

KERATINOLYTIC AND OPPORTUNISTIC PATHOGENIC FUNGI FROM CARPET DUST IN MOSQUES AND RESIDENTIAL HOUSES IN DUHOK, KURDISTAN REGION, IRAQ

Hend A. Hussein, Samir K. Abdullah *

Dept. of Biology, Faculty of Science, University of Zakho, Kurdistan Region, Iraq - hend.hussein@uoz.edu.krd,
samir.abdullah@uoz.edu.krd

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ABSTRACT:

One hundred samples of carpet dusts (50 samples from residential houses and 50 samples from mosques) were collected from different sites in Duhok province during the year 2014 to 2015 for the objective of the study of the occurrence of keratinolytic and other potentially pathogenic fungi using hair baiting method. A total of 24 fungal species (17 species from house dust) and (12 species from mosque dust) in addition to non-sporulating mycelia and yeasts were isolated and identified. The keratinolytic species *Arthroderma cuniculi*, *Chrysosporium tropicum* and *Gymnoascus ressii* were detected. Potentially pathogenic fungi in the genera *Aspergillus*, *Arthrographis*, *Geomyces*, *Microascus*, *Scopulariopsis* and *Neoscytalidium* were also able to colonize and grow on baited hairs.

KEYWORDS: Keratinolytic fungi, opportunistic pathogens, carpet dust, Iraq.

1. INTRODUCTION

Fungi can be deposited on floor carpet through different ways such as food and drink spills, foot traffic or can be air-born transported through heating and cooling systems or carried by people from outside (Augustyniuk-Kram and Dmowska, 2013). Among the important keratinaceous substances that shed by human occupants are hairs and skin scales. These substances are accumulated on the fitted carpets in indoor environments. Keratin is an insoluble proteins macromolecule found in hairs, skin, feathers, nails and horns in human and vertebrates. Keratin degradation has been reported by a variety of organisms including actinomycetes, bacteria and filamentous and non-filamentous fungi (Morihara *et al.* 1967; Zaghoul *et al.* 1998; Scott and Untereiner, 2004). Fungi which are capable of the enzymatic degradation of those polymers are namely found in the order Onygenales of phylum Ascomycetes (Currah, 1985).

Some fungi for example can grow on hairs but are not able to degrade keratin but they utilize the non-keratin lipid fraction of the hair (Griffin, 1960; Filipello Marchisio, 2000).

The distinction between keratinolytic and keratinophilic fungi is based on their degradation or usage of keratin. The term "keratinolytic" is applied to fungi with enzymatic ability to degrade and utilize keratin remains in the environment and therefore are potentially pathogenic to human and animals (Majchrawicz and Dominik, 1969; Filipello Marchisio *et al.* 1994). However, fungi the merely inhabiting keratinaceous substance but lacking keratinolytic activity and nourished by constituents after keratin degradation or associated substance are termed keratinophilic (Filipello Marchisio, 1986). Several studies documented the presence of keratinophilic fungi in floor dust of indoor environments. Ali-shtayeh and Al-Sheikh (1988) investigated the keratinophilic mycoflora of floor dust of 72 class room in 24 kindergarten schools in the city of Nablus (Palestine) using hair baiting technique for isolation. The reported species belong to the genera *Arthroderma*,

Chrysosporium, *Microsporium*, *Trichophyton*, *Geotrichum* and other fungi. Most of the reported species were well know agents of mycoses. Marchantani *et al.* (1983) during their studies on floor dust in Roman primary school in Italy have shown that species of *Chrysosporium* were present in 100 % of the samples, while *Microsporium* and *Trichophyton* species were present in 40 % and 65 % of the samples respectively.

Abdullah and Al-Musa (2000) investigated the incidence of keratinolytic fungi in the floor dust of residential houses in Basrah, Iraq using hair baiting technique. A total of 18 species were isolated, of which 13 species were members of Onygenales and the majority of them were well known as keratinolytic fungi. These were assigned to the genera *Chrysosporium*, *Gymnoascus*, *Gymnoacella*, *Malbranchea* and *Narasimhella*. In another study, Abdullah and Al-Musa (2011) investigated the keratinophilic fungi inhabiting floor dust of Mosques in Basrah, Iraq. Fungi were detected by hair baiting methods assigned to the genera *Chrysosporium*, *Arthroderma*, *Gymnoascella*, *Gymnoascus*, *Oidiodendron* and *Scopulariopsis*, of which *Chrysosporium* was the most frequent genus.

In Kurdistan region of Iraq, apart from the work of Al-Barzanji, (2009) on the general isolation of fungi from indoor dust and study of their correlation with asthma in Erbil, so far, there is no any previous work on the incidence of keratinolytic and other opportunistic pathogenic fungi inhabiting carpet dusts from residential houses and public places, therefore, the aim of this study was the isolation and identification of these fungi using keratin source as a bait.

2. MATERIALS AND METHODS

2.1 Collection of dust samples

A total of 100 dust samples (50 samples each of residential houses and mosques), were collected from different sites in Duhok province (Duhok center, Amadi, Summel, Zakho and Chira cities) during September, 2014 to May, 2015. Dust samples were taken from the surface of fitted carpets using home vacuum

* Corresponding author

cleaner. The samples were stored in sterilized collecting bags at 5 °C and were processed within 1-2 weeks after collection.

2.2 Isolation of fungi

The hair baiting technique (Vanberuseghem, 1952) was adopted for the recovery of keratinophilic fungi and related opportunistic pathogens using a keratinaceous substrates as nutrient sources. Sterile glass Petri dishes were half filled with floor dust sample and horse hairs (5 cm in length sterilized by autoclaving at 121 °C for 15 min) were sprinkled on to the dust sample. A 5 ml volume of sterile water containing 0.5mg /L cycloheximide was added to minimize growth of saprophytic fungi. The Petri dishes were incubated at 28 °C and were checked regularly over a period of 8 weeks for growth of fungi on baited hairs. Sterile distilled water was added to the dishes at different intervals and whenever necessary to keep samples moist. When fungal growth was visible on the hair under a dissecting microscope, isolation cultures were made by lifting a part of the growth with a fine flamed syringe needle and streaking it on Sabouraud dextrose agar. Conidia when present were picked up on an eyebrow hair fixed with nail polish to the top of a syringe needle as described by Abdullah and Hassan (1995).

2.3 Identification of fungi

Pure colonies were established on appropriate media for identification. The majority of the detected species were identified to species level based on morphological and cultural characteristics. Measurements were taken after seven days incubation. Microscopic mounts were made in lacto phenol with or without cotton blue. For recognizing the ornamentations of the conidiophores and conidia, microscopical slides were examined with oil immersion. Keratinolytic and opportunistic pathogenic fungi were identified according to Ellis, (1971; Oorschot (1980); Currah (1985); de Hoog *et al.* (2001); Ellis *et al.* (2007) ; Guarro *et al.* (2012) and Sandoval-Denis *et al.* (2013,2016). For identification of species in the genera *Aspergillus* and *Penicillium*, pure colonies were grown on four media according to Klich (2002) and Samson *et al.*, (2000), which were as follows: Czapeck Yeast Extract Agar incubated for seven days at 25 °C (CYA25), Czapeck Yeast Extract Agar incubated for seven days at 37 °C (CYA37), Czapeck Yeast Extract Agar with 20% Sucrose incubated for seven days at 25 °C (CY20S), Malt Extract Agar incubated for seven days at 25 °C (MEA). Each medium was supplemented with 50mg / Liter chloramphenicol (SDI) to suppress bacterial growth. For each culture four plates were used, two of CYA and one each of CY20S, MEA. Each plate was inoculated at the center and incubated in the dark for seven days. One CYA is incubated at 37 °C. The rest are incubated at 25 °C. All species were identified according to the keys and descriptions provided by Rapper and Fennel (1965), Pitt and Hocking (1997); Klich (2002) and Samson *et al.* (2000,2007) .

3. RESULTS AND DISCUSSION

Data presented in table 1 representing the detected species and their percentage of occurrence in carpet dusts collected from residential houses and mosques using hair baiting method. A total of 24 fungal species (17 species from house dust) and (12 species from mosques dusts) in addition to non-sporulating mycelia and yeasts were isolated and identified. *Aspergillus* was the most common genus represented by 8 species, followed by *Scopulariopsis* (3 species) .Other genera were represented by a single species. Among *Aspergillus* species, *A. flavus* and *A. versicolor* were isolated at high frequency (12% for each), followed by *A.*

ochraceus (8%) and *A. carneus* (6%) from house dust, whereas, *Aspergillus* species isolated from mosque dust showed low isolation frequency between 2-4. %.

Scopulariopsis was the next most common genus, three species of this genus, were identified of which *S. brevicaulis* at 42% and 4% and *S. fusca* at 12% and 6% were from house and mosques respectively. *S. flava* was recovered at 6% from house dust only. The genera *Chrysosporium* and *Gymnoascus* are members of order onygenales and are well known keratinophilic fungi (Kushwaha, 2000; Kunert, 2000)). However several studies showed that keratinolytic activity is not limited to members of onygenales, but also found in other groups of fungi (Rajak *et al.* 1992; Kim, 2003). Ali-Shtaya and Jamous (2000) demonstrated the activity of *Aspergillus carneus*, *A. ochraceus* and *A. versicolor* for their growth on hair with varying intensity of keratinolytic activity. Kim (2003) demonstrated the keratinolytic activity of five *Aspergillus* species viz. *A. flavus*, *A. fumigatus*, *A. niger*, *A. nidulans* and *A. terreus* that could grow on chicken feather and releasing keratinase. Our isolation of *A. carneus*, *A. clavatus*, *A. flavus*, *A. nidulans*, *A. niger* and *A. ochraceus* using hair baiting method as a source of keratin reflecting their ability to degrade this substrate.

Chrysosporium species are primarily soil inhabiting fungi and are predominantly recovered from soil by hair baiting method (Kachuei *et al.* 2012; Deshmukh and Verekar, 2012). Filipello Marchisio *et al.* (1994) demonstrated the keratinolytic activity of *C. keratinophilum*, *C. pannicola*, *C. tropicum* and *Arthroderma cuniculi* by either their ability in developing structures related to surface erosion or by radial penetration when grown on hairs.

Chrysosporium tropicum and *Arthroderma cuniculi* were isolated in the present study from carpet dust of mosques at 2% isolation frequency. Their presence may be attributed to the availability of shed keratinous substrate that comes from activity of humans. Abdullah and Al-Musa (2000) recorded *Chrysosporium keratinophilum*, *C. merdarium*, *C. pannicola*, *C. queenslandicum* and *C. tropicum* from floor dust of residential houses in Basrah, while *C. carnichaellii*, *C. keratinophilum*, *C. merdarium*, *C. pannicola*, *C. queenslandicum* and *C. tropicum* were isolated from floor dust of mosques in Basrah (Abdullah and Al-Musa, 2011). Several species of this genus and their teleomorph, *Aphanoascus* viz. *A. fulvescens*, *A. durus*, *C. crassitunicatum*, *C. tropicum*, *C. keratinophilum*, and *C. pannorum* were isolated from surface sediments of the Shatt Al-Arab River and it is Creeks at Basrah, Iraq (Abdullah and Hassan, 1995).

Chrysosporium species may cause skin infection and onychomycosis in human (Maghazy, 2002; Stebbins, 2004) and in some cases is associated with disseminated human body (Stillwell *et al.* 1984, Liu and Paterson, 2004).

Two species of *Scopulariopsis* (*S. asperula* and *S. flava*) were detected from floor dust of residential house while *S. flava* was detected from floor dust of mosques at Basrah city. The two species were isolated by hair baiting method (Abdullah and Al-Musa, 2000; 2011). *Scopulariopsis brevicaulis* was reported as the most frequently isolated species among the genus from clinical specimens (Sandoval-Denis *et al.* 2013). Other species viz *S. brumptii*, *S. flava* and *S. fusca* are less frequent. *Scopulariopsis* species are known to be opportunistic pathogens involved in superficial tissue infections, non-dermatophyte onychomycosis (Tosti *et al.* 1996; de Hoog *et al.* 2001), and to less extent involved in keratitis (Ragge *et al.* 1990) and in deep tissue infection (Neglia *et al.* 1987; Patel *et al.* 1993; Iwen *et al.* 2012).

Arthrographis kalrae was detected in one occasion from carpet dust of mosques. *A. kalrae* is an opportunistic pathogen and is rarely isolated from clinical specimens. It has been reported as the causal agent of pulmonary infection (Vos *et al.* 2012), causal agent of onychomycosis (Sugiura and Hironaga, 2010) and involved in fungal panophthalmitis and invasive sinusitis (Xi *et al.*

al. 2004). *Neoscytalidium dimidiatum* was isolated in one occasion by hair baiting method from carpet dust of mosques. *N.dimidiatum* is an opportunistic human pathogen causing superficial infections of skin, nail, and nose (Campbell and Mulden, 1997; Madrida *et al.* 2009). It is evident from table 1 that several fungal species that were able to colonize and grow on baited hairs such as *Cladosporium* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., and *Tetracoccosporium* sp. Their detection may be attributed to their ability to grow on substrates naturally present on the

surface of hairs or by capable of using intercellular substances which are easy to digest (Griffin, 1960; English, 1965).

4. CONCLUSION

Dust samples from floor carpets in mosques and residential houses are rich in keratinolytic and other opportunistic pathogenic fungi.

Table 1. Frequency of occurrence of fungi isolated by hair baiting method from dust of mosques and houses.

Species	Houses		Mosques	
	No of positive sample	Frequency%	No of positive sample	Frequency%
<i>Arthroderma cuniculi</i>	-	-	1	2
<i>Arthrographis kalrae</i>	-	-	1	2
<i>Aspergillus carneus</i>	3	6	-	-
<i>A.clavatus</i>	1	2	-	-
<i>A.flavus</i>	6	12	-	-
<i>A.fumigatus</i>	1	2	-	-
<i>A.ochraceous</i>	4	8	2	4
<i>A.nidulans</i>	2	4	-	-
<i>A.niger</i>	-	-	1	2
<i>A.versicolor</i>	6	12	-	-
<i>Chrysosporium tropicum</i>	-	-	1	2
<i>Cladosporium herbarum</i>	6	12	-	-
<i>Fusarium spp.</i>	-	-	6	12
<i>Gymnoascus reesii</i>	2	4	-	-
<i>Geomyces pannorum</i>	-	-	1	3
<i>Microascus paisii</i>	4	8	4	8
<i>Mucor hiemalis</i>	4	8	2	4
<i>Penicillium expansum</i>	2	4	-	-
<i>Rhizopus stolonifer</i>	6	12	-	-
<i>Neoscytalidium dimidiatum</i>	-	-	1	1
<i>Scopulariopsis brevicaulis</i>	21	42	2	4
<i>S.flava</i>	3	6	-	-
<i>S.fusca</i>	8	12	3	6
Sterile mycelium (white)	-	-	3	6
<i>Tetracoccosporium paxiarum</i>	2	4	-	-
Yeast	4	8	4	8

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كورتيا لیکولین:

سەد نمونە ژ تۆزا سەر مەحفیرکان (50 مەحفیرکین مالان و 50 بین مژگەفتان) هاتنە وەگرتن ژ دەفەرین جودا جودا بین پارێزگەها دهۆک د ماوهیی دناقبەرا سالا 2014 هەتا 2015 دا ژ پێخەمەت هەکولینا نەخۆشیین و کەروویین کیراتینی بین پەپوهندیدار و کەروویین دی بین نەخۆشی پەیداکر و دەلیقگر. سەرجه مە 24 رەگەزین کەروویان (17 رەگەز ژ تۆزا مەحفیرکین مالان) و (12 رەگەز ژ تۆزا مەحفیرکین مژگەفتان)، ژێدەباری مایسیلیاییین ب سەر و هەقیرتێش ب رێکا (نێچیرکنا مووی) هاتنە جوودا کەرن. رەگەزین هەلکنا کیراتینی وەك *Arthroderma cuniculi* و *Chrysosporium tropicum* و *Gymnoascus reesii* هاتنە دەستینشانکرن. کەروویین دی بین نەخۆشی پەیداکر و دەلیقگر وەك *Aspergillus*, *Arthrographis*, *Geomyces*, *Microascus*, *Scopulariopsis* و *Neoscytalidium* شیان کولونیان دروست بکەن و ل سەر موویین نێچیری شین بین.

خلاصة البحث:

تضمنت الدراسة جمع 100 عينة من غبار السجاد (50 عينة من دور السكنية و 50 عينة من المساجد) من مواقع مختلفة في محافظة دهوك خلال الفترة من ايلول 2014 وتغابيه ايار 2015 وذلك بهدف دراسة تواجد الفطريات الكيراتينية وغيرها من الفطريات المرضية الإنتهازية المحتملة باستخدام الشعر كطعم كيراتيني. وأظهرت الدراسة عزل 24 نوعاً من الفطريات (17 نوعاً عزلت من غبارسجاد البيوت) و(12 نوعاً من غبار سجاد المساجد) فضلاً عن الخمائر وحبوط فطرية غير متجرئته. تم تحديد الأنواع الكيراتيني *Arthroderma cuniculi*, *Chrysosporium tropicum* and *Gymnoascus reesii* اما الفطريات الممرضة الانتهازية شملت الاجناس *Neoscytalidium* *Aspergillus*, *Arthrographis*, *Geomyces*, *Microascus*, *Scopulariopsis* والتي وجدت ناميه على الشعر.