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# Fungi Associated with the Crop of a Local Fowl: A Public Health Problem

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

This study was aimed at Isolating fungi associated with crop of a local fowl. But series of Research have been conducted on fungal contaminants associated with Broiler Liter , But we Have less reported on fungal contaminants of poultry . The goals of this study were to (1) Describe the nature and Features of litter fungal contaminants and (2) report the frequency of fungi species present.10 new and 20 old samples were collected from 10 farms.In addition, Air samples were also collected, and after various procedures, 14 different fungal genera were detected in new litter. With respect to old litter, 18 species of fungi were detected, with Fusarium the most frequently isolated (41.2%), followed by A. section Flavi. (27.3%), Oudeemansiela sp (9.9%), and Yeast (16.9%).A significant

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positive correlation was found between litter fungal Biomass and total fungal contamination. Different Morphology and quantification of species have important implications in the evaluation of potential adverse health risks to exposed Farm workers and animals. Indescriminate spreading of poultry litter in fields is a potential environmental health concern, since (Rhizopus spp and mucor spp) were isolated.

*Keywords: Rhizopus spp, Oudeemansiela sp, crop and colony forming.*

## 1. INTRODUCTION

"Fungal/mycotic infections are common in all kinds of poultry birds but are less prevalent as compared to bacterial and viral infections. Fungi are eukaryotic organisms, comprising of both yeasts and molds. Fungal diseases of poultry include Aspergillosis, Candidiasis, Dactylariosis, Cryptococcosis, Favus, Rhodotorulosis, Torulopsis, Mucormycoses, Histoplasmosis and Cryptococcosis. Out of these, the first two (Aspergillosis and Candidiasis) are having much importance and impact and the last two (Histoplasmosis and Cryptococcosis) have some zoonotic significance. Fungi produce disease in two ways viz. producing pathogenic signs and lesions of disease by invading, harming and destroying body tissues of the host; and by producing some toxins known as mycotoxins (aflatoxins, ochratoxins, ergot, fusarium toxins etc.) in food grains and feed during crop production, harvesting and storage steps, the intake, consumption and subsequent intoxication of which produce disease, immunosuppressive condition and hampers production potential" [1].

"Over the last few years, the consumption of animal-source food has led to the intensification of livestock production systems . Such demands along with changing management practices influence the distribution and intensity of parasite infections, being linked with zoonotic diseases . Poultry production is mostly confinement structures densely stocked with birds . Thus, maintaining the ideal microclimate and animal hygiene conditions may entail some challenges . Despite mechanical ventilation systems in order to maintain birds' health, microorganisms from animals' bedding are easily accumulated and aerosolized" [2,13-16].

"Feces, leftover food, bedding material, and feathers that characterize poultry litter are a high-quality, low-cost organic soil fertilizer that boosts crop quality and productivity, which explains its extensive use as manure around the world. A proper bedding material, in terms of chemical and physical features as well as microbial counts, is a requirement for efficient broiler production , and wood-based beddings were associated with an improvement in birds' performance" [2,17-20].

"In recent decades, the poultry industry has made tremendous adjustments to meet the increasing demand for a safe supply of meat and eggs and this growth has been accompanied by structural changes within the sector, characterized by the emergence and growth of "land-independent" (industrial) farming establishments, and the intensification and concentration of poultry operations" [3,5-8]. "The production of poultry products results in hatchery wastes, manure (bird excrement), litter (bedding materials such as sawdust,wood shavings, straw, and peanut or rice hulls), and on- farm mortalities, various kinds of packaging for feed and pesticides, used ventilation filters, unused/spoilt medications and used cleaning materials Most of these by-products can provide organic and inorganic nutrients that are of value if managed and recycled properly" [3,9-12]. However, they also give rise to potential environmental and human health concerns as the sources of elements, vectors for insects and vermin, and pathogenic micro-organisms. Specific concerns that are well documented include degradation of nearby surface and/or groundwater, resulting from increased loading of nutrients such as nitrogen and phosphorus (and potassium in some locations). Air quality issues are less well understood and include the effect of ammonia(NH3), hydrogen sulphide(H2S), odour, and dust particulates containing bacteria, bacterial toxins and chicken skin debris emitted from poultry production [3].

## 2. MATERIALS AND METHODS

### 2.1 Poultry Farms

Farms were located in the Oyun,Ilorin East LGA Kwara State Nigeria, and the samples were Taken between June and October 2022. Farms where samples was obtained was actually a mainly broiler chicken producer. A week-old chicks were transferred to a place where they can Grow in a sheds. workers watch over the condition of the birds, feed and water equipment as Instructed by the researcher, and manage vaccines. In some cases, and Transport the matured ones to slaughter. After the reduction in the population Tractor was arranged to carry the manure to the field where will be made used of.

### 2.2 Sample Preparation Techniques and Applications

10 (new) and 20 (old) broiler litter samples were gotten from poultry in well treated Bags, one sample each (12 g, well preserved to , retain the natural water content) was diluted in 200 ml sterilized distilled water, centrifuged for 30 min at 100 rpm, and 0.5 ml of this mixture was spread onto Petri dishes containing malt extract agar (2%) with chloramphenicol (0.05 g/L) and incubated for 7 days at 28C. Fungal identification was carried out by macroscopic and microscopic observation using lactophenol blue staining and atlas identification (c. Viegas et al. 2012). fungi were isolated and identified to attain the results. Reports were recorded in line with Viegas et al., 2012 work as the average count of the three replicas according to C. Viegas et al.,2012, in colony-forming units per gram of litter (CFU/g). In addition to litter samples, Air samples were collected at 1 m height with a flow rate of 140 L/min onto maltextract 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. (C. Viegas et al 2012)

### 2.3 Sterilization of Glassware and Other Materials

All glasswares used for this project were thoroughly washed and sterilized. They were covered with aluminum and oven dried at $150^{o}$c in 20 minutes. The apparatus was properly disinfected using ethanol; media were sterilized inside the autoclave at $121^{o}$c for 15 minutes inclusion. Serial dilution and sub – culturing were all done near the bunsen burner.

### 2.4 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 $37^{o}$c plates were observed after 24hours for lactose and non-lactose fermenter.

### 2.5 Results Analysis and Interpretation

All Data were analysed with SPSS (Statistical Package) 'The Shapiro- Wilk Test were used to test the Normality of the Data, in all tests, the level of statistical significance was set at $p<0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

Table 1 Shows the values several types of litter Used.

Table 2 Marks the Values of frequency and occurrence of the fungi with Fusarium as the Highest value (58.7%) followed by Mucor spp (18.8%) while A. section Nigri appears as the lowest.

Table 3 Marks the values of frequency identified in the Broiler liter with the occurrence of Fusarium spp. shows the highest (41.2%), A.section Flavi shows (27.3%) and yeast with value of occurrence (16.9%), with Oudemansiella shows the lowest value of occurrence (9.9%).

In the older Broiler litter, A. section *Flavi* was the second of the most frequent (27.3%) among *the A. section Flavi genus isolated A. Nomius, A novoparasiticus and A. Pseudonomius* were also identified. *A. section Flavi species* presented different incidences between fresh and old litter.

The values does not have any significant when comparing fungi between different types of Broiler Liter contaminants, Colony forming unit.

### 3.2 Discussion

Different types of isolates were identified from the Broiler liter . Fusarium was the most frequent genus found (58.7%), followed by Rhizopus (.18.8%), mucor spp.(6.7%), and A. Section Nigri (7.1%). In order to ascertain the genus, other genera were also identify, namely, Rhizomcor ., Sporobolomyce ., Byssoclammys ., Rhizopus., Fusarium ., Issatchenka., (Table 3).

**Table 1. Litter samples collected**

**Broiler litter sampled**

| Types of broiler litter | Fresh | Old litter |
|---|---|---|
| Wood Pallet | 4 | 8 |
| Pine Stump Chips | 2 | 2 |
| Peanut Hulls | 2 | 6 |
| Chopped Straw | 2 | 4 |
| | 10 | 20 |

**Table 2. Most frequent fungi genus isolated in new and aged poultry litter**

**Most numerous fungal genera identified in fresh and old broiler litter**

| Species, Fresh | Occurrence | (Percentage %) |
|---|---|---|
| Fusarium spp. | 472,600; | 58.7 |
| Rhizopus spp | 83,096; | 18.8 |
| Mucor spp | 25,124; | 6.7 |
| A. Section Nigri | 67,700; | 7.1 |
| Additional | 183,167; | 8.7 |

**Table 3. Median values, 25th and 75th percentiles, and interquartilile range of total fungal contamination, by litter type**

| Species, Older | Occurrence | (Percentage %) |
|---|---|---|
| Fusarium spp | 862,400; | 41.2 |
| A.Section Flavi. | 971,259; | 27.3 |
| Oudemansiella spp | 201,121; | 9,9 |
| Yeast | 127,277; | 16.9 |
| Additional | 129,089; | 4.7 |

In regard to old litter, 18 several fungal species were found in a total of 3,290,200 isolates. Fusarium. was the most frequently isolated (41.2%), followed by A. Section Flavi (27.3%), Oudemansiella sp . (9.9%), and *yeast* (16.9%). Some others also identified: cremonium, Galactomyces., Clavispora,, and Pestalotiopsis (Table 2).

In the new litter*,* fusarium was the most isolated species identified (58.7%) from *Rhizopus* was also isolated (18.8% )of the samples.However, several organism identified in our work ill Health which can leads to health burden whichis in accordance with the work carried out by(Egbuta MA) which says 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, C. Viegas et al., 2012) but other species, such as cremonium spp., T*richoderma* spp., Fusarium., are opportunistic Pathogens which is related to study done by Ostovick M et al.,2021.

Yeast was Isolated in the older Broiler liter with (16.9%)and it has been said that yeast have been Identified as a beneficial microbes and less piorities has been given to this area which is in accordance to the work carried out by Bryd A et al 2017 iLittle attention has been given to the beneficial effects of fungi (and particularly various yeasts) with regard to food safety and especially with the gastrointestinal tracts of food-producing animals. In cattle, fungi assist in the breakdown of fibrous plant material so the nutrients can be further digested [4]. While in most food-producing species, including poultry, yeasts have been added to the diets to stimulate the immune system [4]. For example, Candida has been shown to bind to numerous pathogens, including Enterobacter, Pseudomonas, Klebsiella, and Salmonella. The binding of these pathogens by this yeast may stimulate the immune system and therefore reduce the pathogen. 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.

Our work shows that Others Oudemansiella spp and other genera which are airborne fungi are present in the farm this in line with other related studies 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. (C. Viegas et al,. 2012). It is noteworthy that only one sample from each litter was taken, and variations in fungal contamination are expected.

Rhyzopus was one of the fungi identified and in this study we find out allergic properties which is related to Bryd et al., 2017 whichrelated with Most of the previous research investigating fungi populations in poultry was related to fungi found in the litter or on the comb of the chicken . Other studies in Europe found similar results with the most frequently isolated fungal species found in poultry farms were Penicillium sp., Fusarium sp., Aspergillus sp., Mucor sp., Rhyzopus sp., and Scopulariopsis sp.. These were airborne fungi found on the poultry farms that were associated with allergies and other fungal diseases. Although these investigations did not evaluate fungi found in the gastrointestinal tract of birds, it does provide an insight into what populations could be taken into the birds that may impact the colonization of the gastrointestinal tract or what fungal populations are passing through the gastrointestinal tract of chickens (Bryd et al ., 2017).

The most Iidentified organism Fusarium is the most cause of pollution which the workers were exposed to from their daily work which is related to other studies with 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, (Viegas et al., 2012).

We found out that Fusarium is a Keratinophilic and Toxigenic fungi isolated from the particle and it was found out that it can cause irritation this is in line with C. Viegas et al ., 2012 studies.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 (*Scopulariopsi*s and *Fusarium* genus) and toxigenic fungi (*Aspergillus*, *Fusarium* and *Penicillium* genus).

## 4. CONCLUSION

Our findings suggest that crop of local fowl comprises of milions of fungi species and some were not yet discovered as pathogenic organism. fungal composition in poultry litter pose a series of health risk disease to animal and HumanLess attention is put to beneficial properties of some of the organisms This work Highlighte the need for further study in this area. Our findings in this study high mark the need for focusing fungi contaminants in fresh and old litter.Focusing on this will shed more lights on the importance of isolated microbes from the farm liter . Thereby producing negative effects on the health of the people.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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