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Fungal carriage in wild birds from Northwestern Türkiye: a descriptive study

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Abstract

Wild birds play a significant role in the dissemination of micro-organisms, including fungal agents, through feathers, skin debris, and feces, both locally and across regions *via* migration. Identifying fungal carriage in wild birds is crucial for understanding the prevalence of pathogenic fungi and developing effective strategies to prevent and control mycotic infections. This study investigated fungal carriage in wild pigeons, doves, water birds, birds of prey, and small passerines in Balıkesir province of Türkiye, an ecologically important region characterized by key wetlands, two national parks, and major migratory bird routes. A total of 46 wild bird samples were analyzed by culture method, as the "gold standard", and several clinically important fungal isolates were obtained and subsequently further analyzed by PCR. Fungi were detected across all bird categories, with 27 birds (58.70%) testing positive for at least one fungal species. In total, 10 distinct fungal taxa were isolated from the wild birds. The most frequently identified organisms were *Mucor* spp. (28.26%) and *Aspergillus niger* (6.52%), followed by *Sporothrix schenckii* (4.35%), *Candida glabrata* (4.30%), *Arthroderma terrestre* (2.17%), *Trichophyton mentagrophytes* (2.17%), *Nannizzia gypsea* (2.17%), *Aspergillus flavus* (2.17%), *Aspergillus fumigatus* (2.17%), *Lichtheimia* spp. (2.17%), and *Beauveria* spp. (2.17%). Prevalence was the highest in birds of prey and small passerines (77.78%), moderate in pigeons and doves (58.82%), and the lowest in water birds (27.27%). These results emphasize the role of wild birds as potential reservoirs and vectors of fungal agents, highlighting the importance of continued surveillance and biosecurity measures to mitigate the risk of environmental and zoonotic transmissions.

Introduction

The instinct for survival drives migratory birds to exploit seasonal opportunities for breeding habitats and food sources, leading them to cross national and international borders. Migratory birds are categorized as local, short-distance, long-distance, vagrant, or nomadic migrants. As they travel, they can carry and spread micro-organisms globally, playing a significant role in the ecology and circulation of pathogenic organisms.[1,2] These birds contribute to the transmission of bacterial, viral, parasitic, and fungal agents, including certain zoonoses, acting as both biological and mechanical vectors.[2-4] Migratory birds could be involved in carriage of microbial pathogens by three mechanisms, including 1) as biological carriers, 2) as mechanical carriers, and 3) as hosts and carriers (transporters) of infected ectoparasites.[1]

Several studies have investigated the roles of different wild bird species in the transmission of fungal agents across various regions of the world.[5,6] In this context, fecal swab samples were collected from a total of 421 wild birds, including woodcocks (*Scolopax rusticola*; n: 105), quails (*Coturnix coturnix*; n: 57), teals (*Anas crecca*; n: 58), garganeys (*Anas querquedula*; n: 30), coots (*Fulica atra*; n: 50), collared doves (*Streptopelia decaocto*; n: 104), and bean geese (*Anser fabalis*; n: 17), being hunted in Balkan countries, such as Romania, Hungary, and Bulgaria. The samples were cultured on Sabouraud dextrose agar and evaluated using conventional culture methods. Yeasts were isolated from 15.70% of the birds sampled, with the highest prevalence observed in *F. atra* (58.80%) and the lowest in *C. coturnix* (1.70%). A total of 131 isolates belonging to 15 yeast species were identified. *Rhodotorula mucilaginosa* was the most frequently isolated species (28.20%), followed by *Naganishia albida* (18.40%), *Candida albicans* (9.20%), *Cutaneotrichosporon cutaneum* (8.40%), *Meyerozyma guilliermondii* (6.10%), *Candida tropicalis* (6.10%), and other species.[7]

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It has been stated that bird feathers play a role in the transmission of various micro-organisms, including bacterial, viral, and fungal agents, to humans and the environment.[8] In one study, feather samples collected from *Coracias cyanogaster, Acridotheres tristis, Serinus canaria, Pycnonotus cafer,* and *Tockus fasciatus* were analyzed using conventional culture methods. The presence of *Microascus* spp. (12.50%), *Aspergillus* spp. (25.00%), *Penicillium* spp. (37.50%), and *Fusarium* spp. (25.00%) was identified.[9] In another study, a total of 378 feather samples from 30 bird species including aquatic (n: 254) and terrestrial birds (n: 124) were examined. Most samples were collected from waterfowl, with some from terrestrial birds. Fungal isolates were identified based on their macroscopic and microscopic morphological characteristics and confirmed by PCR. In total, 736 fungal isolates were obtained from all examined specimens. Keratinophilic fungi, representing 11 different species, were isolated from 71 (18.80%) birds, including 28 (11.70%) waterfowl and 43 (30.70%) terrestrial birds. The most frequently isolated species were *Microascus brevicaulis* (n: 21), *Nannizziopsis vriesii* (n: 20), and *Nanninzzia gypsea* (n: 12). Less frequently detected fungi included *N. gypsea* (n: 7), *Pseudosepedonium* spp. (n: 3), *Pseudogymnoascus pannorum* (n: 3), *Myriodontium* spp. (n: 2), and single isolates of *Chrysosporium tropicum, N. pruinosa, N. lutea,* and *Aphanoascus fulvescens*.[10]

Amid ongoing environmental changes, it is increasingly important for regions without established surveillance programs to develop the capacity to monitor wild birds and contribute to understanding pathogen dynamics in migratory and breeding populations.[11] Currently, there are no scientific data regarding the role of wild birds in the transmission of fungal agents in the Marmara Region, Türkiye's most economically significant and densely populated area.[12] Addressing this gap is crucial for determining the fungal carrier status of wild birds in the region, particularly in Balıkesir province, which has a high human population density and notable economic importance. Therefore, the present study investigates the prevalence and diversity of fungal species carried by wild birds in Balıkesir, as well as potential differences in fungal carriage among bird species and habitats.

Materials and Methods

Study area and sample collection. This study was conducted in Balıkesir province, located in the north-western region of Türkiye (39°38'57.01"N and 27°53' 10"E) and covering approximately 14,583 km^2. The province has coastlines along both the Sea of Marmara and the Aegean Sea and is characterized by a semi-arid climate with hot summers, cool winters, and higher precipitation during the winter season. Climatic conditions are additionally influenced by proximity to the surrounding seas. Balıkesir is bordered by Çanakkale (north), İzmir (southwest), Manisa (south), Kütahya (southeast), and Bursa (east; Fig. 1). As a part of the Marmara Region Türkiye's most densely populated region,[12] the area is known for its ecological diversity and includes major conservation areas, such as Kuş Cenneti (Bird Paradise) National Park and Kazdağı (Mount Ida) National Park.[13-15] Owing to its position along key migratory bird flyways, the province represents a strategically important location for avian research. Ethical approval for the research was obtained with the decision numbered E-72784983-288.04-20982405 from the Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks.

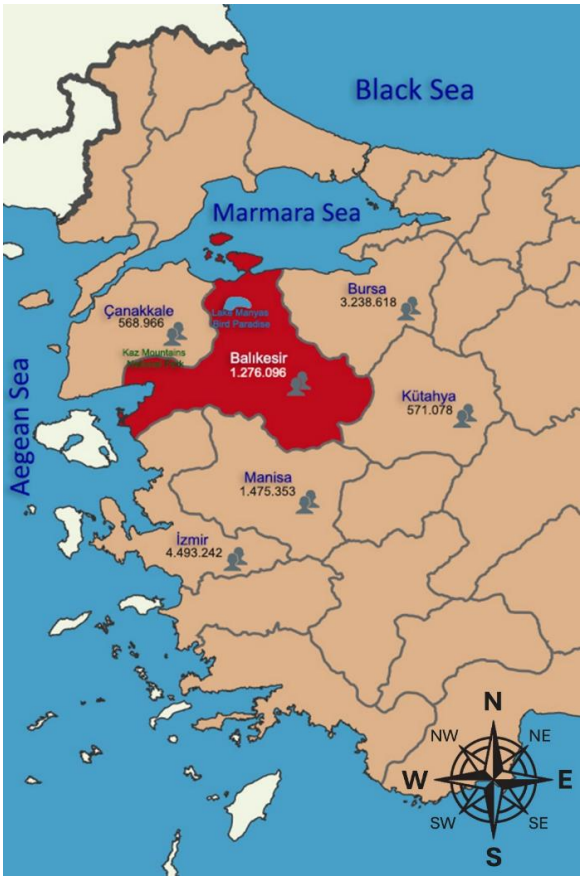

Fig. 1. Geographical location of the region where wild bird samples were collected, and the human population figures of Balıkesir province, Türkiye, and its neighboring provinces.

Samples. This study utilized feather samples collected from 46 wild birds (Table 1). These birds were brought to the Balıkesir Metropolitan Municipality Altıeylül Street Animal Emergency Treatment Center due to the causes, such as exhaustion from migration, trauma, and dependency-related issues. Prior to sampling, all birds underwent a clinical examination to check for symptoms, such as feather loss, itching, skin rashes, dandruff, and swelling. All animals were found to be dermatologically

Table 1. Wild birds and number of samples collected.

Main group	Family	Genus	Species	Sample size
Pigeons and doves	*Columbidae*	*Columba*	*Columba livia*	17
	Columbidae	*Streptopelia*	*Streptopelia decaocto*	
Water birds	*Anatidae*	*Anas*	*Anas platyrhynchos*	11
	Ciconiidae	*Ciconia*	*Ciconia ciconia*	
	Larus	*Larus*	*Larus michahellis*	
Birds of prey	*Accipitridae*	*Accipiter*	*Accipiter gentilis*	9
	Accipitridae	*Accipiter*	*Accipiter nisus*	
	Strigidae	*Bubo*	*Bubo bubo*	
Small passerines	*Hirundinidae*	*Hirundo*	*Hirundo rustica*	9
	Apodidae	*Apus*	*Apus apus*	
	Corvidae	*Pica*	*Pica pica*	
	Corvidae	*Corvus*	*Corvus corone*	

healthy with no signs of skin lesions. The underwing and leg areas were disinfected with 70.00% ethanol and allowed to air dry before sample collection. Approximately eight to ten feather samples were obtained from each bird using a sterile brush. The samples were then placed in sterile plastic containers and immediately transported to the Microbiology Laboratory of the Faculty of Veterinary Medicine at Balıkesir University for further analysis.

Fungal culture. Feather samples were cultured on two sets of Sabouraud dextrose agar (DM200; Mast Group Ltd., Bootle, UK) and two sets of dermatophyte test medium (Acumedia, San Bernardino, USA). The media were prepared in two variants, including one without any supplementation and the other supplemented with chloramphenicol (0.05 mg mL^{-1}; Calbiochem, Darmstadt, Germany) and cycloheximide (0.50 mg mL^{-1}; C7698, Sigma-Aldrich, St. Louis, USA). One set was incubated at 37.00 ˚C and the other at 25.00 ˚C for a period of 5 weeks. Fungal isolates were identified based on their macroscopic and microscopic morphological characteristics, supported by selected conventional tests. Macroscopic examination included evaluation of colony features, such as color (obverse and reverse), shape, elevation, texture, surface appearance, margin, and growth rate. Microscopic features were observed using 40.00 and 100 objective lenses following staining with lactophenol cotton blue (Merck, Darmstadt, Germany) using the wet mount technique. The morphology of spores, hyphae (or mycelium), and reproductive structures was carefully examined. In addition to morphological assessment, various biochemical and carbohydrate fermentation tests were performed, particularly for the differentiation of yeast and mold isolates where applicable. For identification of dermatophytes, hair perforation and urease tests were also carried out following standard protocols. Genus-level identification was achieved by comparing the observed morphological features with standard taxonomic keys and authoritative reference literature specific to filamentous fungi.[15-19]

Polymerase chain reaction. The isolated fungi, including *Aspergillus* species, *N. gypsea*, *Candida glabrata*, and *Sporothrix schenckii*, being identified by conventional culture methods, were further analyzed using PCR. Genomic DNA was extracted from fungal cultures and feather samples using a commercial DNA extraction kit (Quick-DNA™ Fungal/Bacterial Miniprep Kit; Zymo Research, Tustin, USA) according to the manufacturer's instructions. Briefly, fungal cells were mechanically lysed using ZR BashingBead™ lysis tubes with a bead-beating method. Following lysis, the suspension was transferred to a spin column, and DNA binding, washing, and elution steps were performed sequentially. The extracted DNA was stored in elution buffer until PCR analysis. The ribosomal RNA internal transcribed spacer (ITS) region was examined for the molecular identification of fungal species. Amplification was performed using the ITS1 and ITS4 primers including ITS1-F: 5′-TCCGTAGGTGAACCT GCGG-3′ and ITS4-R 5′-TCCTCCGCTTATTGATATGC3′ as described by White *et al.*[20] The PCR amplification of the ITS region of ribosomal RNA was performed in a total reaction volume of 25.00 µL, containing 20.00 - 50.00 ng of genomic DNA, 6.00 pmol of each primer (Sentebiolab Biotech, Ankara, Türkiye), 12.50 µL of 2.00 X Taq DNA polymerase master mix (Ampliqon Inc., Odense, Denmark), and nuclease-free water (UltraPure™; Thermo Fisher Scientific, Waltham, USA) to reach the final volume. Thermal cycling was conducted as follows: an initial denaturation at 95.00 ˚C for 3 min, 35 cycles of denaturation at 95.00 ˚C for 20 sec, annealing at 55.00 ˚C for 30 sec, and extension at 72.00 ˚C for 45 sec, followed by a final extension at 72.00 ˚C for 5 min. Amplification products were separated by electrophoresis on a 2.00% agarose gel, stained with GelRed nucleic acid stain (Biotium, Fremont, USA), and visualized under an ultraviolet transilluminator. Amplification success was evaluated based on the presence, intensity, and expected size of the amplicons.[21-25]

Statistical analysis. The categorical nature of the data was taken into account when comparing fungal carriage rates among bird groups. Fungal carriage was classified as positive or negative, and evaluated across four independent bird groups, including pigeons and doves (n:

17), water birds (n: 11), birds of prey (n: 9), and small passerines (n: 9). Due to the small sample sizes and presence of expected cell frequencies below 5 in some cells, the Fisher-Freeman-Halton exact test (2 × 4 contingency table) was used instead of the conventional chi-square test. A p value of < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS Software (version 30.0; IBM Corp., Armonk, USA).

Results

Fungal culture. Fungal agents were isolated from all wild bird groups examined, including pigeons and doves, water birds, birds of prey, and small passerines. Among the 46 wild birds analyzed, fungal growth was detected in 27 (58.70%) individuals, while no fungal growth was observed in the remaining 19 (41.30%) birds. A total of seven distinct fungal genera were identified from the culture-positive samples. The most frequently isolated fungi were *Mucor* spp. (28.26%) and *Aspergillus niger* (6.52%). Other isolated species included *S. schenckii* (4.35%), *C. glabrata* (4.35%), *Arthroderma terrestre* (2.17%), *Trichophyton mentagrophytes* (2.17%), *N. gypsea* (2.17%), *Aspergillus flavus* (2.17%), *Aspergillus fumigatus* (2.17%), *Lichtheimia* spp. (2.17%), and *Beauveria* spp. (2.17%), (Table 2 and Figs. 2 and 3).

Polymerase chain reaction. Following the PCR with ITS1 and ITS4 primers, ITS amplicons were detected in most samples by agarose gel electrophoresis. Based on comparison with the DNA ladder, product sizes ranged approximately from 580 to 870 bp, with bands most frequently observed at ~600, ~730, and ~870 bp. These sizes broadly overlap with published ITS amplicon ranges reported for *Aspergillus* spp., *N. gypsea*, *C. glabrata*, and *S. schenckii* (Fig. 4). Prevalence estimates for each bird group, together with corresponding sample sizes and 95.00% confidence intervals calculated using the Wilson method are presented in Table 3. Although no statistically significant differences were detected, fungal carriage appeared descriptively lower in water birds and higher in birds of prey and small passerines. The confidence intervals reflect the precision of the prevalence estimates according to the sample size of each group.

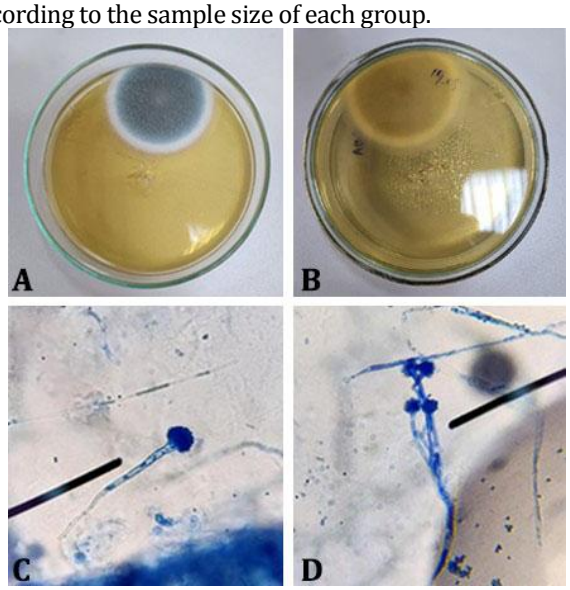

Fig. 2. A) Surface and **B)** Underside views of the *Aspergillus flavus* colony grown on Sabouraud dextrose agar after 7 days of incubation at 25.00 °C. **C** and **D)** The microscopic appearance of *A. flavus* stained with lactophenol cotton blue (40.00 ×).

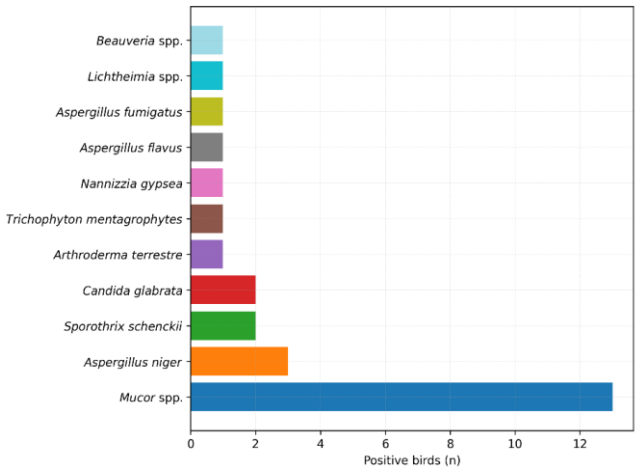

Fig. 3. Fungal species detected in feathers of wild birds.

Table 2. Number (%) of isolated fungi from feather samples of investigated wild birds.

Fungi	Pigeons and doves	Water birds	Birds of prey	Small passerines	Positive birds
***Mucor* spp.**	7 (15.22)	2 (4.35)	3 (6.52)	1 (2.17)	13 (28.26)
Aspergillus niger	1 (2.17)	1 (2.17)	1 (2.17)	-	3 (6.52)
Sporothrix schenckii	-	-	1 (2.17)	1 (2.17)	2 (4.35)
Candida glabrata	1 (2.17)	-	1 (2.17)	-	2 (4.35)
Arthroderma terrestre	-	-	-	1 (2.17)	1 (2.17)
Trichophyton mentagrophytes	-	-	-	1 (2.17)	1 (2.17)
Nannizzia gypsea	-	-	1 (2.17)	-	1 (2.17)
Aspergillus flavus	1 (2.17)	-	-	-	1 (2.17)
Aspergillus fumigatus	-	-	-	1 (2.10)	1 (2.17)
***Lichtheimia* spp.**	-	-	-	1 (2.10)	1 (2.17)
***Beauveria* spp.**	-	-	-	1 (2.10)	1 (2.17)
Total	10 (21.74)	3 (6.52)	7 (15.22)	7 (15.22)	27 (58.70)

A total of 46 birds were examined, of which 27 (58.70%) tested positive for at least one fungal species. Fungal carriage rates were 58.82% (10/17) in pigeons and doves, 27.27% (3/11) in water birds, 77.78% (7/9) in birds of prey, and 77.78% (7/9) in small passerines (Table 3 and Fig. 5). Statistical comparison of fungal carriage rates among the four bird groups showed no statistically significant difference (Fisher-Freeman-Halton exact test; $p > 0.05$).

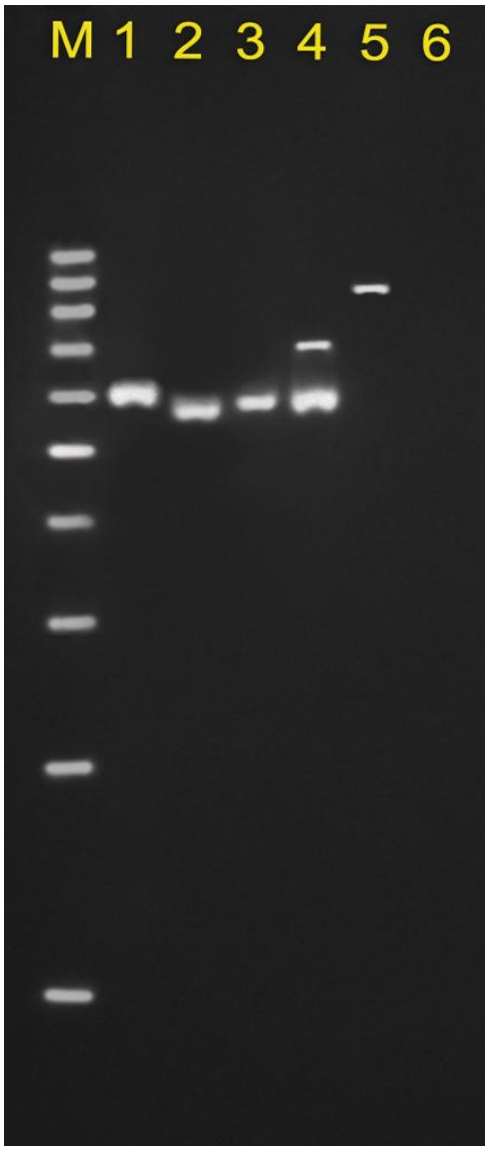

Fig. 4. Agarose gel electrophoresis of ribosomal RNA internal transcribed spacer. Lane M: 100 - 1,000 bp DNA ladder (Thermo Scientific Gene Ruler, SM0241); Lanes 1 - 5: Wild bird samples; Lane 6: Negative control. Based on comparison with the DNA ladder, the estimated amplicon sizes ranged from approximately 580 to 870 bp, with bands most frequently observed at ~600, ~730, and ~870 bp. No amplification was observed in the negative control. Note: Lanes 1 - 3 represent PCR products obtained from fungal cultures, lanes 4 and 5 represent PCR products obtained from feather samples.

Table 3. Fungal prevalence (%) in wild bird groups.

Bird groups	Total	Positive	Negative	Prevalence
Pigeons and doves	17	10	7	58.82
Water birds	11	3	8	27.27
Birds of prey	9	7	2	77.78
Small passerines	9	7	2	77.78
Total	46	27	19	58.70

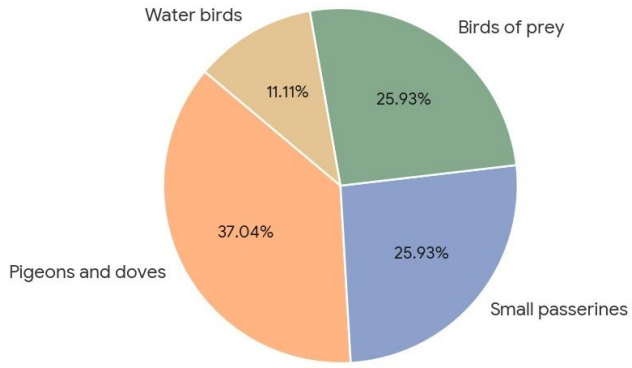

Fig. 5. Comparison of fungal carriage among wild bird groups.

Discussion

As the climate continues to change, global systems are becoming increasingly unstable and unpredictable. This is especially true for many disease systems, including fungal infections being spreading worldwide.[26] Specifically, increasing temperatures, changing precipitation patterns, and extreme weather events are influencing fungal growth, distribution, and virulence. These factors may expand the geographical range of pathogenic fungi, exposing populations to novel, potentially more virulent, or drug-resistant strains. Simultaneously, human factors, such as declining immunity, aging populations, and increased use of immunosuppressive therapies, are enhancing host susceptibility.[2,26,27]

Although fungal diseases are considered less common than viral and bacterial infections, mycotic agents still cause significant illnesses in both humans and animals.[28] Wild birds contribute notably to the dissemination of various micro-organisms, including fungal pathogens, through multiple routes, such as feathers, skin flakes, and fecal matter. This transmission is facilitated not only by endemic, semi-endemic, and exotic bird species residing in specific regions but also by migratory birds traversing diverse geographical areas throughout the year. The movement and biological materials of these birds act as effective vectors for the spread of fungi across ecosystems.[8,29] Moreover, the increasing trend of keeping wild birds as pets has raised public health concerns, as various fungi, such as *Mucor* spp., *Aspergillus* spp., *Candida* spp., *Microsporum* spp., *Trichophyton* spp., and *Rhodotorula* spp., have been isolated from these birds.[30,31] Understanding the factors influencing the epidemiology of

fungal diseases is therefore critical. In this context, several studies have been conducted globally to assess the role of wild birds in fungal carriage. For example, in a study conducted in Iran, 126 wild birds belonging to the *Psittaciformes, Passeriformes,* and *Columbiformes* orders were examined for fungal pathogens, with 83.00% testing positive. The most commonly isolated species were *A. flavus* (29.31%), *C. albicans* (10.34%), and *Cryptococcus neoformans* (1.72%).[31] Another study carried out in Brazil investigated the presence of fungal species in wild birds. Fifty fecal samples were collected from species, such as *Saltator aurantiirostris, Pipraeidea bonariensis*, and *Stephanophorus diadematus*, and analyzed using conventional culture methods. Filamentous fungi were detected in 13 (26.00%) samples, yeasts in 41 (82.00%) samples, and both types in 10 (20.00%) samples. Yeast species included *C. albicans* (31.00%), *Debaryomyces hansenii* (11.00%), *M. guilliermondii* (4.00%), *Diutina catenulata* (4.00%), *Candida intermedia* (2.00%), *Pichia kudriavzevii* (4.00%), *Candida ciferrii* (6.00%), *Candida globosa* (2.00%), *Trichosporon asahii* (2.00%), *Rhodotorula* spp. (2.00%), *Galactomyces geotrichum* (4.00%), *Papiliotrema laurentii* (4.00%), and *Malassezia pachydermatis* (2.00%). The filamentous fungi identified included *Aspergillus* spp. (8.00%), *A. niger* (4.00%), *Penicillium* spp. (6.00%), and *Mucor* spp. (4.00%).[30] Additionally, a total of 240 fecal samples collected from cattle egrets (*Bubulcus ibis*) were analyzed using Sabouraud dextrose agar. The most frequently isolated filamentous fungal genera included *Mucor* spp. (42.30%), *Rhizopus* spp. (26.70%), *Penicillium* spp. (13.30%), *Paecilomyces* spp. (11.40%), and *Scedosporium* spp. (1.95%). Among the yeasts, *Cryptococcus* spp. (2.30%) and *Rhodotorula* spp. (1.90%) were also identified.[5] In the current study, a total of 46 wild birds were examined, with fungal growth detected in 27 (58.70%) individuals, while no growth was observed in the remaining 19 (41.30%). Seven distinct fungal genera were identified in the culture-positive samples. The most frequently isolated fungi were *Mucor* spp. (28.26%) and *A. niger* (6.52%). Additionally, *S. schenckii, C. glabrata*, *A. terrestre, T. mentagrophytes, N. gypsea, A. flavus, A. fumigatus, Lichtheimia* spp., and *Beauveria* spp. were also recovered from the birds (Table 3 and Fig. 3). These findings underscore the potential role of wild birds as reservoirs and vectors of medically important fungi, highlighting the need for ongoing surveillance and greater awareness of zoonotic risks.

Few studies investigating the fungal carrier status of wild birds have reported that prevalence varies among species.[5,30,31] In the present study, although no statistically significant differences were detected, descriptive differences were observed between bird groups, with birds of prey and small passerines exhibiting relatively high rates of fungal colonization, and water birds showing lower prevalence. These patterns may reflect differences in feeding behavior, habitat exposure, and immunological characteristics, while pigeons and doves displayed intermediate prevalence consistent with observations in urban and semi-wild populations. Our findings align with previous studies reporting frequent environmental fungal carriage in small passerines due to the nesting habits and soil contact,[32,33] and general fungal colonization in wild birds, such as cattle egrets.[5] Furthermore, these prevalence patterns have broader ecological implications, as Altizer *et al.*[11] noted, climate change and altered migration routes can influence host pathogen dynamics, increasing bird exposure to environmental fungi and potentially affecting fungal colonization patterns. Overall, these results indicate that fungal colonization occurs at considerable levels in wild and semi-wild bird populations, with certain groups potentially at higher risk, highlighting the importance of monitoring fungal carriage in the context of wildlife health and ecosystem functioning.

Studies have shown that wild birds can harbor a variety of zoonotic mycotic agents, including *Aspergillus* spp., *Cryptococcus* spp., *Candida* spp., *Galactomyces* spp., and *Penicillium* spp., which may pose health risks, particularly to immunocompromised individuals.[6,34] In this context, aspergillosis is a globally distributed opportunistic fungal saprozoonosis affecting humans, animals, and birds. It is primarily caused by several *Aspergillus* species, most notably *A. fumigatus*, followed by *A. flavus, A. niger, A. terreus*, and *A. nidulans*, saprophytic fungi commonly found in the environment.[35,36] Clinical manifestations vary widely, ranging from allergic reactions to severe invasive pulmonary infections, and the disease can be present in both sporadic and epidemic forms, often resulting in significant morbidity and mortality. In a study conducted in Brazil, aspergillosis was diagnosed in 16 (4.48%) out of 357 domestic and wild birds through culture and histopathological analyses.[6] Similarly, Genovez-Oliveira *et al.*,[37] have examined 45 wild bird species, including *Myiarchus ferox, Leptotila verreauxi, Columbina talpacoti*, and *Progne chalybea*, and reported the presence of *Aspergillus* spp. in 3 (6.67%) birds. In the present study, fungi of the *Aspergillus* genus were detected in 5 (10.87%) of the 46 migratory birds examined. Specifically, *A. niger* was determined from 3 (6.52%) birds, *A. fumigatus* from 1 (2.17%) bird, and *A. flavus* from 1 (2.17%) bird. This isolation rate is consistent with previous reports,[6,37] and highlights the potential role of migratory birds in the transmission and dissemination of *Aspergillus* species.

Another important pathogenic genus carried by wild birds is *Candida* spp. A study conducted in Brazil analyzed 19 fecal samples from wild pigeons using conventional culture methods. The results showed that the most frequently isolated genus was the zoonotic *Candida* spp., followed by *Trichosporon* spp., *Rhodotorula* spp., *Cryptococcus* spp., and other fungi.[38] In the present study, *C. glabrata* was detected from 4.30% of the birds,

specifically within the pigeon, dove, and bird of prey groups. This finding supports previous research and highlights the potential of wild birds, particularly urban-dwelling species, to act as reservoirs for opportunistic fungal pathogens with zoonotic potential.

Studies reporting the isolation of dermatophyte species from wild birds remain limited. In a previously published case report, *Nannizzia ripariae* was isolated from the feathers and nests of the sand martin (*Riparia riparia* L.).[39] In another study, *A. terrestre* was isolated from 12 (3.17%) and *N. gypsea* from 7 (1.85%) feather samples out of 378 wild bird species examined during mycological analysis.[10] In the present study, *A. terrestre* was isolated from a bird in the small passerine group, *T. mentagrophytes* from another bird, and *N. gypsea* from a bird belonging to the birds of prey group. These findings are noteworthy as they suggest that wild birds may act as potential carriers in the transmission of dermatophyte species. However, further research is necessary to confirm and expand upon this hypothesis.

Different samples, such as skin, hair, lint, nails, and feces, are commonly used for the isolation of mycotic agents, especially dermatophytes, in living animals.[6,19,38] Feathers and hairs are rich sources of keratin and are considered reservoirs for keratin-degrading micro-organisms. Birds can passively carry keratinophilic fungi on their intact feathers.[40,41] In this study, feather samples collected from wild birds belonging to the groups of pigeons and doves, water birds, birds of prey, and small passerines were used for fungal isolation, resulting in a high isolation rate of 58.70%. This result supports previous studies,[8,10] demonstrating that feather samples, being more accessible and reliable than feces, skin scrapings, and other harder-to-obtain samples, are effective for isolating fungi from wild birds.

The internal transcribed spacer region is one of the standard genetic markers widely used for the molecular identification of fungi due to its high discriminatory power at the species level.[20,21] Amplification using the ITS1/ITS4 primer pair allows the generation of amplicons that may contribute to differentiation among fungal taxa.[21] In the present study, the amplification results obtained with these primers were generally consistent with those derived from conventional culture-based identification. Moreover, the simultaneous detection of two distinct fungal taxa (*e.g.*, *Aspergillus* spp. and *N. gypsea*) within a single feather sample indicates that the ITS1/ITS4 primer set may be useful for detecting mixed fungal populations.

The efficiency of dispersal of pathogenic micro-organisms depends on a wide variety of biotic and abiotic factors affecting the survival of the agent in, or disappearance from, a habitat or ecosystem in a new geographical area.[1] Fungi are incredibly adaptable organisms, with large, malleable genomes allowing them to colonize new geographies and survive as their environment changes.[42] The results of this study suggest that various wild bird species living in the same geographical region may contribute to the spread of different fungal species. In conclusion, such research has the potential to identify natural reservoirs of pathogenic fungi, contributing to a better understanding of their epidemiology and the development of effective prevention strategies.

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Conflict of interest

The authors declare no conflict of interest.

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