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A case of antifungal-resistant ringworm infection in KwaZulu-Natal Province, South Africa, caused by *Trichophyton indotineae*

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Summary

In 2021, a case of an extensive and difficult-to-treat ringworm (dermatophyte) skin infection in a South African woman, a resident of KwaZulu-Natal Province, was reported to the National Institute for Communicable Diseases. Genetic sequencing of a fungus cultured from the patient's skin was performed retrospectively, and a phylogenetic analysis showed the cause of the infection was a recently-described fungus called *Trichophyton indotineae*, an antifungal-resistant dermatophyte. This is the first description of such a case in Africa, and the lack of travel history in the case patient indicates that community transmission is probably occurring in South Africa.

Background

Ringworm (also known as tinea or dermatophytosis) is a common and often highly contagious fungal infection that involves the superficial layers of the skin, hair, and nails. Ringworm infections of the scalp or nails and extensive skin infections need a prolonged course of oral antifungal treatment, rather than topical treatment. *Trichophyton indotineae*, also referred to as *Trichophyton mentagrophytes* genotype VIII, is a recently-described dermatophyte mould that causes extensive and difficult-to-treat infections.[1] Many strains of this particular fungus have genetic mutations that make it resistant to systemic antifungal medicines such as terbinafine.

Large outbreaks of *T. indotineae* infection have been described in South Asia. Infection spreads from person-to-person by skin-to-skin contact or by contact with an infected person's contaminated clothing, combs, towels, and personal items.[2] More recently, clusters of cases have been reported on other continents. These clusters were detected by looking back at fungal culture collections or by prospective diagnosis of cases in people with prior travel to the Indian subcontinent or with epidemiological links to such patients.[3]

In 2021, we diagnosed a case of an extensive and recalcitrant ringworm skin infection in a 30-year-old South African woman who is a resident of KwaZulu-Natal Province. The patient had neither a travel history nor known underlying illnesses or medical conditions. She had sought treatment for an extensive, scaly, inflammatory, and disfiguring superficial skin infection, which had progressively worsened over 12 months, and involved her face, trunk, groin, buttocks, and upper and lower limbs (Figure 1). She had no associated nail or hair involvement. Her husband was being treated with topical steroids for psoriasis. He had travelled to India in 2018 and had a history of ringworm infection, but he did not have skin lesions suggestive of ringworm upon examination by her dermatologist (A.M).

At the time of her presentation and treatment, skin scrapings had been submitted, and a diagnosis of a dermatophyte infection was confirmed by a local diagnostic laboratory, with the causative pathogen suspected to belong to the fungal genus *Microsporum or Trichophyton*. Subsequent to global reports of an emerging antifungal-resistant dermatophyte, further molecular investigations were performed by the National Institute for Communicable Diseases (NICD).

Figure legends

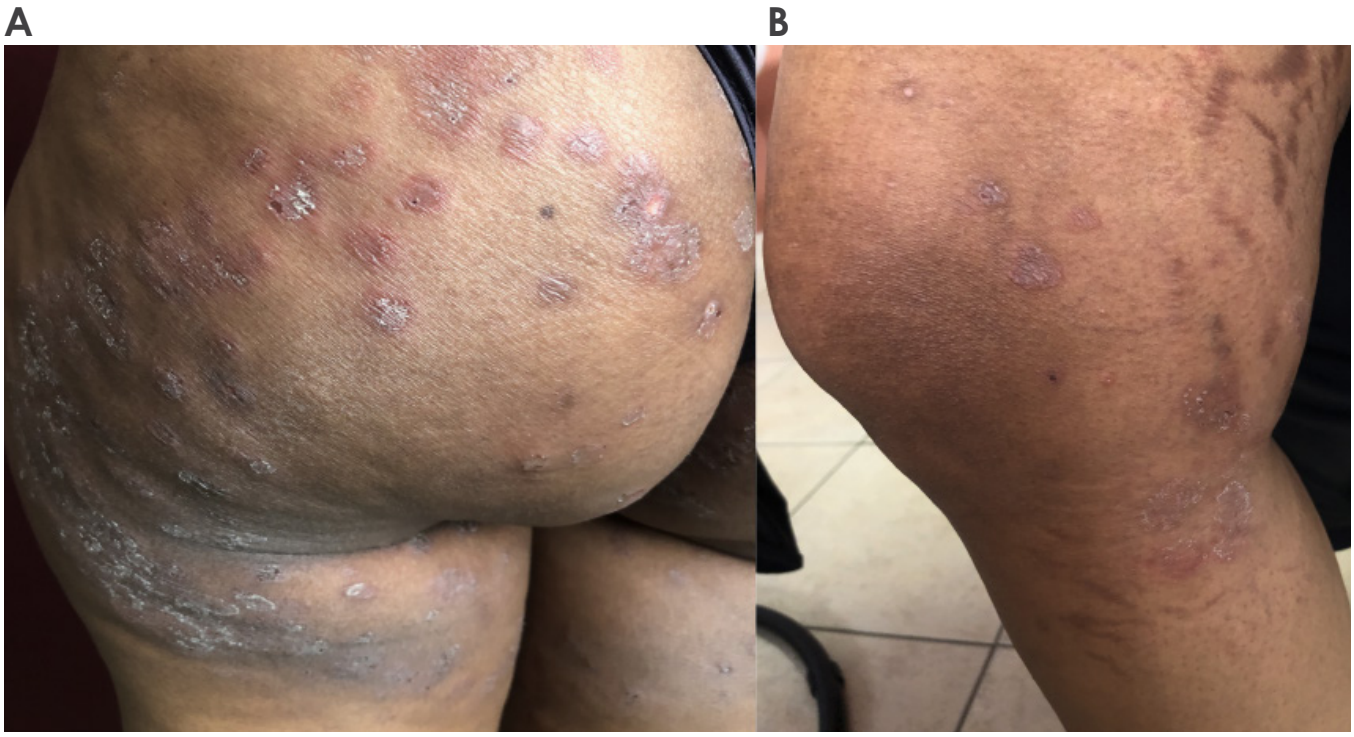

Figure 1. A) *Trichophyton indotineae* (ringworm) infection on the buttocks and thighs of a 30-year old female patient in KwaZulu-Natal Province, South Africa, 2021. B) Annular plaques of tinea and striae on the patient's inner thigh associated with long-term use of topical steroids. (Source: Dr Anisa Mosam)

Methods

The fungus isolated from the patient's skin scrapings was sent to the NICD. After sub-culture onto Sabouraud agar (Diagnostic Media Products, NHLS, Sandringham, South Africa), the fungal colonies were examined macroscopically by observing the following characteristics: surface and reverse colour, texture, topography, and growth rate at different incubation temperatures. Thereafter, a piece of the colony was mounted in lactophenol blue (Diagnostic Media Products) on a glass slide, and hyphae and sporulating structures were observed by light microscopy. DNA from the pure culture was extracted using the Zymo research fungal/bacterial DNA extraction kit (Zymo Research Corp, Irvine, USA). Two panfungal PCR assays targeting the internal transcribed spacer (*ITS*) region and the large subunit (*LSU*) of the multi-copy ribosomal gene were performed.[4,5] Following amplification, Sanger sequencing was performed using the same primers. The NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and MycoBank (https://www.mycobank.org/) databases were used to obtain an initial genus/species identification. Multiple sequence alignment was performed with ClustalW (BioEdit Sequence Alignment Editor) using the *ITS* sequences of different *Trichophyton* species (including *T. indotineae*), and a phylogenetic tree was generated using MEGA software for identification of *T. indotineae* and its relatedness to other *Trichophyton* strains (*T. interdigitale*, *T. mentagrophytes, T. schoenleinii,* and *T. quinckeanum*).

Results

Fungal growth was observed approximately 9 days after inoculation onto Sabouraud agar at 30°C. Macroscopically, white, powdery, flat colonies were observed. The reverse was pale to tan, and the culture grew best at 35°C (Figure 2). The hyphae were septate, and round microconidia and cigar-shaped thin-walled macroconidia were observed microscopically, the latter with narrow attachments to hyphae.

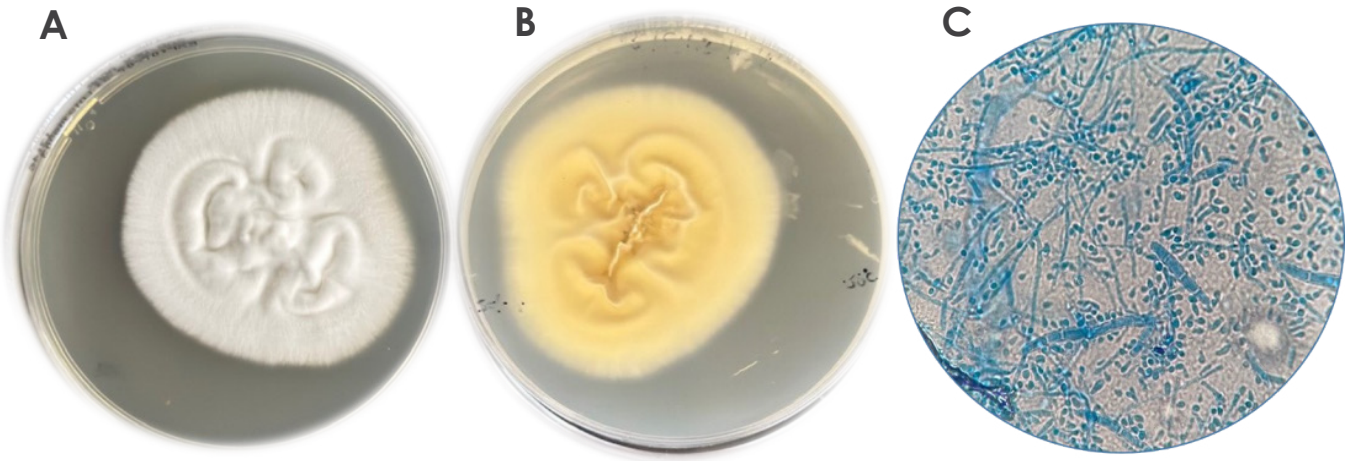

Figure 2. *Trichophyton indotineae* growth isolated from skin scrapings collected from the female patient with ringworm infection. A) Aerial view of white fungal colonies grown at 35°C for 12 days with B) beige pigmentation on the underside. C) Microscopic view of the same colonies at 100x magnification showing septate hyphae and cigar-shaped thin-walled macroconidia and round microconidia.

Based on the sequencing of the *ITS* and *LSU* gene regions, there was a 99% sequence match to both *T. mentagrophytes* and *T. interdigitale*. However, in the phylogenetic tree (Figure 3), the isolate from the patient (MRL clinical isolate 1659) clustered with the *T. indotineae* reference strains from Teikyo University Institute of Medical Mycology, Tokyo, Japan[1] ,and clinical strains from the Czech Republic (NCBI accession number: OM283512).

The patient had sought care from two specialist dermatologists, including A.M. She was treated with a number of oral antifungal medicines at varying dosages and durations, with varying responses. She had an initial partial response to oral fluconazole and itraconazole prescribed by the first dermatologist; however, both medicines were sourced by the patient on the black market and may have had limited potency. She was then treated with oral griseofulvin, followed by oral terbinafine, with no response. She had also obtained a topical over-the-counter cream from a pharmacy containing both an antifungal medicine (clotrimazole) and a moderately potent corticosteroid (betamethasone valerate); she applied liberal amounts of this cream to the affected areas on a daily basis over a long period of time. When she was seen by A.M., the patient was urged to immediately stop this topical cream because corticosteroids worsen fungal skin infections, even though patients sometimes notice an initial response owing to a reduction in inflammation. The infection eventually responded to a prolonged course of oral itraconazole with topical clotrimazole. At the end of this treatment course during November 2021, skin scrapings were negative by microscopy and fungal culture. However, as of 10 October 2023, A.M. noted active areas of infection on the patient's inner thighs and buttocks, though the infection was cleared on other parts of her body.

Treatment with itraconazole and clotrimazole was continued. At this time, her husband still had active psoriasis, but no other skin lesions were observed. Subsequent skin scrapings from the patient's lesions were positive for fungal arthroconidia by microscopy and had the same phenotypic characteristics on culture as the first isolate. This subsequent isolate also clustered with *T. indotineae* clinical isolates on phylogenetic analysis (Figure 3).

Figure 3. Neighbour joining tree of ITS sequences using 1000 bootstraps and Jukes Cantor model. The sequences from the isolates cultured from the case-patient in KwaZulu-Natal Province are denoted as NICD_MRL 1659 (first isolate) and NICD_MRL 1984 (second isolate).

Discussion

Trichophyton indotineae infections have not been previously reported on the African continent. This case, which was initially diagnosed as a *Microsporum*/ *Trichophyton* infection by the diagnostic laboratory, suggests potential local transmission of an antifungal-resistant dermatophyte in South Africa.

Cases of *T. indotineae* infection typically present with large or widespread annular, scaly, erythematous pruritic plaques exhibiting an unusual distribution across the body and are often non-responsive to terbinafine antifungal treatment. [2] However, with the use of steroids, the inflammatory component of the tinea initially responds, which may make conditions on the treating clinician's differential diagnosis such as eczema and psoriasis vulgaris appear to be more likely. This may encourage the continued use of steroids, leading to worsening of the fungal infection. In India, the emergence of antifungal-resistant dermatophytes is, in part, attributed to unrestricted availability of affordable corticosteroid-antifungal-antibacterial fixed-dose combination creams, widely accessible without prescriptions and frequently prescribed for various dermatoses by general clinicians.[5] Although this has not been studied, it is plausible that treatment-resistant dermatophytoses emerged due to alterations in normal skin flora and suppression of the immune system induced by steroid combination creams, creating conditions conducive to such infections.[5] The patient reported here similarly presented with an extensive modified infection. Contact with her husband, who had travelled to India and previously had a ringworm infection, and her long-term use of topical steroids, could have led to her infection.

Despite the patient seeking consultations with dermatologists, an accurate diagnosis of *T. indotineae* infection could not be made without the awareness of this emerging pathogen and the availability of accurate mycological identification methods. Basic culture-based techniques used by most clinical laboratories misidentify *T. indotineae* as *T. mentagrophytes* or *T. interdigitale*. Matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS) is able to correctly identify *T. indotineae* isolates if an updated database is used. Molecular methods such as a panfungal PCR assay combined with phylogenetic analysis or a real-time PCR assay (e.g., DermaGenius 2.0 multiplex real-time PCR kit, Pathonostics, Maastricht, The Netherlands) can also be used for accurate identification.[2,4]

The taxonomy of *Trichophyton* has been changing since 2008, with different strains evolving over time. *Trichophyton mentagrophytes* included both zoophilic and anthropophilic species. *ITS* sequencing led to taxonomic rearrangement with some species within *T. mentagrophytes* moving to *T. interdigitale*, *T. mentagrophytes* (sensu stricto), and *T. erinacei*. Further revision of the taxonomy in 2017 separated the zoophilic and anthropophilic species, with T. interdigitale being considered anthropophilic and *T. mentagrophytes* zoophilic. Sequencing of the ITS gene has identified several genotypes within *T. mentagrophytes,* including genotype VIII, which was initially isolated from India. This genotype is now classified as *T. indotineae* and is morphologically similar to *T. mentagrophytes* but is anthropophilic rather than zoophilic and has a high-level terbinafine resistance (minimum inhibitory concentrations of >0.5 mg/L).[5] In this case-patient, terbinafine was prescribed with no response. There was, however, an initial partial response to a course of itraconazole, although relapse skin lesions have since occurred with positive fungal microscopy and culture.

While *T. indotineae* infections have been reported primarily among patients in India, imported and locally-transmitted infection cases have now been reported globally, including in the America and Europe.[2,4] This situation is concerning because antifungal-resistant dermatophytes have the potential to become predominant causes of infections. In India, *T. mentagrophytes* strains that are resistant to first-line treatment have overtaken *T. rubrum*, now accounting for over 90% of infections.[5] Local transmission in South Africa is a potential concern, as this infection can be progressive and disfiguring, particularly in settings where appropriate testing and antifungal agents are not readily available.

Recommendations

- Cases of extensive ringworm infection or with an unusual body distribution, particularly infections not responding to terbinafine treatment, should be investigated further. Skin scrapings from lesions should be submitted to a diagnostic laboratory for direct microscopy (using potassium hydroxide or a fluorescent stain) and fungal culture.
- Laboratories should refer cultured fungal isolates, especially those identified as *T. interdigitale*, *T. mentagrophytes,* or *Trichophyton* species, to the NICD Mycology Reference Laboratory for molecular identification. Genetic sequencing is needed to confirm the identification, as no in-house PCR assays have been set up.
- In addition, antifungal susceptibility testing (or molecular resistance assays, e.g., mutation analysis of the squalene epoxidase gene) should be performed to confirm susceptibility to antifungal medicines.
- Patients with confirmed *T. indotineae* infections may need prolonged oral antifungal treatment, initially itraconazole 100 mg BD for 4-8 weeks under close supervision. However, if tinea is still clinically apparent, itraconazole may need to be continued for up to 12 weeks to ensure clearance.
- Close contacts should be traced and examined for skin lesions. Close contacts with skin lesions also need treatment to prevent continuing cross-transmission in the household.
- Recognising the potential role of fixed-dose combination medications containing potent topical steroids, it is imperative to establish stringent local regulations, including bans, on their manufacturing, local importation, and distribution.
- Antifungal agents, including itraconazole, should only be accessible to patients following a thorough quality assessment and registration process conducted by the South African Health Products Regulatory Authority (SAHPRA).
- SAHPRA, together with the South African Police Service (SAPS), should rigorously enforce laws and regulations against the unauthorised or illegal sale of sub-standard, counterfeit, or even scheduled drugs by unauthorised facilities and/or unauthorised individuals.
- Awareness of this condition among health professionals and in affected communities is essential to facilitate rapid diagnosis and appropriate treatment of people with this infection and to prevent its further spread.

Ethical considerations

The patient provided informed consent and permission to capture and publish photographs. The University of KwaZulu-Natal Research Ethics Committee granted a waiver for ethics clearance, allowing the publication of the case report (00023882).

Conflicts of interestt

None to declare.

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