

Prevalence of Antifungal-Resistance Among Fungal Agents Isolated in Poultry-Feeds from Poultry Farms in Sokoto, Nigeria.

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Abstract

The aim of this study was to determine the prevalence of fungal agents isolated from poultry feeds for antifungal-resistance. A total of 275 fungal isolates comprising of *Aspergillus* spp, (n = 135), *Penicillium* spp (n = 50), *Rhizopus* spp (n = 35), *Fusarium* spp (n = 20) and *Mucor* (n = 35) isolated from poultry feeds in Sokoto state, Nigeria were screened for resistance to Fluconazole, Nystatin and Voriconazole, using disk diffusion method. The result shows that 12.7% of each fungal genera were susceptible to all the antifungal agents tested. Resistance to at least one or more antifungal agent were expressed by 85.2% of the *Aspergillus* spp., 80% of *Penicillium* spp. and 75% of *Fusarium* spp, with 51.9%, 70% and 50% of them showing multiple drug resistance to two or more antifungal agent tested. Antifungal resistance was found to be expressed by all (100%) of *Mucor* and *Rhizopus* spp respectively, with > 70% showing multiple drug resistance. The resistance was found to be more for fluconazole than Voriconazole. Regular mycological analysis of poultry feeds shall be imposed to ensure quality and safety of poultry feeds in the study area.

Keywords: Antifungal-Resistance Fungal-agents; Multi-drug resistance; Moulds; Poultry-feeds

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Introduction

The indiscriminate use of anti-fungal agents for the prevention of poultry diseases may contribute to the emergence of moulds and yeasts that are resistant to these agents (Reis., et al. 2012). Poultry feed quality is an important pre-requisite for achieving optimal production results and preservation of health condition of the birds (Abo-Shama., et al. 2015). Moulds and mycotoxin contamination of feed and feed ingredients occur worldwide, and because of the ubiquitous nature of these microorganisms they cannot be totally eliminated from feed ingredients (Trenholm., et al. 1988). Presence of mycoflora especially moulds in animal feed indicate a potential threat not only to feed quality but also to the well-being of animals that are being fed with it (Beuchat., et al. 1978).

Although infection with pathogenic yeast such as *Candida albicans* and *Cryptococcus neoformans* are the most common invasive and opportunistic mycotic diseases (Denning., et al. 1998), filamentous fungi such as *Aspergillus fumigatus* are emerging as prominent agents

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causing morbidity and mortality worldwide (Groll & Walsh., *et al.* 2001). Increase in the rate of antifungal-resistant strains of fungal agents and the rate at which diseases such as invasive aspergillosis were reported in poultry and other animals pose a public health concern. Diagnosis and treatment of diseases caused by these agents in animals and humans remain a challenge, and therefore understanding the efficacy and limitations of the available antifungal agents is essential to select the appropriate agent for treatment (Miceli & Lee., *et al.* 2011).

In a study of mycotic agents associated with poultry feeds in Sokoto state, a high rate of feed contaminated by fungal agents such as *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium* and *Mucor* was recorded (Aliyu., *et al.* 2012), and some of the fungal agents involved might be resistant to commonly used antifungal agents such as triazoles. However, some moulds such as *Mucor* spp. and *Fusarium* spp. were reported to be less susceptible to these group of antifungal (Araujo., *et al.* 2015). These antifungal-resistant strains may infect animals, especially poultry and humans causing serious diseases that responds poorly to treatment. To the best knowledge of the authors, there is no documented report on susceptibility profiles of fungal agents isolated from poultry feeds in Sokoto and its environs. Therefore, this research is aimed at determining the susceptibility of selected fungal agents isolated from poultry feeds to three most commonly used antifungal agents in the area.

Materials and Methods

Study area: The study was conducted in Sokoto metropolis and environs. Sokoto township is the capital city of Sokoto State in Nigeria. The state is the second largest livestock producing state in the country (SSIPC 2008). The state is an agric-based with most of the populace (over two million) engaged in rearing of one or more specie(s) of animals especially ruminants and poultry. Poultry production in the state is mostly local with few farmers engaged in medium/large scale production (Maikasuwa and Jabo., *et al.* 2011). Although commercially prepared feeds are predominantly used in the study area, few farmers compounded their feed.

Organism Tested: A total of 275 fungal isolate comprising of *Aspergillus* spp. (n = 135), *Penicillium* spp. (n = 50), *Rhizopus* spp. (n = 35), *Fusarium* spp. (n = 20) and *Mucor* spp. (n = 35) were used for the sensitivity testing. These fungal agents were previously isolated and identified during a 12 month (May 2010 to April 2011) survey of fungal contaminants in poultry feeds in Sokoto and its environs. The isolates were reactivated on Sabroud dextrose agar (SDA) at room temperature for seven days (Saleemi., *et al.* 2010). An inoculants of each fungus sample was prepared by adding 5 millilitre of sterile saline solution to the surface of cultures to harvest the spores. The suspension of each filamentous fungus was adjusted to contain 1×10^6 conidia/ml through a comparison of the turbidity with that of number six (#6) tube of the McFarland turbidity scale, and confirmed using spectrophotometer at 530 nm (Diogo., *et al.* 2010).

Antifungal Agents Used: Two triazole and a polyene antifungal medicaments in the form of Drug-impregnated disks were used in this study. This are Fluconazole (25 µg: CT1806B), Nystatin (100 units: CT0073B) and Voriconazole (1 µg: CT1807B) (Oxoid Ltd. Basingstoke; U.K).

Antifungal Susceptibility Testing of Fungal Isolates: The disk-diffusion technique was performed according to the procedure described in the M44-A method (Sheehan., *et al.* 2004). Mueller Hinton agar (Acumedia Limited; 7101A) was prepared according to instructions from the manufacturers, and 15-20 ml aliquot dispensed in Petri dishes. The plates were then swabbed evenly with a sterile cotton-wool swab dipped into the standardized inoculum of the fungal suspension, and allowed to stay for 5-10 minutes to get absorbed. The drug-impregnated disks comprising of Fluconazole (25 µg:CT1806B), Nystatin (100 units: CT0073B) and Voriconazole (1 µg: CT1807B) (Oxoid Ltd. U.K.) were aseptically placed on the cultured plates in equidistant points and lightly pressed. The plates were incubated at room temperature (22-25°C) for 4 to 7 days to allow for fungal growth. The sensitivity to antifungal agents of each test-fungal agent was determined by measuring the diameter (mm) of the zone of inhibition, using transparent meter rule. Each isolate was classified into: sensitive or resistant based on the reported standard criteria for interpreting inhibition zones as follows; Nystatin ≤ 10 mm, Fluconazole ≤ 14 mm and Voriconazole ≤ 11 mm as adopted from (Nweze., *et al.* 2010) and (Diogo., *et al.* 2010).

Result and Discussions

The frequency of resistance to the antifungal agents tested, as exhibited by the fungal isolates obtained from poultry feed samples is shown in Table 1. Of the 275 fungal isolate tested, 35 (12.7%) were susceptible to all the antifungal agents tested, and 240 (87.3%) had expressed resistance to one or more of the agents. The resistance was observed more with *Mucor* and *Rhizopus* spp (100%) followed by *Aspergillus* spp 118 (85.2%), and the least was found in *Penicillium* spp 37 (74%) respectively. Multi-drug resistance to two or more antifungal agents was observed highest among *Rhizopus* spp (85.7%) followed by *Mucor* spp (71.4%) and least was among *Fusarium* spp (50.0%).

Isolates	Number of Isolates	No. Resistance	%
<i>Aspergillus</i> spp	135	118	87.41
<i>Penicillium</i> spp	50	37	74.00
<i>Rhizopus</i> spp	35	35	100.00
<i>Fusarium</i> spp	20	15	75.00
<i>Mucor</i> spp	35	35	100.00
TOTAL	275	240	87.27

Table 1: Frequency of antifungal resistance expressed by fungal isolates from commercially prepared and self-compounded poultry feeds in Sokoto town and its environs.

Of the 135 *Aspergillus* spp tested, 87.3% were resistant to at least one or more antifungal agent, with more than fifty percent (51.9%) showing multiple drug resistance. More than thirty percent were resistant to only one antifungal agent and 44.4% and 7.4% of the isolates were resistant to two and three antifungal agents respectively. Of the 20 isolates *Fusarium* spp tested, resistant to at least one or more antifungal agents was observe in (--%), and 50.0% showed multiple drug resistance to two antifungal agents (Table 1). All 100.0% the *Mucor* and *Rhizopus* spp tested expressed resistant to at least one or more antifungal agent, with more than 70% of each showing multiple drug resistance. For *Penicillium* spp, 80.0% of the isolates were resistant to at least one antifungal agent, with 70.0% showing multiple drug resistance to two antifungal agents (Table 2).

No. antifungal resisted	Fungal Isolates				
	<i>Aspergillus</i> spp.	<i>Fusarium</i> spp.	<i>Mucor</i> spp.	<i>Penicillium</i> spp.	<i>Rhizopus</i> spp.
1	(n = 135)	(n = 20)	(n = 35)	(n = 50)	(n = 35)
2	60 (44.4%)	10 (50.0%)	20 (57.1%)	35 (70.0%)	20 (57.1%)
3	10 (7.4%)	0 (0.0%)	5 (14.3%)	0 (0.0%)	10 (28.6%)
Total MDR	70 (51.9%)	10 (50.0%)	25 (71.4%)	35 (70.0%)	30 (85.7%)

Table 2: Frequency of multidrug resistant fungal isolates in poultry feed samples collected from Sokoto and its environs.

Among the antifungal agents tested, resistance to Fluconazole was the highest as it was resisted by all (100%) of the isolate tested (Figure 1).

This study evaluated the *in-vitro* susceptibility of commonly used antifungals against 275 filamentous fungi isolated from poultry feeds. The results indicate that high percentages of fungal organisms tested in the present study were resistant to *Fluconazole* (85.0%), but relatively few are resistant to *Veruconazole* (Figure 2). The resistance could be attributed to indiscriminate use of the drugs in the study area, in contrast to the high susceptibility to *Voriconazole* (76.4% of the isolates) which are not commonly used drug in both

humans and animals in the study area. This finding was in agreement with that of Pfaller, *et al.* (2002), which reported approximate 91% susceptibility of *Aspergillus* spp to voriconazole, but *Fusarium* and *Mucor* spp are relatively resistant. In the present study, some moulds such as *Mucor* spp. and *Fusarium* spp. were found to be less susceptible than those reported by Arujo., *et al.* (2015). In USA, the prevalence of Azole resistance in certain fungal species is estimated to be 3-6%. The resistance may be partially attributed to the use of agricultural azoles which protect crops from fungal infections (CDC, 2014). Apart from being resistance to antifungal agents, the fungal species especially *Fusarium* and *Aspergillus* may produce mycotoxins in poultry feeds.

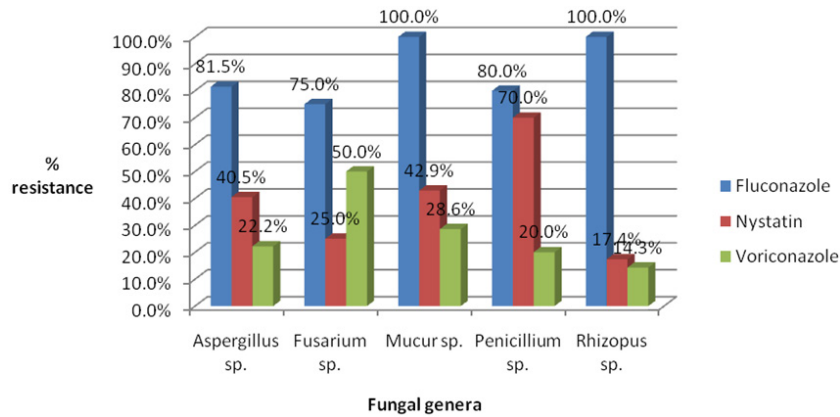


Figure 1: Frequency of antifungal resistance among fungi isolates from poultry feed samples in Sokoto metropolis and its environs.

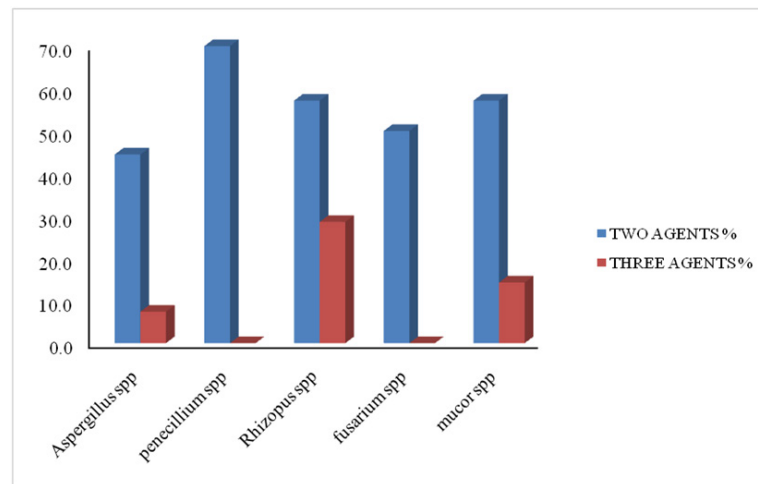


Figure 2: Distribution of multidrug resistant fungal agents by number of antifungal agents.

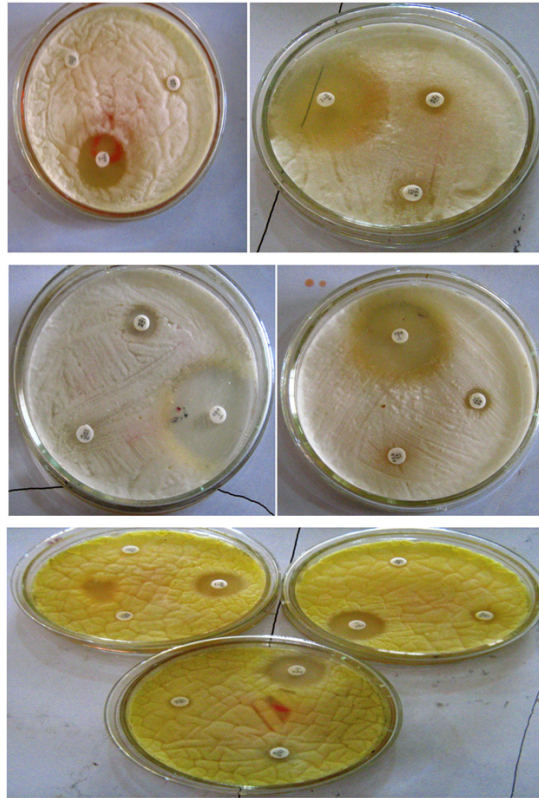


Figure 3: Selected pictures of antifungal susceptibility testing of moulds isolated from poultry feeds in Sokoto metropolis and its environs, Nigeria. The following consistency and trend can be clearly noticed from the pictures: the larger zone of inhibition around Voriconazole disk, followed by a narrow zone of inhibition around Nystatin disk and no zone of inhibition around Fluconazole disk.

Conclusion

In Conclusion, the present study highlighted on the occurrence and prevalence of antifungal-resistance phenotypes of fungal agents in poultry feeds from selected farms in the area of study. Base on the above findings, fluconazole was most resisted to by the fungi tested in contrast to their higher susceptibility to Voriconazole. The increased use of triazoles in prophylactic and treatment of animal and plant diseases in this area may lead to increase antifungal drug-resistant problem, as observed in *Aspergillus* species elsewhere (Arunaloke., *et al.* (2011). There presence is of animal health and public health concern, as they may carry the resistant-gene and transfer it to infected animals and humans.

Regular mycological analysis of poultry feeds shall be imposed to ensure quality and safety of poultry feeds in the study area. There is also the need to investigate further the possible association between antifungal resistance and mycotoxin production.

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