Medical Mycology Case Reports 27 (2020) 59–63

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

Sporotrichosis: The case series in Thailand and literature review in Southeast Asia

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ARTICLE INFO

Keywords:
Sporotrichosis
Southeast asian
Thailand
Sporothrix schenckii

ABSTRACT

Human Sporotrichosis is an infection caused by dimorphic fungus, *Sporothrix schenckii* complex, via direct inoculation. We are herein report proven 2 cases of sporotrichosis along with a literature review about human sporotrichosis in the southeast Asian region. The first case was a 76-year-old female with a non-progressive erythematous plaque at the right ankle. The second case was a 36-year-old female with sporotrichoid lesion for six weeks. Both were treated with itraconazole with an excellent outcome.

1. Introduction

Sporothrix schenckii is a thermally dimorphic fungus in the division Ascomycota, class Pyrenomycetes, order Ophiostomatales, and family Ophiostomataceae. The fungus is distributed over the tropical and subtropical areas with high humidity and mildly high temperatures, and usually resides in abiotic substrates including soil, plants, and organic matter. It was first isolated in 1896 from a 36-year-old male patient who presented with subcutaneous abscesses at right hand and arm by a medical student, Benjamin Schenck [1]. Regarding clinical manifestations, there are 4 forms of sporotrichosis including 1) cutaneous infection, 2) lymphocutaneous infection, 3) extracutaneous and disseminated infections, and 4) mucosal infection [2]. Regarding cutaneous sporotrichosis, there are three distinct clinical types, including fixed cutaneous, lymphocutaneous, and disseminated cutaneous types.

To date, only 2 cases of human sporotrichosis had been reported since 1990 in Thailand [3,4]. We believe that the infection in our country, which locates in the tropical zone, is underreported probably due to under-recognition and no laboratory availability. In the present study, we report the case series of cutaneous sporotrichosis in Thailand and review the English literature of sporotrichosis in Southeast Asia.

2. Cases

2.1. Case 1

A 36-year-old previously healthy Thai female presented with an unhealed ulcer at the left arm for six weeks. Firstly, she noted a small skin lesion at the left forearm, believing that there was like something pinned her. After that, the lesion had slowly grown bigger. After two weeks of illness, she noted that there was a line of growing skin nodules extending proximally from the primary lesion. During her present illness, there were no fever nor weight loss. She had a healthy cat but claimed that she had not bitten or scratched by the cat before the symptom arose. At day, initial physical examination revealed a shallow ulcer at her forearm with multiple small indurated painless subcutaneous nodules located proximally in the typical sporotrichoid pattern (Fig. 1). Gram stain of skin scraping at the base of the ulcer showed many yeasts (2–4 μm) with elongated cells (cigar bodies) (Fig. 2). A diagnosis of lymphocutaneous sporotrichosis was made, and oral itraconazole solution was initiated at day 0. The pathology of the skin biopsy exhibited necrotizing granulomatous inflammation but without asteroid bodies at day +4. The tissue culture later grew *S. schenckii*

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https://doi.org/10.1016/j.mmcr.2020.01.002
Received 1 December 2019; Received in revised form 22 December 2019; Accepted 13 January 2020
Available online 16 January 2020

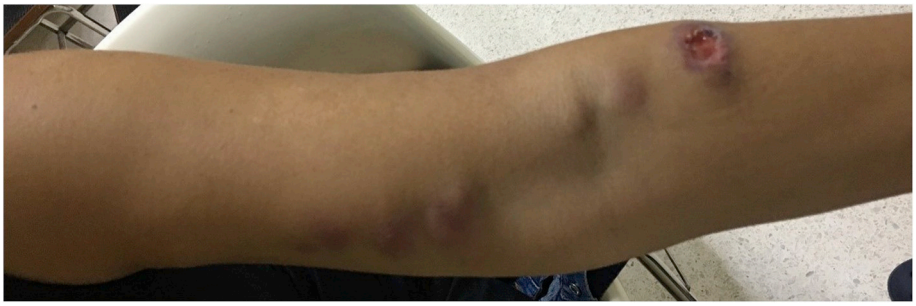

Fig. 1. The lymphocutaneous lesions in the first case.

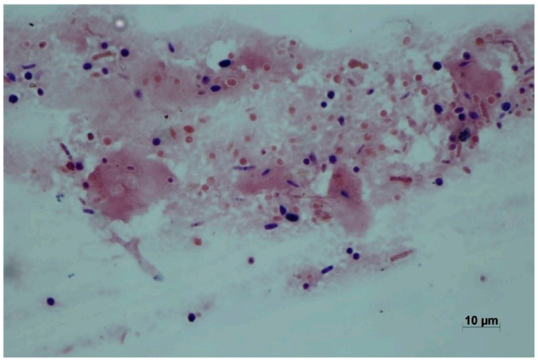

Fig. 2. Tissue Gram stain exhibiting many yeasts (2–4 µm) and elongated cells (cigar bodies).

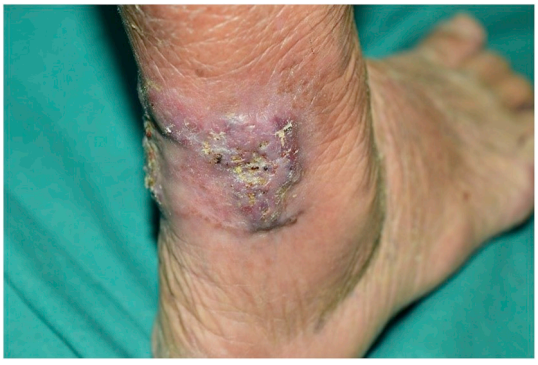

Fig. 3. The nodular plaques in the second case.

which was identified by sequencing the internal transcribed sequence (ITS) region of ribosomal RNA as previously described [5], with GenBank BLAST search showing 99.0% identity to *S. schenckii* CBS359.36 ITS region (Accession number NR_147566; 500 base-pair length of analyzed sequence) at day +21. Additionally, the identification of *S. schenckii* was made using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Vitek II MS). Her skin lesions gradually improved a few weeks after itraconazole treatment and completely resolved at day +90. The patient was followed up until day +360 without recurrence.

2.2. Case 2

A 76-year-old Thai female presented with a growing non-tender indurated erythematous plaque on her right ankle for six months without any history of trauma (Fig. 3). After two months of illness, she came to seek treatment. At day 0, skin biopsy was performed and exhibited non caseous granulomatous inflammation without organisms demonstrated by Gram, acid-fast, and periodic acid Schiff stains. The tissue mycobacterial polymerase chain reaction, as well as fungal and mycobacterial cultures, yielded negative results. She was empirically treated with antituberculous agents (isoniazid, rifampin, ethambutol, and pyrazinamide), but without any clinical improvement after four months of treatment. At day +120, another skin biopsy was performed, and the pathology exhibited pseudoepitheliomatous hyperplasia with dense diffuse mixed polymorphonuclear, mononuclear, and histiocytic infiltration in the dermis with no organisms demonstrated by Gram, acid-fast, and periodic acid Schiff stains. Fortunately, the fungal culture of the tissue grew *S. schenckii* complex at day +141. There was yellow-to-tan creamy yeast (Fig. 4A and B) and mold colonies (Fig. 4C) on blood agar cultures when incubated at 37 °C and 25 °C, respectively. The lactophenol cotton blue stain of the colonies incubated at 25 °C showed branching narrow septate hyphae (1–2 µm in diameter) with slender tapering conidiophores rising at right angles; the apex of conidiophore was swollen and bore small tear-shaped conidia on thread-like denticles, forming rosette-like conidia (Fig. 4D). The species finally turned to be *S. schenckii* which was identified by sequencing the ITS region of ribosomal RNA with GenBank BLAST search showing 99.0% identity to *S. schenckii* CBS359.36 ITS region (Accession number NR_147566; 500 base-pair length of analyzed sequence) at day +162. Additionally, the identification of *S. schenckii* was made using MALDI-TOF MS (Vitek II MS). A diagnosis of fixed-typed sporotrichosis was made, and she was treated with oral itraconazole total three months. At day +260, all of her lesions resolved without recurrence.

For antifungal susceptibility pattern, both clinical isolates were sent to mycology laboratory for antifungal susceptibility testing. Standard powders of itraconazole, voriconazole, caspofungin, and amphotericin B (Sigma-Aldrich, St. Louis, MO) were solubilized in dimethyl sulfoxide (DMSO) or water, according to the manufacturer's recommendations. 96-well plates with gradient concentrations of each drug in 100 µL RPMI 1640 medium (with glutamine, without bicarbonate sodium, and with phenol red as a pH indicator; Sigma-Aldrich, St. Louis, MO) were prepared. The range of concentrations tested for the broth microdilution method was 0.0313–16 µg/ml for all drugs. For the broth microdilution method, we performed the test according to CLSI M38 (2017). Briefly, conidial suspensions were adjusted to approximately 10^6 CFU/ml and 1:50 dilution of the stock solution was made in RPMI 1640 medium to a final concentration of 0.4 × 10^4 to 5 × 10^4 CFU/ml. A volume of 100 µL of this final solution was inoculated in each well of a 96-well plate, containing 100 µL of the drug solution. Plates were incubated at 35 °C. Final readings were performed at 48 hours for *Aspergillus flavus* ATCC 204304 as a quality control strain and at around 50 hours for *S. schenckii*. All experiments were performed in duplicates. Results of antifungal susceptibility were shown in Table 1.

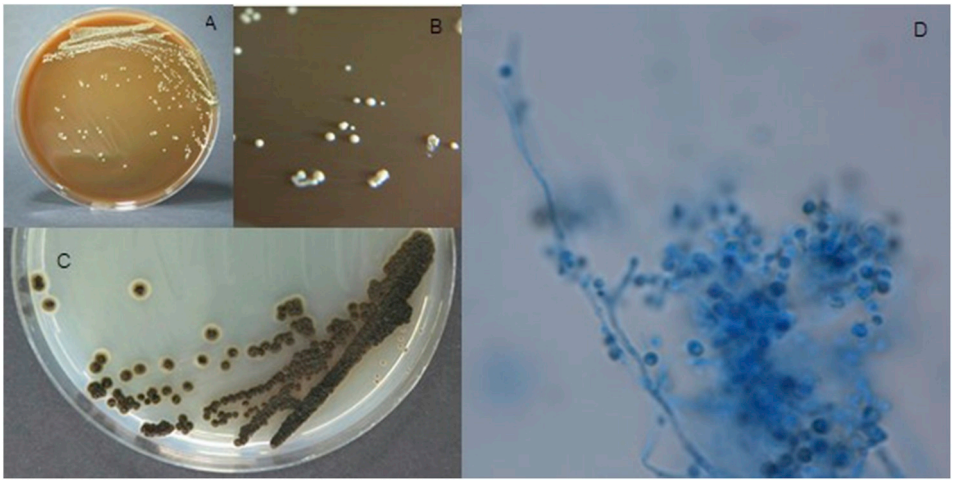

Fig. 4. A and B: the colonies of yeast form incubated at 37 °C. C: the colonies of mold form incubated at 25 °C. and D: lactophenol cotton blue stain showing slender hyphae with rosette-like conidia. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Antifungal susceptibility testing both clinical isolates by broth microdilution methods.

Antifungal agents	MIC (μg/mL)	
	Sporothrix schenckii (Case 1)	*Sporothrix schenckii (Case 2)*
Itraconazole	< 0.0313	0.0625–0.125
Voriconazole	0.5	0.25
Caspofungin	0.25	0.0625
Amphotericin B	0.5	0.5

3. Discussion

We reported two cases of cutaneous sporotrichosis in Thailand. The first case had lymphocutaneous form, and the second case had a fixed-type cutaneous form. The presumed portal of entry in the present study is a direct inoculation due to prominent skin symptoms and signs. To date, only two cases of human sporotrichosis had been reported since 1990 in Thailand [3,4]. We think there will be underreported due to under-recognition and no widely available laboratory.

Regarding of mode of transmission to humans, there are two categories of sporotrichosis including the sapronosis (the most common category) and zoonosis (mainly feline sporotrichosis). Of sapronotic sporotrichosis, the most common causative agent is *S. schenckii*, which is worldwide distributed. In contrast, zoonotic sporotrichosis, the most common causative agent is *S. brasiliensis*, a feline fungus, which is mostly distributed in South America [6,7]. In the first case of the present study, we previously think that the causative agent should be *S. brasiliensis* due to a history of cat exposure. However, it turned out to be *S. schenckii*. Hence, to our knowledge, there has still been no case of sporotrichosis caused by *S. brasiliensis* in Thailand.

Regarding the English literature review, to date, there have been a total of 19 microbiological confirmed cases of sporotrichosis in Southeast Asia (Table 2) [3,4,8–11]. Most of the cases are female (63.2%), and the age group ranges from 23 to 76 years with the mean age of 50.79 ± 17.19 years. The most reported country is Malaysia (73.7%), followed by Thailand (21.1%) and Laos PDR (5.3%). All cases are otherwise healthy without immunocompromised condition except 1 case from Malaysia with lepromatous leprosy. Most patients are housewives (26.3%). Only 9 cases including our first case (47.3%) could remember their preceding risk of exposure to animals, plants or abiotic substrates including trauma from abiotic substrates (33%) and animal exposure (67%). The most common form is lymphocutaneous (52.6%), followed by fixed (31.6%) and disseminated cutaneous infections (15.8%). The longer duration is observed in fixed type (the mean of 5.875 months), compared with a fixed type (the mean of two months) and lymphocutaneous type (the mean of 1.75 months). Most common affect location in lymphocutaneous form is upper extremities (80%). In fixed form, upper extremities were also the most common affect location (60%). The diagnosis of sporotrichosis was made by using the culture (94.4%), direct tissue examination (33.3%) and the molecular method (16.7%). The most common finding pathology is granulomatous inflammation (57.9%), followed by mixed acute and chronic inflammatory cell infiltration with and without granulomata (31.6%). Itraconazole was used as definite treatment (77.8%), followed by potassium iodide solution (10.5%), and itraconazole followed with terbinafine (10.5%). The mean duration of treatment is 4 ± 1.99 (range from 2 to 8) months. The outcome of treatment is very excellent with antifungal alone; the surgical debridement is performed without systemic antifungal treatment in two cases. Only one case died but due to hospital-acquired infection.

Sporotrichosis in Thailand is probably underreported due to under-recognition and no widely available laboratory. In addition, to date, apart from Malaysia there have been handful reported cases of sporotrichosis in Southeast Asia. Most of the patients with sporotrichosis in our case series presented with upper extremities lesion. We assume that it might be related to a history of trauma by handling abiotic substrates and animal exposure, but only few cases could remember preceding history. Treatment with systemic antifungal has an excellent outcome. We encourage the physicians to have an awareness of this infection when taking care of patients presented with chronic cutaneous or lymphocutaneous lesions refractory to antibacterial treatment.

Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Table 2
A summary of all 19 cases of sporotrichosis in Southeast Asia.

Year (Ref.)	Country	Sex	Age	Underlying condition	Infection site	Form	Duration	Occupation (preceding trauma history)	Diagnosis	Tissue pathology	Treatment and duration	Outcome
1990 [3]	Thailand	F	33	None	Left elbow	FC	3 months	Housewife	Tissue culture	NA	Potassium iodide 3 months	Cure
2005 [8]	Lao PDR	F	42	None	Right index finger	LC	1 month	Farmer	Tissue for 18S RNA sequencing	NA	Itraconazole 6 months	Cure
2009 [9]	Malaysia	M	70	None	Left leg	FC (Mass)	1 month	Farmer	Direct tissue examination, tissue culture	Granulomatous and microabscess	Excision No antifungal	Cure
2011 [11]	Malaysia	F	70	None	Face, upper and lower limbs constitutional symptoms	DC	6 months	Retiree/gardening	Direct tissue examination, culture (negative for hemoculture)	Epidermal hyperplasia and granulomatous inflammation in the dermis	Amphotericin B 2 weeks then Itraconazole 8 months	Cure
2012 [10]	Malaysia	F	59	Atrial fibrillation	Left wrist	LC	1 month	Retired teacher (cat bite)	Tissue culture	Vague granuloma with MGC, Mixed infiltrate with lymphocytes and neutrophils.	Itraconazole 11 weeks Switch to terbinafine 4 weeks due to heart failure	Cure
2012 [10]	Malaysia	M	66	NA	Right index finger	LC	1 month	Retired police (thorn prick)	Tissue culture	No granuloma, Mixed infiltrate with lymphocytes, neutrophils.	Itraconazole 16 weeks	Cure
2012 [10]	Malaysia	F	32	NA	Left forearm	LC	2 months	Admin officer	Direct tissue examination, tissue culture	Suppurative granuloma few MGC. Mixed infiltrate with lymphocytes, neutrophils	Itraconazole 24 weeks	Cure
2012 [10]	Malaysia	M	51	NA	Left wrist	LC	1 month	Retired police (cat bite)	Tissue culture	Epithelioid granuloma. No MGC. Mixed infiltrate with neutrophils and lymphocytes	Itraconazole 20 weeks	Cure
2012 [10]	Malaysia	F	56	NA	Right ankle	LC	2 months	Housewife (cat scratch)	Tissue culture	Epithelioid granuloma with MGC. Subcutaneous fat necrosis	Itraconazole 18 weeks	Cure
2012 [10]	Malaysia	F	65	NA	Left cheek	FC	1.25 month	Doctor	Tissue culture	Epithelioid granuloma with MGC. Mixed infiltrate with neutrophils and lymphocytes	Itraconazole 15 weeks	Cure
2012 [10]	Malaysia	M	23	NA	Left hand	FC	12 months	Student (Fish handling)	Tissue culture	No granuloma. Mixed infiltrate with plasma cells, lymphocytes and histiocytes	Excision No antifungal	Cure
2012 [10]	Malaysia	M	28	NA	Right forearm	FC	12 months	Clerk (Pricked by nail)	Tissue culture	Epithelioid granuloma with MGC. Psoriasiform hyperplasia. Infiltrate with Lymphocytes & plasma cells.	Itraconazole 6 weeks	Cure
2012 [10]	Malaysia	M	46	NA	Left index finger	LC	1 month	Waiter (Cat bite)	Tissue culture	No granuloma. Lymphoplasmacytic admixed with MGC, eosinophils & neutrophils	Itraconazole 16 weeks	Cure
2012 [10]	Malaysia	M	61	Lepromatous leprosy with residual deformity	Whole body	DC	3 months	Flower nursery owner	Direct examination, tissue culture	Epithelioid granuloma with MGC. Mixed infiltrate with lymphocytes and neutrophils GMS positive	Amphotericin B 17 days then Itraconazole 2 weeks switch to terbinafine 2 weeks (hepatitis from azoles)	Died (Hospital acquired bacterial infection)
2012 [10]	Malaysia	M	26	NA	Left thenar	LC	1 month	Administrative officer (cat scratch)	Tissue culture	No granuloma. Mixed inflammatory cells infiltration with lymph, plasma cells, foamy macrophages and histiocytes	Itraconazole 14 weeks	Improved (still on treatment)
2012 [10]	Malaysia	F	71	Hypertension, Ischemic heart disease	Face, upper and lower limbs	DC	2 months	Housewife	Direct examination and tissue culture	Granuloma with MGC. Mixed inflammatory cells with neutrophils, lymphocytes and plasma cells. GMS positive	Itraconazole 1 year then recurrence after stop treatment for 3 months Amphotericin B 18 days Itraconazole 16 weeks	Improved

(*continued on next page*)

Table 2 (*continued*)

Year (Ref.)	Country	Sex	Age	Underlying condition	Infection site	Form	Duration	Occupation (preceding trauma history)	Diagnosis	Tissue pathology	Treatment and duration	Outcome
2019 [4]	Thailand	F	54	None	Face	LC	1 month	NA (Chinese herb application)	Tissue culture and ITS gene sequencing	Mixed inflammatory cells, composed of neutrophils, lymphocytes, plasma cells, histiocytes, foam cells and giant cells	Itraconazole 6 months	Cure
2019 *(Case1)*	Thailand	F	36	None	Left arm	LC	1.5 months	Business women (Cat exposure)	Direct examination, tissue culture and ITS gene sequencing	Mixed polymorphonuclear and mononuclear infiltration and granulomatous formation	Itraconazole 6 months	Cure
2019 *(Case 2)*	Thailand	F	76	None	Right ankle	FC	6 months	Housewife	Tissue culture and ITS gene sequencing	pseudoepitheliomatous hyperplasia with dense diffuse infiltration in the dermis, composed of mononuclear cell, polymorphonuclear cell and histiocytes	Itraconazole 3 months	Cure

M: male, F: female, FC: fixed cutaneous type, LC: lymphocutaneous type, DC: disseminated cutaneous type, MGC: multinucleated giant cells, NA: not applicable.

Acknowledgements

We would like to express sincere thanks to Associate Professor Dr. Ariya Chindamporn, Dr. Navaporn Worasilchai, and Siriporn Wongdindam for their invaluable help for fungal isolation, fungal identification, and drug susceptibility.

References

[1] M.B. Barros, R. de Almeida Paes, A.O. Schubach, Sporothrix schenckii and sporotrichosis, Clin. Microbiol. Rev. 24 (4) (2011) 633–654.
[2] S.L. Crothers, S.D. White, P.J. Ihrke, V.K. Affolter, Sporotrichosis: a retrospective evaluation of 23 cases seen in northern California (1987-2007), Vet. Dermatol. 20 (4) (2009) 249–259.
[3] C. Kwangsukstith, N. Vanittanakom, P. Khanjanasthiti, C. Uthammachai, Cutaneous sporotrichosis in Thailand: first reported case, Mycoses 33 (11–12) (1990) 513–517.
[4] N. Taninratapat, C. Srisuttiyakorn, Localized cutaneous sporotrichosis on face in healthy Thai female, Mycopathologia 184 (4) (2019) 539–542.
[5] K. Ruangritchankul, A. Chindamporn, N. Worasilchai, U. Poumsuk, S. Keelawat, A. Bychkov, Invasive fungal disease in university hospital: a PCR-based study of autopsy cases, Int. J. Clin. Exp. Pathol. 8 (11) (2015) 14840–14852.
[6] A. Chakrabarti, A. Bonifaz, M.C. Gutierrez-Galhardo, T. Mochizuki, S. Li, Global epidemiology of sporotrichosis, Med. Mycol. 53 (1) (2015) 3–14.
[7] A.M. Rodrigues, M. de Melo Teixeira, G.S. de Hoog, T.M. Schubach, S.A. Pereira, G.F. Fernandes, et al., Phylogenetic analysis reveals a high prevalence of Sporothrix brasiliensis in feline sporotrichosis outbreaks, PLoS Neglected Trop. Dis. 7 (6) (2013) e2281.
[8] P.N. Newton, W.H. Chung, R. Phetsouvanh, N.J. White, Sporotrichosis, plain of jars, Lao people's democratic republic, Emerg. Infect. Dis. 11 (9) (2005) 1496–1497.
[9] R. Ramli, A.H. Abd Rashid, K.S. Phang, T.M. Khaithir, Sporotrichosis atypical presentation as a soft tissue tumour, Malays. J. Pathol. 31 (2) (2009) 143–145.
[10] M.M. Tang, J.J. Tang, P. Gill, C.C. Chang, R. Baba, Cutaneous sporotrichosis: a six-year review of 19 cases in a tertiary referral center in Malaysia, Int. J. Dermatol. 51 (6) (2012) 702–708.
[11] F.B. Yap, Disseminated cutaneous sporotrichosis in an immunocompetent individual, Int. J. Infect. Dis. 15 (10) (2011) e727–e729.