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Original article

Survival of Dermatophytes in Skin Scales after 10 Years Storage

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Abstract

Background: Survival of *dermatophytes* in skin scales and nail scraps sampled from human lesions could have ecological and epidemiological importance. The aim of this study was to store human skin scale and nail lesions infected with *dermatophyte* agents, at -20°C for a long time and to investigate the re-isolation rate of *dermatophytes* species from such samples after storage.
Materials and Methods: After referral to Medical Mycology Laboratory of Tehran University of Medical Science, the patients were subjected to sampling. Confirmation of *dermatophyte* positive result in direct smears was done with Potassium hydroxide, then culturing on Sabouraud dextrose agar media was performed, and species were identified using standard procedures, and remaining samples were stored at -20°C. After 10 years, samples were re-examined for the presence and re-isolation of intended fungi according to the standard procedures.
Results: From a total of 5 species enrolled in this study, *Epidermophyton floccosum*, *Trichophyton mentagrophytes*, and *Tricholosporum violaceum* survived, and *Trichophyton rubrum* and *Trichophyton verrucosum* lost their viability at -20°C after 10 years. There was a significant difference between the re-isolation rate and the type of *dermatophytes* ($p<.05$). *E. floccosum* had the highest re-isolation rate (66.7%), followed by *T. mentagrophytes* (27%). All of the re-isolated *dermatophytes* were taken from scales samples, but none from nail scraps samples.
Conclusion: Some *dermatophytes* species including *E. floccosum* are able to maintain their ability to survive for a long time in human scale samples in certain time and temperature conditions (10 years at -20 °C) and to grow in appropriate conditions. These findings should be considered in ecological and epidemiological studies.

Keywords: Dermatophyte, Survival, Storage, Scale

1. Background

Persistence and survival of *dermatophytes* especially in skin scales and nail scraps separated and sampled from human and animal lesions could be important in terms of ecological and epidemiological studies. There are some reports on the persistence and survival of this group of keratinophilic fungi in different environmental conditions and temperatures (1-5). To store the isolates of *dermatophytes*, different techniques are used such as lyophilization (6-7); keeping in freezing conditions (8-9), distilled water (10), paraffin oil (11); freezing with liquid nitrogen (12); and the periodic passage (9, 13), all of which require to standardized processes with special procedures. In these methods, fungi are stored by aforementioned processes after growth and isolation in the culture media. In terms of *dermatophytes* survival in skin scales and nail scraps isolated and sampled from human or animal lesions, there are a few studies available, which are about keeping them at room temperature (1, 14).But regarding *dermatophytes* survival in scales and nail scraps and storing them in freezer at -20°C, the information available is limited.

2. Objectives

The aim of this study was to store skin scales and nail scraps infected with *dermatophyte* agents (confirmed by direct microscopic examination, DME, and culturing) and to investigate there-isolation rate of some *dermatophytes* species after storing in freezer at -20°C temperature condition for a long time.

3. Materials and Methods

3.1. Sampling

This laboratory trial study was conducted in Medical Mycology Laboratory of Tehran University of Medical Sciences., The patients were referred to the medical mycology laboratory by specialist physiciant for mycological examinations due to their skin and nail lesions. The patients had been advised to not using anti-fungal drugs and not taking baths in the last two days. They were registered in lab's book, and sampling from their lesions was done. Sterile scalpel was used for skin sampling. Sampling from distal nail lesions, were done from deep parts and nail beds after removing the nail. In the case of proximal nail lesions, the samples were collected from the depth of the nail. In the case of white superficial onychomycosis, the sampling was performed from the surface of the nails.

3.2. Direct Microscopic Examination (DME)

Samples were examined - with DME by 15% Potassium hydroxide (KOH) using an optical microscope (Olympus, Germany) in order to fungal elements observation (hypha, arthrospores, yeast cells, and pseudohyphae).

3.3. Culture and identification

Culture was performed with some other parts of samples on SC (Sabouraud dextrose agar with 0.005% chloramphenicol) and SCC (Sabouraud dextrose agar with 0.05% cycloheximide and 0.005% chloramphenicol) media (E. Merck, Germany) using transplant method. The media were kept at 25-28 and

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37˚ C and checked two time in a weak for evidence of colony growth. In the case of no growth of fungal colony in culture media, they were seen after 4 weeks of incubation. After confirming positive results by DME and culturing, etiological agents of *dermatophytes* were identified according to the standard methods of identification (i.e., morphology of colony and examination of prepared lactophenol cotton blue smear for observing of hyphae structure and its shape, presence and shape of microconidia and macroconidia, other differential methods such as urease test, hair perforation test, , temperature tolerance and temperature enhancement test, *Trichophyton* nutritional media, pigment production, and using of other selective media were performed for identification of species, if were needed) (9, 15-16).

3.4. Storing and re-isolation procedure

The remaining 56 skin scales and nail scraps samples that identified as positive for *dematophytes* were placed inside the micro-tubes without any intervention in terms of adding substances to the micro-tubes and then stored at -20℃. After 10 years, from 2006 to 2016, skin scales and nail scraps were removed from the freezer, and after ensuring about the presence of fungal elements in the stored samples by DME according to the above-mentioned procedures, the rest of the samples were cultured on SCC and incubated at 25-28 and 37 ℃ for up to 4 weeks. During this period, surveying the fungi re-growth and re-identification of positive cultures were performed according to the standard methods listed above (9, 15-16). Also, culture media were examined in terms of possible contamination with other microbial agents affecting the final results in order to repeat the tests if there were any problem.

3.5. Statistical analysis

SPSS software version 22.0 (SPSS Inc., Chicago, IL) was used for data analysis. By Chi-squared and Fisher's Exact Test, resulsof study was assessed with 95 % confidence intervals (CI). In case of *P*value <.05, results were considered as significant, statistically.

4. Results

From a total of 56skin scales and nail scraps samples used for laboratory trials, *E. floccosum* was identified in 21 cases, *T. mentagrophytes* in 26 cases, *T. rubrum* in 6 cases, *T. verrucosum* in2 cases, and *T. violaceum* in 1 case. Out of 56 samples, 46 samples were taken from males, and 10 samples were taken from females. The patients' age ranges were as follows: 1-20, one case; 21-30, 14 cases, 31-40, 14 cases; 41-50, 12 cases; and more than 50, 15 cases. The lowest age was 19 years, and the highest was 75 years while the patients' mean age was 41 years old. Regarding the location of the lesion and biopsy,24 samples were collected from tinea cruris (*T. cruris*), 13 cases from *T. pedis*, 8 cases from interdigital T. pedis,7 cases from toenails *T. unguium*, 3 cases from *T. corporis*, and 1 case from *T. faciei*. The results of there-isolation rate of *dermatophytes* species from skin scales and nail scraps samples cultured after 10 years are shown in Table 1. Re-isolation was observed in 22 out of 56 cases (39.3%). According to the statistical analysis, significant difference was observed between the re-isolation rate and the type of *dermatophytes* (p<.05). *E. floccosum*, *T. mentagrophytes*, and *T. violaceum* maintained their ability to survive; however, *T. rubrum* had lost its ability to re-grow after this period. *E. floccosum* had the highest re-isolation rate (66.7%), followed by *T. mentagrophytes* (27%). The results of the *dermatophytes* species re-isolation based on the sample type (location of the lesions) are shown in Table 1. Also, according to the obtained results, significant difference was seen between the re-isolation rate and the type of sample (p<0.05).All of the re-isolated *dermatophytes* were taken from skin scales, but in none of the nail scraps samples,*dermatophytes* were re-isolated. The highest re-isolation rate was observed in T. cruris samples.

5. Discussion

The ability of *dermatophytes* to persistence and survival in skin scales and nail scraps samples isolated from patients is of special epidemiological importance in terms of laboratory methodological and diagnostic techniques. Samples storage length of time before culturing, environmental impact, and storage temperature have significant diagnostic effects on the persistence and survival of *dermatophytes* in skin scales and nail scraps samples containing *dermatophytes*. According to the reports available on literature, skin, nail, and hair samples infected with *dermatophytes* can be stored for several weeks at room temperature (9, 13). But *dermatophytes* survival in these samples depends on the type of sample (location of the lesions) and their fungal agents. *Dermatophytes* such as *T. rubrum* are non-resistant and quickly destroyed while *E. floccosum* is resistant and can survive for a long time in skins (9, 13). Dvorak et al. (1969) showed that the maximum survival rate of *T. rubrum, T. mentagrophyte*, *T. verrucosum*, and *E. floccosum* in skin scales were 7, 9, 20, and 26 months, respectively (1). Also, according to a study conducted by Rosenthal et al. (1962) on hair, the maximum survival rate of *T. violaceum* and *T. verrucosum* in hair was at refrigerator temperature (17). Knudsen (1980) reported that after 2 weeks of keeping scale samples at room temperature, the first fungus, among the *dermatophyte* fungi, losing its ability to grow was a strain of *T. rubrum* with a maximum durability of six months while the durability of *T. mentagrophytes* and *E. floccosum* species continued to eight months after sampling (14). The present study showed that by freezing samples at -20°C for 10 years, *E. floccosum*, *T. mentagrophytes*, and *T.violaceum* were able to maintain their ability to survive; however, *T. rubrum* had lost its ability to re-grow after this period. Regardless of *T. violaceum* which was present in one case and positive in terms of re-isolation, *E. floccosum* re-isolated from 14 scale samples out of 21 samples infected by *E. floccosum* (66.7%), had the highest re-isolation rate, indicating the high resistance of this fungus at -20°C (Table 1). Also, between species comparison showed that *E. floccosum* re-isolated from 14 cases out of 56 samples cultured primarily (25%), had the highest re-isolation rate (Table 1) (p <.05). Although in some studies, *E. floccosum* was reported to be sensitive to cold and refrigerator temperature, the present study showed that this sensitivity was low, and its resistance and survival rate was higher than its sensitivity (more than 50%). Thus, regarding *dermatophytes* survival in scales in different temperature conditions, it is evidenced that *dermatophytes* residing in patients' scales separated and released in environment are able to persist not only in normal conditions but also in hard conditions such as low temperature in cold regions and also to play a role in the transmission of infection to others. These findings are of special epidemiological and ecological importance.

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Table 1. Frequency of *dermatophytes* re-isolation in skin scales and nail scraps samples based on the dermatophyte species and type of samples (location of lesions or type of tinea).

Lesion \ Species		*E.floccosum*	*T.mentagrophytes*	*T.rubrum*	*T.verrucosum*	*T.violaceum*	Total of each rows	In total
*T. cruris	Specimens No.	16	6	1	-	1	24	24/56
	Reisolations No. (%)	12(75)	3(50)	0	-	1(100)	16(66.7)	16/56 (25/100)
*T. pedis	Specimens No.	1	10	1	1	-	13	13/56
	Reisolations No. (%)	0	3(30)	0	0	-	3(23.1)	3/56 (5.3/100)
*T. pedis (interdigital)	Specimens No.	1	6	1	-	-	8	8/56
	Reisolations No. (%)	0	1(16.7)	0	-	-	1(12.5)	1/56 (1.8/100)
*T. unguium (toenail)	Specimens No.	-	4	2	1	-	7	7/56
	Reisolations No. (%)	-	0	0	0	-	0	0
*T. corporis	Specimens No.	2	-	1	-	-	3	3/56
	Reisolations No. (%)	1(50)	-	0	-	-	1(33.3)	1/56 (1.8/100)
*T. faciei	Specimens No.	1	-	-	-	-	1	1/56
	Reisolations No. (%)	1(100)	-	-	-	-	1(100)	1/56 (1.8/100)
Total of each columns	Specimens No.	21	26	6	2	1	56	56/56
	Reisolations No. (%)	14(66.7)	7(27)	0	0	1(100)	22(39.3)	22/56 (39.3/100)
In total	Specimens No	21/56	26/56	6/56	2/56	1/56	56/56	-
	Reisolations No. (%)	14/56 (25/100)	7/56 (12.5/100)	0	0	1/56 (1.8/100)	22/56 (39.3/100)	

*T= Trichophyton, E= Epidermophyton , *T = Tinea*

In statistical analysis, there was a significant difference between the rate of re-isolation and the type of samples (location of the lesion biopsied) ($p<.05$). Regardless of *T. faciei* which was one sample and positive in terms of re-isolation, the re-isolation rate in 16 out of 24 *T. cruris* samples was in the highest rate (66.7%). The fungus re-isolation rate from *T. cruris* samples was also in the highest rate in comparison with the skin scales and nail scraps taken from other sites of the body such as *T. pedis*, *interdigital T. pedis*, toenails *T. unguium*, and *T. corporis* (16 cases out of 56 primarily cultured samples) (28%)while in none of the nail scraps cases, *dermatophytes* re-isolation was observed (Table 1). According to the different studies' findings and available literature, when patients initially refer to the laboratory, and samples are taken, *dermatophytes* growth and isolation in nail scraps samples are in a lower rate in comparison to other samples (9, 13, 16, 18). Thus, after keeping the nail scraps in -20°C for 10 years, the lack of *dermatophytes* re-isolation in these samples wasn't unexpected. The high re-isolation rate in *T. cruris* samples can be attributed to the fact that the predominant etiological agent in this type of tinea was *E. floccosum*, which was present in 16 out of 24 *T. cruris* samples (66.6%). Due to high resistance to improper temperature conditions and passing the time, *E. floccosum* was able to re-grow and be re-isolated again in 12 out of 16 cases (75% re-isolation) of *T. cruris* samples infected by this *dermatophyte* species (Table 1). This finding is of special importance so that in high prevalence rate of *T. cruris*, its etiological agent should be taken into account, that is, if *E. floccosum* is identified as the predominant species, its resistance to some conditions even to hard conditions such as low temperature and also its role in the transmission of infection to others and its physiological characteristics could be considered as one of the reasons for the high prevalence and incidence of this type of dermatophytosis.

Although *T. rubrum* was re-isolated in none of the samples, this finding is related to the low resistance and persistence rate of this *dermatophyte* species, not to the type of sample. This species re-grew and was re-isolated in none of the *T. cruris*, *T. pedis*, interdigital *T. pedis*, toenails *T. unguium*, and *T. corporis* cases (Table 1). According to the other studies' findings, this species is highly sensitive and loses its ability to survive in a short period of time after storing samples taken from the lesions (1, 9, 13, 14, 17, 19).

Apart from fungi standard storage methods such as lyophilization; keeping in freezing conditions, distilled water, and paraffin oil; freezing with liquid nitrogen; and the periodic passage, all of these methods make use of fungus grown spores and colonies rather than taken samples such as scales and nail scraps(6-13); hence, the use of skin scales and nail scraps or even hair instead of fungus colonies and spores without any laboratory intervention for future studies in coming years, are of special methodological importance. Due to *smal* stored sample size in this study (56 samples) as well as low number of fungal etiological agents in some samples (e.g. *T. violaceum* 1 case, *T. verrucosum* 2 cases), we cannot confidently comment about the validity of this fungi storage method. In this method, although the re-isolation rate of fungi like *E. floccosum* was higher than the others (66.7%), it was lower than the reports from other

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aforementioned methods; however, it had higher recovery rates in comparison with paraffin oil method (11).

6. Conclusion

This study showed that some of the *dermatophytes* species including *E. floccosum* are able to maintain their ability to survive for a long time in human skin scale samples in certain time and temperature conditions (10 years at -20°C) and to grow in appropriate conditions. These findings should be taken into account in ecological and epidemiological studies. Therefore, the need for a comprehensive study with a large sample size is felt in order to determine whether storage method used in this study for skin scales and nail scraps containing *dermatophytes* can be used as an effective storage method for this group of fungi. Also, more research on storage conditions including temperature, humidity, and gaseous environment may lead to the method improvement in order to maintain *dermatophytes* for a long time in samples taken from the lesions.

Conflicts of interest

There was no conflict of interest in the present study.

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Authors' Contributions

All of the authors contributed to this study.

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