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Isolation and Identification of Pathogenic Fungi from Domestic Chicken Droppings in Diyala Province, Iraq

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ABSTRACT

Background and Objective: Some of significant zoonotic fungal pathogens are associated with birds, especially poultry. The purpose of this study was to isolate and identify pathogenic yeast and fungi strains from domestic chicken feces in different locations in Diyala Governorate.

Methods: Fresh fecal and fecal mixed with soil samples were obtained at random from different surrounding environments, such as cages, and residential coops. Specimens were collected with swabs or with labeled plastic spoons using sterile techniques, yielding a total of 50 samples. The Mycology Laboratory, College of Veterinary Medicine, University of Diyala performed processing and microbiological analysis. For yeasts isolation, the samples were cultured on SDA. Fungal species were identified using morphological and microscopic analyses.

Results: Each of the 50 samples tested positive, with the following distribution: Candida spp.: 30 isolates (60%). Rhodotorula spp.: 5 isolates (10%). Cryptococcus spp.: 3 isolates (6%). Filamentous fungi: 12 isolates (24% of cases). Aspergillus species: 10 isolates (20%) including 7 isolates of A. niger and 3 of A. flavus. Rhizopus spp.: 2 isolates (4%).

Conclusions: These data suggest the relevance of poultry excreta as reservoirs of potential pathogenic fungi for human infections. Additional research is needed to evaluate related public health risks.

Introduction

Mycotic infections are frequent in birds as well. Two of the more common mycotic infections in birds include respiratory aspergillosis and gastrointestinal candidiasis [1]. Conversely, *Candida species* are opportunistic yeasts from mucosal surfaces, most frequently encountered as avian microflora (gastrointestinal tract) but also in cases of dysbiosis or extreme immunosuppression when these *Candida species* can create more invasive disease [2]. These fungi are found in the environment from soil and water to plantoid and animal secretions; thus, their zoonotic relevancies render them important to both animal and human health [3].

Birds are a diverse reservoir of pathogenic fungi; about half of all bird species carry what can infect a human [4]. While many infection cases are anecdotal, those on farms and at rehabilitation centers indicate the potential for infectious transfer compounded by environmental exposure and a lack of biosecurity [5]. The gastrointestinal tract of many birds provides an ideal niche for fungi as they feed off nutrients and microbial byproducts [6]. Therefore, this means entry occurs nasally and via skin (feathers), as ectoparasites, as well as in crops and the gastrointestinal tract. The latter means feathers and feces present a potential fecal-oral route. In addition, *Cryptococcus neoformans* is a yeast that causes disease in humans. This is a type of yeast associated with pigeon droppings, and there is a clinical connection to this leading to cryptococcal meningitis in humans [7,8].

Yet it's fascinating, therefore, that the same qualities of these fungi found in birds that keep them temperature regulated—their naturally high body temperature (40–42°C)—are why healthy birds do not manifest the fungi and remain asymptomatic transmitters [4]. Yet they manifest pathologically in mammals, especially those with weakened immune responses, and thus, the One Health implications of avian fungal illnesses are clear [9]. For example, if humans inhale it, they are susceptible to a diagnosis of invasive pulmonary aspergillosis; *Candida auris* is a more recent multidrug-resistant yeast, and its parent fungi is zoonotic in nature and connected to transfer on farms [10].

Yet, there is still a possibility of misdiagnosis due to the nonspecific clinical signs in birds and the inability to culturally assess properly via outdated means [11]. The advent of more accurate assessment via molecular diagnostics—from PCR to MALDI-TOF—exists unless, of course, the antifungal susceptibility/resistance is assessed first [12]. For example, with triazole, there are triazole-resistant strains of *Aspergillus* found in avian populations, often associated with the use of fungicides in avian feed for preventative purposes [13].

Chickens (*Gallus gallus domesticus*) represent one of the most eaten animals worldwide and contribute to international food sustainability; however, the increased levels of intensive farming make these creatures susceptible to fungal infections [14]. In addition, their excrement provides fertilizer, contaminating many of these pathogens in an environmental, epidemic-like fashion—and raising zoonotic probabilities [8].

The study was designed to isolate and identify fungal species in domestic chicken poop from Diyala Province. The results will contribute to biosecurity, diagnostics, and mitigation of risks for avian and human health through identification of common fungi and their zoonotic potential. Insights are particularly important to address gaps in One Health frameworks in resource-limited pastoral or agricultural settings.

MATERIALS AND METHODS

Sample Collection:

From multiple chicken houses, we retrieved two samples: fresh chicken droppings and soil mixed with chicken droppings. For most samples, we just had clean plastic spoons (Disposable plastic spoons) to scoop up the fresh droppings and sealed them in ziplock bags instantly. When we couldn't easily scoop up a sample with a spoon, we rubbed a clean swab over the droppings until it was visibly coated with material that would go in the sample. This way, we collected 50 different samples altogether.

Sample Preparation:

In the lab, we took each chicken dropping sample and precisely weighed one gram on our scale. We placed that inside a small test tube, which already contained 5 ml of clean water. Next, we leave it alone for an hour, mixing it up by hand every 15 minutes to ensure thorough combining.

So, we took 2 minuscule tubes, each containing 900 µl of water. Next, we took 100 µl of our original mixture and placed it in tube 1, a 10x dilution. So, then we took 100 µl from that tube and transferred it to the second one; thus, it was 100 times more diluted from our sample.

For the samples we collected using swabs, we added a bit of water (100 µl) into a tube with a round bottom. Then we plunged the dirty end of each swab into the water and stirred it up to

release the sample from the swab and into the water.

Isolation and Identification of Fungi:

To initially purify the *fungal* species, we decided to culture on Sabouraud dextrose agar (SDA). SDA possesses an acidic pH that inhibits the growth of many bacterial species while allowing for the growth of fungi. We also added chloramphenicol (100 mg/L) to the agar for antifungal selectivity since this antibiotic would inhibit the growth of any unwanted bacterial contaminants. To minimize potential contamination, inoculated plates were sealed with parafilm and stored in the refrigerator for future use. Stains were used for identifying isolation. Therefore, the Gram stain and the lactophenol cotton blue stain contributed to determining the identification, in addition to morphology and arrangement beyond budding yeast. Furthermore, India ink capsule staining of the wet mounts was done and observed microscopically to see if any encapsulated strains existed, including *Cryptococcus* spp., which has a large capsule that shows as a clear halo around the cell with this particular stain.

Results:

All the 50 samples taken from the poultry farm in the various areas of Diyala Province were found to be colonized by fungi. The percentage of fungal contamination equals the total number of examined samples at 100%. Yeast occurs as the dominant isolated fungi since 80% (n = 40) were yeasts and 20% (n = 10) were filamentous fungi. The predominant yeast was *Candida* spp. (60%, n = 30), followed by *Rhodotorula* spp. (10%, n = 5) and *Cryptococcus* spp. (6%, n = 3). As for the filamentous fungi, the predominant was *Aspergillus* spp. (20%, n = 10), which consists of *A. niger* (14%, n = 7) and *A. flavus* (6%, n = 3) identified by macroscopic and microscopic characteristics, and *Rhizopus* spp. (4%, n = 2) (fig1).

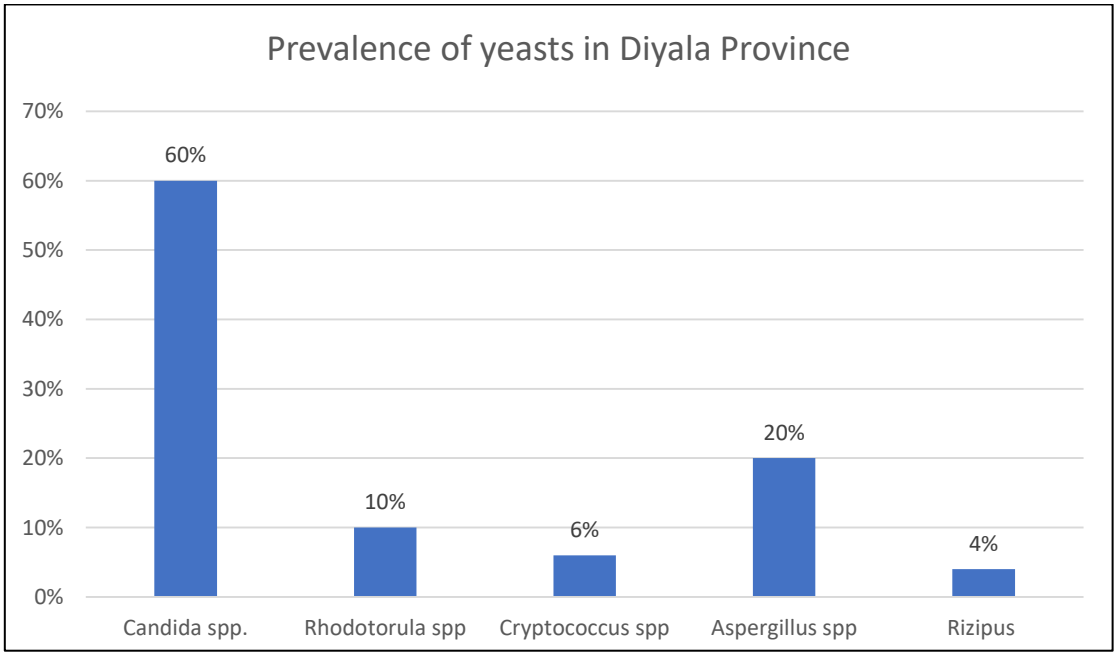

Figure 1: Percentage distribution of fungal from samples collected from poultry droppings.

All isolates were grown on Sabouraud dextrose agar (SDA) and incubated at 37°C. Yeast colonies were smooth and creamy in appearance, while filamentous fungus was pigmented with more expected coloration and morphology of hyphae and conidia. Therefore, the Candida and Cryptococcus isolates were confirmed via Lactophenol Cotton Blue mounting for the

presence of budding yeasts. Capsules were determined for all Cryptococcus spp. via India ink. Aspergillus spp. were determined via the detection of septate hyphae and conidiophores with vesicles; Rhizopus spp. were determined by viewing non-septate hyphae and rhizoids.

The evaluation of phenotypic characteristics of the fungal isolates on Sabouraud dextrose agar (SDA) occurred as deviations. For instance, Candida spp. (n = 30) presented on SDA as white to creamy (SDA appears yellowish brown), smooth and wrinkled, convex after 48 hours at 37 degrees Celsius with a standard yeast-like scent and morphology. A 40× LCB microscopically

stained image after 48 hours at 37 degrees Celsius demonstrated budding blastoconidia, which was expected for Candida spp (Fig2).

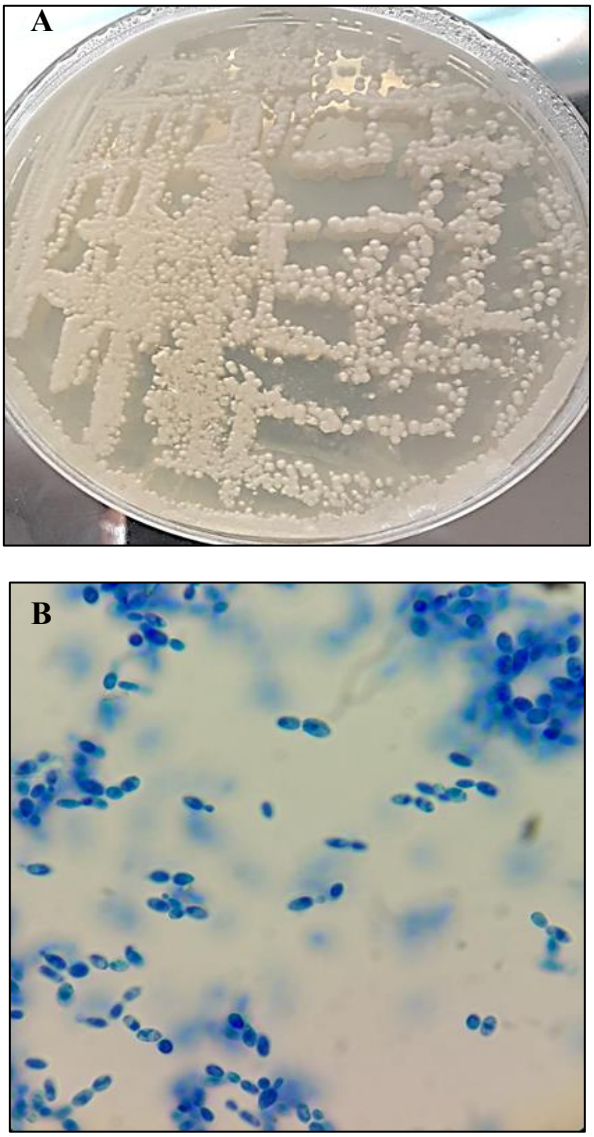

Figure 2: (A) Colonies of Candida spp. cultured on SDA at 37ºC for 48 hrs. (B) staining of Candida spp with Lacto phenol cotton blue (100X).

Cryptococcus spp. (n = 3) shall mucoid colonies with flaming capsules that was highlighted by India ink dyes. Higher magnification showed capsular halos in fresh preparations viewed in phase-contrast microscopy, an important distinguishing feature of Cryptococcus as compared to other yeasts (Fig3).

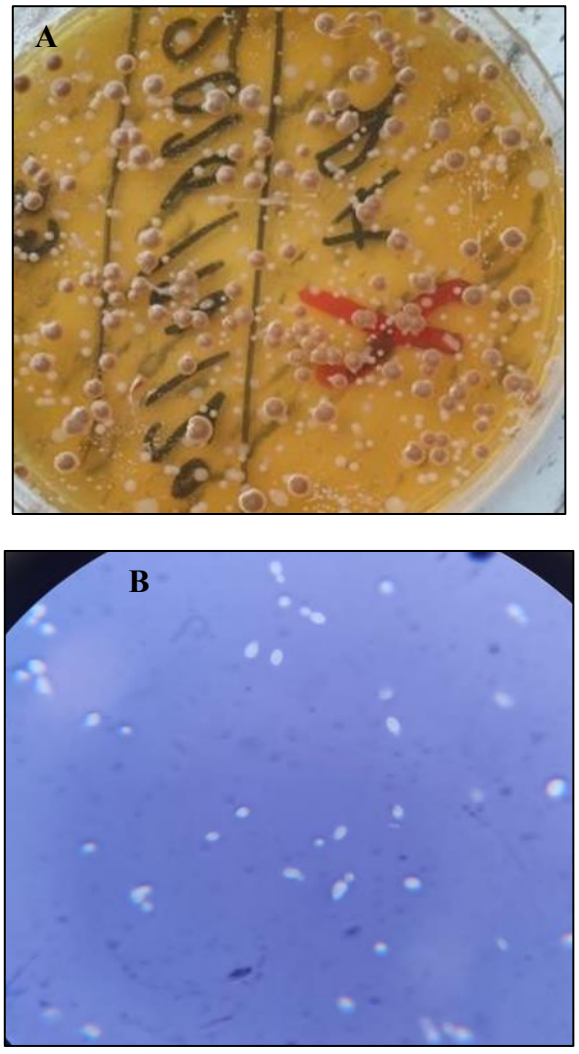

Figure 3: (A) Colonies of Cryptococcus spp. cultured on SDA at 37ºC for 48 hrs. (B) Cryptococcus spp. capsule in India ink stained smear.

In contrast, Rhodotorula spp. (n = 5) grew rapidly in thin, mucoid colonies with pigmentation varying from coral red to orange-yellow. LCB staining showed that these colonies were glistening, soft, and with no pseudohyphae, consistent with the non-dimorphic trends of Rhodotorula spp (Fig 4).

Figure 4: Colonies of Rhodotorula spp. cultured on SDA at 37ºC for 48 hrs.

Filamentous fungi, including Aspergillus spp. (n = 10) that formed powdery, pigmented colonies with green (A. flavus) or black (A. niger) conidial heads. On microscopic examination, septate hyphae and vesiculate conidiophores were observed. Rhizopus spp. (n = 2): different morphology (cotton-like growth, non-septate hyphae and rhizoids) more typical of zygomycetes. These results highlight the value of integrated macroscopic and microscopic approaches to identifications of avian-associated fungi (Fig 5).

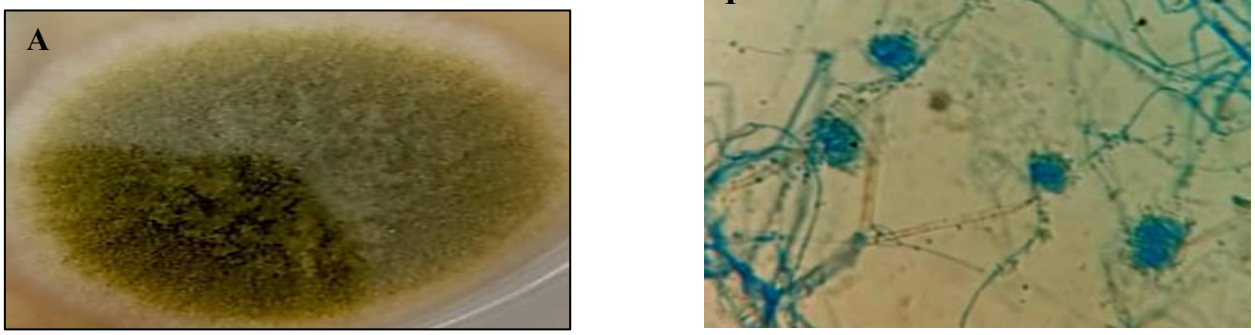

Figure 5: Colonies of (A) A. flavus, (B) A. niger (C) Rhizopus spp cultured on SDA at 25ºC for 7 days. Staining of (D) A. flavus (E) A. niger (F) Rhizopus spp with Lacto phenol cotton blue (40X).

Discussion

The following diagnostic criteria necessary for diagnosis were determined by morphological identifications of the fungal isolates grown on Sabouraud dextrose agar (SDA). The *Candida* spp. were white/creamy (n=30), smooth/wrinkled, yeasty in smell, non-opaque, cultured at 37 degrees C for 48 hours. Also, budding blastoconidia were seen upon microscopic examination with lactophenol cotton blue (LCB). Thus, the morphological characteristics of *Candida* spp. were confirmed [15, 16]. The fact that they are all uniform morphologically increases the ability to differentiate species as fungi are usually differentiated through phenotypic expression in culture [17].

Three of the *Cryptococcus* spp. were identified due to the presence of mucoid colonies. Capsules were revealed through India ink staining. The observation of capsular halos was noted under phase-contrast microscopy, a distinguishing feature of *Cryptococcus* that is not found with any other yeasts [18, 19]. The five *Rhodotorula* spp. were identified due to non-dimorphic features with moderately growing and mucoid forming colonies growing slowly but rapidly growing colonies of coral red to orange-yellow [20].

There were filamentous fungi as well. The majority isolated were *Aspergillus* spp. (n = 10). Macroscopically, these powdery, pigmented colonies appear as conidial heads—green for *A. flavus* and black for *A. niger*. Microscopic confirmation of septate hyphae and conidiophores with vesicles was noted for clear characteristic identification [21]. Also noted was cotton growth (*Rhizopus* spp., n = 2). This zygomycetes was confirmed through microscopic identification of non-septate hyphae and rhizoids [21]. Thus, it's clear that the macro and micro-observations from this experiment confirm successful differentiation via a multifactorial approach, corroborating the literature of such findings in places with birds [15, 20]. Relating fungi to chickens and their human/living environment in Diyala province helps differentiate between normal microbiota establishment and the potential for zoonotic transfer.

Fungal growth establishment occurred at a 100% positive rate from processed 50 samples. 80% of the isolated fungi were yeasts with Candida spp. as the most isolated. Therefore, these fungi can transfer to the environment where poultry and humans live. For example, *Candida* spp. has been noted in proper studies as an opportunistic fungi found in many different environments, and if it is found in this study, that means the environment it grows in has a critical factor of its existence [22, 23]. The fact that yeasts were more prevalent than filamentous fungi could be attributed to the temperature therein of poultry houses and expected nutrients and other factors therein [24].

The filamentous fungi detected and especially *Aspergillus* spp. are of concern because they can produce mycotoxins, which can be toxic to poultry and humans [25, 26]. Particularly worrisome is the identification of *A. niger* and *A. flavus* as both of these species are well-known for producing aflatoxins, which are extremely toxic and carcinogenic [27]. The distinctive growth traits noted in Sabouraud dextrose agar (SDA) also help validate the identification of these fungi, as the morphological characteristics align with those described in the existing literature [22].

The identities of the fungi were further confirmed through microscopic examination, with techniques such as Lactophenol Cotton Blue and India ink staining also revealing key structural characteristics such as budding in yeasts and the presence of capsules in *Cryptococcus spp* [22, 23]. Candida spp. in poultry environments is a matter of concern in terms of zoonotic transmission, especially in settings with high levels of interactivity between poultry and humans [28]. That underscores the importance of improving biosecurity in poultry farms to reduce health risks related to fungal pathogens.

Conclusion

This investigation uncovered a wide variety of fungi in poultry regions of Diyala province with yeast *Candida species* accounting for 80% of the isolates. Every one of the 50 samples showed positive fungal growth which illustrates these microorganisms are omnipresent in poultry environments. The manifestation of these fungi, especially *Aspergillus species* possessing the ability to produce deleterious mycotoxins, is hazardous for the health of both poultry and humans which is a profound concern. These opportunistic fungi mark the need for effective biosecurity practices in poultry farms in order to diminish the possibility of zoonotic diseases. This work documents noteworthy aspects of the microbiological ecosystems, and set the stage for subsequent interventions to control fungal pathogens in poultry environments.

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Declaration of interests

The author declares that there are no conflicts of interest.

Funding Sources

The author alone provided funding for this study.

Publication consent

Data and material availability

The data that was analyzed and generated during this whole study are put right into the published research.

Author Contributions

The author is involved in every step of the manuscript's construction.

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عزل وتحديد الفطريات المسببة للأمراض من فضلات الدجاج المنزلي في محافظة ديالى، العراق

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الملخص

المقدمة والهدف: ترتبط بعض الفطريات الممرضة ذات الأصل الحيواني المنشأ ارتباطًا وثيقًا بالطيور، ولا سيما الدواجن. تهدف هذه الدراسة إلى عزل وتحديد سلالات الخمائر والفطريات الممرضة من براز الدجاج المنزلي في مواقع متعددة ضمن محافظة ديالى، العراق.

طرائق العمل: جُمعت خمسون عينة براز طازج، وأخرى ممزوجة بالتربة، بشكل عشوائي من بيئات مختلفة محيطة بالدواجن، شملت الأقفاص والحظائر السكنية، باستخدام تقنيات تعقيم قياسية وبوساطة مسحات وملاعق بلاستيكية معقمة. أُجريت عمليات العزل والتحليل الميكروبيولوجي في مختبر الفطريات بكلية الطب البيطري، جامعة ديالى، حيث زُرعت العينات على وسط السابورود ديكستروز (SDA) لغرض عزل الخمائر، وتم التعرف على الأنواع الفطرية باستخدام التحاليل المورفولوجية والمجهرية.

النتائج: أظهرت النتائج أن جميع العينات الخمسين كانت موجبة لوجود فطريات، بتوزيع كما يلي: تم عزل 30 عزلة (60%) من جنس المبيضات، و5 عزلات (10%) من *جنس* رودوتورولا، و3 عزلات (6%) من جنس المكورات الخفية. كما تم تسجيل 12 عزلة (24%) من الفطريات الخيطية، تضمنت 10 عزلات (20%) من جنس الرشاشيات، منها 7 عزلات تعود إلى النوع الرشاشيات السوداء، و3 عزلات إلى النوع الرشاشيات الصفراء، بالإضافة إلى عزلتين (4%) من جنس ريزوبس.

الاستنتاج: تشير هذه النتائج إلى أن فضلات الدواجن قد تمثل مستودعًا بيئيًا مهمًا للفطريات الممرضة التي قد تشكل مصدرًا محتملاً للعدوى الفطرية لدى الإنسان، مما يستدعي إجراء المزيد من الدراسات لتقييم المخاطر الصحية العامة المرتبطة بها واقتراح التدابير الوقائية المناسبة.

الكلمات المفتاحية: فطريات، خميرة، دجاج، فضلات، المبيضات.