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Research Article

DNA Sequencing Accurately Diagnosed 146 Cases of Superficial Mycosis

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Abstract

Objective: Traditional fungal detection methods, such as fungal microscopy and cultivation, often have drawbacks such as high false negative rates and time-consuming cultivation. Using molecular biology methods for diagnosis can not only be used for identifying fungal strains in cultured colonies, but also for diagnosing diseased tissues, which can shorten the diagnosis time. There is a lack of systematic research on the clinical characteristics, susceptibility factors, and the composition and distribution of pathogenic fungi of superficial mycosis in Shiyan area. In order to understand the relevant situation of superficial mycosis and pathogenic fungi in this area, this study conducted a molecular epidemiological investigation on 146 patients with superficial mycosis who visited our outpatient department. Methods: From January 2022 to December 2022, the typical clinical manifestations of outpatient visits in our department were collected. 146 cases of superficial fungal patients with positive fungal microscopy were cultured and DNA was extracted. PCR technology was applied to compare the products in GeneBank after the amplification of ITS region. Results: A total of 23 pathogenic strains were obtained, including 112 strains of *Trichophyton rubrum* (76.71%), 5 strains of other dermatophytes (3.42%), 6 strains of *Candida* species (4.11%), 4 strains of *Aspergillus* species (2.74%), 8 strains of *Cladosporium* species (5.48%), and 11 strains of other fungi (7.53%). Conclusions: DNA sequencing combined with traditional fungal microscopic culture is helpful for more accurate diagnosis of superficial mycosis.

Keywords

Superficial Mycosis, *Trichophyton rubrum*, DNA Sequencing

1. Introduction

Superficial mycosis, as one of the main clinical skin diseases, often has a long course, relapse repeatedly, has a certain degree of infectivity, and affects aesthetics and causes varying degrees of distress to patients [1]. Superficial mycosis accounts for more than 25% of outpatients in dermatology [2], with a global prevalence rate of 20% - 25% [3], and its distribution and pathogenic fungi vary according to regional environment, age, gender, occupation, etc. even in the same region, its pathogenic spectrum is constantly changing, this has had a certain impact on the prevention and control of diseases. The traditional diagnostic methods mainly rely on direct microscopic examination, fungal culture to identify the pathogenic fungi, but they are time-consuming. Molecular biology diagnosis can not only be used for fungal identification of cultured colonies, but also for the diagnosis of diseased tissues, which can shorten the diagnosis time. There is a lack

of systematic research on the clinical characteristics, susceptibility factors, and the composition and distribution of pathogenic fungi of superficial mycosis in Shiyan area, in order to understand the relevant situation of superficial fungal diseases and pathogens in the region, 146 patients with superficial mycosis were analyzed.

2. Materials and Methods

2.1. Clinical Data

All 146 patients were from the dermatology outpatient department of Taihe Hospital in Shiyan City, with a visit time from January 2022 to December 2022. They were clinically diagnosed as superficial mycosis (except for tinea versicolor) and all fungal direct microscopy tests were positive. Basic information such as name, gender, age, occupation, and medical history were recorded, further fungal cultivation and molecular biology identification had been carried out.

2.2. Methods

The collected specimens included dandruff, nail shavings, and broken hair. Inoculated all specimens onto Sabourg glucose agar (SDA) medium, with 2 tubes per specimen. Incubated in a constant temperature incubator at 25 ℃. Observed colony growth on days 7, 14, 21, and 28, respectively. If no growth was observed on the 4th weekend, it would be considered negative. Preliminary identification of growing colonies based on their morphological characteristics, conidia, and hyphae.

2.3. Molecular Biology Assay

For all specimens cultured, fungal deoxyribonucleic acid (DNA) was extracted using QIAamp DNA Mini Kit (QIAgen Technology Co., Ltd., Germany, item number 51304) to extract the DNA of the strains. Please refer to the instructions of the kit for specific methods. When conducting polymerase chain reaction (PCR), internal transcribed spacers (ITS) are used as primers. The primer sequence is as follows: ITS1

5'-TCCGTAGGTGGAACCTGCGGG-3', ITS4 5'-TCCTCCGCTTATTGATATGC-3'. The reaction system consisted of 20 μl, and the reaction conditions were: pre denaturation at 95 ℃ for 3 minutes; Then performed 40 cycles under the following conditions: denaturation at 95 ℃ for 30 seconds, annealing at 58 ℃ for 45 seconds, and extension at 72 ℃ for 2 minutes; After the loop ended, the final amplification process was completed by extending for 8 minutes at 72 ℃. The amplification products were sequenced, and the original sequence obtained from sequencing was input into the The National Center for Biotechnology Information (NCBI) website (http://www. Ncbi. nlm. nih. gov/last) for comparison.

2.4. Data Analysis

Statistical Package for the Social Sciences (SPSS) 17.0 statistical software was used for analysis, and bilateral tests were used. The positive rates of each group were compared using a chi square test, and $P<0.05$ indicates a statistically significant difference. When there were differences in positive rates among multiple groups and pairwise comparisons are required, the corrected test level should be applied.

2.5. Ethical Considerations

All procedures were approved by the Institutional Ethics Committee of Taihe Hospital.

3. Results

3.1. The Specific Clinical Diagnosis, Age, and Gender Distribution of Patients Are Shown in Table 1

In this study, the number of male patients was higher than that of female patients, but there was no statistically significant difference in incidence between males and females in different parts ($P>0.05$) (see Table 1).

Table 1. Clinical diagnosis, age, and gender distribution of patients with superficial fungal disease.

Clinical diagnosis	n	Gender				Age (years)						
		male	female	χ^2	P	0~10	11~20	21~30	31~40	41~50	51~60	61~
Tinea cruris	62	46	16	2.907	0.088	0	11	21	13	10	5	2
Onychomycosis	25	14	11	1.474	2.225	1	5	11	3	2	1	2
Tinea corporis	21	17	4	2.317	0.128	2	2	6	3	4	2	2
Tinea manuum	15	10	5	0.000	0.984	0	0	3	4	4	4	0

Clinical diagnosis	n	Gender				Age (years)						
		male	female	χ^2	P	0~10	11~20	21~30	31~40	41~50	51~60	61~
Tinea pedis	11	5	6	1.442	0.230	1	2	2	4	0	1	1
Tinea facial	9	5	4	0.122	0.727	2	0	1	3	1	1	1
Tinea capitis	3	0	3	3.403	0.065	3	0	0	0	0	0	0
Total	146	97	49			9	20	44	30	21	14	8

3.2. Superficial Fungal Diseases and Their Pathogen Distribution (Table 2)

Table 2. Distribution of Superficial Fungal Diseases and Pathogens (n,%).

Stains	Tinea capitis (%)	Tinea facial (%)	Tinea corporis (%)	Tinea cruris (%)	Tinea manuum (%)	Tinea pedis (%)	Onychomycosis (%)	Total
Trichophyton rubrum	1 (33.33)	4 (44.44)	19 (90.48)	53 (85.48)	13 (86.67)	9 (81.81)	13 (52.00)	112 (76.71)
Other dermatophytes	2 (66.67)	1 (11.11)				1 (9.09)	1 (4.00)	5 (3.42)
Candida species				2 (3.23)			4 (16.00)	6 (4.11)
Aspergillus species				1 (1.61)	1 (6.67)		2 (8.00)	4 (2.74)
Cladosporium species		1 (11.11)	1 (4.76)	4 (6.45)	1 (6.67)		1 (4.00)	8 (5.48)
Other pathogenic fungi		3 (33.33)	1 (4.76)	2 (3.23)		1 (9.09)	4 (16.00)	11 (7.53)
Total	3 (2.05)	9 (6.16)	21 (14.38)	62 (42.47)	15 (10.27)	11 (7.53)	25 (17.12)	146

Other dermatophytes: Trichophyton violaceum 2 stains, Trichophyton interdigitale 1 stain, Microsporum canis 2 stains

Candida species: Candida albicans 2 stains, Candida glabrata 2 stains, Candida parapsilosis 1 stain, Candida metapsilosis 1 stain

Aspergillus species: Aspergillus sydowii 2 stains, Aspergillus versicolor 2 stains

Cladosporium species: Cladosporium cladosporioides 4 stains, Cladosporium sphaerospermum 2 stains, Cladosporium halotolerans 1 stain, Cladosporium tenuissimum 1 stain

Other pathogenic fungi: Curvularia tuberculata 2 stains, Talaromyces funiculosus 2 stains, Plectosphaerella cucumerina 1 stain, Parengyodontium album 1 stain, Chaetomidium pilosum 1 stain, Hortaea werneckii 1 stain, Lecanicillium antillanum 1 stain, Didymella glomerata 1 stain, Arthrinium arundinis 1 stain

4. Discussion

In recent years, with the wide application of broad-spectrum antibiotics, glucocorticoids and immunosuppressants, the widespread development of organ and bone marrow transplantation, the prevalence of pet raising and the rise of the beauty industry, the incidence rate and treatment rate of superficial mycosis have been rising. However, due to differences in age, gender, climate, living environment, occupation, and location of onset, it is difficult to accurately evaluate the diagnosis, treatment, and prognosis of superficial fungal diseases [4]. Therefore, analyzing various related factors, understanding the incidence patterns and pathogenic characteristics of superficial fungal diseases, is of great significance for the prevention and treatment of such diseases.

From the perspective of disease distribution, tinea cruris had the highest proportion (42.47%), followed by onychomycosis

(17.12%), tinea corporis (14.38%), and tinea manus (10.27%). The statistical results of multiple epidemiological investigations were not completely consistent [5, 6], but most of them were mainly tinea corporis, tinea pedis, or onychomycosis. The affected areas were mostly located in enclosed environments and areas with abundant sweat glands, which were conducive to the growth of fungi. Most of the superficial fungal diseases in this region were tinea cruris, onychomycosis, tinea corporis, etc. The incidence rate of tinea pedis was low. Considering that it was convenient to apply topical drugs to the feet, patients often apply topical drugs containing antifungal drugs, resulting in a low detection rate.

From the distribution of pathogenic fungi, dermatophytes were the most common genus of pathogenic fungi in this region (80.14%), with *Trichophyton rubrum* being the most common species (76.71%), and other dermatophytes accounting for 3.42%; *Aspergillus* species accounts for 2.74%, *Candida* species accounts for 4.11%, and other pathogenic fungi only account for 7.53%. Except for pityriasis versicolor and Malassezia folliculitis, the main pathogenic fungi of various superficial mycosis was *Trichophyton rubrum*, which is consistent with domestic and foreign reports [7-12]. Some studies had shown that the genus *Cladosporium*, which previously caused damage to crops, had been found in human superficial fungal skin lesions [13, 14]. In this survey, *Cladosporium* species accounted for 5.48%, higher than *Candida* species and *Aspergillus* species, indicating that the pathogenic species of superficial mycosis are constantly changing.

In this study, the proportion of male patients (66.44%) was higher than that of females (33.56%), which is consistent with some reported results [15, 16]. This may be related to factors such as strong sweat gland secretion in males, long-term physical labor, and lack of attention to hygiene. It might also be related to some female patients being diverted to obstetrics and gynecology clinics for treatment. However, there was no statistically significant difference in the incidence of diseases between men and women in different parts, which might be related to insufficient sample size.

In this study, the age of the affected population was mostly concentrated between 21 and 30 years old, accounted for 30.14%, 31 to 40 years old accounted for 20.55%, 41 to 50 years old accounted for 14.38%, and the proportion of middle-aged and young patients exceeds 70%. Considering that young and middle-aged people are the main workers of the society, they are engaged in more physical labor, and the secretion of sebaceous glands is strong, which is conducive to the growth of fungi, and they pay more attention to beauty, have a higher degree of concern about themselves, have a strong desire for treatment, and show a higher rate of medical treatment, which is consistent with the report of Das S et al. [15, 17, 18]. The peak incidence of tinea scalp had shifted forward, all occurring in the age range of 0-10 years old, which was considered to be related to underdeveloped scalp sebaceous glands and lack of free fatty acids in children. Moreover, children have more outdoor activities, have more

opportunities to come into contact with soil, leaves, and pets such as dogs and cats, and have poor hygiene awareness [19-21]. Elderly patients are prone to diabetes, chronic bronchitis or other basic diseases, their immune function is low, and they are more likely to be infected with fungal diseases [22]. However, elderly patients do not pay enough attention to the beauty of their skin, hair, and toenails compared to middle-aged and young people, so the proportion of elderly patients is actually lower.

5. Conclusion

To sum up, the distribution of superficial mycosis in Shiyan area generally conformed to the epidemiological trend of superficial mycosis in China, but it also had its own characteristics, which was manifested by the high incidence rate of tinea cruris, fungal culture products from diseased areas had been found to contain rare fungi that infected the human body, such as *Cladosporium*, which had previously caused damage to crops. In the future, it is necessary to further strengthen the epidemiological monitoring of fungi in the local area, expand the sample size, and combine clinical manifestation to control the incidence of false positives and false negatives. In response to the constantly changing spectrum of pathogenic fungi, timely adjustment of treatment plans is needed to provide assistance in formulating prevention and control measures for superficial mycosis in the region.

Abbreviations

SDA	Sabourg Glucose Agar
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
ITS	Internal Transcribed Spacers
NCBI	The National Center for Biotechnology Information
SPSS	Statistical Package for the Social Sciences

Author Contributions

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Conflicts of Interest

All the authors do not have any possible conflicts of interest.

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