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Isolation and identification of *Aspergillus fumigatus* from feline respiratory infection in Baghdad province

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Abstract---*Aspergillus fumigatus* considered to be the most important species to cause respiratory infection cases in both humans and animals especially in cats in the last decades. In this study, we focused on the isolation and identification of *Aspergillus fumigatus* by collecting 40 samples in deferent veterinary clinics and stray cats in Baghdad city, during the period (October 2021 to January 2022), all samples were cultured on Sabouraud dextrose agar and malt extract agar. The isolates identified by the laboratory methods, it's depend on macroscopic and microscopic appearance. The results showed that (40) swaps taken from the pharynx of infected cats, included: *Aspergillus fumigatus* 16 (40%), *Aspergillus* spp. 7 (17.5%), *Aspergillus niger* 8 (20%), *Penicillium* spp. 5 (12.5%), *Cryptococcus* spp. 3 (7.5%), *Fusarium* spp. 1 (2.5%), and the presence of infection in female (62.5%) more than male (37.5%), this study indicated that the virulence and normal habitat of *Aspergillus fumigatus* make it the most important pathogen to cause respiratory infection and allergen in cats.

Keywords---*Aspergillus fumigatus*, fungal infection, feline respiratory infection.

Introduction

Aspergillus spp. are saprophytic filamentous fungi that are commonly found in soil, where they thrive as saprophytes, with an occasional potential to infect living

hosts, including plants, insects, birds, and mammals (1). *Aspergillus* spp. are responsible for a broad spectrum of illnesses, from saprophytic colonization of the bronchial tree to rapidly invasive and disseminated diseases. Invasive aspergillosis remains a major cause of morbidity and mortality in immunosuppressed patients with profound granulocytopenia secondary to hematological malignancies, or solid organ and bone marrow transplantation (2). The three principal entities encompassed by the term are: Allergic bronchopulmonary aspergillosis (ABPA), Aspergilloma (fungus ball) and Invasive (systemic) aspergillosis (IA) (3). Aspergilli are known for their ability to secrete a variety of biologically active chemical compounds including antibiotics, mycotoxins, immune-suppressants, and cholesterol lowering agents (4). *Aspergillus fumigatus* is one of the most ubiquitous fungi found in soil and organic debris worldwide. It is found in large quantities in the garden and greenhouse soil, and is a primary inhabitant of compost heaps (5). The adhesins, hydrolases, and toxic molecules of *A. fumigatus* which are putatively involved in this invasive process (6). Animal hosts are much more exposed than humans to infectious propagules, both in husbandry and in natural ecosystems. For reasons that could not always be explained, an increasing number of recalcitrant fungal diseases in animals have emerged during the last decades. Similar to disease in humans, aspergillosis in cats can be classified by anatomic location, invasiveness, duration of infection, host immune status, pathology and pathogenesis. The most common site of disease is the respiratory tract, reflecting the primary inhalational route of infection. Respiratory involvement is usually confined to the upper respiratory tract as chronic fungal rhinosinusitis (FRS). However, invasive pulmonary aspergillosis (IPA) can occur as a focal infection or as part of disseminated invasive aspergillosis (DIA). Focal invasive infections of the gut or urinary bladder have also been described (7). Therefore, the aims of this research were to isolate the *Aspergillus fumigatus* from respiratory infection of cats in Baghdad province.

Materials and Methods

Samples collection

A total of forty samples were collected from different veterinary clinics cases in Baghdad province (Al- Rusafa); and from the stray cats during (October 2021 to January 2022). Swabs were collected from the pharynx of infected cats in both sex with clinical signs (coughing, respiratory sounds, sneezing and lacrimation).

Isolation and identification of *Aspergillus fumigatus*

Samples were placed directly on the Sabouraud-Dextrose Agar and malt extract agar through sterilizer swab and incubation for 5 days at 37°C. The isolation and identification of *Aspergillus fumigatus* depend on morphological features according to (8).

Macroscopic Appearance

Macroscopic appearance was set by examination of the shape, color, Consistency, edges of the colony, and other evident characteristic arrangements of colonies of the *Aspergillus* spp.

Microscopic Appearance

Detection of the cell shape, size and arrangement of *Aspergillus fumigatus*. The sample was examined by light microscope with one drop of lacto phenol cotton blue stain, and examined under a 40 X lens.

Statistical analysis

All data were analyzed by ANOVA in the SPSS software (9).

Results and Discussion

Isolation and identification of Fungi from cat's respiratory infection cases

Forty pharynx sample that grew on Sabouraud Dextrose Agar gave primary positive results for presence of fungus spp. The results of (40) swaps taken from the pharynx of infected cats, included: *Aspergillus fumigatus* 16 (40%), *Aspergillus* spp. 7 (17.5%), *Aspergillus niger* 8 (20%), *Penicillium* spp. 5 (12.5%), *Cryptococcus* spp. 3 (7.5%), *Fusarium* spp. 1 (2.5%) as in table (1).

Table (1) The Number of Fungi Isolate from cat'spharynx

Fungal spp.	Isolation No.	Percentage%
<i>Aspergillus fumigatus</i>	16	40.0
<i>Penicillium</i> spp.	5	12.5
<i>Aspergillus niger</i>	8	20.0
<i>Aspergillus</i> spp.	7	17.5
<i>Cryptococcus</i> spp.	3	7.5
<i>Fusarium</i> spp.	1	2.5
Total	40	100%

The percentage of incidence of infection relative to gender show increase in female (62.5%) than the male (37.5%) as in table (2).

Table (2) The Number of Fungi isolate relative to gender

NO. of isolates	Species	NO. Male	NO. Female
16	<i>Aspergillus fumigatus</i>	5	11
5	<i>Penicillium</i> spp.	3	2
8	<i>Aspergillus niger</i>	2	6
7	<i>Aspergillus</i> spp.	1	6
3	<i>Cryptococcus</i> spp.	3	0
1	<i>Fusarium</i> spp.	1	0
Total 40		37.5%	62.5%

Isolation of *Aspergillus fumigatus* on Sabouraud-Dextrose Agar

Aspergillus fumigatus were described based on morphological characteristics of the culture medium in both macroscopic and microscopic appearance.

Macroscopic Appearance

Aspergillus fumigatus was cultivated on Sabouraud's Dextrose agar, the surface growth is velvety, blue-green in color with a narrow white border and white to pale yellowish reverse at 37 °C for 4 days after inoculation as in figure (1).

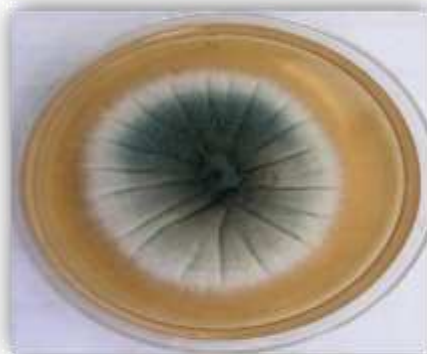


Figure (1) Macroscopic appearance of *Aspergillus fumigatus* colonies on Sabouraud Dextrose agar after 4 days at 37 °C.

Microscopic appearance

Microscopic examination showed green conidia, produced in chains based on greenish phialides, the vesicles are subclavate using lactophenol cotton blue as in figure (2).



Figure (4) Microscopic appearance of *Aspergillus fumigatus* which appear the vesicles and conidia.

Malt extract agar

Aspergillus fumigatus grew in malt extract agar and produce deep green-blue color with powdery appearance and white border, after incubation 4 day at 37 °C. As in figure (3).



Figure (3) *Aspergillus fumigatus* on malt extract agar at 4 days at 37°C

The present study showed that the highest fungus isolated was *Aspergillus fumigatus* as 16 (40%), due to difference reasons such as the specific adhesion molecules and *A. fumigatus* produces an immuno suppressive toxin (gliotoxin) that inhibits macrophage phagocytosis, *A. fumigates* also have thermotolerance particularity and special invade mechanisms, this result confirmed with previous studies by reported that *A. fumigatus* is most virulence strain (10, 11), The present of *Aspergillus* spp. In second highest percentage due to its nature which is opportunistic mold and don't need high nutrient requirement for sporulation, wherever its widespread in air, decaying material, house walls, these investigate agree with (12) when she isolate *Aspergillus* spp from milk and its products. *Fusarium* spp. 1(2.5%) it is a common ground saprophyte and an important pathogen, and has the capability of surfacing adherence, and produce proteases and collagenases, and can cause local or disseminated infection due to produced toxin, (13) reported the first case infection in cat by fusarium spp. The reasons for increase the percentage of infection in female (62.5%) than male (37.5%) due to hormonal disorders, pregnancy and estrus cycle, the researcher (14) found the percentage of infection in female more than male when they isolated dermatophytoses from feline and canine (Suryasa et al., 2021; Suryasa, et al., 2022).

Conclusions

This study provide that *Aspergillus fumigatus* was the most pathogenic fungi casing respiratory infection in cats and most species spread between cases recorded.

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