Original Article

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# Detection of *Trichophyton* spp. from footwear of patients with tinea pedis

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**Summary** **The prevalence of tinea pedis (also known as athlete's foot) in Japanese workers as well as contamination of their footwear by pathogenic filamentous fungi were investigated. Health checks by a dermatologist at a factory located in the Kanto region (Japan) led to a clinical and morphologic diagnosis of tinea pedis in 9 of 19 workers. Scales obtained from the feet and dust obtained from the protective footwear (safety shoes) worn daily in the factory were obtained from these nine subjects and tested using a mycological culture technique. Scales obtained from six of the nine subjects indicated pathogenic filamentous fungi, not only *Trichophyton* spp., but also *Acremonium*, which causes symptoms similar to tinea pedis or onychomycosis. Similarly, culture of the dust obtained from the safety shoes yielded pathogenic filamentous fungi in six of the nine subjects, and in four samples *Trichophyton* spp. was also identified. These findings suggest that cultivable *Trichophyton* spp. can be detected in approximately 40% of the safety shoes of workers with tinea pedis. The risk of reinfection by pathogenic filamentous fungi is likely increased by wearing dermatophyte-contaminated shoes.**

***Keywords:*** Tinea pedis, dermatophyte, reinfection, microbial pollution

## 1. Introduction

Although recently developed antifungal agents have a high capacity to cure tinea pedis, the population of patients with tinea pedis has not decreased – at least in Japan (*1*). Some potential reasons for the steady number of patients with tinea pedis despite the availability of effective antifungal agents are: 1) viable dermatophytes remain in the infected lesions when the application of therapeutic agents is discontinued prior to eradication of the dermatophytes, 2) reinfection by dermatophytes from other sites, such as a patient's onychomycosis, and 3) reinfection by dermatophytes remaining in the environment.

In the present study, we focused on the third possibility – reinfection from dermatophytes in the environment. Several reports indicate that viable dermatophytes are distributed in the houses in which individuals with tinea pedis live; cultivable dermatophytes are detectable from various areas in the house (*2*), such as from room-dust (*3*) and bath mats (*4*). Dermatophytes are also detected at high frequency in shoes deposited by prisoners in USA upon entry into prison (*5*). Dermatophytes were isolated from the footwear using tape strips in 41% of 34 patients with clinically active tinea pedis (*6*). Recently, we found viable dermatophytes attached to the shoes of patients with tinea pedis visiting a dermatology clinic (*7*). These findings suggest that reinfection from not only the indoor environment, but also from footwear is responsible for the steady number of patients with tinea pedis, as predicted by Bonifaz (*8*).

We hypothesized that the detection ratio of viable *Trichophyton* spp. from worker's shoes, who didn't recognize one's infection of dermatophytes, might be high because their footwear is generally not washed or sanitized. Recently, dermatologic examinations of workers' feet was performed as part of a health support program for a factory. In parallel with this health examination, we checked the inside of the workers' safety shoes for fungal contamination. Here we report that viable *Trichophyton* spp. was detected in the safety shoes in 4 of 9 cases in which the workers were diagnosed with

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dermatophyte infection by a professional dermatologist.

## 2. Materials and Methods

### 2.1. *Dermatologic examination and collection of fungi from shoes*

A clinical dermatologist experienced with medical treatment for dermal mycosis performed the dermatologic examination. He performed an examination of the feet of 19 workers (men) and collected desquamated skin scales to check for fungal filament using the KOH test. The skin samples of KOH-test positive participants were cultured as described below. In this examination procedure, their shoes were donated for collection of materials adhering to the inside of the shoe. To protect the privacy of the individuals, an opt-out policy was instituted; a notice regarding the opt-out policy was posted in the factory and the study was conducted with consent from all of the factory workers.

The methods in detail of these tests were as follows. By scraping the affected area, a large amount of scales was collected on slide glass. KOH test solution (20%) was dropped on the scales. The KOH-treated preparation on the slide glass was heated by exposure to the upper edge of the flame of an alcohol lamp for approximately 1 min to properly clear the keratin. After microscopic confirmation, KOH-positive specimens were cultured on Mycosel agar plates (Becton Dickinson, Franklin Lakes, NJ, USA).

### 2.2. *Method for collecting dermatophytes from inside the footwear*

Most of the workers wear safety shoes while at work, changing into personal shoes before leaving the factory at the end of the day. The work shoes were not washed and were worn almost every day. To collect fungi from the shoes, sealing tape for enzyme-linked immunosorbent assay (ELISA, Sumitomo Bakelite Co., Ltd., Japan) (*7*) was cut into a circle that was slightly smaller in size (φ = 7.5 cm) than the inner diameter of a petri dish (φ = 9 cm). The circular piece of tape containing both fungi and dust was incubated at 30°C on a Mycosel agar plate, so that the surface containing the dust and fungi was in contact with the medium surface. After 24 h culture, the tape was removed and culture was continued at 30°C. Six days after culture, we selected white fungus from the petri dishes where many fungal colonies were densely grown, gently touched the fungus with the tip of a micropipette, and cloned the fungus into a fresh agar plate. The cloning procedure was repeated at least twice.

### 2.3. *Morphologic observation of fungi*

Fungi-Tape (Scientific Device Lab. Inc., Des Plaines, IL, USA) was pressed against the surface of the fungal colony to adsorb hypha and spores. The staining solution, Myco Perm Blue (Scientific Device Laboratory Inc., USA), was dropped on a slide glass, and Fungi-Tape with the hypha attached was adhered onto the slide glass, and stained. After staining, it was observed with a microscope and photographed.

### 2.4. *Determination of nucleotide sequences of the DNA of the filamentous fungi and identification of the species*

Using the obtained DNA as a template, the nucleotide sequence of the Internal Transcribed Spacer 1 (*9*) region located between 18S and 5.8S ribosomal DNA was amplified by polymerase chain reaction (PCR) using the primers shown below (*9*).

Internal Transcribe Spacer 1 primer sequences: #18SF1: AGGTTTCCGTAGGTGAACCT; #58SR1: TTCGCTGCGTTCTTCATCGA

The PCR conditions were as follows: After dissociating double stranded DNA at 94°C for 10 min, we performed 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s. The temperature was then reduced to 4°C. The PCR product was purified using a QIAquick PCR Purification Kit (Qiagen, Germany). To determine the base sequence, samples were prepared with the two primers using the BigDye Terminator cycle sequence (Thermo Fisher Scientific, Waltham, MA, USA) method, and the base sequence was determined using an ABI sequencer 3130 (Applied Biosystems, Foster City, CA, USA). The sequence was obtained using the SEQUENCHER v4.10.1 (Gene Codes Corporation, Ann Arbor, MI, USA) and assembled from both directions. The resulting sequence was compared with the existing fungal base sequence using NCBI's BLAST, allowing for identification of the fungal species.

## 3. Results

Of 19 men, 9 were diagnosed with tinea pedis by clinical and mycological examination by a dermatologic expert. The diagnosis was made on the basis of not only clinical symptoms, but also the microscopical detection of filamentous fungi from scales. Table 1 shows the prevalence of tinea pedis, the age distribution of the subjects, and the rate of symptom awareness in 19 male subjects on the basis of the examination and inquiry results of the examinees. No person under the age of 35 had tinea pedis. Table 1 shows that the infection rates tended to be higher with increased age (maximum age 61). Although tinea pedis seemed to preferentially affect older people compared with younger people, the mean age of those without tinea pedis (43.9 ± 16.1 years old) is not significantly different from that of subjects with tinea pedis (52.4 ± 10.1 years old).

For 9 of the 19 participants of the dermatology health examination found to have filamentous fungi

**Table 1. Characteristics of male workers who underwent a dermatology medical checkup**

| Items | *n* | Age (average) | Age (distribution) | Rate of men with symptom awareness related to tinea pedis |
|---|---|---|---|---|
| Person undergoing medical examination | 19 | 47.9 ± 14.0 | 19-61 | 26.3% |
| Healthy person | 10 | 43.9 ± 16.1 | 19-59 | 0% |
| Tinea pedis | 9 | 52.4 ± 10.1 | 36-61 | 55.6% |

**Table 2. Identification of white filamentous fungi isolated from patient foot scales and dust from their footwear**

| No. | Age | From patient's scales | From shoes |
|---|---|---|---|
| 1 | 56 | *T. mentagrophytes* | *T. rubrum, T. mentagrophytes* |
| 2 | 36 | *T. interdigitale* | ND |
| 3 | 60 | ND | ND |
| 4 | 40 | *T. interdigitale* | *T. interdigitale, Acremonium screlotigenum* |
| 5 | 57 | *T. rubrum* | *T. rubrum, Acremonium spp* |
| 6 | 59 | *T. interdigitale* | *Fusarium solani* |
| 7 | 61 | ND | *Arthrographis kalrae* |
| 8 | 61 | *Acremonium sclerotigenum* | *Acremonium sclerotigenum* |
| 9 | 42 | ND | *T. rubrum, Acremonium screlotigenum* |

ND, not detected

on the basis of the KOH-test, we cultured scraped and sliced keratinized samples on agar medium. After the primary culture, the samples prepared from six of the nine participants positive for filamentous fungi were re-culture for cloning. Genetic methods were used to identify the samples as *Trichophyton interdigitale* (*n* = 3), *T. rubrum* (*n* = 1), *T. mentagrophytes* (*n* = 1), and *Acremonium sclerotigenum* (*n* = 1; Table 2). Questionnaires completed by the workers diagnosed with filamentous fungi in the skin revealed that four of the nine subjects (44.4%) were unaware of their dermatologic infection (subjective symptoms) or did not know they had a skin abnormality (Table 1).

Filamentous fungi collected from the right and left shoes of the nine people diagnosed with tinea pedis were cultured. The plates were cultured for 1 week at 30°C. A wide variety of fungal colonies was observed on the cultured agar plates, including *Acremonium*, *Alternaria*, *Aspergillus*, *Arthrographis*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Trichophyton*. We cloned small white colonies thought to be *Trichophyton*, which has a relatively slow growth rate. After two rounds of cloning the obtained colonies, microscopic observation and DNA analyses were performed. Three species of *Trichophyton* detected in the shoes were *T. rubrum*, *T. mentagrophytes*, and *T. interdigitale* (Table 2). The same species of fungi was identified in the scales and shoes obtained from four patients (Nos. 1, 4, 5, and 8; Table 2).

Figure 1 shows the typical images of *T. interdigitale* isolated from the scales (Figure 1A) and shoe (Figure 1 B) of patient No. 4. We wish to note that *T. mentagrophytes* was detected from the scales of the left foot and the left shoe of patient No. 1, and *T. rubrum* was detected from both the left and right shoes of the same patient. *T. rubrum* was also detected in the shoe of patient No. 9, but could not be cultured from the scales (Table 2). *Acremonium sclerotigenum* was detected in both the scales and shoes from patient No. 8, and

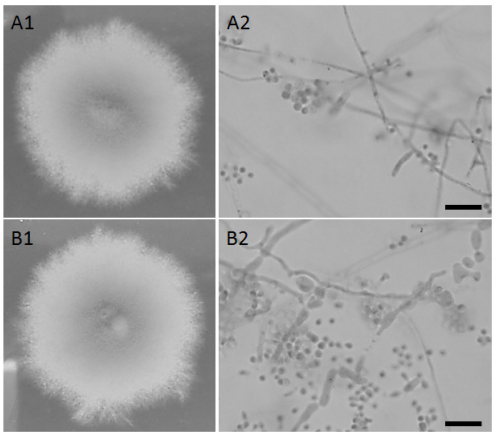


**Figure 1. Typical images of *Trichophyton* obtained from the scales of the feet and dust from the shoes of examinee No. 4 in Table 2. (A)** *T. interdigitale* from scales, **(B)** *T. interdigitale* from the dust of the shoe, 1; colony images grown on agar medium, 2; microscopic images of hyphae and conidia stained by MYCO PERM BLUE (Scientific Device Lab. Inc.). Bar represents 10 μm.

*Acremonium* was also detected from the shoes of three other workers (No. 4, 5, and 9; Table 2).

## 4. Discussion

The dermatologic examination at the factory in the present study revealed two major findings. First, 9 of 19 workers in the factory were diagnosed with tinea pedis (47%). This number is more than double that reported in Japanese in 1999/2000 (18.6%) (*1*), in 2006 (24.1%) (*1*), and 2007 (21.6%) (*10*). Second, the rate of detection of dermatophytes from the shoes of the workers with tinea pedis was also high (4 of 9, 44%).

The dermatologic examination in this study was conducted by an experienced dermatologist. Of 19 workers examined, 9 (47.4%) were diagnosed with tinea pedis and had a high infection rate; the youngest subject was 36 years old and none of the infected

subjects was under 35 years of age. We found a higher tinea pedis–positive rate in comparison with a previous study reported by Watanabe *et al*. (*1*). But, they also reported that persons between 50 and 60 years old having tinea pedis comprised 35% (1999/2000) and 36% (2006) of the subjects (*1*).

Although information regarding the existence of viable pathogenic fungi in footwear is very limited, we think that the detection rate of *Trichophyton* spp. in the shoes may be dependent on several factors. In our preceding study, the detection rate of *Trichophyton* spp. from the shoes of patients visiting the dermatology clinic for diseases other than tinea pedis was very low (7%), even the rate of patients with tinea pedis was high (*7*). We noted that the one of the reason for low detection rate may result from short wearing period of the patients visiting the clinic. In this study reported here, we studied workers who wore safety shoes almost every day for more than one year had the highest detection rate of *Trichophyton* spp. (44%) among the studies (*5*-*7*): The difference may depend on the wearing period.

The rate of *Trichophyton* spp. detected in the shoes may also be related to the severity of the tinea pedis-symptoms of the person wearing the shoes. In this connection, Knudsen reported that 41% of the footwear worn by patients with tinea pedis are dermatophyte-positive (*6*).

*Trichophyton* spp. was detected from shoes at a relatively high rate in this study, suggesting that fungal detection methods should be improved. Compared with skin samples, shoe dust was more often contaminated with mold, which grew faster, making it difficult to determine the causative *Trichophyton* spp. of tinea pedis among the various species found in the shoes. Actually, in this study, multicolored fungal colonies began to form on the plate on day 4 of culture, but *Trichophyton* spp. was not detected until after day 7 of culture. A method that could selectively cultivate *Trichophyton* spp. alone would enable a higher rate of collection of *Trichophyton* spp. from shoes. We plan to develop a selective nutrient media and clarify the culture temperatures to more efficiently detect *Trichophyton* spp.

*Trichophyton* spp. detected from inside the shoe might derive from a patient's scales through the socks or from contamination by the outside of the socks, because all persons providing the shoes tested in this study wore cotton socks. All subjects changed into their safety shoes from their personal shoes in the morning, but wore the same socks in both the work and personal shoes. With regard to dermatophyte penetration through socks, not only nylon stockings, but also cotton socks are reported to transmit *Trichophyton* spp. (*11*). *Trichophyton* spp. may attach to the outside of the socks from the environment, e.g., when they take their shoes off at home.

If the same species of *Trichophyton* are obtained from the scales and from the shoes, it is highly likely that the origin was the same. Cases in which *Trichophyton* spp. was detected only in the shoes may be due to contamination of the shoes from the environment or the detection efficiency of *Trichophyton* spp. in the scales of the patient by the KOH method may depend on the area of the foot selected for scraping.

Filamentous fungi other than *Trichophyton* spp. were also detected. *Acremonium*, which is not normally considered to be a common cause of tinea pedis with the exception of a recent report (*12*), was detected from patient No.8's scales. We think that *Acremonium* was the cause of infection in this patient due to the fact that the scales were obtained after the patient's skin was almost entirely disinfected with ethanol and the same filamentous fungus was collected from the patient's shoe. In addition to *Acremonium*, several kinds of fungi that cause skin mycoses and onychomycosis were detected and identified among the shoes, *e.g.*, *Arthrographis* (*13*) and *Fusarium* (*14*), as shown in Table 2. The rate of infection increases, especially in immunocompromised persons, when mold, including *Trichophyton* spp., grows abundantly in environments such as shoes.

The findings of this study suggest a high risk of reinfection by tinea pedis from footwear and an increased risk of spreading *Trichophyton* spp. from shared footwear. Frequent eradication of the fungi inside of shoes to prevent infection by other dermatophytes as well as reinfection by tinea pedis is important, as well as for preventing the spread of *Trichophyton* spp. in other environments, such as the home.

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**References**

1. Watanabe S, Harada T, Hiruma M, Iozumi K, Katoh T, Mochizuki T, Naka W; Japan Foot Week Group. Epidemiological survey of foot diseases in Japan: results of 30,000 foot checks by dermatologists. J Dermatol. 2010; 37:397-406.
2. Katoh T. Dermatomycosis and environment. Jpn J Med Mycol. 2006; 47:63-67.
3. Shimmura Y. Isolation of dermatophytes from human cases of dermatophytosis and from house dust. Jpn J Med Mycol. 1984; 26:74-80.
4. Sano T, Taniguchi H, Yokozeki H, Katoh T, Nishioka K. The dissemination and adhesion of dermatophytes and

a treatment to decrease collecting them on the various materials in daily life. Jpn J Dermatol. 2005; 115:1315-1319.
5. Ajello L, Getz ME. Recovery of dermatophytes from shoes and shower stalls. J Invest Derm. 1954; 22:17-24.
6. Knudsen EA. Isolation of dermatophytes from footwear with adhesive tape strips. J Med Vet Mycol. 1987; 25:59-61.
7. Ishijima SA, Hiruma M, Yamada A, Abe S. Detection and identification of *Trichophyton* living cells in footwear of patients with tinea pedis. Med Mycol Res. 2017; 8:17-23.
8. Bonifaz A, Vázquez-González D, Hernández MA, Araiza J, Tirado-Sánchez A, Ponce RM. Dermatophyte isolation in the socks of patients with tinea pedis and onychomycosis. J Dermatol. 2013; 40:504-505.
9. Makimura K, Mochizuki T, Hasegawa A, Uchida K, Saito H, Yamaguchi H. Phylogenic classification of *Trichophyton mentagrophytes* complex strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. J Clin Microbiol. 1998; 36:2629-2633.
10. Naka W. Foot Check 2007. J. JOCD (Journal of the Japan Organization of Clinical Dermatologists). 2009; 26:27-36. (in Japanese)
11. Watanabe K, Taniguchi H, Nishioka K, Maruyama R, Katoh T. Preventive effects of various socks against adhesion of dermatophytes to healthy feet. Jpn J Med Mycol. 2000; 41:183-186.
12. Chan GF, Sinniah S, Idris TI, Puad MS, Abd Rahman AZ. Multiple rare opportunistic and pathogenic fungi in persistent foot skin infection. Pak J Biol Sci. 2013; 16:208-218.
13. Ramli SR, Francis AL, Yusof Y, Khaithir TM. A severe case of *arthrographis* kalrae keratomycosis. Case Rep Infect Dis. 2013; 2013:851875.
14. Diongue K, Diallo MA, Ndiaye M, Seck MC, Badiane AS, Ndiaye D. Interdigital tinea pedis resulting from *Fusarium* spp. in Dakar, Senegal. J Mycol Med. 2018; 28:227-231.

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