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Original Article

A Case of an *Ex Vivo* Onychomycosis Model Introduced with a Cultured Colony of *Kocuria koreensis* from a Diabetic Ingrown Toenail

Tomotaka Sato[1], Akiko Kasuya[2], Hisashi Kobayashi[3], Yasuhiko Asahina[1], Tomohiro Suzuki[1], Kazuhiro Aoyama[1], Kazuto Yamazaki[4], and Takashi Yaguchi[5]

[1] *Department of Dermatology, Teikyo University Chiba Medical Center*
[2] *Office of Clinical Laboratory Technology, Keio University Hospital*
[3] *Department of Plastic and Reconstructive Surgery, Teikyo University Chiba Medical Center*
[4] *Department of Pathology, Teikyo University Chiba Medical Center*
[5] *Medical Mycology Research Center, Chiba University*

Abstract

A 57-year-old male patient with > 10-year history of type 2 diabetes presented with a left big toenail deformity and pain. A physical examination revealed a white and yellow-to-brown patch on the nail as well as thickening and ingrowth of the nail plate. The nail plate was opened using nippers, and a fungal culture revealed *Trichophyton interdigitale* with yellow yeast. The yeast isolate was identified as *Kocuria koreensis*, a Gram-positive aerobic coccoid with keratinolytic properties that is part of the normal flora of the skin. We created an *ex vivo* onychomycosis model of *T. interdigitale* infection of the human nail by placing a sterilized normal nail on the cultured slant. *K. koreensis* initially spread over the normal nail, and *T. interdigitale* then penetrated the nail plate. After one year and six months, a spiral ingrown nail developed. A histopathological examination of the spiral revealed onychomycosis with superficial and deep abscesses of Gram-positive cocci infection. We performed PCR from paraffin-embedded material, and the sequences obtained were identical to those of *T. interdigitale* and *K. koreensis.* These results suggest that the development of onychomycosis by *T. interdigitale* is introduced and accelerated by *K. koreensis*, and the symbiosis of these microorganisms is suspected in the nail. This *ex vivo* model has a number of limitations. Therefore, further research on co-infected cases is needed to confirm this hypothesis.

Key words : *ex vivo* onychomycosis model, ingrown toenail, *Kocuria koreensis, Trichophyton interdigitale*

Introduction

Foot care education during the treatment of diabetes is important for patients to avoid limb amputation surgery. Furthermore, the visualization of microbiological events occurring under the nail is informative for patients and care workers. Diabetic ingrown toenails with onychomycosis increase the risk of diabetic gangrene. Onychomycosis is a fungal infection of the nail plate, and secondary infections may also occur[1)]. The present experimental onychomycosis model using a normal human nail is of importance because onychomycosis due to *Trichophyton interdigitale* may be introduced and accelerated by *Kocuria koreensis*, a Gram-positive aerobic coccoid that has keratinolytic properties, is part of the normal flora of the skin, and is also isolated from Korean fermented seafood made from comb pen shells[2)].

Case

A 57-year-old diabetic male with glycemic control (HbA1c 6.7%) presented with pain in the left big toe. He had diabetes for more than 10 years, and HbA1c was controlled between 10.2 and 6.7%. A white and yellow-to-brown patch on the nail as well as thickening and ingrowth of the nail plate were observed. We suspected dermatophytoma (Fig. 1A). The nail

Address for correspondence: Tomotaka Sato, MD, PhD
Professor, Department of Dermatology, Teikyo University Chiba Medical Center, 3426-3 Anesaki, Ichihara, Chiba 299-0111, Japan
Received: 17 March 2021, Accepted: 13 September 2021
E-mail: zxg03117@nifty.ne.jp

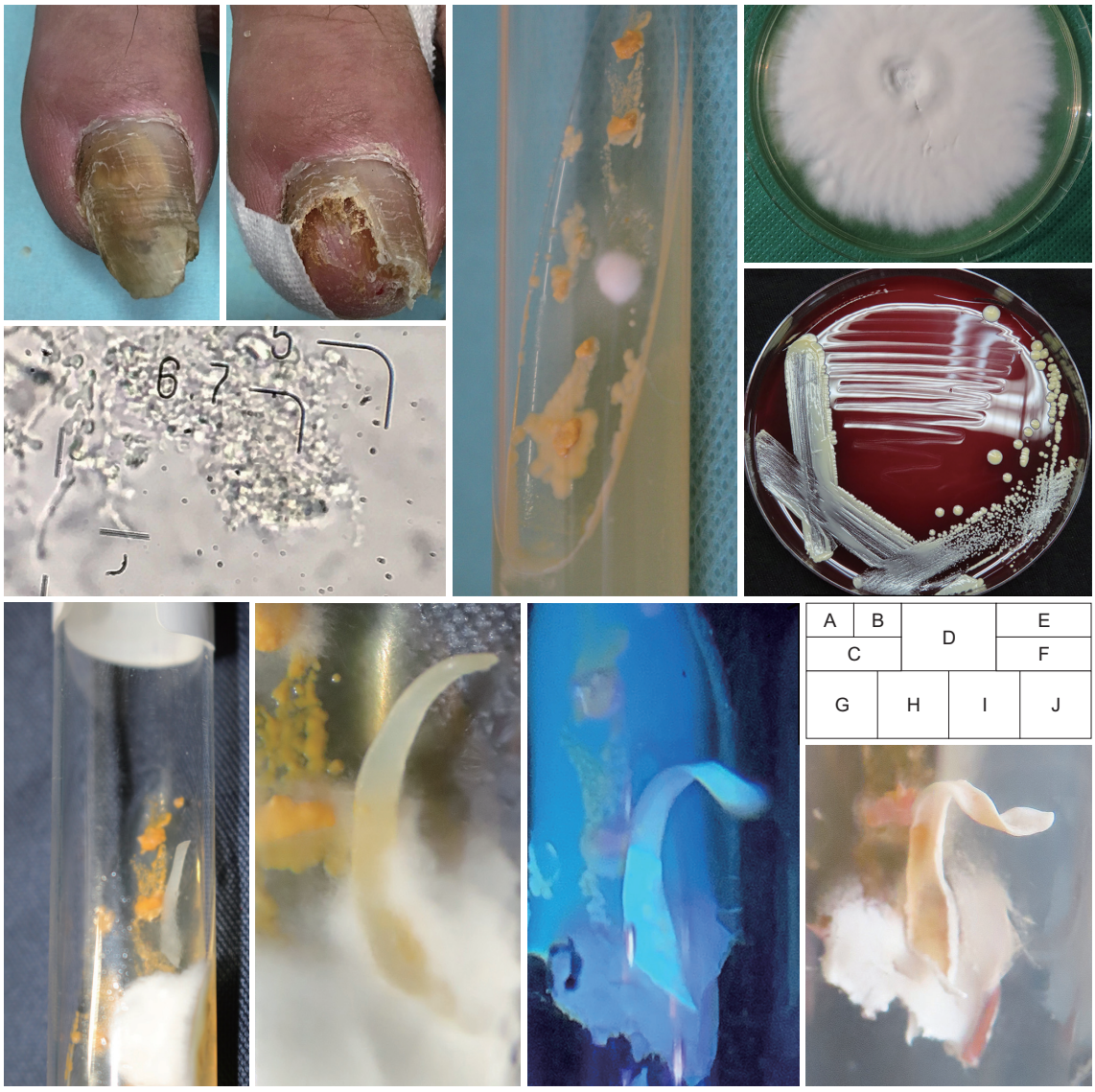

Fig. 1. Diabetic ingrown toenail with onychomycosis.

A: Diabetic left big toenail with onychomycosis.

B: The nail plate was opened.

C: A KOH direct examination showing dermatophytoma.

D: Sabouraud dextrose agar slant with cotton-like white fungal colonies with yellow yeast.

E: *Trichophyton interdigitale.*

F: *Kocuria koreensis.*

G: *Ex vivo* onychomycosis model.

H: Yellow yeast spread over the normal nail on day 30.

I: Ingrown nail after one year with violet fluorescent fungal balls under UVA light.

J: Spiral ingrown nail after one year and six months.

plate was opened using nippers (Fig. 1B), and a KOH direct microscopic examination revealed dermatophytoma (Fig. 1C). The patient was administered cefdinir at 300 mg/day for three days, which resulted in the disappearance of pain, as well as oral terbinafine for six months. A fungal culture on a Sabouraud dextrose agar (SDA) slant revealed a cotton-like white fungal colony with yellow yeast (Fig. 1D). The fungal isolate was identified as *T. interdigitale* (Fig. 1E) by a ribosomal RNA sequencing analysis of the internal transcribed spacer (ITS) region and preserved at Chiba University as IFM 65664 through the National Bio-Resource Project, Japan. Blood agar plate colonies were identified by a 16S ribosomal RNA sequencing analysis with 1462/1464 (= 99.9%) homology to *K. koreensis* (Fig. 1F).

We created an *ex vivo* onychomycosis model of *T. interdigitale* infection of the human nail by placing a sterilized normal nail on the cultured slant and observing it each day at room temperature (Fig. 1G). The yellow yeast initially spread

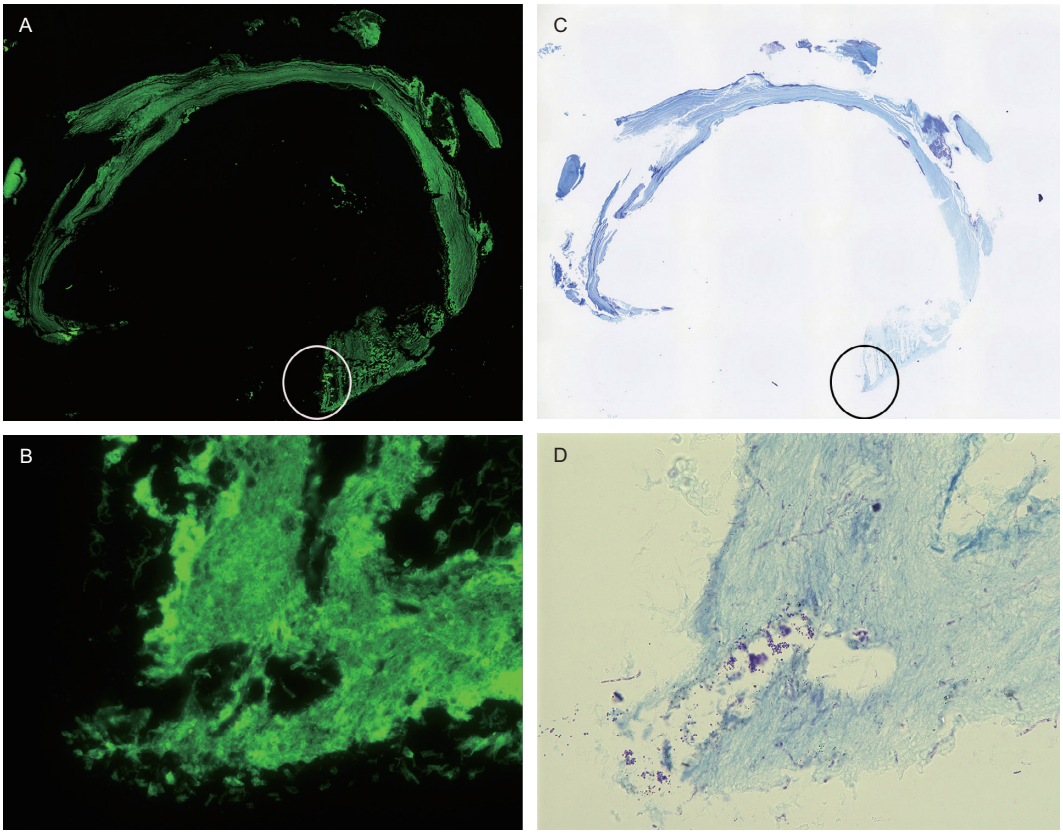

Fig. 2. Histopathological findings in the *ex vivo* onychomycosis model.
A: Fungiflora Y staining revealed whole-thickness dermatophyte infection.
B: Dermatophytoma in the center of the distal edge.
C: Gram staining revealed nail deformity with Gram-positive cocci infection.
D: Gram-positive cocci infection in the center of the distal edge.

over the normal nail from the lateral edge near the culture agar to another lateral edge, and the filamentous fungus then penetrated the nail plate. High air humidity induced fungal and bacterial growth. Water droplets due to high humidity were observed on the glass test tube wall. The filamentous fungus spread to the glass test tube wall and existed with the yellow yeast on the agar (Fig. 1H). It then spread to another nail fold, and yellow fluorescent colonies and fungal balls were observed under UVA light (Fig. 1I). After one year and six months, the slant agar became dry and broken because of the consumption of the agar by the fungus and yellow yeast growth; however, a spiral ingrown nail developed (Fig. 1J). A histopathological examination by Fungiflora Y staining (fluorescent staining) of the spiral nail revealed onychomycosis. Under fluorescence microscopy, whole-thickness dermatophyte infection was noted (Fig. 2A), and dermatophytoma was confirmed (Fig. 2B). A histopathological examination by Gram staining of the spiral revealed superficial and deep abscesses of Gram-positive cocci infection (Fig. 2C). Gram-positive cocci infection was also detected in the center of the distal edge (Fig. 2D). We performed PCR using the ribosomal DNA extracted from paraffin-embedded material and sequenced the ITS regions of rDNA and 16S ribosomal RNA. The sequences were identical to those of *T. interdigitale* and *K. koreensis*. As a normal control of the clipped nail deformity, we observed a clipped normal nail for one year and six months and found no nail deformity (Fig. 3).

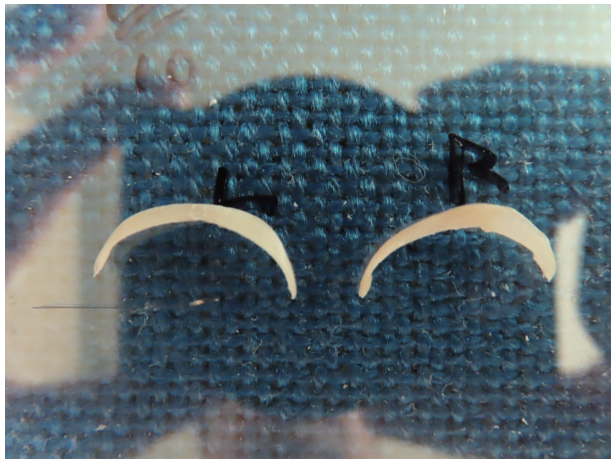

Fig. 3. Two clipped normal nails observed for one year and six months.
No nail deformity was observed.

Discussion

The genus *Kocuria* was initially proposed by Stackebrandt et al. (1995). *Kocuria* spp. are Gram-positive, aerobic, non-motile, and coccoid actinobacteria belonging to the family Micrococcus, which is a standard antibiotic reference strain sensitive to all common antibiotics comprising 18 species that are part of the normal flora of the skin and oral cavity. *Kocuria* spp. is a microorganism that is present in fermented foods and beverages and may grow in various environments, including that of the sake production process in Japan[3]. Keratins are insoluble in water and exhibit high resistance to typical proteolytic enzymes. *Kocuria* spp. have keratinases and are candidate keratinolytic bacteria in the valorization of chicken feather waste in leather processing industries[4]. The inherent ability of *K. marina* DAGII to produce cryptoxanthin (CRX) was previously reported for industrial and pharmaceutical purposes[5]. In the case of carotenoderma, carotenoids, such as CRX, fluoresce under fluorescence microscopy because of affinity to the superficial horny layer[6]. We suspected penetration of the nail by *T. interdigitale*, introduced by *K. koreensis,* in this onychomycosis model. Human nail fragments were used as a model of onychomycosis in the present study and by Nakashima et al[7]. In summary, we herein demonstrated that the development of an ingrown nail from a normal human nail by *T. interdigitale* may be induced by *K. koreensis* from a diabetic onychomycosis patient. In a dermatophyte culture, SDA with cycloheximide and chloramphenicol is used to prevent bacterial contamination; therefore, this phenomenon was previously undetected. However, this scenario remains highly speculative and, thus, further research is warranted to confirm this hypothesis. This *ex vivo* model has a number of limitations; we only observed one case and did not examine an *ex vivo* model without *K. koreensis* infection. Further research on co-infected cases is needed to confirm this hypothesis.

The development of onychomycosis with *K. koreensis* requires further investigation.

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Conflicts of interest

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