FUNGI ASSOCIATED WITH THE CROP OF A LOCAL FOWL : A PUBLIC HEALTH PROBLEM

ABSTRACT

This study was aimed at Isolating fungi associated with crop of a local fowl. Although numerous studies have been conducted on microbial contaminants associated with various stages related to poultry products processing, only a few reported on fungal contamination of poultry litter. The goals of this study were to (1) characterize litter fungal contamination and (2) report the incidence of fungi species present. Ten fresh and 20 aged litter samples were collected from 10 poultry farms. In addition, 27 air samples of 25 litters were also collected through impaction method, and after laboratory processing and incubation of collected samples, quantitative colony-forming units (CFU/m3) and qualitative results were obtained. Twelve different fungal species were detected in fresh litter and Penicillium was the most frequent genus found (58.9%), followed by Alternaria (18.8%), Cladosporium (6.1%), and Aspergillus (6.7%). With respect to aged litter, 19 different fungal species were detected, with Penicillium sp. the most frequently isolated (41.3%), followed by Rhodotorula sp. (38.3%), Oudeemansiela sp (8.8%), and. Asperillus (6.9%). A significant positive correlation was found between litter fungal contamination (CFU/g) and air fungal contamination (CFU/m3). Litter fungal quantification and species identification have important implications in the evaluation of potential adverse health risks to exposed workers and animals. Spreading of poultry litter in agricultural fields is a potential public health concern, since (Rhodotorula and Fusarium genus) as well as toxigenic fungi (Aspergillus, Fusarium, and Penicillium genus) were isolated.

KEYWORDS: Asperillus spp, Oudeemansiela sp, crop and colony-forming

1. INTRODUCTION

Exposure to certain fungi is known to produce adverse human health effects through three specific mechanisms: (1) generation of a harmful immune response such as allergy or hypersensitivity pneumonitis, (2) direct infection by fungal organism, and (3) toxicirritant effects from mold by-products such as mycotoxins (Norred 1993; Bondy and Pestka 2000; Bush et al. 2006).

Soil represents the main reservoir of fungi (Chmel et al. 1972). Some soil fungi are potential pathogens to both humans and animals (Ajello 1980). Soils rich in keratinous materials are more conducive to growth and occurrence of eratinophilic fungi (Mercantini/" et al. 1980; 1986), which may be considered a potential pathogen (Rippon 1982).

Comment [H1]: All scientific names should be italicized e.g *Penicillium*
If the species level of the fungi is not known, then its advisable its written as *Penicillium* sp

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In fact, the richness in keratin residues constitutes a permanent or occasional reservoir for keratinophilic fungi and a source of potential infection for humans and animals (Filipello 1986). Various investigators have examined keratinophilic fungi distribution in soil, and these fungi have attracted the attention of dermatologists and mycologists due to their association with human and animal mycoses (Papini et al. 1998; Rippon and Garber 1969; Mirocha et al. 1972; Mitra et al. 1982). In addition, fungi produce secondary metabolites, like mycotoxins, in response to environmentalchanges. Mycotoxins may be pro-inflammatory,
immunosuppressive, or carcinogenic (Bondy and Pestka 2000; Jarvis 2003). The different chemical groups of mycotoxins include aflatoxins, fumonisins, ochratoxins, rubratoxins, and trichothecene toxins, all with different biologic
properties (Norred 1993; Clark and Snedeker 2006: Jarvis 2003; Chung et al. 2003). Poultry/broiler litter is a bedding material, usually straw in Europe, mixed with excreta, spilled feed/water and feathers, whose quality is important for boiler welfare and productivity as it absorbs moisture, dilutes faecal content, and serves as a thermal insulator and protective cushion between broilers and the floor (Meluzzia, A et al; 2009, Saboleki, et al; 2020, Ritz CW, et al; 2020). However, it is also known to favour fungal growth (Arne et al; 2011) which is why its quality should be monitored continuously, mostly by moisture assessment. In the initial fattening period, the level of broiler litter moisture is 10 – 50%, which by the end of this period rises to 25 – 50%. Recommended level span between 30% and 40% [Benito 1991 Zuzuls S. et al; 2017, Aberu VMN, et al; 2011] other recommended conditions include neutral PH, low ammonia production, and loose, crust – free litter. High moisture leads to litter caking that favors ammonia release, where as low moisture favors respiratory problems due to high levels of dust that carries microorganism, including fungal spores [Viegas C et al; 2012].

2.MATERIALS AND METHODS

2.1 Poultry Farms

The selected poultry farms were located in the Oyun,Ilorin East LGA Kwara State Nigeria, and the litter samples were collected between January and May 2022. Selected farms were dedicated to broiler chicken production, where birds are bred to reach slaughter weight as rapidly as possible. Day-old chicks were transferred from hatcheries to the growing farms, where they are housed in single-story sheds. Farm staffs monitor the condition of the birds daily, adjust feed and water equipment as necessary, and administer vaccines. In some cases, the same workers also catch and transport mature chickens to slaughterhouse. After depopulation the manure-removal contractor accumulated manure and loaded it onto trailers for transport to fields where it is used as fertilizer.

Comment [H3]: Citations in this format should be written as;(Papini *et al.*,1998)
Note all et al should be italicized

Comment [H4]: Citations in this format should be written as (Norred, 1993)
Clark and Snedeker, 2006)

Comment [H5]: Follow the examples made in previous comment to correctly write the citations
Note: Initials of names doesn't appear in citations.

Comment [H6]: The followings were suggestion on how the subsections under the materials and methods should be presented
- Description of Study Area
- Sample collection and preparation
- Sterilization of glass wares and other materials
- Preparation of Media
- Isolation of fungi from poultry filters
- Identification of fungal isolates

Samples Collection, Preparation and Analyses

Ten fresh (new) and 20 aged litter samples were collected from 10 poultry farms in sterilized bags (Table 1). Each litter sample (10 g, not oven-dried prior to processing, retaining the natural water content) was diluted in 100 ml sterilized distilled water, agitated for 30 min at 100 rpm, and 0.2 ml of this suspension was spread onto triplicate Petri dishes containing malt extract agar (2%) with chloramphenicol (0.05 g/L) and incubated for 5–7 d at 27.5◦C. Fungal identification was carried out by macroscopic and microscopic observation using lactophenol blue staining and atlas identification (Hoog et al. 2000). Isolated fungi were identified to the species level. Results were reported as the average count of the three replicas, in colony-forming units per gram of litter (CFU/g). In addition to litter samples, 27 air samples of 25 litters were also collected through impaction method.

Air samples were collected at 1 m height with a flow rate of 140 L/min onto malt extract agar (MEA) supplemented with the antibiotic chloramphenicol (0.05%). After laboratory processing and incubation of the collected samples, quantitative (colony-forming units [CFU]/m3) and qualitative results were obtained with identification of the isolated fungal species.

2.2 Sterilization of Glassware and Other Materials

All glasswares ~~is~~ used for this project were thoroughly washed and sterilized. They were wrapped with aluminum foil and sterilized in hot air oven at 150°c for 20 minutes. The work bench was properly disinfected using cotton wool soaked in 70% ethanol; media were sterilized inside the autoclave at 121°c for 15 minutes inclusion. Serial dilution and sub – culturing were all done near the bunsen ~~burses~~ burner.

2.3 Coliform Count

Spread plate method was also used for coliform using MacConkey agar. 0.1ml of the mixture was taken from 10^{-3} and spread on the MacConkey Agar using a spreader. The plates were incubated for 24hours at 37°c plates were observed after 24hours for lactose and non-lactose fermenter.

2.4 Identification of Fungal Isolates

The identification of fungal isolates was based on microscopic and macroscopic examination. Macroscopic examination was based on color and nature of the hyphae. In microscopic examination, the technique of James and Natalie (2001) was adopted for identification of unknown isolated fungi using cotton blue in lactophenol stain. A drop of the stain was drop on a clean grease free slide, a small portion of the mycelium was placed on

Comment [H7]: The litter types from where the samples were collected should be stated under this heading.

Comment [H8]: This should be explain under isolation of fungal from poultry litters

Comment [H9]: The purpose of adding chloramphenicol should be stated (to prevent bacterial growth)

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the stained glass slidespread very well on the slide with the aid of athe needle. A cover slip was gently placed applied with little pressure to eliminate a bubbles. The slides were mounted and observed with X4, X10 and X40 object lens and a camera attached to the microscope upon observation.

2.5 Statistical Analysis

Statistical analysis was performed using SPSS 20.0. Significant differences of the total CFU/g between different litter type were tested by the Kruskal–Wallis test and tendencies by median values, 25th and 75th percentiles, and interquartile range. The correlations between total CFU per gram and litter age and between
litter fungal contamination and air fungal contamination from the analyzed poultry farms were conducted using the Spearman correlation coefficient

3.RESULTS AND DISCUSSIONS

3.1 RESULTS

Twelve different fungal species were detected in fresh, new litter in a total of
830,500 isolates from all the analyzed poultry farms. Penicillium was the most frequent genus found (58.9.%), followed by Alternaria (18.8%), Cladosporium (6.5%), and Aspergillus (6.7%). In addition to these, other genera were also isolated, namely, Rhizomcor sp., Sporobolomyce sp., Byssoclammys sp., Rhizopus sp.,
Fusarium sp., Exophiala sp., Issatchenka sp., and Syncephalastrum sp. (Table 2).
With respect to aged used litter, 19 different fungal species were detected in a total
of 2,290,500 isolates. Penicillium sp. was the most frequently isolated (41.3%), followed by Rhodotorula .sp(38.1%), Oudemansiella sp.. (8.8%), and *Aspergillus* sp. (6.9%). Besides these fungal genera, other fungi were also identified: cremonium sp., T*richoderma* sp., Fusarium sp., Galactomyces sp., Clavispora sp., *Phoma* sp., *Paecilomyces* sp., and Pestalotiopsis sp. (Table 2). *Paecilomyces .sp* (80.5%) was the most frequently isolated species among paecilomyces general.
In the new litter, Aspergillus fumigatus was the most frequent species identified (33.6%) from Aspergillus genus, and *A*. flavus was also isolated in 9.9% of the samples. Besides these two species, and among Aspergillus genus, others were also found, namely, *A. candidus* and *A. clavatus-nanicus*.

TABLE 1. Litter Samples Collected

Litter type	New	Aged
Pine shavings	4	8
Straw	2	2

Wood shavings with rice hulls	2	6
Wood shavings	2	4
Total	10	20

TABLE 2. Most Frequent Fungi Genus Isolated in New and Aged Poultry Litter

Fungi, new litter	Frequency (n; %)
Penicillium sp.	472,500; 58.9
Aspergillus sp.	25,000; 6.7
Alternaria sp.	83,000; 18.8
Cladosporium sp.	67,000; 6.1
Others	183,000; 9.5
Fungi, used litter	**Frequency (n; %)**
Penicillium sp.	862,500; 41.3
Rhodotorula. sp.	971,000; 38.1
Oudemansiella sp	201,000; 8.8
Aspergillus sp.	127,000; 6.9
Others	129,000; 4.7

In the aged litter, *Aspergillus versicolor* was the most frequent (74.1%) among *Aspergillus* genus, but *A. fumigatus*, *A. flavus*, and *A. niveus* were also identified. *Aspergillus* species presented different incidences between new and aged litter.

Statistically significant differences were not found when comparing total fungal contamination (CFU/g) between different litter types.

TABLE 3. Median Values, 25th and 75th Percentiles, and Interquartilile Range of Total Fungal Contamination, by Litter Type

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Litter type	Median (totalCFU/g)	25th Percentile (totalCFU/g)	75th Percentile (totalCFU/g)	Interquarile range (totalCFU/g)
Pine shavings	200,500	90,000	300,000	210,000
Straw	285,000	113,000	1,785,000	1,672,000
Wood shavings with rice hulls	156,250	9500	818,750	809,250

Wood shavings	830,000	612,500	3,200,000	2,587,500

4.DISCUSSION

isolates from all the analyzed poultry farms. Penicillium was the most frequent genus found (58.9%), followed by Alternaria (18.8%), Cladosporium (6.1%), and Aspergillus (5.7%). In addition to these, other genera were also isolated, namely, Rhizomcor sp., Sporobolomyce sp., Byssoclammys sp., Rhizopus sp.,

Fusarium sp., Exophiala sp., Issatchenka sp., and Syncephalastrum sp. (Table 2).

With respect to aged used litter, 19 different fungal species were detected in a total of 2,290,500 isolates. Penicillium sp. was the most frequently isolated (41.3%), followed by Rhodotorula.sp (38.1%), Oudemansiella sp . (8.7%), and *Aspergillus* sp. (6.9%). Besides these fungal genera, other fungi were also identified: cremonium sp., T*richoderma* sp., Fusarium sp., Galactomyces sp., Clavispora sp., *Phoma* sp., *Paecilomyces* sp., and Pestalotiopsis sp. (Table 2). *Paecilomycet* Lilacinous (80.5%) was the most frequently isolated species among *Scopulariopsis* general.

In the new litter, Aspergillus fumigatus was the most frequent species identified (33.6%) from Aspergillus genus, and *A*. flavus was also isolated in 9.9% of the samples.Nevertheless, several fungal genera isolated in our study pose a health risk both as pathogens or producers of mycotoxins, which can cause severe poisoning in humans and animals (. Egbuta MA), *Aspergillus* spp. in particular. *Aspergillus fumigatus* (section *Fumigati*) is the most pathogenic fungus affecting poultry, accounting for 95 %of all cases of aspergillosis, but other species, such as cremonium sp., T*richoderma* sp., Fusarium sp., Galactomyces sp., Clavispora sp., *Phoma* sp., *Paecilomyces* sp., and Pestalotiopsis sp.. Besides birds, *Aspergillus* species are known opportunisticpathogens in humans, (Ostovick M.et al2021 immunocompromised patients in particular. *Aspergillus fumigatus* accounts for over 80 % of diseases, including invasive pulmonary aspergillosis, aspergilloma, and different forms of hypersensitivity disorders such as allergic asthma, allergic sinusitis, pneumonitis, and allergic bronchopulmonary aspergillosis. . Another species with emerging evidence of a broad spectrum of infections that are difficult to treat are the *Fusarium* spp., which can cause onychomycosis, skin infections, and keratitis. We did not find any species from the *Aspergillus* section *Fumigati* in our litter samples, regardless of the season, while *Fusarium* spp. was detectedin the winter(Ostovick M.et al2021

Litter fungal concentration ranged from 10^2 to 10^5 (Fulg, which is consistent with previous report [Viegas et al; 2012, Yardimei et al, 2008, Dennis et al, 1973, Bacon et al; 1977] and increased with time (Fattening weeks), as expected [Bacon et al; 1977] we found no significant seasonal differences at the beginning and the end of the fattening periods or in the median fungal concentration for either period (Table 1). This is because fungal concentration highly correlated with litter moisture and PH throughout the study which did

Comment [H13]: Results should not be repeated discussion section.. findings should be compared with other related works and give reasons for disparities in this study compared to theirs/ give reason why there are differences within variables in your research.

not significantly differ between the season the correlation between fungal growth and litter moisture and PH was also reported by Hutson [Hutson 1966] and Schefferle (Schefferle 1965) but there are report to the country as well [Lovelt et al; 1971] according to Arne et al. (Arne et al; 2011) the impact of variation in litter moisture and PH on fungal population density remains a controversial issue, although wet and soil areas can intensify fungal growth.

Other studies performed in poultry farms across Europe reported similar results. Rimac et al. (2010) in Zagreb noted the most prevalent fungi were species from the genera *Penicillium* sp., *Fusarium* sp., *Aspergillus* sp., *Mucor* sp., *Rhyzopus* sp., *Scopulariopsis* sp., *Mucor* sp., *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. These fungi were also the predominant genera in other study in agricultural facilities in Ukraine and Poland (Tsapko et al. 2011). Others investigators also concluded that airborne fungi present in poultry facilities include *Cladosporium* sp., *Aspergillus* sp., *Penicillium* sp., and, less commonly, *Alternaria* sp., *Fusarium* sp., *Geotrichum* sp., and *Streptomyces* sp. (Sauter et al. 1981; Lee et al. 2006). It is noteworthy that only one sample from each litter was taken, and variations in fungal contamination are expected.

Nevertheless, the prevalent genera found are common in several studies not only in poultry farms (Kotimaa et al. 1991; Anbu et al. 2004; Rimac et al. 2010; Sauter et al. 1981; Lee et al. 2006), but also in other agricultural settings (Tsapko et al. 2011).

Some specific species found in poultry litter and also in environmental samples from the same poultry farms (Viegas et al. 2012b), such as Rhinzomcor Variabilis, need to be considered with caution, since exposure to this species is associated with cases of occupational allergy (Ponikau et al. 1999) and also is known to produce onychomycosis (Tosti et al. 1996).

Contribution from this species to the fungal load in litter and air is high, and data obtained show an enhanced risk to poultry workers and a public health concern.

The levels of microbial contamination of the bedding material in two broiler houses were determined at the beginning of the broilers' fattening period – prior the introduction of the flocks, and in seven-day intervals throughout the 35 days of fattening.

The most frequent genus found was *Penicillium*, followed by *Alternaria*, *Cladosporium* and *Aspergillus* (*Viegas et al., 2012*). Fungal contamination of poultry litter (CFU/g) is in direct correlation with fungal contamination of the air (CFU/m3), which poses health risks to exposed workers and animals. If inhaled,

dust particles containing fungal spores may cause irritation, or even allergic and/or toxic respiratory diseases (*Viegas et al., 2012*). Moreover, spreading of poultry litter as fertiliser on agricultural land is a potential public health concern due to the possible dissemination of

keratinophilic (*Scopulariopsis* and *Fusarium* genus) and toxigenic fungi (*Aspergillus*, *Fusarium* and *Penicillium* genus).

5.CONCLUSION

Our findings suggest that crop of local fowl consist of total fungal concentrations as much as fungal composition in broiler litter. They have also confirmed that fungal concentrations much depend on litter moisture,,pH and Temperature can be useful in the assessment and control of potential adverse effects on poultry and poultry farm workers. Our General knowledge ~~Knowlledge~~ in this study highlight the need for monitoring fungal contamination in new and aged litter. Litter fungal species identification play important roles in the estimation of potential health risks to exposed workers and animals. Spreading of poultry litter in agricultural fields is a potential public health concern, . Litter is dry and prone to generate particles spreading all the potential pathogenic fungi to the environments consequently producing adverse human health effects.

Comment [H14]: This statement holds no validity, since there is no result supporting this in this research.

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Comment [H15]: Bush, R., Portnoy, J., Saxon, A., Terr, A. and Wood, R. (2006). The medical effects mold exposure. *Journal of Allergy Clinical Immunology*, 17: 326-333

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