the health impacts were not fully known for many decades (Stratton *et al*., 2003). In the early 1980s, unknowingly contaminated therapeutic proteins from human plasma caused widespread transmission of viruses such as human immunodeficiency virus (HIV) to people with haemophilia who received these treatments (Rosendaal *et al*., 1991). As a result, public trust in the plasma industry's ability to safely make these therapies declined (Butler, 1994). However, other sources of contamination may result in poor handling of vaccine products such as poor storage, poor handling etc. These conditions could make the vaccine ineffective and encourage the growth of bacterial or fungal organisms.

Fungi play a significant role in feeds, drugs and vaccines causing disease in animals, often influencing sensory qualities, which can be desirable in some contexts but harmful in others due to the production of secondary metabolites, such as mycotoxins. Fungal contamination can lead to various unwanted sensory effects, including gas formation, off-flavours, unpleasant odours, proteolysis, and lipolysis (Vivier *et al*., 1994; Maraz and Kovacs, 2014). *Penicillium* and *Aspergillus* species are common contaminants in feeds and other products such as vaccines (Gandomi *et al*., 2009), with *Aspergillus* in particular contributing to economic losses due to poor appearance, off-flavours, and health risks from toxic secondary metabolites. Certain mycotoxins, such as Strigmat and Ocystin, are even linked to liver cancer. Fungal toxins in the feed are a major issue in poultry farming causing a detrimental effect on the performance and health of poultry. Mould and mycotoxin contamination of feeds occur worldwide, and they cannot be eliminated from the feed ingredients (Ghaemmaghami *et al*., 2016)

The genera of most concern globally are *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp., (Wolf-Hall and Nganje, 2017). Some fungal species, such as *Aspergillus* spp, can also invade animal tissues and produce mycotoxins (Richard, 2007). The growth of these fungi on raw materials as well as vaccines may cause detrimental effects such as suppressing the immune system, reduced performance, increased feed conversion ratio, poultry mortality, and economic losses. The spread of fungal infections is related to several factors such as geographic location, storage conditions, processing of various vaccines and moisture. This case report described the laboratory diagnosis of *Aspergillosis* in poultry and humans and the detection of fungal contaminants in poultry vaccines in Jos, Plateau, Nigeria

## CASE HISTORY AND DIAGNOSTIC

Case 1: A poultry farmer with a flock size of one thousand (n=1000), 20-week-old pullets reported to the Central Diagnostic Laboratory (CDL) of the National Veterinary Research Institute (NVRI), Vom, which a chief complaint of high mortality, and submission of carcasses for postmortem. Clinical history showed that the birds were fed with commercial feed (Chukun Feed®) and borehole water (untreated). Vaccination history showed that the birds were up to date. However, seromonitoring was not carried out to check for protective antibody titre. The carcases of the dead birds were necropsied and samples (lungs, liver, and spleen) were collected and seeded on Sabouraud Dextrose Agar (SDA) (Plate 1).

Case 2: A grain seller with a prolonged history of persistent dry cough, weight loss, and history of hospitalization at the Jos University Teaching Hospital (JUTH), was advised to visit the Dermatophilosis Laboratory of the National Veterinary Research Institute, Vom. Further enquiries showed that the patient had been under several antibiotic treatments and without significant improvement. At NVRI, the sputum sample was aseptically collected and cultured on Sabouraud Dextrose Agar.

Case 3. Four different poultry vaccines (A, B, C and D) were submitted to the CDL of NVRI and taken to the Dermatophilosis Laboratory for possible check of fungal contamination.

### Fungal isolation and identification

The microbiological examination of samples (liver, lungs, kidney, sputum and vaccines) was conducted using standard mycological culture techniques. These included the use of Sabouraud Dextrose Agar (SDA) (Himedia) containing chloramphenicol (0.05 mg/ml) and cycloheximide (0.5 mg/ml). The seeded SDA plates were sealed with masking tape and incubated at room temperature (25 ± 2°C) for 6 weeks hours. The cultured SDA plates were regularly observed for differentiated colonies. The growth rate, obverse, and reverse pigmentation of the recovered colonies were reported as described by Kane *et al.* (1997). Microscopically, the cellular morphology of the fungal cultures was identified using Lactophenol cotton blue staining. In cases 1 and 2, the fungal organism revealed a characteristic colonial morphology of *Aspergillus fumigatus* and *Aspergillus niger*. Macroscopically, *A. fumigatus* appeared as a velvety growth, downy or powdery, showing different

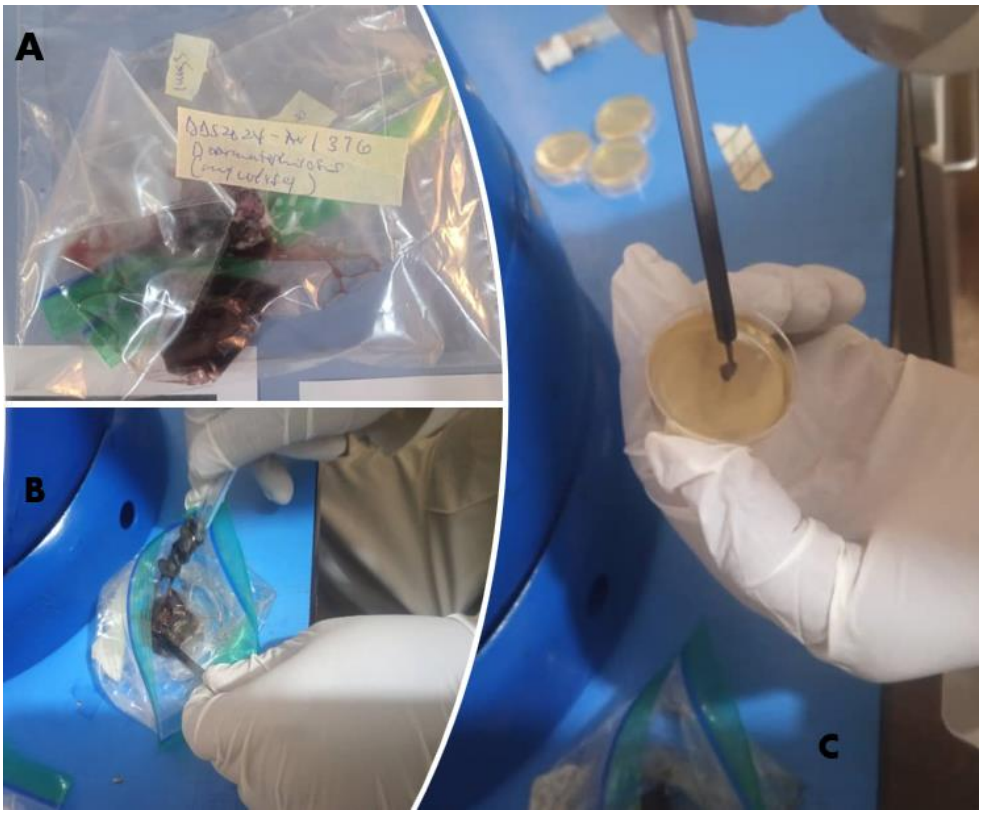


**Plate 1.** Showing submitted samples seeded on SDA for fungal isolation.

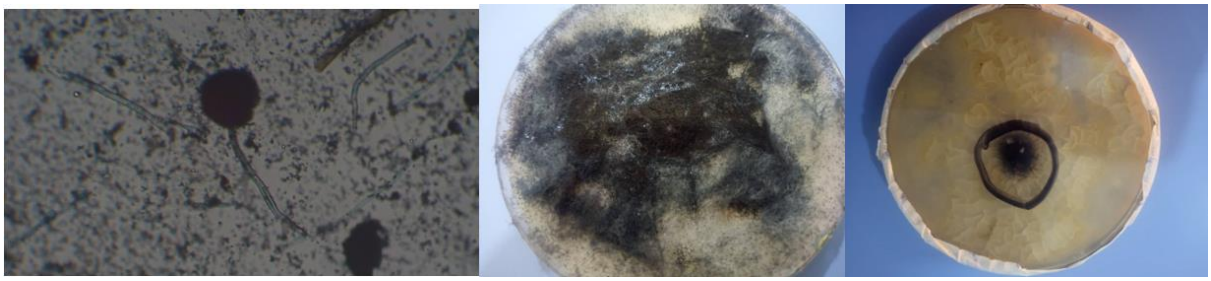

**Plate 2.** Cellular and macroscopic morphology of *A. niger* isolated from sputum.

shades of green or most commonly seen blue-green to a grey-green with a narrow white border and tan to pale yellow on the reverse side of the plate, that darkens with age. Additionally, *Aspergillus niger*, appeared as a white to yellowish felt-like mat of mycelia, quickly turned black as conidia developed the pigment aspergillin during maturation, and the reverse remains white to pale in colour. Microscopically, isolates of *A. fumigatus* showed a conidiophore, vesicles, metulae, phialides, and conidia, while that of *A. niger* revealed a septae, hyaline hyphae, with long conidiophores carrying vesicles at the apex (Plate 2). For case 3, the cultured plate revealed four different fungal organisms including *Penicillium spp., Fusarium spp., Aspergillus flavus and Aspergillus niger* respectively (Plate 3 and 4). The *Penicillium spp* colony appeared dark green at the centre of the colony, however, the edges of the colony were white to greenish white. The mycelium of the isolate had high-branched networks of multinucleated cells located on a septum lacking hyphae. Conidiophores are at the end of each branch accompanied by green spherical constricted units called conidiospores. *Fusarium* spp. showed rapidly growing hyaline Molds that are white and develop colouration in the centre with a light outer edge as they mature. This colouration can vary widely, from pink or violet to tan or orange, blue-green, or blue-brown. The reverse of the colony on the clear medium

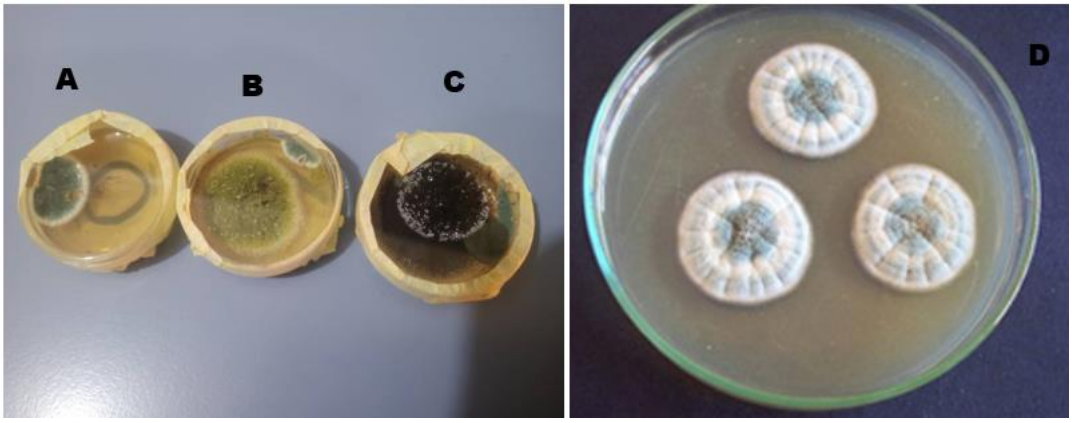


**Plate 3.** Surface view of *Fusarium spp* [A], Aspergillus *flavus* [B], Aspergillus *niger* [C], and d) *Penicillium spp* [D] on Sabouraud dextrose agar with chloramphenicol, gentamicin and cycloheximide added.

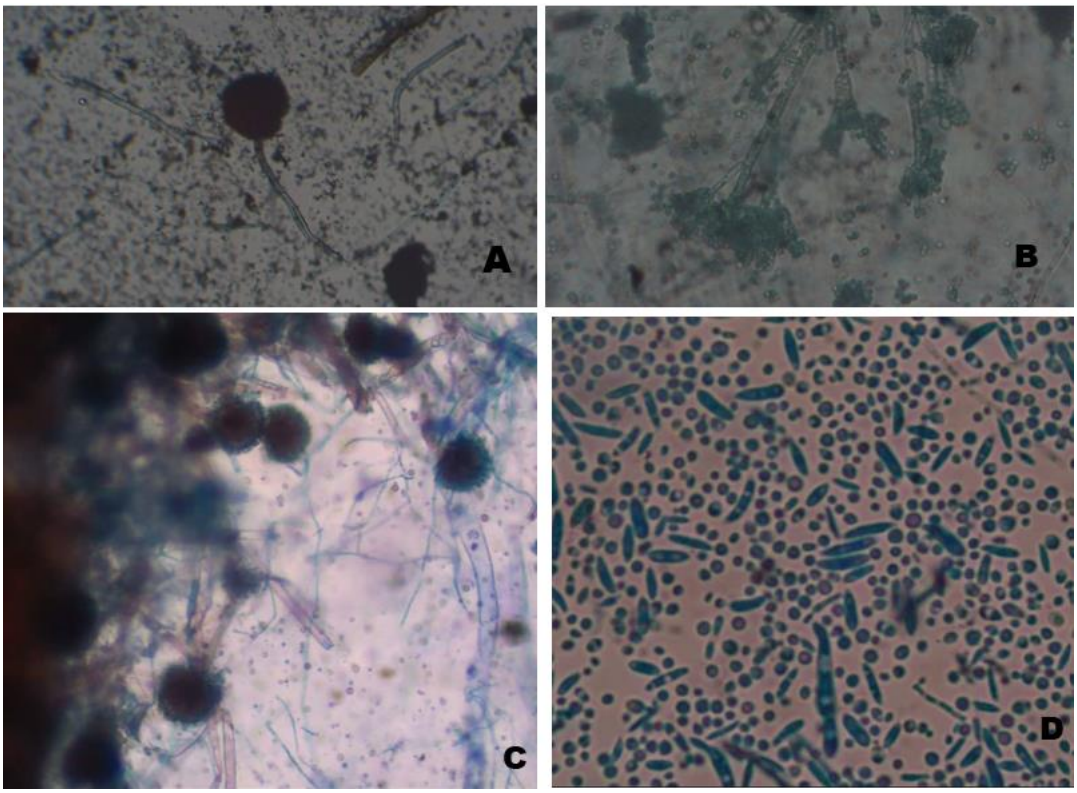


**Plate 4.** Microscopic characteristics showing conidiophores, mycelium, hyphae of [A] *Aspergillus niger*, [B] *Penicillium* spp [C] *Aspergillus flavus*, and [D] *Fusarium* spp., under the microscope using lactophenol cotton blue stain at X40.

is typically light but may also develop pigmentation. Microscopically, the hyphae of *Fusarium* spp. are septate, and both microconidia and macroconidia are formed *Fusarium* sp. microconidia are short and oval and may have one septation. *Aspergillus flavus* colonies have dark green conidia except for the colony edge which was greenish yellow. Sometimes, the colony appeared downy or powdery in texture. Microscopically, *A. flavus* appeared to have radiating conidial heads. While the conidiophores appeared thick-walled and rough. The conidia were

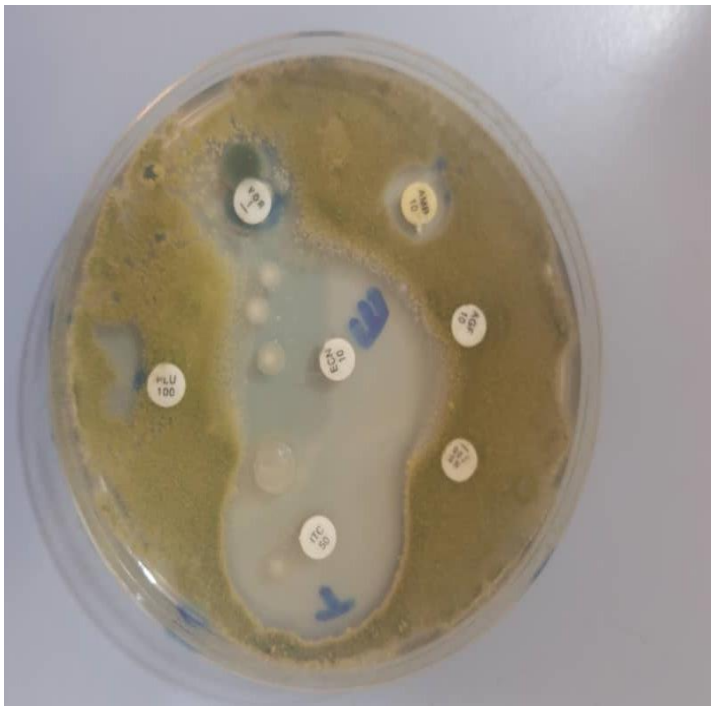


**Plate 5.** Antifungal susceptibility profiles of the fungal agent isolated from human sputum.

globose with a thin wall. In all the cases, molecular characterisation was not performed due to paucity of funds.

## Antifungal susceptibility testing

Antifungal susceptibility testing was conducted on SDA using the disc diffusion method, with selected antifungal agents including Amphotericin B (10 µg), Anidulafungin (10 µg), Voriconazole (1 µg), Itraconazole (50 µg), Fluconazole (100 µg), Econazole nitrate (10 µg) and nystatin (100 µg). Briefly, a few fungal colonies were suspended in normal saline to a McFarland Standard 0.5 ($1.5 \times 10^8$ CFU/mL) and seeded on SDA to create a uniform lawn using a spreader. Antifungal agents were then placed on the plates and incubated at room temperature for 24 to 72 hours, after which the zones of inhibition were observed and recorded. The antifungal susceptibility test showed that the *A. niger* isolated from human sputum was resistant to nystatin, fluconazole and Anidulafungin, but susceptible to amphotericin B, voriconazole itraconazole, and econazole (Plate 5).

## DISCUSSION

Fungal mycoses pose a significant threat to ecosystem health. It is a major impediment of the poultry industry and an important source of respiratory problems in humans. The condition in poultry is most often associated with practices that place undue stress on birds, such as overcrowding, poor litter management, rearing birds in poorly ventilated pens and improper feed formulation and storage (Bitrus *et al*., 2024). In these cases, the major fungal organism isolated were *Aspergillus niger, A. fumigatus*, and *A. flavus* respectively. Others included *Penicillium* species and *Fusarium* spp. This agreed with the report of Ameji *et al*. (2020) where contamination of feed and water by *Aspergillus* species was reported. It is important to note that *Aspergillus* species are ubiquitous in nature and thus, their conidia are easily dispersed and can be found in and on many habitats. Furthermore, the findings of this case report were in agreement with previous reports of isolation of *Aspergillus* species from tissue samples of infected birds (Fagbohun *et al.,* 2020; Oladele *et al.,* 2022). One striking finding of this case report was the accurate diagnosis of a case of *Aspergillus niger* from the sputum of a human patient showing clinical symptoms of a consistent and prolonged dry cough that defied antibiotic treatment. Antifungal susceptibility test showed that the *A. niger* was susceptible to amphotericin B, voriconazole itraconazole, and econazoles respectively and the patient showed remarkable recovery after antibiotic treatment. The diagnosis of a case of *A. niger* in a human patient underscores the need for the adoption of a multidisciplinary approach in diagnosis and treatment of diseases. Because the One-Health model has proven to

be effective and also reduced the cost of treatment and unnecessary hospitalization (Erkyihun and Alemayehu, 2022). Furthermore, the isolation of the *A. niger* from the sputum of the human patient could be attributed to occupational hazards, because the patient was a grain seller and there is a possibility that he inhaled the fungal hyphae in the course of doing his day-to-day business. It is important for stakeholders to create platforms for awareness creation regarding occupational hazards.

This case report also observed the isolation of fungal agents (*Penicillium*, *Fusarium* and *Aspergillus* spp) from vials of four different poultry vaccines. The isolation of fungal species like *Penicillium*, *Fusarium*, and *Aspergillus* from poultry vaccines raises significant concerns about the sterility and quality control in vaccine production. These fungi are known contaminants in agricultural and industrial settings, often causing diseases and spoilage. When these fungi are isolated from vaccines, they indicate potential contamination that could compromise vaccine efficacy, harm animal health, and even lead to zoonotic risks in cases where contamination affects human handlers. Furthermore, fungal presence can degrade vaccine components, reducing their effectiveness and making the vaccine less likely to induce the desired immune response in poultry. Additionally, contaminated vaccines could introduce pathogenic fungal strains to poultry, leading to secondary infections, compromised immunity, and increased mortality rates. Certain *Aspergillus* and *Fusarium* species produce mycotoxins, which can have harmful effects on poultry and potentially humans if ingested through poultry products. Inhalation of spores by farm workers handling contaminated vaccines could pose respiratory health risks. Poor vaccine quality can lead to vaccine failure, outbreaks of preventable diseases, increased veterinary costs, and significant economic losses for poultry farmers and the industry.

## Conclusion

This case report described the importance of accurate laboratory diagnosis in achieving efficient treatment and reducing losses associated with increased cost of treatment. Also, it emphasized the importance of One-Health as a good model for achieving improved treatment options. Furthermore, the isolation of *Penicillium*, *Fusarium*, and *Aspergillus* species from poultry vaccines is a red flag indicating potential contamination issues within the production and storage chain. Such contamination has serious implications, from compromised vaccine efficacy to potential zoonotic risks and economic impacts. Ensuring strict environmental controls, sterility testing, and adherence to good management practices in vaccine production and handling can mitigate these risks and enhance the safety and efficacy of poultry vaccines.

## CONFLICT OF INTEREST

The authors of this case report declared no competing interests concerning its publication.

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