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Original Research Article

Evaluation of Indoor and Outdoor Fungal Flora of Two Poultry Farms in Akungba-Akoko and Ayegunle-Akoko

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Abstract

Good quality of air in poultry farms is a prerequisite for healthy animals and farm workers. Air acts as a good dispersal medium for microbes and its quality is a reflection of the environment. The focus of this study was to evaluate indoor and outdoor fungal flora of two poultry farms (Akungba-Akoko and Ayegunle-Akoko) and to determine effect of some antifungal agents on all isolates. Identity of each fungus was determined by comparing the morphology and microscopic characteristics of each fungus with those in compendium of soil fungi. Air flora sampling was performed both inside and outside premises of farms, and an open-air method was used. Antifungal activity was performed using two antifungal drugs (ketoconazole and nystatin). A total of twenty-three (23) fungal species were obtained belonging to fourteen (14) genera; *Aspergillus, Penicillium, Eurotium, Monascus, Alternaria, Cryptococcus, Curvularia, Chrysonilia, Microsporum, Cunninghamella, Bipolaris, Acremonium, Fusarium* and *Trichoderma*. Genera such as *Curvularia, Chrysonilia, Cunninghamella* and *Cryptococcus* were not inhibited by antifungal drugs used across all concentrations, while nystatin inhibited 74% of these fungi using concentration of 5 mg/ml with 25 mm being highest zone of inhibition. Ketoconazole' s highest zones of inhibition were found with *Alternaria tenuissima* (26 mm), *Trichoderma harzianum* (25 mm) and *Aspergillus acidus* (25 mm). The study revealed presence of some toxicogenic fungi in poultry environments. Therefore, this study recommends preventive measures like provision of adequate ventilation system, regular cleaning of poultry farms, use of clean equipment, and use of antifungal drugs such as ketoconazole and nystatin (to be used at low concentration) to inhibit and control infections in poultry farms.

Keywords: Indoor and outdoor poultry environments; animal and human health; fungal flora; antifungal activity.

INTRODUCTION

Pollution is one of the problems facing community health today. It affects the health of both animals and humans. Pollution interferes with human health, quality of life, natural functioning of ecosystem and their physical environment (Fabian *et al.*, 2005). Pollution is caused by a wide range of biotic and abiotic entities that include chemical or particulate droplets to biological contamination of the air by airborne microorganisms (bio-aerosols). Air can be polluted by the addition of harmful substances to the atmosphere resulting in damage to the environment, human and animal health and quality of life (Hart *et al.*, 2008). Poultry production is based on chickens is farmed in large numbers because more than seventy billion chickens are killed for consumption annually (Sanders, 2018).

Poultry farms are among the polluted areas with large quantities of different microbial components (bio-aerosols) such as bacterial and fungal cells, their spores and fragments of mycelium as well as their toxins (Karwowska, 2005). Their concentrations change in atmosphere based on environmental conditions (Kasprzyk, 2008). Examples of bio-aerosols are *Alternaria alternata, Aspergillus fumigatus, Cladosporium* spp, and different species of the following genera; *Fusarium, Mucor, Penicillium, Rhizopus* and *Trichoderma* that can be derived from soil, dust, feed and bedding and birds themselves.

Microbial contaminants in poultry farms are assisted by contaminated feed, litter, inadequate ventilation, poultry droppings, and improper personal hygiene of workers. Most microorganisms are saprophyte, but some airborne microorganisms may be

pathogenic. Also, some poultry birds are constantly exposed to pathogenic bioaerosols when there is an infected bird in the poultry house (Radon *et al*., 2002). Microbial survival is mainly determined by temperature and humidity of the environment. Microbial contaminant of air, litter, and surfaces in poultry farms can be attributed to high flocks' number and presence of microbial sources (Witkowska and Sowińska, 2017). The presence of microorganisms such as *Alternaria* and *Cladosporium* in poultry houses indicates the microbes from environment, including soil, can spread to farm to farm buildings.

Presence of pathogenic microorganisms outside poultry buildings are caused by defective ventilation system (Lonc and Plewa, 2011). Broiler houses can be infested by fungi of the genera *Penicillium*, *Aspergillus* and *Fusarium*, which are the main fungi producing pathogenic mycotoxins. Other toxigenic fungi species, such as *Alternaria*, *Cladosporium*, *Trichoderma*, and *Rhizopus* have also been identified in poultry buildings (Brodka *et al*., 2012), thus, this study aimed at comparing indoor and outdoor fungal flora of some poultry farms in Akungba-Akoko and Ayegunle-Akoko. This will enhance our understanding on how their growth can be avoided or inhibited in a given poultry farm and its environment, thereby preventing of disease that might be caused by these fungi.

MATERIALS AND METHODS

Study area

The poultry farms are located within two towns in northern part of Ondo State, Nigeria. The towns (Akungba-Akoko and Ayegunle-Akoko) are situated in the tropical rain forest region of Nigeria. These selected sites were chosen based on their location and differences in their population density.

Sample collection

Potato dextrose agar (PDA) medium was prepared according to manufacturer's instruction. An open-air method was used by placing plates in both indoor and outdoor of the poultry farms. Each sample was collected after 5 min of exposure and time of sampling was kept uniform. After exposure, the plates were transported to Microbiology laboratory of Adekunle Ajasin University, Akungba-Akoko. The plates were incubated at 28^{o}C for 5 days.

Fungal staining

Two drops of lactophenol cotton blue was placed at the center of a grease-free microscopic glass slide. A pure mycelial mat of each fungus was carefully picked using a sterile inoculating wire, placed on the stain and gently teased-out. The glass slide was covered with a cover slip and viewed using a compound microscope. Each fungus was identified on the basis of colour, texture, topography of culture's surface, smell of colony, and microscopic features (e.g., the presence of macroconidia and microconidia) (Chapin, 2007)..

Antifungal activity

Ketoconazole and nystatin were the two antifungal drugs used. Concentrations of ketoconazole used were 5 mg/mL and 0.05 mg/mL while nystatin's concentrations were 2 mg/mL and 0.5 mg/mL. After PDA preparation, each agar plate was divided into two halves; a cork borer (7 mm diameter) was used to make a well at the center of one half of the plate (the well was filled with 100µL of each antifungal agent and allowed to diffuse into the medium for 1 h) and a 7 mm diameter mycelial mat of each fungus was placed on the other half of the plate. The plate was then incubated at 28^{o}C and examined after 5 days. Zones of inhibition observed were measured in mm.

RESULTS

The result shows that indoor fungal load (60%) was higher than the outdoor fungal load (40%) in the two poultry farms used for the study. Although some were found both indoor and outdoor of the farm, 14 genera were identified indoor while 9 genera were identified outside, this could be linked to lack of ventilation, high level of waste from the bird in the poultry which can cause diseases due to toxigenic potential of species like *Aspergillus fumigatus* and *Aspergillus flavus* that were found, thereby posing risk to the bird, human or the environment. *Aspergillus* species (60%) were mostly isolated which is followed by *Eurotium* species (40%), *Penicillium* species (30%) and others at 1% each (Table 1).

The results revealed that the concentrations of 5 mg/ml and 2 mg/mL inhibited the growth of most of the fungal isolates but at 0.05 mg/mL and 0.5 mg/mL concentrations had no inhibition except for *Aspergillus fumigatus* (20 mm) and *Monascus ruber* (12.5 mm) (Table 1). Thus, both antifungal drugs were effective against *Aspergillus fumigatus*, even at lower concentration.

Table 1: Antifungal Activity

S/N	Organism	Ketoconazole		Nystatin	
		5 mg/mL	0.5 mg/mL	2 mg/mL	0.5 mg/mL
1.	*Acremonium strictum*	18	0	0	0
2.	*Alternaria tenuissima*	26	0	25	0
3.	*Aspergillus acidus*	25	0	25	0
4.	*Aspergillus clavatus*	0	0	14	0
5.	*Aspergillus flavus*	0	0	0	0
6.	*Aspergillus fumigatus*	15	0	20	20
7.	*Aspergillus niger*	0	0	18	0
8.	*Bipolaris australiensis*	16	0	16	0
9.	*Chrysonilia sitophila*	0	0	0	0
10.	*Cryptococcus laurentii*	0	0	0	0
11.	*Cunninghamella bertholletiae*	0	0	0	0
12.	*Curvularia geniculata*	0	0	0	0
13.	*Eurotium amstelodami*	19	0	18	ND
14.	*Eurotium chevalieri*	18	0	18	0
15.	*Eurotium herbariorum*	0	0	8	0
16.	*Fusarium avenaceum*	15	0	12	0
17.	*Microsporum canis*	15	0	15	0
18.	*Monascus ruber*	18	13	15	13
19.	*Penicillium aethiopicum*	0	0	12	0
20.	*Penicillium chrysogenum*	0	0	14	0
21.	*Penicillium citrinum*	15	0	15	0
22.	*Penicillium expansum*	18	0	12	0
23.	*Trichoderma harzianum*	25	18	25	0

KEY: ND = not determined

Table 2: Comparison of fungi found in both indoor and outdoor environment of the poultry farms

Genus	Species	Indoor	Outdoor
Aspergillus	*fumigatus*	+	-
	niger	+	+
	acidus	+	-
	clavatus	+	-
	flavus	+	+
Eurotium	*amstelodami*	-	+
	chevalieri	-	+
	herbariorium	-	+
Penicillium	*chrysogenum*	+	-
	aethiopicum	+	-
	citrinum	-	+
	expansum	+	-
Acremonium	*strictum*	+	-
Alternaria	*tenuissima*	+	+
Monascus	*ruber*	+	-
Cryptococcus	*laurentii*	+	-
Curvularia	*geniculata*	-	+
Chrysonilia	*sitophila*	+	+
Microsporum	*canis*	+	-
Trichoderma	*harzianum*	+	-
Bipolaris	*australienisis*	-	+
Fusarium	*avenaceum*	-	+
Cunninghamella	*bertholletiae*	-	+

KEY: + = organism present, - = organism not present

DISCUSSION

The results showed that twenty-three (23) fungi were isolated from the two locations both from indoor and outdoor of the poultry units and the genera include; *Aspergillus, Penicillium, Eurotium, Monascus, Alternaria, Cryptococcus, Curvularia, Chrysonilia,*

Microsporum, Cunninghamella, Bipolaris, Acremonium, Fusarium and *Trichoderma.* Three fungal genera (*Aspergillus, Eurotium* and *Penicillium*) were found in most of the samples collected. These findings agree with the report of Nichita and Tirziu (2008) that isolated eight fungal genera from poultry house air (*Aspergillus* and *Penicillium*). Also, Okiki and Ogbimi (2010) found *Aspergillus, Penicillium, Trichoderma, Fusarium,* and *Cryptococcus* in poultry farms.

Indoor fungal load was higher than those of the outdoor in the two poultry farms. Fourteen fungal genera were obtained from indoor environment with *Aspergillus* being the most common species while outdoor environment revealed eleven genera of fungi; *Eurotium* being the most isolated (Table 2). The result of indoor fungal load corroborates a report in Zagreb where higher fungal counts were reported for indoor air flora (Rimac *et al.,* 2010). Rimac *et al.,* (2010) reported that species belonging to genera *Aspergillus* and *Penicillium* were the most prevalent. Moreover, some fungal species identified in our study (*Penicillium* and *Aspergillus*) have been reported to cause hypersensitivity reactions in humans, with clinical manifestations such as allergic rhinitis, asthma and extrinsic alveolitis (Ostro *et al.,* 2001). Some of these organisms are common pathogens *Curvularia geniculata, Aspergillus* spp, *Cryptococcus laurentii* while some are beneficial. Examples include; *Trichoderma harzianum,* and *Penicillium chrysogenum.*

Indoor particle concentrations involve complex combinations of numerous factors, such as emissions sources, ambient conditions, building structure and materials, work activities, ventilation and air exchange rate, which also affect particle size distributions. The results obtained in our study are similar to those of other studies (Elen *et al.,* 2000; Donham *et al*., 2000). *Alternaria* and *Trichoderma* isolated are capable of producing toxins known to cause immunosuppression, allergies, inflammation for respiratory tract and they may have impact on growth parameters of birds (Vučemilo *et al*., 2007).

Antifungal assay revealed that *Curvularia geniculata* and three other genera were not inhibited by ketoconazole and nystatin both at 5 mg/mL and 2 mg/mL concentrations. *Curvularia geniculata* is a fast growing fungus commonly found in soil, it is fungus causing plant and animal infection (Davis *et al.,* 2005). Similarly, *Cryptococcus laurentii* was not inhibited by the antifungal agents, a yeast infection, the fecal matter of health birds has been identified as an important repository for *Cryptococcus* fungi, and the main route of infection is inhalation of airborne yeast. *Chrysonilia sitophilia* grows rapidly within a short period of time; the findings revealed it was not inhibited by the antifungal drugs.

The following fungi were inhibited by the antifungal agents used (ketoconazole and nystatin) at concentration as low as 5mg/mL and 2mg/mL; *Penicillium citrinum, Monascus ruber, Trichoderma harzianum,* and *Aspergillus fumigatus* with varying zone of inhibition. This showed that ketoconazole and nystatin can be used for the control of some of these organisms even at lower concentration. However, those fungi that have been isolated but were not susceptible to the antifungal drugs can be prevented by search for effective, inexpensive and environmentally friendly methods of lowering fungal contamination especially the pathogenic ones. Cumielowiec-Korzeniowska *et al.,* (2005) evaluated the effectiveness of a prototype container biofilter in eliminating organic air pollutant in chick hatchery. The biofilter bed was composed of sallow peat (30%), fermented horse manure (10%), fibrous peat (30%), treated compost (10%), and wheat straw (20%), the tested devices decreased the levels of all pollutants by 66% on average. Similarly, Manafi *et al.,* (2011) have used high grade sodium bentonite in diet to reduce toxicity of aflatoxin in broiler.

CONCLUSION

The study revealed that poultry farms are significant reservoirs and emitters of microbiological (e.g., fungi) contaminants. Indoor environment was more contaminated than outdoors. The type and concentrations of bioaerosols produced in poultry farms are determined by various factors; bird species, stocking density, season, time of day, stage of the production cycle, temperature, moisture content and the physicochemical parameters of litter, sampling site, ventilation efficiency, and farm management system. If poultry farms are not in good shape, farm workers and animals can be exposed to pathogenic organisms. Therefore, preventive measure like regular cleaning of the poultry houses and used of clean equipment should be adopted as control measure to prevent presence of pathogenic fungi in poultry farms.

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