Incidence of Microascus/Scopulariopsis Species Complex (Microascales:Ascomycota) in Fitted Carpet Dust From Residential Houses and Mosques in Duhok Province, Iraq

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Received: June 12 , 2017 Accepted: August 27, 2017 Online Published: September 1, 2017

doi: 10.23918/eajse.v3i1sip55

Abstract: One hundred samples of carpet dusts (50 samples from residential houses and 50 samples from mosques) were surveyed for the presence of the potentially pathogenic fungi Microascus/Scopulariopsis species complex (Microascales:Ascomycota). The samples were collected from different sites in Duhok province,Kurdistan region,Iraq, during September, 2014 to May, 2015. Four species of Microascus (M.brunneosporus, M.cirrosus, M.pyramidus and M.paisii) and three species of Scopulariopsis (S.asperula, S.brevccaulis and S.flava) were identified. Acaulium acremonium (=Scopulariopsis acremonium) was also detected. The diagnostic features of the reported species based on micro-morphological features of their reproductive structures were provided. M.brunneosporus represents a new record for the Iraqi mycobiota. The identified species were reported in several publications as potentially pathogenic to human.

Keywords: Microascus, Scopulariopsis, Acauliim, Carpet Dust, Iraq

1. Introduction

Typical indoor environments such as houses, workplaces and places of worships (mosques) support the growth of a variety of organisms including fungi. The presence and activity of these fungi depends on several factors particularly humidity, temperature and available nutrient sources (Burge et al., 1984).

Several studies have shown that the floor dust accumulated on fitted carpets are a suitable niche for the growth of several dermatophytes, related keratinophilic fungi, potentially pathogenic fungi and mycotoxin producing fungi (Beguin and Noland,1996; Bahkali and Parvez,1998; Abdullah and Al-Musa, 2000, 2011; Al-Musa and Abdullah, 2001; Engelhart et al. 2002; Singh et al. 2009 and Al-Humiany, 2010). The aim of the study was to isolate and identify potentially pathogenic fungi in Microascus/Scopulariopsis species complex in dust from fitted carpets in mosques and residential houses in Duhok province.

2. Materials and Methods

2.1. Collection of Dust Samples

Dust samples (n=100) were taken from the surface of fitted carpets by the help of home vacuum cleaner from residential houses and mosques in Duhok province during September, 2014 to May, 2015. The samples were stored in sterilized collecting bags at 5°C and were processed within 1-2 weeks after collection.

2.2. Isolation Methods

2.2.1. Hair Baiting Technique

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 onto the dust sample (Vanbreuseghem, 1952). A 5ml volume of sterile water containing 0.5mg /L Cycloheximide was used to prevent saprophytic fungi. The Petri dishes were incubated at 25 ˚ C and were checked regularly over a period of 8 weeks for growth of fungi. Sterile distilled water was added to the dishes at different intervals and whenever, it is 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 streaked 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).

2.2.2. Dilution Plate Method

Initial dilution was made by mixing 1 g of floor dust (as dry weight base) with 9 ml sterile distilled water in a test tube and 1 drop of Tween 80 was added and shacked thoroughly for 10 mints. Serial dilutions up to 10-3 were made. Aliquots of 1 ml from 10-3 dilution were added to sterile Petri dishes (in triplicates) and about 20 ml of Sabouraud's Dextrose Agar (SDA) medium amended with 0.5 g/l cycloheximide and 0.25g/l chloromphenicol was pored over and were incubated at 25˚C. Isolates from these colonies were subcultured on fresh appropriate media for identification.

2.3. Identification of Fungi

The detected species were identified to species level based on morphological and cultural characteristics following keys and descriptions provided by De Hoog and Guarro, (1995); Guarro et al., (2012) and Sandoval-Denis et al., (2016 a, b).

3. Results and Discussion

Four species of Microascus Zukel have been identified during this study.

1-Microascus brunneosporus Sandoval-Denis, Gene & Guarro (Fig.1. A, B, C)
2-M.cirrosus Curzi (Fig.2A, B)
3-M.pyramidus G.L. Barron and J.C. Gilman (Fig.2,C,D)
4-M.paisii (Pollacci) Sandoval-Denis, Gene & Guarro (Fig.1.D)
=Torula paisii Pollacci
= Scopulariopsis paisii (Pollacci) M.Ota

=S.brumptii Salv.-Dural
Their diagnostic features are presented in Table (1).

Microascus brunneosporus Sandoval-Denis, Gene & Guarro is newly reported in Iraq. The species was recently discovered by Sandoval-Denis et al. (2016b) and was isolated from bronchoalveolar lavage fluid. To the best of our knowledge, our finding probably represents the second isolation of the species in the world.

The distinctive feature of the species produces ellipsoidal to allantoidal ascospore (5-7x2-3 um) in size and conidiophores are absent or as based single cell bearing 1-3 annelides with subglobose to ellipsoidal smooth walled conidia arranged in long chains.

Microascus paisii (Pollacci) Sandoval-Denis, Gene &Guarro is newly redefined in Microascus based on molecular analysis (Sandoval-Denis et al., 2016a).There is a confusion in naming this fungus. It was known as anamorphic genus under different Scopulariopsis names (S.paisii and S.brumptii) and considered as the asexual morph of Microascus.

Torula paisii is linked to M.cirrosus as asexual morph. More recently, phylogentic analysis showed that the ex-type of T.paisii belongs to Microascus lineage well supported sub clade together with several strain of S.brumptii.

Three species of Scopulariopsis Bainier have been identified.

1-S. asperula (Sacc.) S.Hughes (Fig.3.A)

2-S.b revcaulis (Sacc.) Bainier (Fig.3.B)

3-S .flava (Sopp) F. J. Morton & G. Smith. (Fig.3.C)

Their diagnostic features are presented in Table (2).

Scopulariopsis was erected by Bainier (1907) for a fungus characterized by producing annelidic conidiogenesis with mostly thick-walled, conidia with truncate base arranged in long dry chain. Mating different isolates in culture as well as by molecular methods demonstrated that sexual morph of Scopulariopsis belongs to the ascomycete genus Microascus (Abbott et al., 1998; Issakainen et al., 2003). Microascus together with other fungi with annelidic conidiogenesis was accommodated with family Microascaceae, order Microascales (Lumbasch and Huhndrof, 2007). The genus is characterized by producing perithecial ascomata with dark peridium of texture angularis and cylindrical or papilate neck. Ascospores are dextrinoid when young, asymmetrical, reniforms triangular or lunate forming along cirrhosis at the ascomatal ostiole (Guarro et al., 2012).

A recent study carried out by (Sandoval-Denis et al., 2016a) revealed that Microascus / Scopulariopsis were polyphyletic with species distributed into several distant lineages. However, most species of Microascus / Scopulariopsis clustered into a single large lineage that comprised of four sub lineages corresponding to three distant genera, Microascus, Pithoascus and Scopulariopsis and a fourth newly described genus Pseudoscopulariopsis (Sandoval-Denis et al., 2016a).

Genus: Acaulium Sopp

A.acremonium (Delacr) Sandoval-Denis, Guarro & Gene (Fig.3.D)

=Scopulariopsis acremonium (Delacr) Vuill

This species was assigned of Scopulariosis (S.acremonium) but more recently included in genus Acaulium and excluded from Microascus /Scopulariopsis on the basis of DNA phylogenetic analysis (Sandoval-Denis et al., 2016b). The distinction of Acaulium from Scopulariopsis is difficult based on morphology. However, Acaulium species are able to grow at low temperature and sporulate abundantly at 15 C, whereas sporulation in Microascus and Scopulariopsis is low at temperature below 25 C. (Sandoval-Denis et al., 2016b).

Table (1): Diagnostic features of Microascus species

Species	**Ascomata** **Shape and size (*um*)**	**Asci (*um*)**	**Ascospores(*um*)**	**Conidiophore and conidiogenus cell (*um*)**	**Conidia (*um*)**
M. brunneosporus	Globose 110-200 with cylinidrical neck upto40	Ellipsoide or ovoidal 11-14*7-8	Ellipsoide to allantoide 5-7*2-3 , light yellow brown	Absent or abasal single cell, anneidic	Subglobose to ellipsoidal 4-5*2.5-5 With truncat bee arranged in chain
M.cirrosus	Globose 140-220 with along cylindrical neck	Nearly spherical to obovate 9-12*8-11	Broadly reniform 5-8*3-4	Annelidic 10-20*2-3.5	Broadly clavate, pale yellow 4-6*3.5-5.5
M.pyramidus	Black flask-shaped 125-250,neck 100-200 long with osteolar hairs	Ovoid 13-18*9-12	Triangular as quadrangular in lateral viens 5-6.5*5.5-7	Conidiophore finely roughened	Pale grey-brown obovate 4.5-5.5*3-4
M.paisii	Absent	Absent	Absent	smooth-walled	broadly ellipsoidal to short clavate 4–6 *2–4.5

Table (2): Diagnostic features of Acaulium and Scopulariopsis

Species	**Colony diameter(mm)** **PCA**	**Color**		**Conidia**		
		Colony	**Reverse**	**Shape**	**Wall**	**Size(um)**
A.acremonium	20-40	White to pale buff	Pale brown	Ovoidal	smooth	7.5-13*5-6
S. asperula	50-60	Avellaneous to vinaceous brown	Cream to brownish	Spherical to broadly ovoidal	Smooth	5-8*5-7
S.brevcaulis	50-60	Whitish ,powdery to felty ,soon becoming avellaneous	Cream to brownish	Spherical to obovoidal or bullet-shaped	Rough	5-8*5-7
S.flava	20-45	White ,floccose to fasciculata	Yellow to brownish	Spherical to slightly ovoidal	Finely to coarsely roughened	5-8*5-7

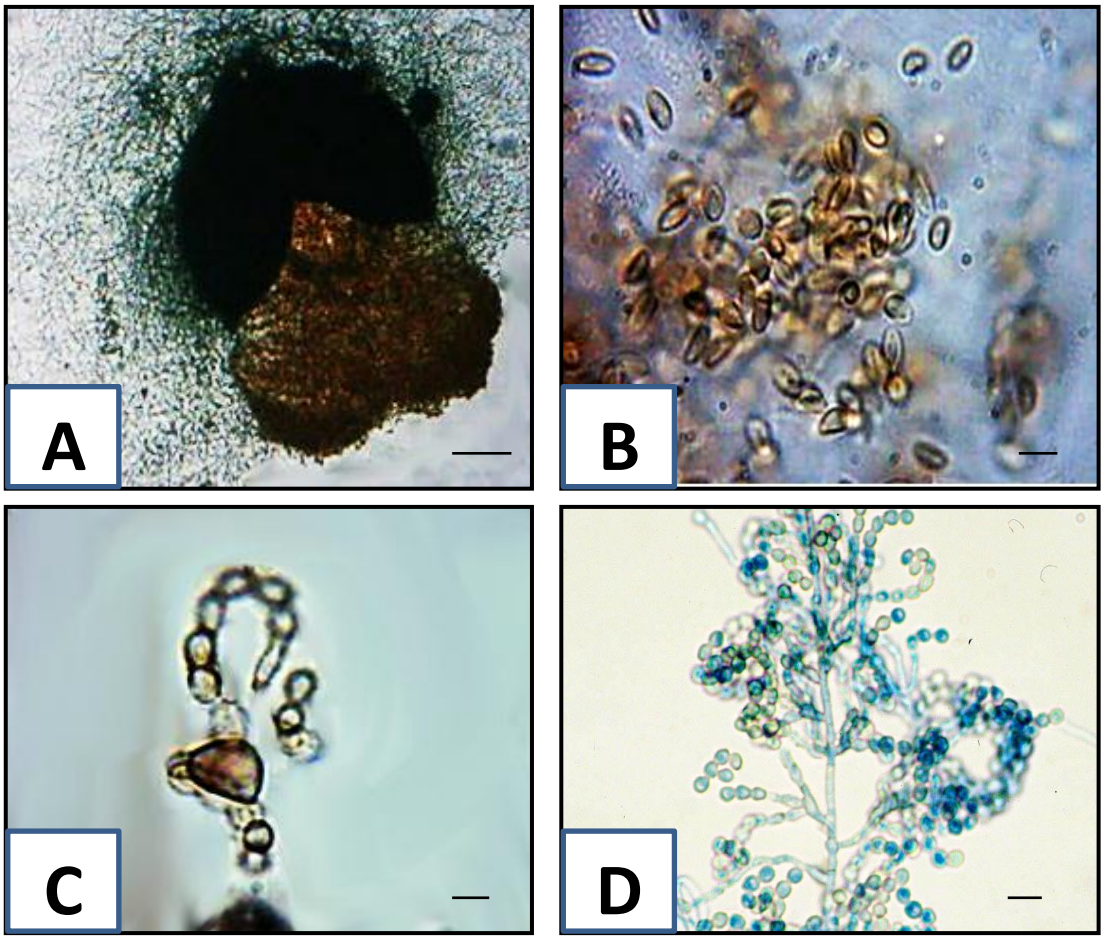

Figure (1): Microascus brunneosporus (A- Perithecia, B-Ascospores, C-anamorph of Scopulariopsis (Conidia), D- Scopulariopsis anamorph of M.paisii.

Bar (A) = 50 um, (B,C) = 5 um, (D) = 10 um

Several species of Microascus and Scopulariopsis have showed resistance to cycloheximide and were frequently isolated from floor dusts (Abdullah and Al-Musa, 2000, 2011) and from sludge (Awad and Kraume. 2011).

Microascus and Scopulariopsis species were reported as potentially pathogenic to humans. M.cirrosus has been reported as a causative agent of onychomycosis (De Vroey et al., 1992; Elewski, 1998). Scopulariopsis species were reported as the most non-dermatophyte fungi involved in nail infections (Issakainen et al., 2003). S.brevicaulis was reported as etiologic agent of fungal keratitis (Del Preto et al., 1994), and as a causative agent of subcutaneous mycosis in immunocompromised patients (Martel et al., 2001).

4. Conclusion

Carpet dust in residential houses and mosques are rich in Microascus/Scopulariopsis species complex. Four species of Microascus, three species of Scopulariopsis and one species of Acaulium have been isolated and identified. Acaulium acremonium and Scopulariopsis brunneosporus are

recorded for the first time in Iraq.

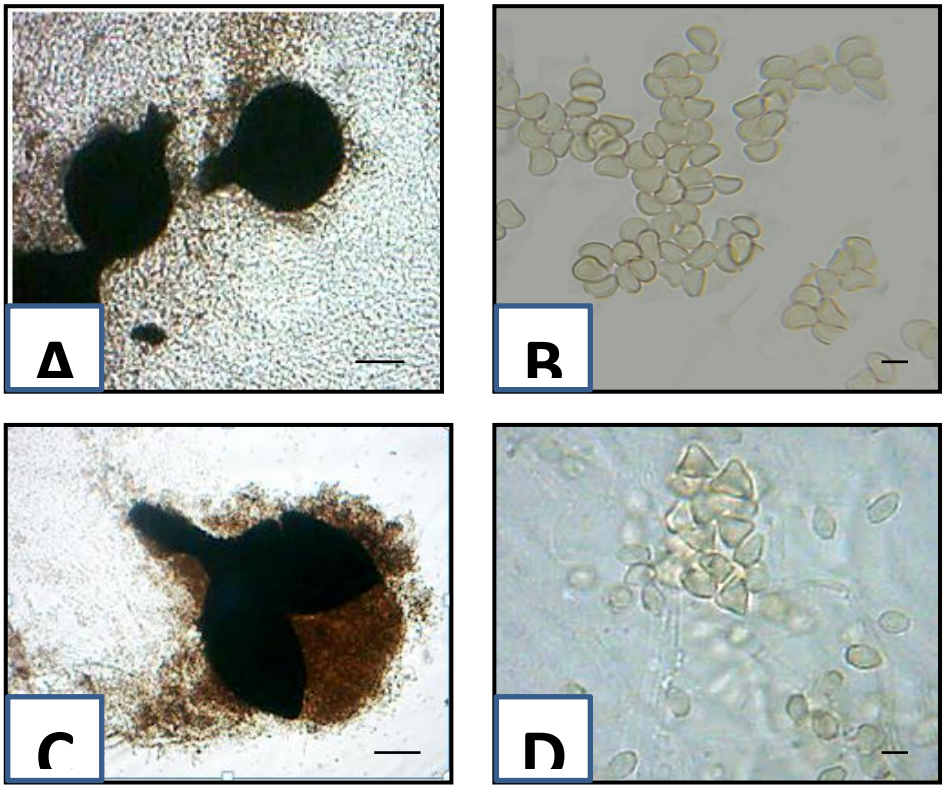

Figure (2): M.cirrosus (A- Perithecia B- Ascospore), M.pyramidus (C- Perithecia, D- Ascospores). Bar (2A, C) = 50 um, (2B, D) = 5 um

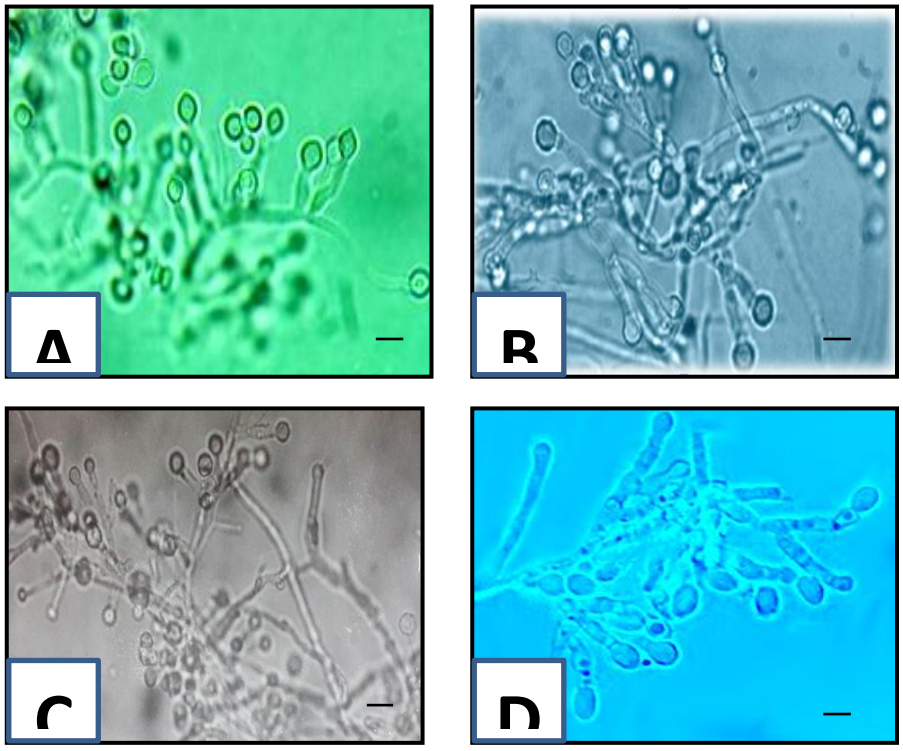

Figure (3): (A) Scopulariopsis asperula, (B) S. brevicaulis, (C) -S.flava, (D)-Acaulium acremonium, Bar (A- D) = 10um

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