 doi:10.20944/preprints201807.0236.v1

Characterization of Keratinophilic Fungal Species and Other Non-Dermatophytes in Hair and Nails Samples in Riyadh, Saudi Arabia

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Running title: Mycological analysis of hair and nails of workers males in Central Saudi Arabia

Disclosure of potential conflicts of interest: No conflict of interest to disclose.

Research involving Human Participants and/or Animals: IRB approval was submitted for research involving obtaining samples from human subjects.

 doi:10.20944/preprints201807.0236.v1

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Abstract

Abstract

Background: The presence of fungal species on the surface skin and hair is a known finding in many mammalian species and humans are no exception. Superficial fungal infections are sometimes a chronic and recurring condition that affects approximately 10-20% of the world's population. However, most species that are isolated from humans tend to occur as co-existing flora. This study was conducted to determine the diversity of fungal species isolated from the hair and nails of workers in the central region of Saudi Arabia where there are not many observational studies on the mycological species.

Materials and Methods: Male workers from Riyadh, Saudi Arabia were recruited for this study and samples were obtained from their nails and hair for mycological analysis which was done using Saboraud's agar and sterile wet soil. Fungal isolates were examined microscopically.

Results: Twenty four hair samples yielded a total of 26 species from 19 fungal genera. Chaetomiumglobosum was the most commonly isolated fungal species followed by Emericellanidulans, Cochliobolusneergaardii and Penicilliumoxalicum. Three fungal species were isolated from nail samples, namely, Alternariaalternata, Aureobasidiumpullulans, and Penicilliumchrysogenum. Most of the isolated fungal species (17 of the 26 or 65.38% of the isolated fungal species) have not been thoroughly characterised nor morphologically classified.

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Conclusion: This study demonstrates the presence of previously undiscribed fungal species that contribute to the normal flora of the skin and its appendages and may have a role in their pathogenies.

Keywords: keratinophilic fungi, non-dermatophytes, fungal flora, hair, nails

Introduction

Superficial fungal infections are a chronic and recurring condition, and approximately 10%-20% of the world's population is affected during their lifetime [(1)]. Fungal infections of the skin, hair and nails are commonly caused by dermatophytes called dermatophytosis, ringworm or "tinea" that are acquired via direct contact of other people, infected animals or fomites [(31)] · Dermatophytosis can either be caused by true dermatophytes (*Microsporum*, *Trichophyton* and *Epidermophyton*), yeasts (*Candida*), or moulds (e.g., *Aspergillus*, *Alternaria*, and *Fusarium*) [(2)]. Most attention conferred to fungi and the human body details pathological relationship during which fungi act as infectious parasites, however, not much attention is brought to the possible fungal commensals that coexist with humans as part of their flora.

Several studies have been conducted on fungal isolates on human hair and nails from various geographical locations. In Northern Greece, dermatophytes were the most common isolates including *Trichophyton rubrum* (53.9%), *Trichophyton mentagrophytes* (17.6%), and *Microsporum canis* (22.5%) [(3)]. In northern Egypt, the most prevalent species included *Aphanoascus*, *Aspergillus*, *Penicillium*, *Paecilomyces* and *Chrysosporium*[(4)]. In Turkey, *Trichophyton rubrum*, *T. mentagrophytes*, *M. canis*, *M. gypseum*, *Epidermophyton floccosum*, *T. verrucosum* and *T. violaceum* were commonly isolated from the hair and nails of

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students [5]. In Northeast India, *T. rubrum* (47.54%), *T. mentagrophytes* (22.95%) and *M. gypseum* (3.27%) were the common isolates [6]. These studies demonstrate the wide variety of fungal species that exist as normal flora or possibly as colonizing non-pathological organism.

Although, dermatophytic infections are a commonly encountered problem in Saudi Arabia, very few studies are available from the region about the specific species that cause these infections and even fewer exist describing the species (7,8). One study conducted in 2009 among patients clinically diagnosed with dermatophytic infections in an Eastern province of Saudi Arabia showed a variety of species including *Epidermophyton floccosumTrichophyton rubrum*, *Trichophyton schoenlenii*, and the non-dermatophytes *Candida albicans* and *Fusarium* [7]. In another study conducted among patients in Central Saudi Arabia, *T. mentagrophytes*, *Candida spp*. and *Aspergillus spp*. were found to be the most likely isolated species causing onychomycosis i.e. fungal infections of the nails [1].

The heterogeneity of the distribution pattern of dermatophytes in different parts of the world has been attributed to various factors, including climate, lifestyle, and the prevalence of immunodeficiency diseases in the community, as well as the reluctance of patients to seek treatment because of embarrassment or the minor nature of disease unless the condition became sufficiently serious to affect the quality of life [7,8]. Even fewer studies have attempted to understand the heterogeneity of the commensal fungi of the region due to their relative non-pathogenicity compared to the bacterial flora of the human body. Hence, this study was conducted to determine the diversity and distribution of the commensal mycology from people living in Saudi Arabia in an attempt to characterize, classify and document these species and further understand their biology. We have also

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attempted to assess the potential capacity for pathogenicity that some commensal fungi may have by qualifying and assessing their capacity for keratin breakdown. The ability of some species to utilize keratin as an energy source i.e. keratinophilic fungi may aid in furthering our understanding of the interactions that fungi have with humans [9].

Materials and Methods

Twenty-four workers in Riyadh, Saudi Arabia were recruited as participants between January and March of 2016. Their jobs included construction, menial work and operation of gas and petrol stations. Most of the recruited workers had spent at least a year in Saudi Arabia. We informed the participants of the aim and objectives of the study and obtained written informed consent. The study protocol was reviewed and approved by the Princess Nourah bint Abdulrahman University Research Ethics Committee IRB No. H-01-R-059. Hair and nail samples were obtained from each worker using sterile instruments and collection bags. Hair samples were of clippings and did not include the roots.

I. Mycological analysis of human hair samples:

1) *Plating on Sabouraud`s Dextrose Agar (SDA)*

Hair samples were individually placed on the surface of Sabouraud`s Dextrose agar (SDA) which contained 20 g/L glucose, 10 g/L peptone, 20 g/L agar, and 40 g/L chloramphenicol according to the procedure described by Ellis *et al*. in 2007. Chloramphenicol was incorporated into the medium to suppress bacterial growth. The cultures were incubated at 28°C for 1-3 weeks during which the growing fungi were examined and identified. Pure cultures of fungi were kept on slants containing the same medium for preservation and revision [9] .

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2) Plating on sterile wet soil

To identify keratin-utilizing fungi (i.e. keratinophilic fungi capable of parasitizing keratin-containing tissue and thus potentially capable of causing hair, skin and nail diseases). A medium of About 1 kg of a clayey soil sample was autoclaved twice and distributed into sterile plastic Petri plates (30 grams/plate). Five ml of sterile distilled water was added to each. Fragments of hair samples were distributed on the soil surface, the plates were then incubated at 28°C and rewetted with sterile water as required, as described by Moubasher [(10)]. The plates were then examined for fungal growth and the fungi appearing on hair fragments were obtained and streaked on an SDA medium for further identification. Slant cultures of fungal strains were also prepared for preservation.

II. Mycological analysis of human nails samples:

Nail samples were placed on the surface of SDA, the same medium as that was used for the hair samples. The samples were incubated at 28°C for 1-3 weeks, during which the growing fungal cultures were identified and examined.

III. Imaging of fungal species

Wet slide preparations of fungal isolates were made using lactophenol cotton blue stain (LPCB). Fungi were examined under low and high magnification with an Axiostar binocular research microscope (Carl Zeiss Microscopy, GmbH, Germany). Images were taken with a Canon Power shot G6 digital camera (Canon, New York, USA).

IV. Identification of fungal cultures

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Fungi were identified based on their macroscopic and microscopic features using the following references: [10–14]

Results

A total of 24 male workers participated in the study. The mean age was 34.1 ± 5.8 years and their ages ranged between 23 to 50 years. Most of the workers were originally from South Asian countries including Bangladesh, Nepal and India but had spent at least a year working Riyadh, Saudi Arabia.

Twenty out of 24 (83.3%) hair samples obtained grew fungal cultures when incubated and examined. The total number of isolates including those grown on SDA and in soil cultures was 49. Of all the isolates, we found 26 species attributed to 19 fungal genera were identified from these isolates. Isolates that grew dark sterile mycelia and budding yeasts were included in those samples found to be positive for fungal growth. The results of the isolated fungal species and genera can be found in Table 1.

Table 2 presents which of the isolate were cultured on SDA and which were able to grow in soil media and thus are considered keratinophilic, the species that only grew on SDA media and those that grew on both. The number of isolates per sample ranged from 1 to 9 with the majority of samples yielding 1 or 2 isolates (7 samples for each). Three samples yielded 3 isolates while the remaining positive samples produced 4, 6 or 9 isolates (one sample for each). *Chaetomium globosum* was the most commonly isolated fungal species accounting for 14.29% of the isolated fungi, followed by *Emericella nidulans* (8.16%). *Cochliobolus neergaardii* and *Penicillium oxalicum* (each recorded from 6.12% of the samples).

Of the 26 samples isolated, 9 isolates grew on wet soil including *Chrysosporium keratinophilum* and *Chaetomium globosum.* This indicates their

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capacity to be pathogenic and produce infections by breaking down the keratin of hair, nails or skin. Three fungal species were isolated from the nail samples including *Alternaria alternata, Aureobasidium pullulans and Penicillium chrysogenum.*

Figure 1 demonstrates fungal growth on a human hair fragment that showed the characteristic dark flexuous conidiophores and ellipsoidal conidia of *Cochliobolus neegaardi*, the dark geniculate conidiophore and cylindrical conidia of *Cochliobolus spicifer*, and the ellipsoidal conidia with transverse septa of *Embellicia chlamydospora.*

Figure 2 shows the pigmented conidiophore of *Emericella nidulans*, the hyphae and polyphialides of *Fusarium chlamydosporum* and the black, shining, smooth-walled conidia of Nigrospora oryzae.

Figure 3 shows the conidiogenous cells and conidia *of Nodulisporium acervatum*, the long metulae and cylindrical phialides of Penicillium oxalicum, the rebranched conidiophores of *Penicillium chrysogenum* and the dark pycnidium of *Phoma herbarum.*

Figure 4 shows the dark phialides and conidiophores of *Stachybotrys chartarum* and the geniculate and solitary muriform conidia of *Ulocladium botrytis*.

Figure 5 shows the following: Image A. *Alternaria alternate* with branched chains of dark conidia and transverse and longitudinal septa. Image B. shows *Aspergillus sydowii* with hyaline vesiculate conidiophores, biseriate conidial heads, metulae and phialides producing chains of echinulate conidia. Image C. Shows the pigmented conidiophores of *Aspergillus ustus* with conidial heads and rough conidia. Image D shows growth of *Chaetomium globosum* on a human hair fragment showing dark perithecial ascoma and ascospores.

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Figure 6 shows the following: Image A: *Chaetomium globosum* with dark subglobose perithecial ascomata with lateral and terminal hairs. Dark olive-brown lemon shaped ascospores are produced. Image B. Fungal growth on human hair fragment plated on wet sterile soil. Image C. Growth of *Chrysosporium keratinophilum* on human hair showing hyaline hyphae, ovoid spores and degenerated hair fragments. Image D shows growth of *Curvularia papendorfii* on a human hair fragment.

Discussion

Human skin, which includes support structures such as hair and nails, supports the growth of a varied fungal flora, not only dermatophytes and yeasts but also other species of moulds. In this study, we were able to show the diversity of fungal flora of 26 species from 19 genera, both common and uncommon.

Chaetomium globosum was the most commonly isolated fungal species. *Chaetomium globosum* produces mycotoxins, particularly chaetoglobosins A and C when cultured on building materials. These fungi are usually found indoors and on wooden products. They are the most common cause of fungal infection amongst construction workers and have been termed as "Sick building syndrome" [(15).] *Emericella nidulans* which was found 8.16% of our isolates has been recently found to cause endophthalmitis after cataract surgery which does not improve with vigorous topical and intravitreal therapy [(16)]. *Cochliobolus neergaardii* is a fungi that is associated with *Oryza sativa* seed (rice) and is usually found in the Asian temperate zones such as Saudi Arabia and the Arabian peninsula, and has been known to cause devastating disease epidemics on food crops, such as rice, wheat and maize [(17).] *Penicillium oxalicum* was found in 6.12% of our samples. *Penicillium oxalicum* is a

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potentially allergen and is found mostly in organic waste recycling facilities. Exposure to spores of *Penicillium oxalicum* may provoke adverse health effects such as allergic rhinitis, bronchial asthma or extrinsic allergic alveolitis [18].

Of the 26 fungal species isolated, 17 or 65.38% of them were potentially pathogenic to humans. This included *Alternaria alternata*, which is found in dates and can cause allergies [19]. Onychomycosis is documented to result from *Aspergillus sydowii* and *Ulocladium botrytis* [20,21]. Fungal endophthalmitis from *Aspergillus terreus* and *Emericella nidulans* [16,22]. pulmonary infections from *Aspergillus ustus* and *Stachybotrys charatarum* [23,24], infection of the lymphatic system from *Aureobasidium pullulans* [25], haemorrhagic pneumonia from *Cladosporum cladosporioides* [26], perinephric abscesses from *Fusarium chlamydosporum* [27] and intestinal disseminated disease from *Penicillium chrysogenum* [28]. Some of the remaining isolates are known plant pathogens while others are relatively unknown. The large percentage of the isolated species that could potentially cause a human infection should be seriously considered. Mycological infections usually recieve less attention than bacterial and viral infections, but the potential for these fungi to infect humans with their added ubiquity should be taken seriously [29].

Furthermore, in comparison to other studies conducted on fungal isolates from hair and nails in different parts of the world, the diversity of the isolates from this study indicated more non-dermatophytes. This has been found in other studies such as those conducted in Northern Greece [3], Turkey [5], India (6) and the Czech Republic [30]. The non-dermatophytes characterized in our study have also shown more diversity and were more prevalent in our samples. This diversity in fungal isolates

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supports the hypothesis that heterogeneity of the distribution may be due to differences in climate and lifestyle [7,8,31].

Conclusions

A diverse population of potentially pathogenic and non-pathogenic non-dermatophyte fungal species was isolated from the hair and nails of Saudi Arabian workers. They were characterized and identified microscopically. The presence of these fungal species, their distribution amongst human hosts, their contributions to the normal flora of the skin and its appendages and their possible pathogenies warrant further study. Identifying these species and describing them morphologically with high definition images makes this study the first of its kind in the region.

Acknowledgments:

I would like to thank Prof . Ahmad M. Moharram Vice Director of Assiut University Mycological Center (AUMC) and his team for their great effort in taking pictures of the fungi.

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Table 1. Frequency of fungal isolates from human hair samples of 24 male workers.

Fungal species	n	%
1. *Alternaria alternata* (Fries) Keissler	1	2.04
2. *Aspergillus niger* van Tieghem	1	2.04
3. *Aspergillus sydowii* (Bainier & Sartory) Thom and Church	1	2.04
4. *Aspergillus terreus* Thom	1	2.04
5. *Aspergillus ustus* (Bainier) Thom and Church	1	2.04
6. *Aureobasidium pullulans* (de Bary) Arnaud	2	4.08
7. *Chaetomium globosum* Kunze	7	14.29
8. *Chrysosporium keratinophilum* (Frey) Carmich. **	2	4.08
9. *Cladosporium cladosporioides* (Fresenius) de Vries	2	4.08
10. *Cochliobolus spicifer* Nelson	2	4.08
11. *Cochliobolus neergaardii* Danquah	3	6.12

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12. *Curvularia papendorfii* van der Aa	1	2.04
13. *Embellisia chlamydospora* (Hoes, Bruehl & Shaw) Simmons	2	4.08
14. *Emericella nidulans* (Eidam) Vuillemin	4	8.16
15. *Emericella variecolour* Berkeley & Broome	1	2.04
16. *Fusarium chlamydosporum* Wollenweber & Reinking	1	2.04
17. *Nigrospora oryzae* (Berkeley & Broome) Petch	1	2.04
18. *Nodulisporium acervatum* (Massee) Deighton	1	2.04
19. *Penicillium chrysogenum* Thom	1	2.04
20. *Penicillium glabrum* (Wehmer) Westling	1	2.04
21. *Penicillium oxalicum* Currie & Thom	3	6.12
22. *Phoma herbarum* Westend.	1	2.04
23. *Stachybotrys charatarum* (Ehrenberg) Hughes	1	2.04
24. *Ulocladium botrytis* Preuss	1	2.04

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25. Dark sterile mycelium	2	4.08
26. Budding yeasts	5	10.20
TOTATL	49	100.00

** (Teleomorph= *Aphanoascus fulvescens* (Cooke) Apinis)

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Table 2. Fungal species isolated from human hair on SDA (a) and sterile wet soil (b).

Fungal species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total
1. *Alternaria alternata*													a												1
2. *Aspergillus niger*			a																						1
3. *Aspergillus sydowii*														a											1
4. *Aspergillus terreus*																						a			1
5. *Aspergillus ustus*													a												1
6. *Aureobasidium pullulans*				a										a											2
7. *Chaetomium globosum*		b					a	a									b		a	a				a	7
8. *Chrysosporium keratinophilum*													b	b											2
9. *Cladosporium cladosporioides*				a					b																2
10. *Cochliobolus spicifer*									b											a					2

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11. Cochliobolus neergaardii											a		b					a						3
12. Curvularia papendorfii													b											1
13. Embellisia chlamydospora							a						a											2
14. Emericella nidulans								a	a			a											a		4
15. Emericella variecolor									a															1
16. Fusarium chlamydosporum													a											1
17. Nigrospora oryzae									a															1
18. Nodulisporium acervatum							a																	1
19. Penicillium chrysogenium			a																					1
20. Penicillium glabrum										a														1
21. Penicillium oxalicum		a				a				a														3
22. Phoma herbarum									a															1
23. Stachybotrys charatarum													b											1
24. Ulocladium botrytis															a									1

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25. Dark sterile mycelium															a	b								2
26. Budding yeasts				a									a		a		a			a				5
Number of isolates/sample		2	2	3		1	3	2	6	2		1	4	9		3	2	1	2	2	1	1	1	1	49

: Hair samples showing negative results are highlighted (four samples)

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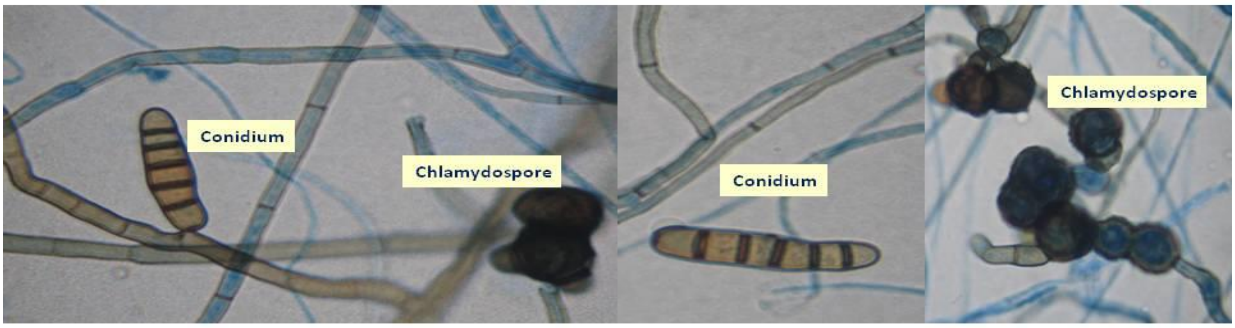
Figure 1. Fungal species isolated from the hair of workers

Cochliobolus neergaardii: Dark flexuous conidiophores and broadly ellipsoidal , 3 septate conidia

Cochliobolus spicifer: Dark geniculate conidiophores and cylindrical 3 septate conidia.



Embellisia chlamydospora: Ellipsoidal dark conidia with 5 thick transverse septa

Embellisia chlamydospora: Dark septate conidium (left) and chlamydospores of variable size and shape (right)

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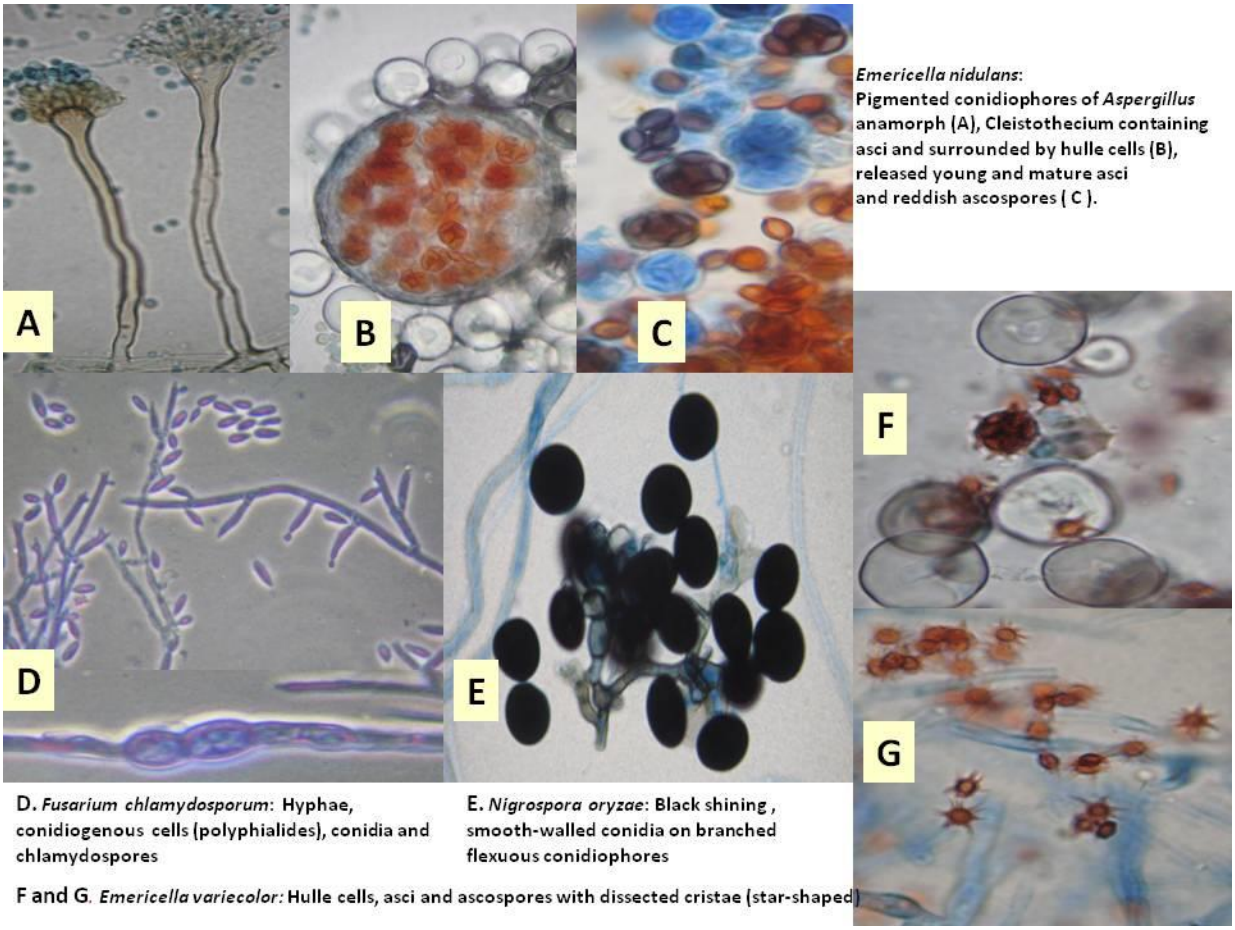
Figure 2. Fungal species isolated from the hair of workers



Emericella nidulans: Pigmented conidiophores of *Aspergillus* anamorph (A), Cleistothecium containing asci and surrounded by hulle cells (B), released young and mature asci and reddish ascospores (C).

D. *Fusarium chlamydosporum*: Hyphae, conidiogenous cells (polyphialides), conidia and chlamydospores

E. *Nigrospora oryzae*: Black shining , smooth-walled conidia on branched flexuous conidiophores

F and G. *Emericella variecolor:* Hulle cells, asci and ascospores with dissected cristae (star-shaped)

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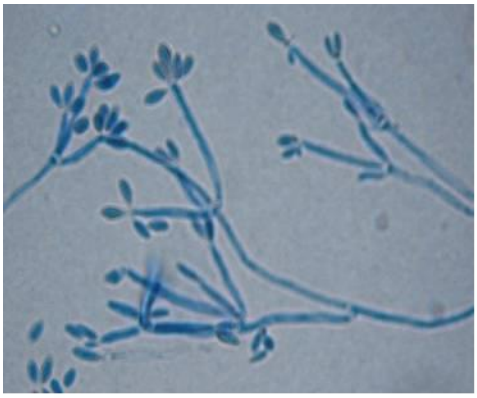
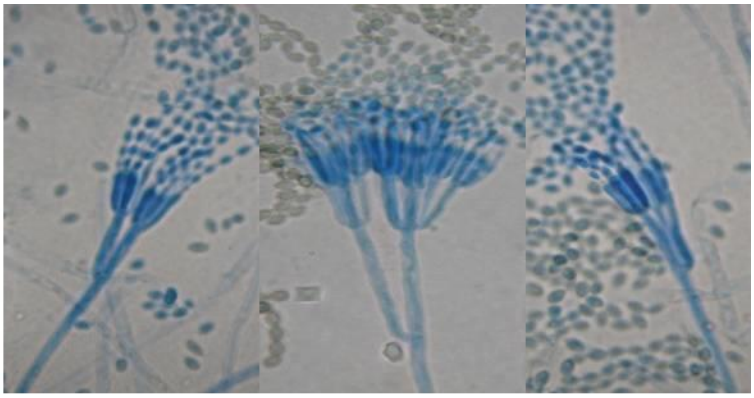
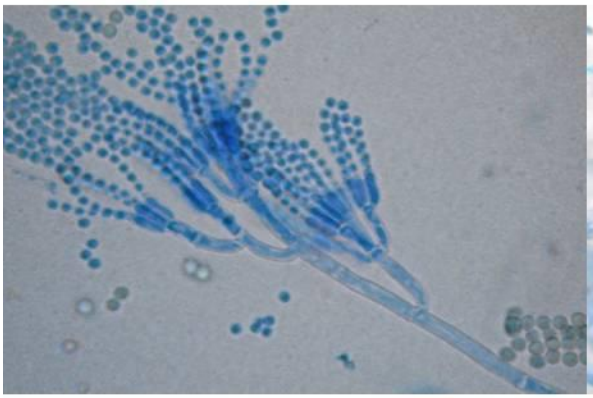
Figure 3. Fungal species isolated from the hair of workers



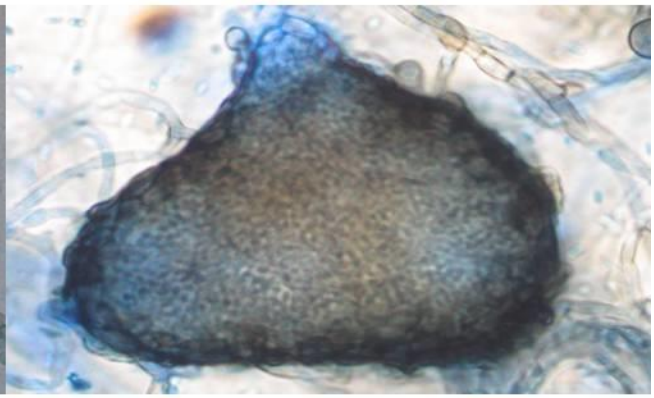
Nodulisporium acervatum: Conidiophores, conidiogenous cells and conidia



Penicillium oxalicum: Conidiophores with long metulae and cylindrical phialides producing chains of strongly elliptical smooth-walled conidia.



Penicillium chrysogenum: Rebranched conidiophore, metulae, phialides and conidial chains



Phoma herbarum: A dark coloured pycnidium and hyaline ellipsoidal conidia

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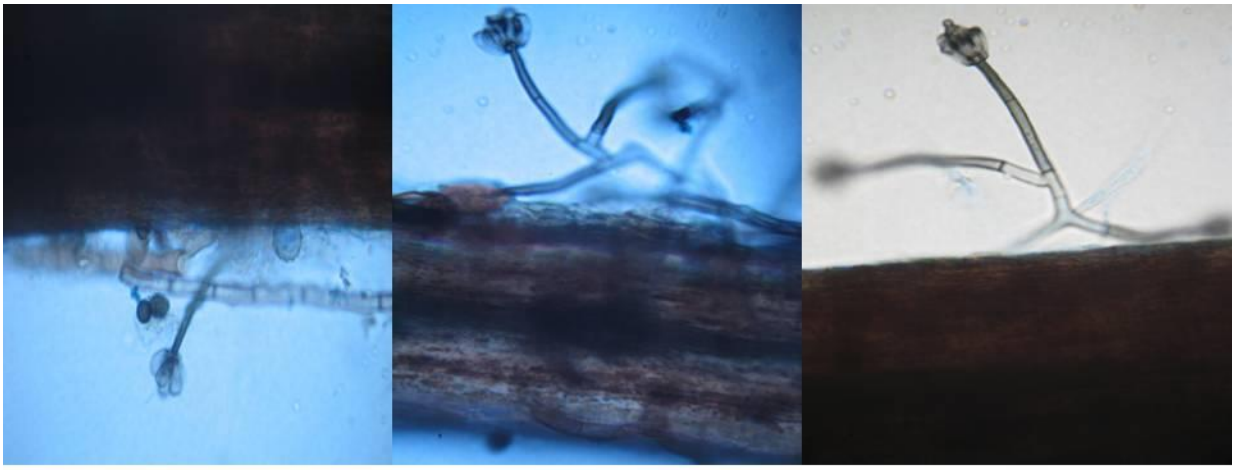
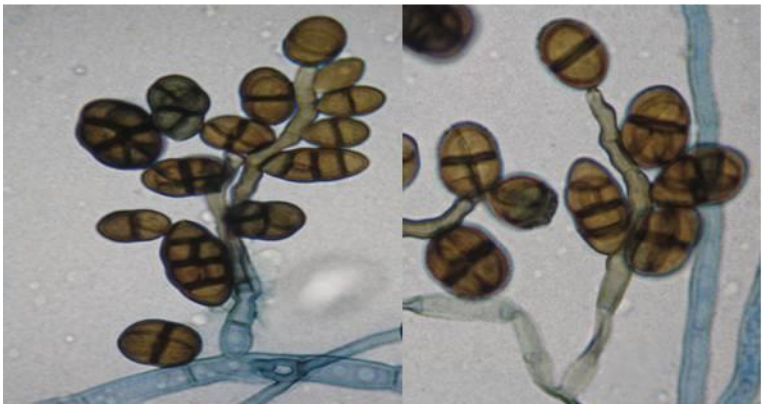
Figure 4. Fungal species isolated from the hair of workers



Growth of *Stachybotrys chartarum* on human hair fragment showing dark phialides and conidiophores.



Ulocladium botrytis:
Dark conidiophores (geniculate)
and solitary muriform conidia

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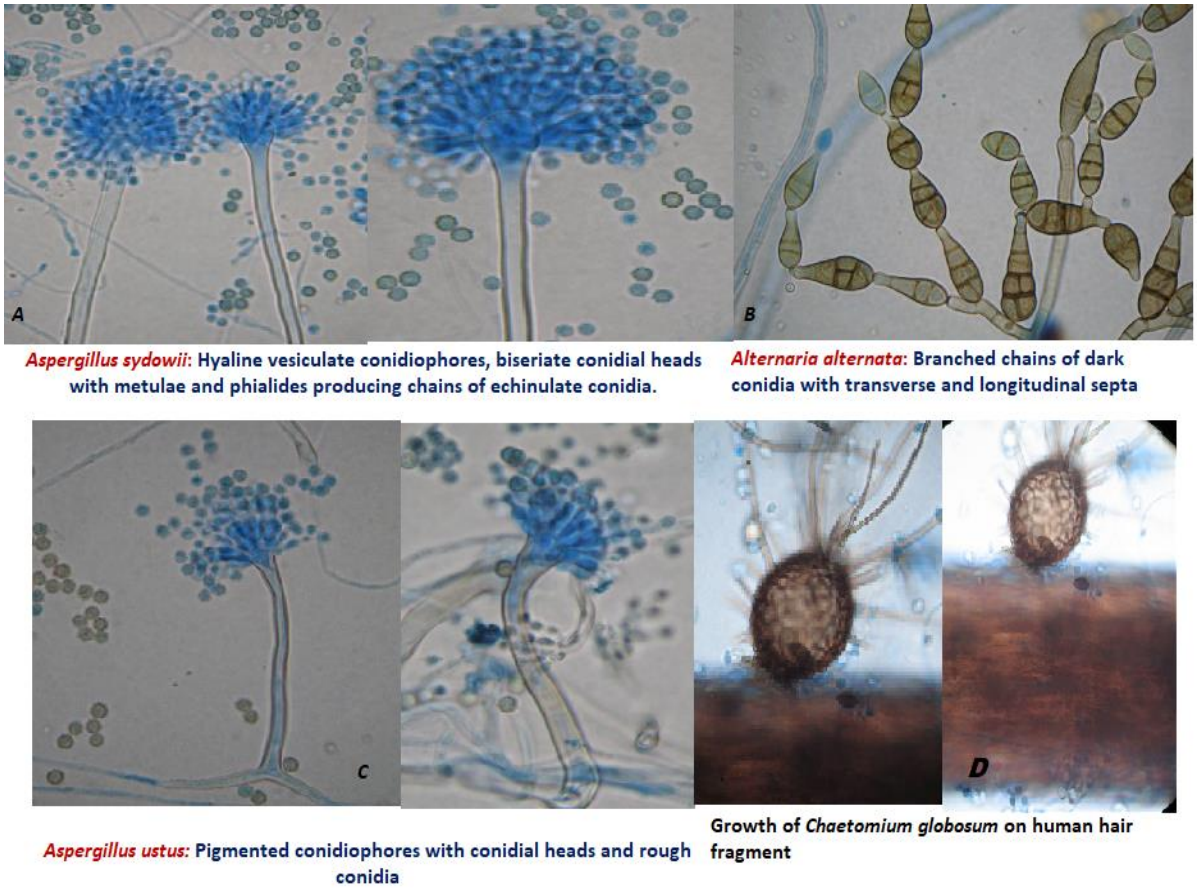
Figure 5. Fungal species isolated from the hair of workers



Aspergillus sydowii: Hyaline vesiculate conidiophores, biseriate conidial heads with metulae and phialides producing chains of echinulate conidia.

Alternaria alternata: Branched chains of dark conidia with transverse and longitudinal septa

Aspergillus ustus: Pigmented conidiophores with conidial heads and rough conidia

Growth of *Chaetomium globosum* on human hair fragment

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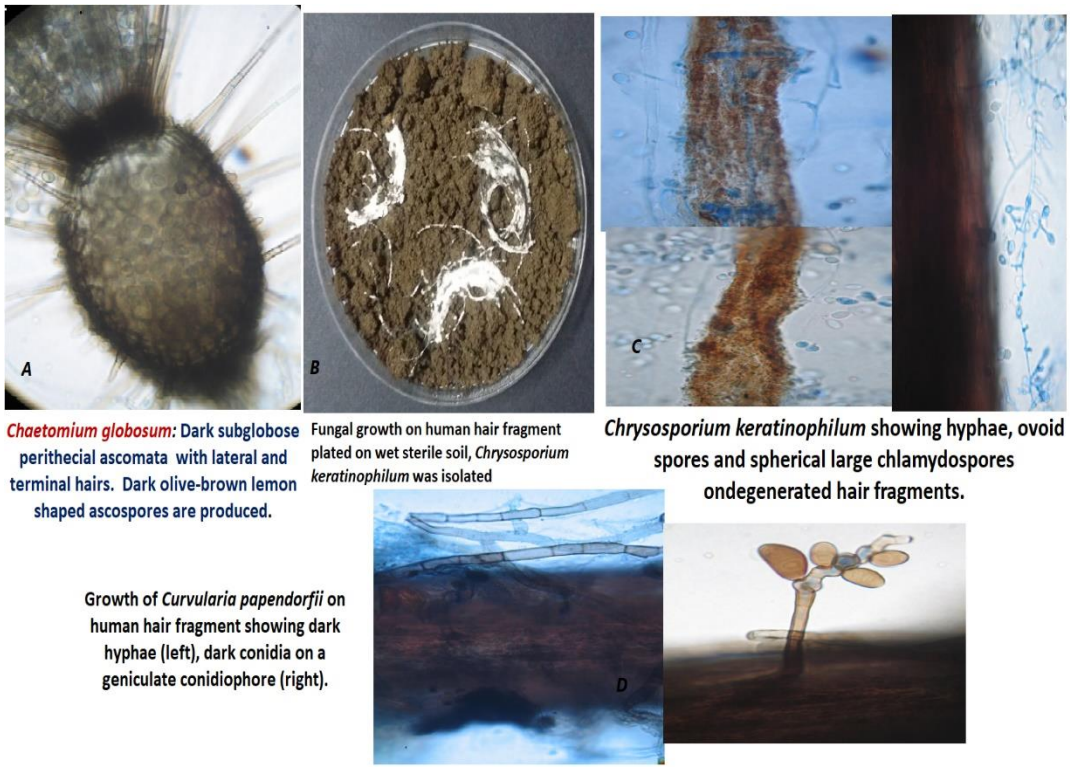
Figure 6. Fungal species isolated from the hair of workers



Kingdom of Saudi Arabia
Ministry of Education
Princess Nourah Bint Abdulrahman University
(048)

المملكة العربية السعودية
وزارة التعليم
جامعة الأميرة نورة بنت عبد الرحمن
(٠٤٨)

Institutional Review Board

مجلس المراجعة المؤسسي

IRB Registration Number with KACST, KSA: H-01-R-059

November 2, 2017
IRB Log Number: 17-0165
Project Title: Characterization of Keratinophilic Fungal Species and Other Non-Dermatophytes in Hair and Nails Samples in Riyadh, Saudi Arabia
Category of Approval: **Special Consideration-Manuscript**

Dear Dr. Suaad AlWakeel,

Thank you for submitting your case for PNU Institutional Review Board consideration and review. The case was evaluated considering the national regulations that govern human subjects' protection when involved in research activities. The IRB had determined that your case although the study was conducting prior obtaining IRB approval, the study was conducted with strict adherence to ethical principal that governs human subjects research. Review of submitted documents and study findings supports the committee decision to issue a special consideration approval letter justified by the following reasons:

- The study was conducted with strict adherence to ethical principles that governs human subjects' research.
- The study findings were unique and novel.

Please note that this letter is valid for the purpose for submitting for journal publication only.

We wish you well as you proceed with the study. Should you have additional questions or require clarification of the contents of this letter, please contact me.

Sincerely Yours,

Ebtisam AlMadi

جامعة الأميرة نورة بنت عبد الرحمن
مجلس المراجعة المؤسسي
Institutional Review Board (IRB)
02 NOV 2017

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