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TRICHOPHYTON SCHEONLEINII MOLECULAR TYPING BY PCR-RFLP OF THE RIBOSOMAL DNA NON-TRANSCRIBED SPACER REGION

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Abstract:

Objective: *Tinea capitis is a common children's disease in the southern part of Xinjiang province, China. Trichophyton schoenleinii is the only causative agent of tinea favosa, which is a relatively common infection in southern Xinjiang.*

Materials: *In the previous study, the molecular epidemiological study of Trichophyton schoenleinii showed that the strains from Xinjiang have different genotypes compared to the strains from mainland China. In this study, we investigated the genetic diversity of 33 T. schoenleinii strains isolated from favus children in Hotan, a famous area in old SilkRoad, which is located at southern Xinjiang.*

Results: *The sequences of rDNA NTS regions of 33 strains were identical. Genotyping study of these strains by PCR-restriction fragment length polymorphism (RFLP) of ribosomal dna (rDNA) non-transcribed spacer (NTS) region used restriction enzyme DdeI showed that 33 strains divided into 10 genotypes.*

Conclusion: *Our result indicated that different geno typicstrains of T. schoenleinii were prevalent among the children in Hotan, and PCR-RFLP analysis of DNA NTS region is a reliable tool for geno typing study of T. schoenleinii.*

Keywords: *Trichophyton scheonleinii; NTS region; PCR-RFLP; Phylogeny; Sequencing.*

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INTRODUCTION:

Tinea capitis is a widespread scalp infection in children [13]. Deng et al. [2] investigated the matter of the cause of favus cases. Their findings concluded that *Trichophyton schoenleinii* is an anthropophilic dermatophyte; also, this is the main case of the 95% cases that result in favus. Xinjiang is the largest and western province of China. Tinea capitis has been endemic in school children throughout the south of this province, especially in Hotan and Kashgar area. *Trichophyton schoenleinii* is the main cause of Tinea favosa, which is an anthropophilic dermatophyte [27]. There was no report of tinea favosa in mainland China in recent decades, but it is relatively common in Hotan and Kashgar. This epidemiological study of tinea capitis was conducted in southern Xinjiang, including Kashgar, to assess the control of the disease, but it was concluded that the disease was not controlled well yet in those endemic areas [15]. Until now, there were no research studies conducted to ascertain the causative agent of tinea capitis in Hotan. The recent study showed that the causative agents of tinea capitis in Kashgar were different from other places of China and the causative agents migrating very slowly [2]. There are different factors which have an impact on predominant pathogens that cause tinea capities. These factors are the geography or region, the environment of the place, the weather, climatic conditions, the occupation of the local people, ethnic groups that make up the locality, and lifestyle of the people. The climate of Hotan is dry and hot, seldom rainfalls. The etiologic agents that are found in Hotan are not the same as the ones that are found in land China, and this is mainly because of the climate. In the previous genotyping study of *T. schoenleinii* strains from Xinjiang and mainland China by PCR-RAPD was shown that Xinjiang's strains have different genotypes compared to the strains from mainland China [11]. The genetic diversity of Trichophyton tonsurans was shown by PCR-RFLP of the ribosomal DNA non transcribed spacer region [11]. In this study, we applied the PCR-RFLP of the ribosomal DNA nontranscribed spacer region for the genotyping study of *T. schoenleinii* strains isolated from tinea favosa children in Hotan.

MATERIALS AND METHODS:

Strains

Thirty-three strains were used in this study listed in Table 1. All strains were isolated from 4 to 14 years old tinea capitis children's hair or scales. The lesions seriousness was variable, 10 cases were favus and 23 were serious gray patch.

Strains identification

Morphological identification

Clinical specimens were inoculated on PDA and incubated at 25°C for 2 weeks until visible growth of fungal colonies.

Molecular biological identification

DNA extraction kit:

DNA was extracted with extraction kit (Biospin Fungus Genomic DNA Extraction Kit, BioFlux). Briefly, a small amount of fungal colonies was suspended in LE buffer, and adding DA buffer, centrifuging 1,400 g for 3 minutes; then, when the impurity is made redundant, the DAN is bound to the bio spin membrane. There is also an E binding buffer, in this membrane, that contains proper salt iron, as well as pH conditions. There were proteins that were denatured, and contaminants, which were eliminated with the help of a washing procedure. With the help of the Elution buffer, the DNA was then removed from the membrane.

Sequencing of rDNA ITS region:

According to White et al. [17], universal primers like ITS1 and ITS4 were used to amplify the ITS region. Sequencing was done with an ABI PRISM 3100 sequencer (Applied Biosystems/HITACHI,Foster City, CA). The reaction content and reaction condition was omitted.

Amplification of NTS region

The rDNA NTS region is amplified by using primers (F: 5'TAGACCGTCATAAGACAG3', andR: 5'GAGACAAGCATATGACTAC3').

Reaction contents 251 µl. PCR set (Dongsheng Biotech Co. Ltd), 2X Taq Mix 1 µl, dNTPs 1 µl, each primer (20 pmol/1 µl), template DNA 5 µl, and nucleasefree water 16 µl. PCR conditions are as follows: The initial denaturation takes place at a temperature of 94°C, and this happens in the duration of 3 minutes. The denaturation consists of 35 cycles, which has a temperature of 94°C and it continues for 30 seconds. The next step annealing occurs at primer dependent temperatures (55°C–68°C), respectively, for 30 seconds and 72°C for 1 minute, final extension at 72°C for 10 minutes.

RFLP reaction

NTS amplicons were digested Dde I : Buffer D 10X Buffer 1 × 1 ml, Bovine Serum Albumin,Acetylated 1 × 150 µl, Dde1 [PROMEGA (BEIJING) BIOTECH CO., LTD] 1 × 200 u, MULTICORETM 10X Buffer 1 × 0.25 m. The 20 µl of NTS amplicon was digested by Dde I at 37°C for 4 hours, electrophoresed on 2% agarose gel, observed under ultraviolet illumination, and finally analyzed by the U.S. Bio-Rad's Quantity one 4.6.5 software.

RESULTS:

Morphological identification

Macroscopically colonies growing rather slowly, waxy, later becoming velvety, folded, cerebriform and heaped with age, often cracking and splitting the agar, and whitish to creamcolored. Reverse unpigmented, while microscopically it looks like Antlerlike hyphae, with dichotomously branched, swollen tips (favic chandeliers) present in the submerged margin of fresh cultures.

Molecular biological identification

The ITS sequence data compared with the *T. schoenleinii* strain in GenBank, and there is only one base pair diversity in two strains and all other strains showed identical ITSsequences with the strains deposed in GenBank with accession No. AB663206.

Amplification of NTS region

The NTS region of 33 isolates yielded the amplicons approximately 1.3 kb.

PCR-RFLP results

Dde I digestion and Quantity one 4.6.5 software analysis showed that 33 *T. schoenleinii* strains were divided into 10 genotypes, naming A, B, C, D, E, F, G, H, I, and J (Fig. 1). Results show that genotypes A and B account for the same 15.15% revealing the highest percentage among the total 10 types; both A and B includes five strains each, and these two types spread in different villages in Hotan area. While the next two genotypes C and D consisting of four strains, each made up to 12.12% each. Further results concluded that genotypes E, F, G, and H, all these four genotypes, consists of three strains, and the percentage is 9.09%, respectively. Genotype I contains three strains, thus accounts for 6.06% and the last one genotype J contains one strain so presenting the lowest percentage of 3.04% (Table 2). The 10 isolates from favus patients showed genotypes A (four strains), C (2), D (1), E (2), and G (1). The isolates from gray patch cases showed variable genotypes

DISCUSSION:

Hotan is located in the south of Xinjiang, southwest of Taklamakan desert, which is a place in the famous Silk Road. There is rarely any rainfall in this region, which keeps the weather hot and dry. Over the last 30 years, it has been observed that the frequency of Tinea capitis in Hotan is high; this means that it is a common fungal infection in the region [15]. According to Deng et al. [2], Ilkit [5], and Zaraa et al. [28], tinea favosa is a fungal infection, which originates in the scalp. Usually, this infection impacts children from the age of 4–14 and is hardly ever found in adults. This infection of the scalp is caused by tinea favosa, which arises from an anthropophilic dermatophyte called *Trichophyton schoenleiniis*. According to Li et al. [8], the element that plays a significant role as a host defense against *T. schoenleinii* is NLRP3 inflammasome. Researchers also say that if NLRP3 is manipulated, then it can be a fresh approach to control the disease that is caused by *T. schoenleinii* infection. The causative agents of tinea capitis in south Xinjiang were reported as *T. violaceum*, *M. ferrugineum*, *T. schoenleinii*, *T. tonsurans*, *T. verrucosum*, and *T. mentagrophytes*; in order of the frequency of species number, they were identified both morphologically and biologically [1]. *Trichophyton schoenleinii* described as an only causative agent of tinea favosa in all books and references, but a recent study showed that this species can be isolated from both of favus and greypatch types [1]. The same evidence was found in our most recent study; *T. schoenleinii* can be isolated in favus and greypatch type. Historically, J. L. Schönlein (1790–1864) discovered the etiology of favus in the mid-19th century [3]. This specific pathogen causing tinea favosa found most commonly in Eurasia and Africa [4]. *Trichophyton schoenleinii* should appear on the list of human disease agents, even though it has been eliminated worldwide, except a few pockets; it needs to be completely eliminated. In Hotangray, patch type of tinea capitis is caused by *T. schoenleinii*, which now is endemic in the middle east [16], Asia and Africa. It has been predicted that it comes from camels, who belong to environments that are similar to deserts, in fact, studies [12] shows that when *T. schoenleinii* are compared with isolates that are linked to camels, they contain similar genes, and this means that there is a close relationship between both species. The presence of *T. schoenleinii* and camels in the arid climate of Xinjiang match with this theory. *Trichophyton schoenleinii* is now rare throughout northwestern Europe and Greece [9,10] showing that, for the last two decades, *T. schoenleinii* was isolated rarely from tinea capitis patients. There is no report about cases caused by *T. schoenleinii* in mainland China, though tinea capitis caused by this species is not yet well controlled in Xinjiang. The pathogenicity and characteristics of this pathogen are may be related to the location and geographical environments of Xinjiang. Some other factors are also considered like living habits and health conditions.

Previously studies suggested that when humans came into contact with each other, the infection transferred [7]. It can be a common way of transmission in the southern part of Xinjiang, as well as in Hotan. Most probably due to lack of enough awareness and the poor living conditions; local residents mostly work in the field and grow up in an extended family, which promotes the etiology of a contagious disease like tinea capitis. Hotan as a second biggest area in south

Xinjiang is most prevalence place of tinea capitis in Xinjiang province; *T. schoenleinii* was frequently isolated in Hotan. The genotyping study of *T. schoenleinii* isolated from Xinjiang by PCR-RAPD method revealed that the strains showed low genetic variation compared to the stains from Japan [11]. According to Jackson et al. [6], for the purpose of strain differentiation of some dermatophyte, the region of NTS was chosen, and this study prompted us to utilize the region for genotyping of *T. schoenleinii* as well. In the present study, we applied the PCR RFLP method of rDNA NTS region for genotyping of 33 *T. schoenleinii* strains, which were isolated in the Hotan area. As clinical manifestation of typical favus, 10 patients showed marked inflammatory cup-shaped crusts and matted hairs over the scalp and other 23 patients showed grey patch with multiple scaly lesion, patchy hair loss, stubs of broken hairs, and variable inflammation response. Our results showed that 33 strains divided into 10 genotypes, all strains from two clinical types were showed intraspecies diversity, no correlations were found among the strains genotypes, clinical types, and the villages of patients, and maybe multiple strains transmitted in a family or closely related population. Same genotypic strain can cause different clinical manifestations. Strains transmitted person to person in Hotan area may lead to the original flow carrying of this dermatophyte species, prompting the epidemiological characteristics of this pathogensare more complicated, and perhaps one of the reasons that tinea capitis is beyond the control, still residing in the rural, crowded and low socioeconomic areas, like Hotan. In the present study, we made it possible to achieve the strains typing of 33 *T. schoenleinii* isolates from Hotan area, southern Xinjiang, China by PCR-RFLP analysis of the NTSregion of rDNA. When it comes to the molecular typing of *T. schoenleinii*, this technique can be taken as viable and simple.

CONCLUSION:

Studies done in the past reveal that this infection was transmitted through human contact. Our results reveal that human contact could be the reason for this transmission in the south part of Xinjiang and Hotan. Also,our results indicate that there are different genotypic strains for *T. schoenleinii*, which were found among the children in Hotan and PCR-RFLP analysis of DNA NTS region is a reliable tool for genotyping study of *T. schoenleinii*.

Future studies should focus on finding the different ways through which awareness can be raised among the affected regions, on how to curb this infection. As these regions witness extended families living together, there could be something done to explain these families, what they could do to protect their children from this infection.

Zhan et al. [18], did a study on tinea capitis and the epidemiological changes in it over the course of 60 years, of economic growth that took place in China. They wanted to find out if there is a connection between the pattern of the disease and the socioeconomic status, along with the etiological agents and their variability. They found a different source of infection that is the modern lifestyle of people and how they have included pets in their life. The pets can become a source of this infection, which eliminates the human contact transmission as it is discussed in this study. However, even Zhan et al. [18], agrees that the human transmission mode is more prevalent, particularly in those areas where the public and health facilities are not good.

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Conflict of Interest

All the authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

This article does not contain any studies with animals performed by any of the authors.

Table 1. Isolates used in this study and genotypes.

Strain NO	Type	Strain NO	Type
XYZ30031	B	XYZ30046	F
XYZ30032	G	XYZ30047	C
XYZ30033	C	XYZ30048	B
XYZ30034	D	XYZ30049	A
XYZ30035	A	XYZ30050	D
XYZ30036	B	XYZ30051	F
XYZ30037	J	XYZ30052	I
XYZ30038	D	XYZ30053	C
XYZ30039	B	XYZ30054	I
XYZ30040	E	XYZ30055	A
XYZ30041	G	XYZ30056	E
XYZ30042	C	XYZ30057	G
XYZ30043	E	XYZ30058	A
XYZ30044	D	XYZ30059	F
XYZ30045	A	XYZ30025	B
XYZ30026	H	XYZ30027	H
XYZ30028	H		

XYZ fungal collection of the first affiliated hospital of Xinjiang Medical University.

Table 2. The percentage of different genotypes.

Type	NO of strains	Percentage	Type	NO of strains	Percentage
A	5	15.15	F	3	9.09
B	5	15.15	G	3	9.09
C	4	12.12	H	3	9.09
D	4	12.12	I	2	6.06
E	3	9.09	J	1	3.04
Total	33	100			

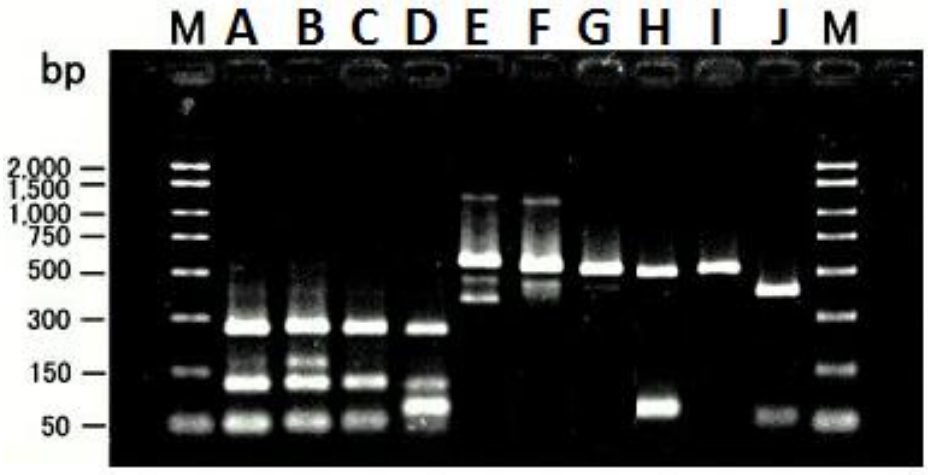

Figure 1. Gel electrophoresis of *T. schoenleinii* strains digested with restriction enzyme Dde I, M**:** Molecular maker, type A XYZ30049, type B XYZ30031, type C XYZ30033, type D XYZ30038, type E XYZ30043, type F XYZ30051, type G XYZ30057, type H XYZ30026, type I XYZ30052, and type J XYZ30037.

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