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The Hygienic Importance of Fungi Colonizing the Sheep Wool in Erbil/ Iraq

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Abstract

Aims: The study was conducted to identify a non-dermatophytic fungi associated with wool hairs in Erbil city. The common and dominate fungal genera were recorded, as well as the hygienic importance of all isolates were reviewed.

Materials and Methods: Wool samples were collected during February 2017. Pieces of wool hair were cultured on Sabouraud's dextrose agar. Cultured plates were incubated in room temperature. The growing fungi were counted and were identified microscopically. The occurrence % (O%), and the frequency of occurrence % (FQ%) for the identified genera were calculated.

Results: A 22 fungal isolates belong to 16 genera beside one isolate of Actinomycetes were recognized. The Hyphomycetes represented in (10 genera 62.5%), they were (*Alternaria, Aspergillus* (5 sp), *Scytelidium, Chrysosporium, Curvelaria, Cladosporium, Penecilium, Scpulariopsis, Fusarium and Ulocladium. Two genera belong to each of Ascomycetes and Zygomycetes (12.5%) include (Chaetomium, Pseudallescheria) and (Mucor, Rhizopus)* respectively Basidiomycetes and Coelomycetes represent one genus (6.25%), they were *Rhodotorula* and *phoma*. *Aspergillus* showed the highest O% and FO% (88%; 32.9%) followed by *Cladosporium* (36%; 13.8%).

Conclusion: The results showed that: 1-Hyphomycetes is predominant and Aspergillus is the common genus. 2-All the recorded fungi had an effects on human health. According to the previous studies their infections ranging from mild skin mycoses to serious deep infections. 3-A 81.25% of the recorded fungal genera in this study were previously isolated from upper respiratory tract of individuals have asthma and allergic symptoms in Iraq. 4-It is the first recorded of *Rhodotorula mucilaginosa* from wool samples in Iraq.

Keywords: Sheep wool; Fungi; Rhodotorula; Non dermatophyti; Occurrence percentage; Iraq

Introduction

Sheep wool and the coat of several domestic animals are a suitable habitat for saprophytic and parasitic fungi [1-3]. Keratin, the essential protein of wool and other animal coats support growth of several microorganisms including fungi [4]. Keratin enhance the growth of the dermatophytes as well as the keratinophilic nondermatophytes, therefore animal coats were regarded as a source of different infectious fungi [5,6].

A several diseases may cause by the non-dermatophytic fungi inhabiting sheep wool. *Phoma*, *Chaetomium*, *Fusarium*, *Mucor*, *Scopulariopsis*, *Microasus*, *Aspergillus*, *Penicillium*, *Curvilaria* involve in cutaneous and subcutaneous infections, they were also recorded as agents of sinusitis, keratomycosis and otitis infections [7-11].

The saprophytic genera *Aspergillus*, *Penicllium*, and *Alternaria* which were commonly isolated from the environment and animal coats are well known as a main etiologic agents of pulmonary infections [12]. They are beside *Cladosporium* have a significant relation with human respiratory tract allergy [12-14]. In his review on keratin degradable fungi.

Blyskal [15] had recorded 290 fungal isolates which have the ability to utilize keratin, out of which a 275 are non-dermatofytic fungi. He listed a 79 etiologic fungal isolates from sheep wool only.

Isolation of opportunistic and/or pathogenic fungi from sheep wool was a target of several previous studies in Arab countries. In Basrah/ south of Iraq, a 77 species related to 13 genera were recorded [16]. In Yemen a 32 species belong to 14 genera were recognized [17], while 26 genera were counted in Libya [18], and from 30 samples of sheep wool a 15 genera were recorded in Taif/KSA [3].

The current study aimed to estimate the non-dermatophytic fungi associated with sheep wool in Erbil city /north of Iraq and discuss their hazards on human health.

Materials and Methods

The cross sectional study was followed in this work, so a limit number of samples were used during a short period. The calculated data highlighted for farther works related to human health and wool industry.

Fifty sheep wool samples were collected randomly by nylon bags during February 2017. They were brought to mycology lab and were kept in refrigerator until testing.

A duplicates plate of Saouraud's dextrose agar (SDA) medium was used. Medium was supplemented by (15 mg/L) Chloramphinicol to prevent bacterial growth. Direct plate method was followed, and in each pla2ccte, a five pieces of sheep hair wool were put on the surface of the culture medium. The Petri-dish plates then were incubated at 2± 2. During four weeks, the Petri dishes were checked occasionally by dissecting microscope. The phenotypic identification but not the genotypic one was followed here. The developing fungi were identified by direct microscopic observation, using the lacto-phenol ± cotton blue stain. A pure culture for a number of isolates was prepared before they were identified perfectly. To confirm the primary identifying of fungal isolates, the following references were used [8,19,20].

The occurrence% and the frequency of occurrence% were calculated for each genus by the following formulas:

Occurrence% (O) = (no. of samples in which the genus occurred)/(no. of total samples) × 100

Frequency of occurrence % (FO) = {no. of (species/genus) colonies}/{no. of total (species/genus) colonies} ×100

The importance value index [IVI] of the isolated species was calculated by standard equations with modification [21]. IVI= relative density (RD) + relative frequency (RF) + relative dominance (RD). According to (RD), the isolated species from wool of sheep were grouped in four categories (sub dominant, common, and rare).

Results

The fungal community of tested samples

A twenty two fungal isolates belong to 16 genera (plate 1) were recognized in the fifty wool samples. Hyphomycetes represented the highest occurrence (46 samples, 92%), followed by Zygomycetes (10 samples, 20%), Basidiom ycetes (6 samples, 12%), Ascomycetes and Coelomycetes (4 samples, 8%) (Figure 1). A previous studies revealed that Hyphomycetes is the abundant group in such specific habitat [3,16,18].

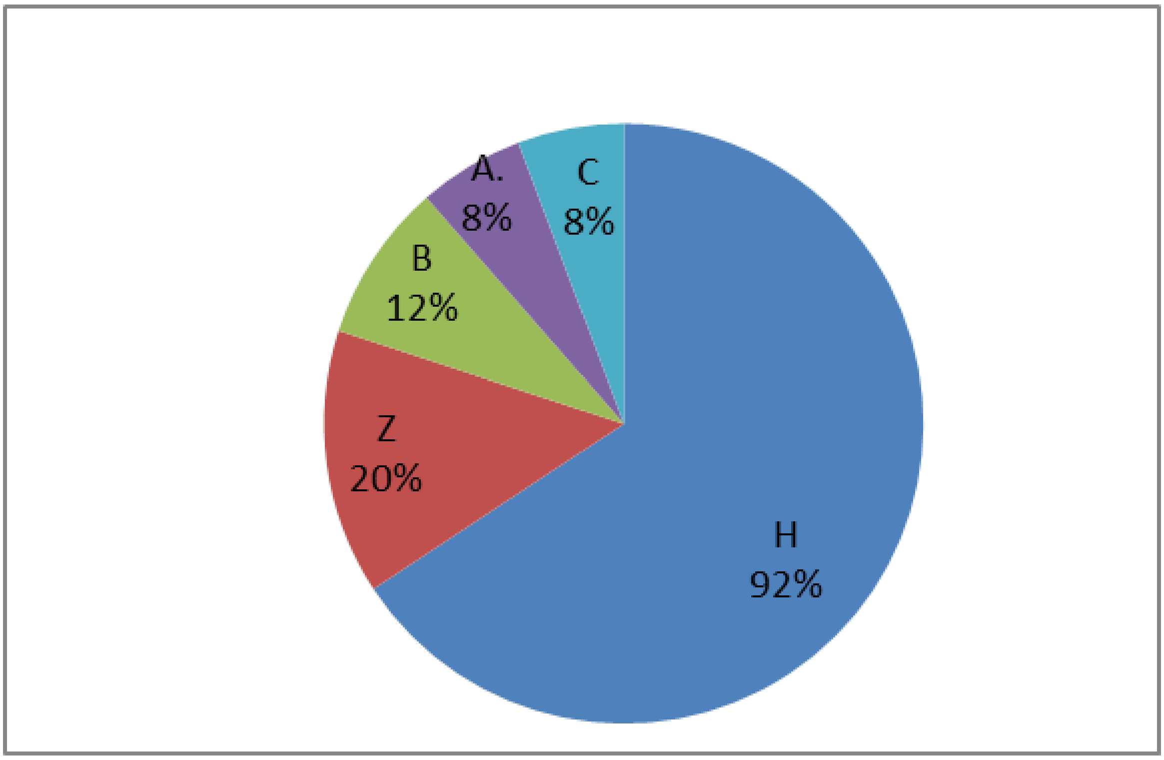

Figure 1: The percentage of occurrence for the taxonomic groups H=Hyphomycetes, Z=Zygomycetes, A=Ascomycetes, B=Basidiomycetes, C=Coelomycetes.

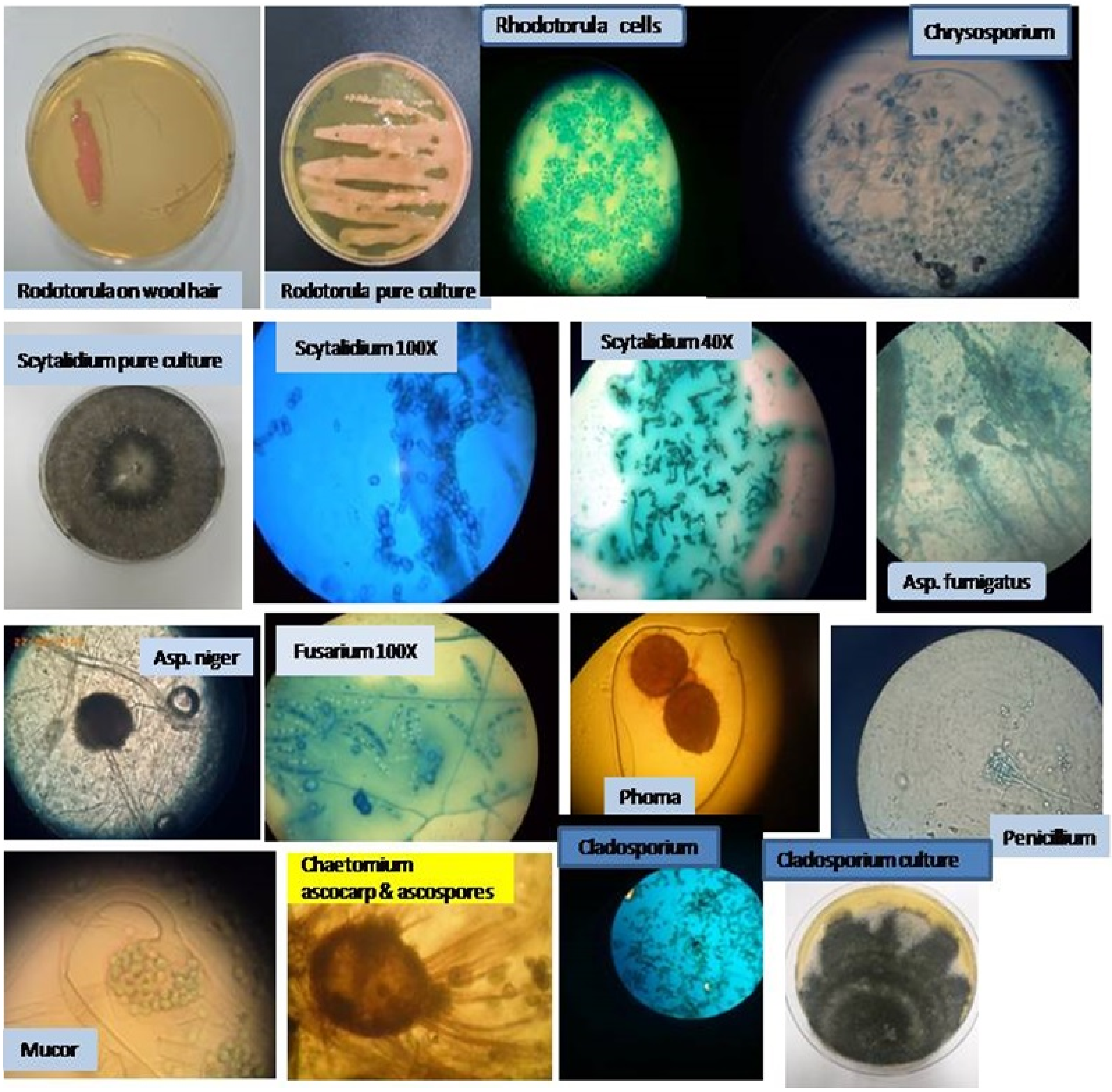

Plate 1: The fungal isolates which developed on wool threads.

No.	Fungi	No.	F O%	O%	R D%	IVI	TG
1	*Cladosporium cladosporoides*	24	13.87	36	12	61.87	H
2	*Aspergillus fumigates*	28	16.18	32	14	62.18	H
3	*Aspergillus niger*	15	8.67	26	7.5	42.17	H
4	*Penicillium sp.*	11	6.35	18	5.5	29.85	H
5	*Rhodotorula mucilaginosa*	10	5.78	14	5	24.78	B
6	*Pseudallescheria boydii*	9	5.2	14	4.5	23.7	A
7	*Rhizopus stolonifera*	8	4.62	12	4	20.62	Z

8	*Chrysosporium indicum*	6	3.46	12	3	18.46	H
9	*Aspergillus terreus*	5	2.89	10	2.5	15.39	H
10	*Aspergillus flavus*	6	3.46	10	3	16.46	H
11	*Alternaria alternate*	7	4.04	10	3.5	17.54	H
12	*Fusarium sp.*	10	5.78	10	5	20.78	H
13	*Scytilidium dimidiatum*	6	3.46	8	3	14.46	H
14	*Mucor cercinelloides*	5	2.89	8	2.5	13.39	Z
15	*Ulocladium charatarum*	4	2.31	8	2	12.31	H
16	*Phoma herbarum*	4	2.31	8	2	12.31	C
17	*Chaetomium indicum*	4	2.31	8	2	12.31	A
18	*Aspergillus nidulans*	3	1.73	6	1.5	9.23	H
19	*Curvularia clavata*	3	1.73	6	1.5	9.23	H
20	*Scopulariopsis candida*	5	2.89	4	1.5	8.39	H
Total isolates		173					

Table 1: The isolated fungi from sheep wool and their taxonomic group (TG), occurrence % (O%), frequency of occurrence (FQ%), relative density (RD), and importance value index(IVI) (B=Basidiomycetes) (A= Ascomycetes) (Z=Zygomycetes) (H= Hyphomycetes) (C= Coelomycetes).

According to the Frequency of occurrence (FQ), the Hyphomycetes are represented by ten genera (FQ 62.5%) (Table 1) while only two genera, belong to each of Zygomycetes and Ascomycetes (FQ 12.5%), and one genus only related to each of Basidiomycetes and Coelomycetes (FQ 6.25%) (Table1) The current results agree with several previous studies which explained the dominance of Hyphomycetes in sheep wool [3,16-18].

There are two main critical factors determine the colonizing of wool, the first is the affinity of fungus to utilize keratin and the second is the resistance of fungus to the lethal effect of ultra violet and dryness. The natural reservoir of the [20] fungal isolates (Table 1) is either the soil or shrubs and grasses. The open grazing of sheep increase the number of fungal isolates as well as the diversity of fungi which inhibited their coats.

Cladosporium cladosporoides occurred in 18 samples, its occurrence and frequency of occurrence are (O% 36 and FO% 13.87) (Table 1) *Cl. cladosporoides* is dominant in sheep's wool and in other animal's keratinous coat [3,16,18,22-24]. The strong affinity of *Cl. cladosporoides* to utilize keratin [24] and in producing of melanin pigment in its vegetative and reproductive structures increase its chance to survive in the wool samples [25,26].

Five species of *Aspergillus sp.* occurred in samples (total occurrence=88%, FO=32.9%) [44]. The dominance of this genus in sheep wool was previously reported by several workers [3,16-18,24]. *Aspergillus* is a cosmopolitan fungus establish a several habitats, it is dry tolerant, and has an active efficiency to utilize different substrates including keratin which assist its occurrence in high level on wool samples [20, 22-24].

A. fumigatus and *A. niger* appeared in [13,16] samples respectively (occurrence 32% and 26%), they are the highest among the five isolated Aspergilla (Table 1).

Penicillium, Chrysosporium, Alternaria, Scopulariopsis which were recorded here are commonly isolated from sheep's wool and other domestic animals [3,16,24]. They had been reported as keratinophilic fungi and can degrade keratin in nature [13]. *Rhodotorula* mucilaginous (O=14% and FO=5.78%) was isolated for the first time from sheep's wool in Iraq. It is the only *Basidiomycetes* identified in the present study, *R. mucilaginous* were commonly isolated from the environment [28]. The fruiting bodies of *Pseudallescheria* were recognized in two samples only (O=14% and FO=5.2%). Presence of Pseudallescheria on sheep's wool was provoke by its ability to utilized keratin. The ascomata with its anamorph *"Scedosporium"* were commonly isolated from soil by keratin baits, that confirm a keratenophilic affinity of *Pseudallescheria* [29]. The two *Zygomycetes Mucor* and *Rhizopus* were isolated by [3,16-18] from sheep's wool and hairs of goats. Both fungi have the activity to degrade keratin [30,31].

Scytalidium, Curvularia, Fusarium, Ulocladium, and Chaetomium (Table 1) which developed on wool pieces have the ability to use keratin as a nitrogen source, and they were isolated by different types of keratin baits from soil [31-34], as well as they were recorded on keratinous materials [16,17,34].

Among the twenty fungal isolates (Table 1) there are a several melanoid fungi, they include *Aspergillus fumigates, A. niger, A. nidulans, A. terreus, A. flavus, Cladosporium cladosporoides, Scytilidium dimidiatum, Curvularia clavata, Chaetomium indicum, Ulocladium charatarum, Alternaria alternate, Scopulariopsis candida, and Phoma herbarum*. The melanin pigment provide an active protection against ultra violet radiation and keep the vitality of fungal cells, as well as it was regard as a virulence factors [29].

Generally the occurrence% of isolated genera can be grouped into three categories (Table 2) the high occurrence % group include *Aspergillus* and *Cladosporium* (88% and 36% respectively), and the low occurrence% group include *Chaetomium, Scopulariopsis and Alternaria* (4%). The rest eleven genera (the third group) showed a moderate occurrence% ranging from [8%-16%].

SN	Fungi	Occurrence%
1	*Aspergillus**	88%
2	*Cladosporium*	36%
3	*Penicillium**	16%
4	*Rhodotorula*	12%
5	*Rhizopus**	12%
6	*Pseudallescheria*	12%
7	*Chrysosporium**	12%
8	*Mucor*	8%
9	*Scytalidium*	8%
10	*Curvularia*	8%
11	*Fusarium**	8%
12	*Ulocladium**	8%
13	*Phoma*	8%
14	*Alternaria**	4%
15	*Chaetomium**	4%
16	*Scopulariopsis**	4%

Table 2: The occurrence % of the isolated genera in wool samples. [*] Fungi which were isolated previously from nonwoven wool.

The hygienic importance of fungal isolates

The twenty two fungal isolates which were recognized in the current study had been recorded previously as etiologies of several infections, and their hazards are ranging from mild infection to deep lethal mycoses [8,19,27].

Globally, there are 4.3 million individuals death/year due to respiratory infections, among them, there are undefined fungal infections [36]. Among 299 isolates had the keratinolytic property may be wool inhabitants, there are a 107 etiologic fungi to human [15]. It is worthy to mention that a majority of fungi which were isolated in this study are well known as respiratory allergens that may accidently cause human disease [8,37,38]. *Aspergillus* and *Cladosporium* had the highest (IVI) (Table 1) in the same time they are the main fungal allergen. The wool of sheep as a source of respiratory fungal infections increase significantly in our region because of the improper method of sheep breeding, since their yards are in a public locations within the city boundary, besides that, the farmers follow the open grazing method instead of a particular feeding location. From the other side the long dry season is an important predisposing factor which increases air transmission of fungal structures. Thirteen fungal genera out of the sixteenth (Table 2) were previously isolated from the upper respiratory tract of 100 individuals had asthma and allergic symptoms in Basrah (south of Iraq) [38].

The impact of fungi associated with wool are one of the occupational hazards for the workers in wool industry, particularly that processing of wool enhance fungal growth [39]. Ten species of Aspergillus, six species of *Penicillium, Chrysosporium, Fusarium, Rhizopus, Chaetomium, Alternaria, Ulocladium, and Scopulariopsis* were previously isolated from nonwoven wool by several researchers [39]. These genera equal 56.25% of those listed in current study (Table 2).

The five species of Aspergillus (Table 1) are the most important allergens, as well as they have the ability to cause different pulmonary infections [8, 35-38].

Aspergillus, Cladosporium, Penicillium and Alternaria were found to be the most prevalent fungi in asthmatic patients [38]. Moreover hey were listed as a causative agents of cutaneous, subcutaneous and systemic infections beside otitis, keratitis and pheohyphomycoses [8,40,41].

Rhodotorula musilaginosa are responsible for different infections concerning with skin, and nails, it also cause keratitis and fungemia [42]. *Mucor and Rhizopus*, the two zygomycetes isolates were associated with a number of infections known as mucormycosis [37,43]. The black fungi *Alternaria, Curvilaria and Ulocladium* were listed previously as etiologic agents of pulmonary and tissue infections [8,40,44,45]. *Chaetomium* was recorded as toxin allergenic agent [46,47] it was also isolated from nail as well as other cutaneous infections. *Pseudallescheria* and *Sceosporium* (anamorph) are common as invasive pathogen and they caused a wide range of disease include cutaneous, subcutaneous, and systemic infections [48-50].

Phoma mostly is a phytopathogenic fungi, but during the two previous decades a several human diseases due to *Phoma* were reported [51]. There are a few reports related to the pathogenicity of Scytalidium, they include onychomycosis and skin infections [19] beside a number of case reports of pulmonary and deep infections [52-54]. *Scopulaiopsis* is a keratinophilic fungus, it cause a several cutaneous lesions and nail infections [8,55]. It may have a high relationship with asthma and allergy cases with other indoor air borne fungi [38].

Although the investigated isolates were a saprophytic fungi which had a low virulence, but nowadays there are a continuous increasing of infections caused by saprophytes and they had been a real threat to human health. The high incidence of diseases caused by opportunistic fungi coincides with increasing of immunosuppressive diseases, organ transplantation, and increasing the antifungal resistance strains.

Conclusion

According to the above data, it seems that:

The fungal community of the wool tested samples are highly divers.

The isolated fungi are an important risk factors, they involve in several human infections particularly as a pulmonary allergens.

Aspergillus fumigates and Cladosporium cladosporoides have the highest importance value index, both fungi are well-known human allergens.

It is worthy to note that [60%] of the recorded fungi in the current study are characterized as dematiaceous (melanoid).

Results of the current work must be taken into account from the Directorate of Human Health and Veterinary Health to set instructions for control sheep breeding.

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