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Isolation and molecular identification of fungi from nearby human environments

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ABSTRACT

In this study, samples were collected from household environments, banknotes, insects, and domestic animals. Fungi isolated from insects accounted for 14% of the total isolates, with *Cladosporium sphaerospermum* at 11%. In the pet environment, *Penicillium* spp. yeast had the highest incidence (42%) among isolates from the mouth and skin of cats, followed by *Saccharomyces* at 27%. In geckos, the most frequently isolated fungi were *Cladosporium* spp., followed by *Rhodotorula glutinis* yeast. Samples from cockroaches, flies, and banknotes were cut into five parts and distributed on Petri dishes for fungal cultivation. Fungi isolated from banknotes included *Aspergillus flavus* (25%), Penicillium spp. (16.5%), *Fusarium* spp. (16.5%), and *Cryptococcus* (16.5%). Selected isolates were identified by PCR and deposited in GenBank: *Aspergillus flavus* (KY693973.1, 100% identity), *Cladosporium sphaerospermum* (OR958629.1, 94%), *Penicillium* spp. (MN105322.1, 100%), and *Candida membranifaciens* (EF362753.1, 100%).

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Introduction

The home environment is an ecosystem consisting of humans and the insects in it, which may interfere in one way or another with human life in that house; some insects and animals that live with us in the house may be carriers of many diseases and pathogen such as microorganisms and viruses, but it is typical to raise pets in the house from cats, dogs, and birds, which are a repository of fungi where human contact with pets such as cats and dogs leads to the transmission of fungal infection, transmissions can occur through the skin, mucous membranes through scratches, or any other direct contact with animals, since opportunistic fungi exploit weakened human immunity and fungal diseases are standard transmission worldwide. This may exist in the home environment on those insects and domestic animals (Abdel-Azeem et al. 2020, Mohamedet al. 2021, Laranjeira et al. 2022).

There are also in the home environment some exotic animals such as some types of reptiles, especially the gecko (geckos), scientific name *Palmatogecko rangei*, an animal crawling animal of the order of squamous, and has many species and forms and is spread throughout most parts of the earth desert, swamps, and gardens and prefers places and warm areas to live in addition to animals that are raised optionally. Such as cats and bulbs, in another study (Al-Shibly & Alzamily 2020), they took swabs from different areas of this animal from the mouth, skin and legs and planted them on the medium of PDA was fungus *Aspergillus niger* with a rate of about 40% of the rest of the isolates and then from the fungus *Rhizopus stolonifer* and *Penicillium digitatum*. The appearance rate was approximately 20%. The gecko is an animal that raises concerns among the population because of its presence in dirty places, such as heavy streams, dirty corners, and around the remains of food; and what is less attractive is

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that the gecko is a carrier of many pathogens, it transmits the infection to food in the case of food preparation or storage of food.

One of the household pollutants is the cockroach, which has become widespread and resistant to pesticides due to its role in transmitting contaminated fungi and causing diseases worldwide (Nasirian, 2017). In a study conducted in the American city of Ohio, approximately 105 cockroaches were randomly collected, and surface swabs were taken from their bodies in toilet areas and kitchens. Fungi and bacteria were isolated, with Candida albicans being the most frequently detected fungus among the isolates (Hassan et al., 2022).

With the presence of these pathogens in the home environment, contamination by fungi and other microorganisms exposes humans to potential risks. Fungi are notable for their morphological characteristics, including filamentous structures and yellow, white, or gray coloration. The surface of fungal colonies can be smooth or cottony, and they produce spores that are either single or arranged in chains. Fungi also possess virulence factors, such as the secretion of proteolytic enzymes including keratinase, lipase, and protease, which allow them to degrade keratin in human nails, skin, and hair, penetrate tissues, and survive on them. Additionally, they have surface proteins that facilitate adhesion to keratinized cells, promoting infection and colonization of the skin, and they produce resistant conidia (Mini & Mathew, 2024).

Therefore, the present study was designed to explore fungi that exist in everyday environments surrounding humans and that may pose potential health risks. By collecting samples from domestic animals, insects, birds, reptiles, and even banknotes, we sought to identify the types of fungi most commonly present in these settings. In addition, we aimed to determine which culture medium provides the most effective conditions for isolating and accurately detecting these fungal species.

Materials and Methods

Sampling and isolation of fungi

Fifteen samples were collected from each source under investigation. Samples were collected from the skin and mouth of cats (three replicates each), the outer bodies of cockroaches (three replicates), the skin and mouth of geckos, and the outer bodies of flies. Direct isolation technique according to Abdel-Azeem and Salem (2012) was used. Banknotes were cut into five sections and placed on Petri dishes. All sampling was performed using sterile swabs, and the sampling areas were sterilized beforehand with 70% ethanol. Swabs were then transferred to the laboratory and inoculated onto Sabouraud Dextrose Agar (SDA) under sterile conditions in a laminar flow hood for the isolation of fungi from all sources (domestic animals, insects, and household surfaces).

Phenotypic identification

Isolated taxa were identified morphologically up to the species level on standard media based on the phenotypic characters and relevant identification keys for *Penicillium* spp. (Pitt 1979); for *Aspergillus* spp. (Abdel-Azeem et al. 2020) and for miscellaneous fungi (Domsch et al. 2007). For identification of yeast taxa Kurtzman et al. (2011) was consulted.

Molecular Identification of the most frequent taxa

The recovered fungi were selected and prepared for DNA extraction. Each isolate was transferred to 250 mL conical flasks containing 100 mL Potato Dextrose Broth (PDB) and incubated in a shaker at 150 rpm for four days at 28 °C. The mycelia were then harvested, washed, and subjected to filtration before being used for DNA extraction with the Geneaid Plant Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan).)

Molecular identification of fungi

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The ITS region is highly variable among different fungal species, making it ideal for species-level identification, while remaining relatively conserved within the same species, allowing consistent primer binding. A large number of ITS sequences are available in public databases such as GenBank, which facilitates accurate comparison and species identification. The ITS region is recognized as the official fungal barcode marker by the Consortium for the Barcode of Life (CBOL).

The PCR reaction mixture included the following primers ITS1 and ITS4: Forward 5′-TCCGTAGGTGAACCTGCGG-3′ and Reverse 5′-TCCTCCGCTTATTGATATGC-3′. PCR amplification was performed using the following program: an initial denaturation at 95 °C for 6 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 58 °C for 1 minute, and extension at 72 °C for 1 minute. A final extension was carried out at 72 °C for 5 minutes. The amplified DNA was then confirmed by agarose gel electrophoresis (Khalil, 2020).

The DNA implied by fungal universal primer showed Apure bund in size 300 bp by filaments fungi and 550n bp yeast. After completing the steps of the process of identifying the fungus molecularly, the Figure (3) shows the process of gel electrophoresis. The PCR reaction test was performed to Microgen in the United States of America to sequence DNA using a device (genetic analyzer) and the NCBI-Genbank-Blast Alignment tool data analysis database was used to confirm the identity of the fungus. The PCR technique has been utilized in numerous biological subjects by different authors (Zedan & Al-Amer 2022, Muhammed et al. 2024)

Following amplification, the DNA bands were excised from the gel and sent to South Korea for sequencing using the Genetic Analyzer 3130 (Macrogen Biotech). The resulting nucleotide sequences were submitted to the DNA Data Bank of Japan (DDBJ) for further analysis (Rampini et al., 2016). Molecular identification of the recovered isolates were performed by comparing its ITS1and ITS4 rDNA region sequence data with data on reference strains deposited in GenBank.

Results and Discussion

According to Table 1, the fungi recovered from insects were *Aspergillus flavus*, *Candida* spp., *Cladosporium* spp., and *Nigrospora* spp. The highest incidence was observed for *Aspergillus flavus* (34%), followed by *Candida* spp. (26.8%), while *Cladosporium* spp. and *Aspergillus* spp. had the lowest appearance (2.4%).

According to Table 2, the fungi isolated from domestic animals included *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium* spp., *Candida* spp., *Rhodotorula glutinis*, *Trichophyton verrucosum*, *Nigrospora* spp. and *Penicillium* spp. The fungus with the highest percentage of appearance was *Penicillium* spp. (33.6%).

This common fungus has about 300 species, can produce a wide variety of enzymes, and generates a significant number of conidia. It can be found in soil and air when conditions are suitable for growth. Candida spp. (21.6%) was the next most frequent, while *Aspergillus niger*, *Aspergillus flavus*, *Penicillium* spp., and *Trichophyton verrucosum* had the lowest incidences (0.8%).

These results are similar to a study conducted in Poland (Dworecka-Kaszak et al. 2020), which assessed the most common causative agents of skin diseases and compared the prevalence of animal fungal infections. The fungi most frequently isolated from animals with skin lesions were *Malassezia pachydermatis* (29%) and *Candida* yeasts (27%), including *Microsporum canis*, which was the majority (59%). *Malassezia pachydermatis* accounted for 80% of isolates in animals with otitis externa, and more than 19% of positive fungal cultures were obtained from the outer ear canals. *Candida* yeasts were also identified as part of the etiology of fungal transmission from pets. One cause of otitis is fungi transmitted from home-bred animals (Mokhtar, 2022).

Table1. Percentage of fungal isolates obtained from insects incubated at 27 °C for one week on SDA medium.

S	Insect Name	Place of Isolation	Isolated Fungi	Number of Isolates	Percentage of Impressions%
1	*Periplaneta americana* (Cockroach)	From under the wing	*Aspergillus flavus*	14	34%
			Candida spp.	11	26.8%
			Cladosporium spp.	3	7.3%
			Nigrospora spp.	4	9.3%
2	*Musca domestica* (Fly)	From the body of the fly	*Aspergillus flavus*	4	9.7%
			Candida spp.	4	9.7%
			Cladosporium spp.	1	2.4%
Total number of isolates			41		

Table 3 shows the effect of different culture media on the growth of fungal isolates; it is evident that the highest and most intense growth occurs on SDA (Sabouraud Dextrose Agar). SDA is a selective medium for fungal cultivation, primarily used to isolate skin fungi, yeasts, and various pathogenic and non-pathogenic fungi. The traditional formulation relies on a pH of 5.6 to inhibit bacterial growth and may include antibiotics.

Table 4 shows the fungi isolated from banknotes after incubation at 27 °C for one week on SDA medium (Fig. 1). The occurrence rate of fungi was as follows: *Aspergillus flavus* (25%), *Penicillium* spp. (16.5%), Fusarium spp. (16.5%), and Cryptococcus yeast (16.5%) respectively.

Table 2 Percentage of fungal isolates obtained from domestic animals incubated at 27 °C for one week on SDA medium.

S	Animal name	Place of isolation	Isolated fungi	Number of isolates	Percentage of Impressions%
1	*Palmatogecko rangei* (Gecko)	Skin Mouth	*Cladosporium spp.*	16	12.8
			Trichophyton verrucosum	2	1.6
			Rhodotorula glutinis	3	2.4
			Aspergillus niger	13	14.4
2	*Pycnonotus lucogenys* Nightingale	Mouth	*Cladosporium spp.*	3	2.4
			Rhodotorula glutinis	4	3.2
			Trichophyton verrucosum	1	0.8
3	*Felis catus* (Cat)	Skin Mouth	*Candida spp.*	27	21.6
			Cladosporium spp.	2	1.6
			Penicillium spp.	1	0.8
			Aspergillus flavus	1	0.8
			Aspergillus niger	1	0.8
			Nigrospora spp.	9	7.2
			Penicillium spp.	42	33.6
	Total number of isolates		125		

Table 3 Fungal growth (cm) on three types of media at 27 °C for one week.

Fungus	PDA	SDA	SDA Rose Bengal Agar
Candida spp.	1.4	1.9	1.1
Nigrospora spp.	1.9	5.4	1.4
Trichophyton verrucosum	1.4	1.4	0.9
Cladosporium spp.	2.2	3.4	0.9
Aspergillus flavus	1.2	2.4	0.9

Table 4 Isolated taxa from banknotes incubated at 27 °C for one week on SDA medium.

Isolated Fungi	Number of isolates	Percentage of impressions%
Penicillium spp.	21	%16.5
Aspergillus flavus	32	%25
Fusarium spp.	21	%16.5
Trichoderma spp.	12	%9.4
Alternaria spp.	11	%8.6
Cryptococcus	13	%10
Saccharomyces spp.	9	%7
Candida spp.	9	%7
Total number of isolates	128	

In figure 2, the results show the most prominent taxa of fungi recovered from domestic animals, including the fungal species that were microscopically examined and partially identified. The sequence analysis confirmed *Aspergillus flavus*, deposited in GenBank under accession number KY693973.1, with 100% identity. *Cladosporium sphaerospermum* was deposited under accession number OR958629.1, showing 94% identity. *Penicillium* spp. and *Candida membranifaciens* were deposited under accession numbers MN105322.1 and EF362753.1, both with 100% identity.

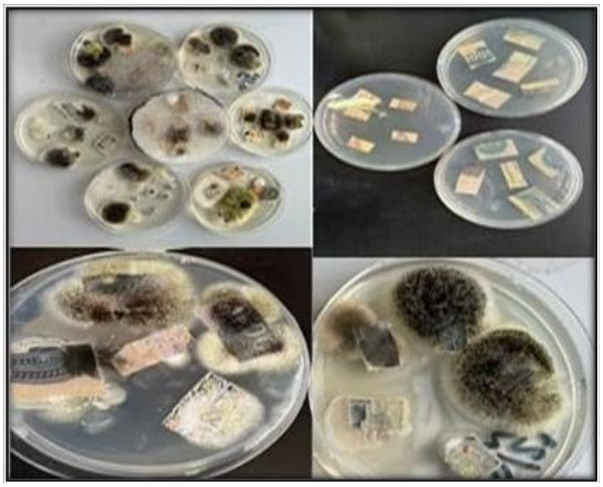

Fig 1. Recovered fungi from banknotes on the isolation medium.

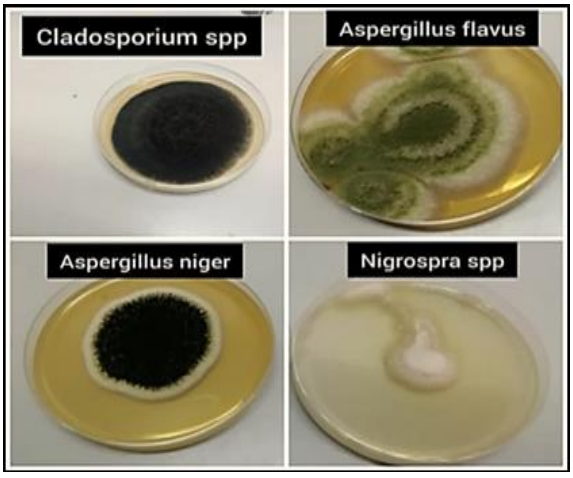

Fig 2. Common fungal taxa recovered from the home environment.

One of the recovered taxa is *Aspergillus flavus*, a cosmopolitan species belonging to the genus *Aspergillus*, which includes more than 350 species (Abdel-Azeem et al., 2020). Some isolates of *A. flavus* produce aflatoxins, which are secreted during fungal growth and can cause poisoning in humans and animals (Khalifa et al., 2022). The percentage of *Aspergillus* in this study is consistent with other relevant studies. For example, Hassan et al. (2022) reported that *Aspergillus* was more prevalent than other fungi isolated from the outer bodies of cockroaches collected from kitchens, similar to findings by Davari et al. (2012).

Fungal isolation from house flies (Diptera: Muscidae) in slaughterhouses and hospitals in South Texas has also been reported, with genera such as *Cladosporium cladosporioides*, *Aspergillus* spp., and *Penicillium griseofulvin* identified (Ysquierdo et al., 2017).

In another study, fungi isolated from geckos and cockroaches included Geotrichum sp., Penicillium sp., and *Aspergillus* sp. Three fungi isolated from cockroaches (K1, K2, K3) were identified as *Aspergillus* sp., except for K3, which was *Penicillium* sp. These findings highlight the presence of pathogenic fungi that could potentially be used for the biological control of harmful insects in agriculture (Rosa et al., 2021).

In a study on *Musca domestica*, 17 fungal genera were isolated on SDA medium, many of which can cause allergies in humans and animals. The most commonly recovered fungus was *Cladosporium cladosporioides* (Rosa et al., 2021). Fungi isolated from domestic animals in the current study included *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium* spp., *Candida* spp., *Rhodotorula glutinis*, *Trichophyton verrucosum*, *Nigrospora* spp., and *Penicillium* spp., with *Penicillium* spp. showing the highest occurrence (33.6%). This widespread fungus produces numerous conidia, can form various enzymes, and contains more than 300 species, commonly found in air and soil under suitable growth conditions. It was followed by *Candida* spp. (21.6%), while the lowest incidence was observed for *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton* verrucosum, and Penicillium spp. (0.8%). Al-Shibly and Alzamily (2020) also reported 20% occurrence of Penicillium isolated from geckos in homes.

Fungi isolated from the home environment in China included *Basidiobolus ranarum* and Beauveria bassiana from dead Asian geckos in water containers (Yang et al., 2022). In Indonesia, fungi were isolated from the outer shells of gecko eggs (*Eublepharis macularius*) incubated for four days at 25 °C, including *Fusarium* spp., *Cephalosporium* spp., *Rhizopus* spp., and *Aspergillus* spp. (Joseph et al., 2022). Mokhtar (2022) reported that fungi isolated from animals included *Aspergillus flavus* and *Aspergillus nidulans*, which may be transmitted to humans and cause infection.

The effect of different isolation media on fungal growth was evaluated, with the highest and most intense growth observed at the center of SDA. SDA is a selective medium primarily used to isolate skin fungi, yeasts, and various pathogenic and non-pathogenic fungi. Its traditional formulation relies on a pH of 5.6 to inhibit bacterial growth, often supplemented with antibiotics. Fungal colonies of SDA reached a diameter of 3.4 cm.

Fungi were also isolated from banknotes incubated at 27 °C for one week on SDA. The frequency of occurrence rates were *Aspergillus flavus* 25%, *Penicillium* spp. 16.5%, *Fusarium* spp. 16.5%, and *Cryptococcus* yeast 16.5%. Studies indicate that lower-

denomination paper currency harbors more pathogens due to frequent handling compared to higher denominations (Sahab et al., 2012). Fungi isolated from banknotes may contribute to the spread of infections, influenced by environmental factors such as heat, humidity, and hygiene practices within the community (Uneke & Ogbu, 2007).

Conclusions

In this study, we found that household insects and pets can carry fungi that live close to humans, potentially spreading fungal pathogens. By modifying the SDA medium with rose Bengal dye, we were able to reduce unwanted fungal growth and isolate the fungi more effectively. These results highlight that our homes and the animals around us can harbor fungi, emphasizing the importance of hygiene and careful monitoring to reduce potential health risks.

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Ethical Approval

The study was approved by Department of Biology, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq (REC 4S/113 on 23/5/2024 and 4S/115 on 28/5/2024). This was done following the ethical standards of the 1964 Helsinki Declaration and its later comparable ethical standards or amendments, as well as the ethical standards of the national and/or the institutional research committee. The maneuver was explained, and written consent was taken from all couples before starting the study. This work included fungi collected from animals.

Conflict of Interest:

All authors declare that they have no conflict of interest.

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