DR CHI-CHING TSANG (Orcid ID : 0000-0001-6705-2866)
PROFESSOR PATRICK CY WOO (Orcid ID : 0000-0001-9401-1832)

Article type : Original Article

Clinical characteristics, rapid identification, molecular epidemiology and antifungal susceptibilities of *Talaromyces marneffei* infections in Shenzhen, China

Susanna K. P. Lau[1,2,3,4,5,*,#], Fanfan Xing[5,*], Chi-Ching Tsang[1,*], James Y. M. Tang[1], Yen-Pei Tan[1], Haiyan Ye[5], Ricky W. T. Lau[5], Jonathan H. K. Chen[1], Simon K. F. Lo[5], Patrick C. Y. Woo[1,2,3,4,5,#]

[1]Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

[2]State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong

[3]Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong

[4]Collaborative Innovation Centre for Diagnosis and Treatment of Infectious Diseases, The University of Hong Kong, Hong Kong

[5]Department of Clinical Microbiology and Infection Control, The University of Hong Kong–Shenzhen Hospital, 518053 Shenzhen, China

[*]These authors contributed equally to this article.

Short title: *Talaromyces marneffei* infections in Shenzhen

Keywords: *Talaromyces marneffei*, talaromycosis, rapid identification, molecular epidemiology, antifungal susceptibilities

[#]Corresponding authors: Patrick C. Y. Woo and Susanna K. P. Lau, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 19/F, Block T, Queen Mary Hospital, Pokfulam, Hong Kong. Phone: +852-2255-4892. Fax: +852-2855-1241; E-mails: pcywoo@hku.hk (Patrick C. Y. Woo) and skplau@hku.hk (Susanna K. P. Lau)

SUMMARY

Although case series of talaromycosis have been reported in China, their detailed clinical and microbiological characteristics have never been systematically profiled. In this study, we report the clinical characteristics, molecular epidemiology, rapid identification and antifungal susceptibilities of talaromycosis in The University of Hong Kong–Shenzhen Hospital in Shenzhen. Seven cases of talaromycosis were observed since commencement of hospital service in 2012. Three patients were local Shenzhen residents, whereas the other four were immigrants from other parts of China. Two patients were HIV-negative, but with underlying diseases requiring immunosuppressive therapy. Two of the seven patients succumbed. All the seven isolates were successfully identified as *T. marneffei* by MALDI–TOF MS using Bruker database expanded with in-house generated *T. marneffei* mass spectra. MLST showed that the seven strains belonged to six different, novel sequences types. Phylogenetic analysis of the concatenated five-locus sequence revealed that the seven strains were scattered amongst other *T. marneffei* strains. The MICs of itraconazole, isavuconazole, posaconazole

and voriconazole against the seven clinical isolates were low but MICs of anidulafungin were high. Underlying diseases other than HIV infection are increasingly important risk factors of talaromycosis. MALDI–TOF MS is useful for rapid identification. Highly diverse *T. marneffei* sequence types were observed.

INTRODUCTION

Talaromyces marneffei, previously named as *Penicillium marneffei*, is the most important thermally dimorphic fungus causing respiratory, skin and systemic mycosis in China and Southeast Asia.[1,2] After its discovery in 1956, only 18 cases of human diseases were reported until 1985.[3] The appearance of the HIV pandemic saw the emergence of the infection as an important opportunistic mycosis in HIV-positive patients. About 8% of AIDS patients in Hong Kong are infected with *T. marneffei*.[4] In northern Thailand, *T. marneffei* infection is the third most common indicator disease of AIDS, following tuberculosis and cryptococcosis.[5] Besides HIV-positive patients, *T. marneffei* infections have been reported in other immunocompromised patients, such as transplant recipients, patients with systemic lupus erythematosus, those on corticosteroid therapy, with anti-interferon-gamma autoantibody, and receiving anti-CD20 monoclonal antibodies or kinase inhibitors.[6] Apart from China and Southeast Asia, imported cases of *T. marneffei* infections have also been reported in non-endemic countries.[2]

Although case series of *T. marneffei* infections have been reported in China, the clinical characteristics, molecular epidemiology and antifungal susceptibilities have never been systematically profiled. In 2007, we described a multilocus sequence typing (MLST) scheme for *T. marneffei* and characterised the strains in Hong Kong.[7] Recently, we also reported the use of matrix-assisted laser desorption ionisation–time of flight mass spectrometry (MALDI–TOF MS) for identification of *T. marneffei*[8] and the susceptibilities of *T. marneffei* strains from Hong Kong against

amphotericin B, itraconazole, posaconazole and voriconazole.[9] In this study, we report the clinical characteristics, molecular epidemiology, rapid identification and antifungal susceptibilities of *T. marneffei* infections in Shenzhen, a Southern Chinese metropolitan city with a large immigrant population from other parts of China.

MATERIALS AND METHODS

Patients and fungal isolates. This was a retrospective study conducted over a six-year period (1 July 2012 to 30 June 2018) in The University of Hong Kong–Shenzhen Hospital. This 1,400-bed multi-specialty hospital was established in 2012 and provides primary to tertiary medical services to the residents of Shenzhen city in both inpatient and outpatient settings. Shenzhen is a Special Economic Zone with an estimated population of nearly 11 million people including a large migrant population from other regions in China. Geographically, it is located in the Guangdong Province, immediately north to Hong Kong. Affected by the policy of the government in mainland China, Shenzhen has been one of the fastest growing cities in the world during the 1990s. Clinical details of all patients with *T. marneffei* infections were retrieved and analysed. Ethics approval for this study was provided by the Institutional Review Board of The University of Hong Kong–Shenzhen Hospital. All *T. marneffei* strains recovered were identified by conventional phenotypic tests and MALDI–TOF MS (as described below); and were maintained on Sabouraud's dextrose agar (SDA; Difco, BD Diagnostics Systems, USA) supplemented with chloramphenicol (50 μg/mL; Calbiochem, USA).

MALDI–TOF MS. MALDI–TOF MS was performed using the ethanol-formic acid extraction method according to the manufacturer's instruction and our previous publication[8] with slight modifications. Briefly, *T. marneffei* strains were cultured on SDA in the yeast phase at 35°C for 7–12 days. For each strain, after colony maturation, cells were harvested with a sterile cotton swab and suspended in 300 μL of autoclaved distilled water. After washing the cells were resuspended in 300

µL of autoclaved distilled water and 900 µL of absolute ethanol (Merck, Germany). The mixture was then vortexed and centrifuged at 16,100 × *g* for 2 min. The supernatant was then removed, and the pellet was air-dried. Subsequently, the pellet was resuspended in 30–50 µL of 70% formic acid (Merck) and an equal volume of acetonitrile (Merck), followed by centrifugation at 16,100 × *g* for 2 min. Using the IVD Bacterial Test Standard (BTS) (Bruker Daltonics, Germany) as a control, one microliter of the supernatant was transferred to an individual spot on the MSP 96 polished steel BC targets plate (Bruker Daltonics), and was air-dried. Each spot was further overlaid with the α-cyano-4-hydroxycinnamic acid (HCCA) matrix (Sigma-Aldrich), and was air-dried. The target plate was then loaded into the microflex LT system (Bruker Daltonics), where spectra with *m*/*z* values of 2,000 to 20,000 Da were obtained with an accelerating voltage of 20 kV in linear mode. Fungal identification was achieved by comparing the mass spectra generated in this study with those in the reference database Filamentous Fungi Library 1.0 (Bruker) expanded with in-house generated *T. marneffei* mass spectra obtained in our previous study.[8]

Molecular typing. MLST for *T. marneffei* was performed as we described previously.[7] Briefly, DNA extraction was achieved by firstly disrupting fungal cells with glass beads (Sigma-Aldrich, USA) using TissueLyser II (Qiagen, Germany) and then the released fungal DNA was purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's protocol. One microlitre of the extracted DNA was used for PCR-amplification, utilising the AmpliTaq Gold DNA polymerase (Applied Biosystem, USA), of the five *MP1* homologues (*MP1*, *MPLP4*, *MPLP7*, *MPLP10* and *MPLP13*) used for MLST. The primers used for PCR were described previously.[7] After agarose gel electrophoresis, the PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's protocol. Both strands of the PCR products were sequenced by the ABI PRISM 3130*xl* Genetic Analyzer (Applied Biosystems), using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the PCR primers. The nucleotide sequences of the five gene loci used for MLST were aligned with those of other *T. marneffei* strains using MUSCLE 3.8.[10] Dendrogram reconstruction was performed with Sequence Type Analysis and Recombinational Tests

(START) 2 0.9.0 beta by the unweighted pair group method with arithmetic mean (UPGMA)[11] and MEGA 6.0.6 by the maximum likelihood method using the substitution model Tamura 3-parameter (T92) with estimated proportion of invariable sites (I).[12] Sequence types were assigned according to the five-locus MLST scheme previously published.[7] Lineage analysis was performed using eBURSTv3.[13,14]

Antifungal susceptibility testing. Antifungal susceptibility testing was performed using the broth microdilution method according to both the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standard Institute (CLSI) methodologies.[15,16] The antifungal drug tested included amphotericin B (Cayman Chemical, USA), anidulafungin (TargetMol, USA), isavuconazole (TargetMol), itraconazole (Sigma-Aldrich), posaconazole (Sigma-Aldrich) and voriconazole (TargetMol). For the EUCAST methodology, the test range for all the drugs was 0.008–4 μg/mL; whereas for the CLSI methodology, the test range for amphotericin B, isavuconazole, itraconazole, posaconazole and voriconazole was 0.03–16 μg/mL and that for anidulafungin was 0.015–8 μg/mL. Results were read after three days of incubation. *Candida parapsilosis* ATCC 22019^T and *Pichia kudriavzevii* (synonym: *Candida krusei*) ATCC 6258^T were used as quality controls.

Nucleotide accession numbers. The *MP1*, *MPLP4*, *MPLP7*, *MPLP10* and *MPLP13* sequences of the seven *T. marneffei* isolates were deposited in the DDBJ/ENA/GenBank databases with the accession numbers LC428220–LC428254 (Table S1).

RESULTS

Clinical characteristics. A total of seven cases of *T. marneffei* infections were observed in the last six years, since the commencement of service of the hospital in 2012 (Table 1). Only one case was observed each in 2014, 2015 and 2016, while four cases occurred in 2018, in line with the gradual

opening of more beds and expansion of various services in the hospital. The male to female ratio was 6:1, with a median age of 44 (range 30–75) years. All seven patients were of the Han ethnic group. Three (Cases 1–3) of the seven patients were local residents of Shenzhen, whereas the other four (Cases 4–7) went to Shenzhen to work. These four patients were also from Southern China, including Guangdong (Case 4), Chongqing (Case 5), Guangxi (Case 6) and Jiangsu (Case 7) (Fig. 1). Four patients (Cases 1, 4, 5 and 6) were HIV-positive and two (Cases 3 and 7) were HIV-negative. These two patients had other underlying diseases requiring immunosuppressive therapy. The HIV status of the remaining one patient (Case 2) was indeterminate. The initial result was equivocal but unfortunately a second blood sample was not taken as the patient died rapidly after admission. Two (Cases 2 and 7) of the seven patients succumbed.

MALDI–TOF MS. All the seven clinical isolates were successfully identified as *T. marneffei* by MALDI–TOF MS using the Bruker database expanded with in-house generated *T. marneffei* mass spectra, with top match scores ranging from 2.29–2.63.

Molecular typing. MLST characterisation showed that the seven Shenzhen strains belonged to six different sequences types (ST-36–ST-41). Their allelic profiles were listed in Table 2. A new *MPLP13* allele (allele 6) was identified for strain PM61. This allele was almost identical to allele 4,[7] except for a G→C transversion substitution at position 70 (overall 99.8% sequence identity). The six sequence types observed in the present study were novel and different from those previously identified in *T. marneffei* strains from Hong Kong.[7] Moreover, the sequence types for five of the Shenzhen strains (ST-36–ST-39 and ST-41) were unique; whereas the last sequence type (ST-40) was shared by two Shenzhen strains. Phylogenetic analyses of the concatenated five-locus sequence revealed that the seven Shenzhen strains did not cluster together and were scattered throughout the tree (Fig. 2). Lineage analysis defined two main clonal complexes and 17 singletons. Clonal complex 1 consisted of 23 sequence types, including the novel sequence types ST-36, ST-37, ST-38, ST40 and ST41; whereas there were two sequence types in clonal complex 2, namely ST-23 and ST-

33. ST-38 was predicted as the primary founder and ST-14, ST-15, ST-27 and ST-36 were predicted as the sub-group founders for clonal complex 1 while no sequence type was predicted as primary or sub-group founder for clonal complex 2 (Fig. 3).

Antifungal susceptibility testing. The *in vitro* susceptibilities of the seven *T. marneffei* strains to six different antifungal drugs are listed in Table 3. The minimum inhibitory concentrations (MICs) of triazole agents, including itraconazole, isavuconazole, posaconazole and voriconazole, determined using both the EUCAST and CLSI methodologies against the seven clinical isolates were generally in congruence and were low (≤0.008–0.25 μg/mL). In contrast, anidulafungin possessed high MICs against the *T. marneffei* strains (2–>4 μg/mL in most cases), except that the MICs determined using the CLSI methodology against isolates PM60 and PM62 were 0.5 μg/mL. As for amphotericin B, the MICs determined using the CLSI methodology were mostly 2–8-fold higher than those obtained using the EUCAST protocol, except for strain PM63 whose MIC by EUCAST method was one $\log_2$ concentration higher than the MIC by CLSI protocol (Table 3).

DISCUSSION

Shenzhen is a unique Special Economic Zone of China with a significant proportion of the population being migrants from other parts of the country. The establishment of The University of Hong Kong–Shenzhen Hospital, which is financed by the mainland Chinese government but administered mainly by The University of Hong Kong, has given us an unprecedented unique opportunity to study and understand infectious diseases in mainland China. Among the seven patients with *T. marneffei* infections in the present series since the opening of the hospital six years ago, only three were locally born Shenzhen residents. The other four were from Heyuan in Guangdong Province, Guangxi Province, Chongqing (one of China's four direct-controlled municipalities geographically located next to the Sichuan Province) and Xuzhou in Jiangsu Province (Fig. 1). Since Guangdong Province, Guangxi

Province and Chongqing city are all in Southern China where *T. marneffei* infection is endemic, it is difficult to distinguish whether the *T. marnffei* infections in these three patients were acquired in Shenzhen or their hometowns.

Underlying diseases other than HIV infection are increasingly important for *T. marneffei* infection. Traditionally, HIV infection is the single most important underlying condition resulting in *T. marneffei* infection.[5] With the advancement of antiretroviral therapy, a significant proportion of HIV infections, particularly in developed countries, are relatively under reasonable treatment.[17] At the same time, with the advancement of underlying of more secondary immunodeficiency conditions, other underlying conditions, some with previously undescribed specific molecular mechanisms, associated with *T. marneffei* conditions are increasingly recognised. Some notable examples include anti-interferon-gamma antibody[18] and target therapies for haematological conditions such as anti-CD20 monoclonal antibodies and kinase inhibitors.[19] Patients with impaired interferon-gamma immunity due to autosomal dominant gain-of-phosphorylation STAT1 mutations[20] were observed. Recently, we have also described the first report of *T. marneffei* infection in a patient with autoimmune hepatitis receiving prednisolone treatment.[21] In the present series, two of the seven patients were HIV-negative. One of them (Case 3) had systemic lupus erythematosus and Sjogren's syndrome and was on methylprednisolone, mycophenolate mofetil and hydroxychloroquine. The other one (Case 7) was a patient with myelodysplastic syndrome diagnosed in 2016 when the patient presented with disseminated *Mycobacterium kansasii* infection. In 2017, the patient was put on prednisone because of adrenocorticoid insufficiency. In 2018, the patient was admitted because of recurrent fever and pneumonia and *T. marneffei* was isolated from his bronchoalveolar lavage. One month later, acute myeloid leukaemia was diagnosed and the patient died rapidly despite treatment.

MALDI–TOF MS is useful for rapid identification of *T. marneffei* and the seven *T. marneffei* strains are susceptible to isavuconazole in addition to itraconazole, voriconazole and posaconazole. Our recent study showed that based on the Bruker original database combined with BDAL v4.0.0.1 and Filamentous Fungi Library 1.0, MALDI–TOF MS failed to identify the 60 *T. marneffei* strains grown in mould and yeast phases. However, when the combined database was expanded with inclusion of spectra from ~20 *T. marneffei* strains in mould and/or yeast phases, all the remaining ~40 *T. marneffei* strains grown in mould or yeast phases were correctly identified to the species level with scores >2.0. In the present study, all the seven strains of *T. marneffei* isolated from patients in Shenzhen were accurately identified by this expanded database. As for antifungal susceptibility and treatment, *T. marneffei* is known to be susceptible *in vitro* to various antifungal agents including amphotericin B, itraconazole and terbinafine [22,23]. Besides itraconazole, voriconazole is another azole also effective and well-tolerated for the treatment of talaromycosis.[23,24] Posaconazole and isavuconazole, two triazoles with broad anti-fungal activities, may offer additional advantages over itraconazole and voriconazole especially in critically ill patients with organ dysfunction, as no renal or hepatic dosage adjustment is required. Recently, we reported promising activities of posaconazole against *T. marneffei*.[9] In this study, we observed that the activity of isavuconazole is comparable to posaconazole against the seven *T. marneffei* strains. With its very good bioavailability and absorption not affected by food and gastric pH, isavuconazole is an alternative antifungal agent for the treatment of *T. marneffei* infections.

Molecular typing, using our published MLST scheme based on *MP1* and its four homologues,[7] showed that the seven *T. marneffei* strains are of six different sequence types. In 2007, we observed that the nucleotide sequences of some selected housekeeping genes were highly conserved.[7] Therefore, we have designed an MLST scheme using five lineage-specific genes.[7] Using this scheme, 44 *T. marneffei* strains collected in Hong Kong were assigned to 35 sequence types, resulting in an MLST scheme with discriminatory power of 0.9884.[7] In the present study, all the seven *T. marneffei* strains belonged to newly described sequence types, most of which were unique.

Similar to the situation in Hong Kong,[7] no predominant *T. marneffei* sequence types were circulating in Shenzhen. No separate clustering was observed for the seven Shenzhen clinical isolates amongst all the *T. marneffei* strains, which did not suggest any epidemiological relationship between the sequence types and the geographical origins of the strains (Fig. 2). Lineage analysis revealed that the sequence types could be grouped into two main clonal complexes, in contrast to the four 'BURST groups' identified in our previous study.[7] Detailed examination on the two new clonal complexes obtained in the present study showed that clonal complex 2 actually corresponded to the previous 'BURST group 4'; whereas clonal complex 1 was formed as a result of the merging of the former 'BURST groups 1, 2 and 3', which were connected by the novel sequence types ST-36 and ST-38 (Fig. 3). It is of note that when sequences of the five MLST loci were also retrieved from the DDBJ/ENA/GenBank databases for the *T. marneffei* ex-type strain ATCC 18224^{T} (isolated from a Chinese bamboo rat [*Rhizomys sinensis*] in Vietnam),[25] it was found that this ex-type strain possessed novel alleles for *MP1* (allele 12), *MPLP10* (allele 6) and *MPLP13* (allele 7), leading to one additional new sequence type (ST-42) (Table 2), which is a new singleton as shown by lineage analysis (Fig. 3), suggesting that it is not closely related to any of the human isolates in the present and our previous studies.[7] Molecular typing for more *T. marneffei* strains from rodent origins could help delineate the relationships between clinical and murine isolates more clearly.

ACKNOWLEDGEMENTS

This work was partly supported by the General Research Fund (No. 17104318), University Grants Committee, Hong Kong; Health and Medical Research Fund (No. HKM-15-M07 [commissioned project]), Food and Health Bureau, Government of the Hong Kong Special Administrative Region, Hong Kong; as well as the Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Ministry of Education, China.

CONFLICT OF INTEREST

Patrick C. Y. Woo has provided scientific advisory/laboratory services for Gilead Sciences, Incorporated; International Health Management Associates, Incorporated; Merck & Corporation, Incorporated and Pfizer, Incorporated. The other authors report no conflicts of interest. The funding sources had no role in study design, data collection, analysis, interpretation, or writing of the report. The authors alone are responsible for the content and the writing of the manuscript.

REFERENCES

1. Deng Z, Ribas JL, Gibson DW, Connor DH. Infections caused by *Penicillium marneffei* in China and Southeast Asia: review of eighteen published cases and report of four more chinese cases. *Rev Infect Dis.* 1988; 10:640–652.

2. Vanittanakom N, Cooper CR, Fisher MC, Sirisanthana T. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. *Clin Microbiol Rev.* 2006; 19:95–110.

3. Deng Z, Connor DH. Progressive disseminated penicilliosis caused by *Penicillium marneffei*: report of eight cases and differentiation of the causative organism from *Histoplasma capsulatum*. *Am J Clin Pathol.* 1985; 84:323–327.

4. Low K, Lee SS. The pattern of AIDS Reporting and the implications on HIV surveillance. *Public Health Epidemiol Bull.* 2002; 11:41–49.

5. Supparatpinyo K, Khamwan C, Baosoung V, Sirisanthana T, Nelson KE. Disseminated *Penicillium marneffei* infection in Southeast Asia. *Lancet.* 1994; 344:110–113.

6. Chan JFW, Lau SKP, Yuen K-Y, Woo PCY. *Talaromyces* (*Penicillium*) *marneffei* infection in non-HIV-infected patients. *Emerg Microbes Infect.* 2016; 5:e19.

7. Woo PCY, Lau CCY, Chong KTK*, et al.* MP1 homologue-based multilocus sequence system for typing the pathogenic fungus *Penicillium marneffei*: a novel approach using lineage-specific genes. *J Clin Microbiol.* 2007; 45:3647–3654.

8. Lau SKP, Lam CSK, Ngan AHY*, et al.* Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for rapid identification of mold and yeast cultures of *Penicillium marneffei*. *BMC Microbiol.* 2016; 16:36.

9. Lau SKP, Lo GCS, Lam CSK*, et al. In vitro* activity of posaconazole against *Talaromyces marneffei* by broth microdilution and Etest methods and comparison to itraconazole, voriconazole, and anidulafungin. *Antimicrob Agents Chemother.* 2017; 61:e01480-16.

10. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 2004; 32:1792–1797.

11. Jolley KA, Feil EJ, Chan MS, Maiden MCJ. Sequence type analysis and recombinational tests (START). *Bioinformatics.* 2001; 17:1230–1231.

12. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol Biol Evol.* 2013; 30:2725–2729.

13. Feil EJ, Li BC, Aanensen DM, Hanage WP, Spratt BG. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. *J Bacteriol.* 2004; 186:1518–1530.

14. Spratt BG, Hanage WP, Li B, Aanensen DM, Feil EJ. Displaying the relatedness among isolates of bacterial species – the eBURST approach. *FEMS Microbiol Lett.* 2004; 241:129–134.

15. Arendrup MC, Meletiadis J, Mouton JW*, et al. EUCAST Definitive Document E.Def 7.3.1 Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Antifungal Agents for Yeasts*. 2017.

16. Clinical and Laboratory Standards Institute (CLSI). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition. CLSI Document M27-A3*. Wayne: CLSI; 2008.

17. Geretti AM, Tsakiroglou M. HIV: new drugs, new guidelines. *Curr Opin Infect Dis.* 2014; 27:545–553.

18. Chan JFW, Trendell-Smith NJ, Chan JCY*, et al.* Reactive and infective dermatoses associated with adult-onset immunodeficiency due to anti-interferon-gamma autoantibody: Sweet's syndrome and beyond. *Dermatology.* 2013; 226:157–166.

19. Chan JFW, Chan TSY, Gill H*, et al.* Disseminated infections with *Talaromyces marneffei* in non-AIDS patients given monoclonal antibodies against CD20 and kinase inhibitors. *Emerg Infect Dis.* 2015; 21:1101–1106.

20. Lee PPW, Mao H, Yang W*, et al. Penicillium marneffei* infection and impaired IFN-γ; immunity in humans with autosomal-dominant gain-of-phosphorylation STAT1 mutations. *J Allergy Clin Immunol.* 2014; 133:894–896.e5.

21. Wong SCY, Sridhar S, Ngan AHY*, et al.* Fatal *Talaromyces marneffei* infection in a patient with autoimmune hepatitis. *Mycopathologia.* 2018; 183:615–618.

22. McGinnis MR, Nordoff NG, Ryder NS, Nunn GB. In vitro comparison of terbinafine and itraconazole against *Penicillium marneffei*. *Antimicrob Agents Chemother.* 2000; 44:1407–1408.

23. Liu D, Liang L, Chen J. In vitro antifungal drug susceptibilities of *Penicillium marneffei* from China. *J Infect Chemother.* 2013; 19:776–778.

24. Supparatpinyo K, Schlamm HT. Voriconazole as therapy for systemic *Penicillium marneffei* infections in AIDS patients. *Am J Trop Med Hyg.* 2007; 77:350–353.

25. Capponi M, Sureau P, Segretain G. Pénicilliose de *Rhizomys sinensis*. *Bull Soc Pathol Exot.* 1956; 49:418–421.

LEGENDS TO FIGURES

Fig. 1 Geographical locations of the hometowns of the patients reported in this study in mainland China.

Fig. 2 Phylogenetic trees showing the relationship of the seven Shenzhen clinical isolates to other *Talaromyces marneffei* strains, inferred from the concatenated five-locus (*MP1*, *MPLP4*, *MPLP7*, *MPLP10* and *MPLP13*) sequence data using the (a) unweighted pair group method with arithmetic mean and (b) maximum likelihood method. The scale bars indicate the estimated numbers of substitutions per base. Nucleotide accession numbers are listed in Supplementary Table S1. The seven Shenzhen strains are highlighted in bold red whereas the ex-type of *T. marneffei* (strain ATCC 18224^{T}) is highlighted in bold blue. For (b), numbers at nodes indicate levels of bootstrap support calculated from 1,000 trees and are expressed as percentage. Only nodes that were well supported (≥70% bootstrap support) have their bootstrap values shown.

Fig. 3 Lineage analysis of the new sequence types (STs) reported in this study (in bold) in comparison to those previously assigned.[7] Each number represents an ST, and each line connects STs that are identical for at least four of the five multilocus ST loci. There are two main clonal complexes, with the predicted primary and sub-group founders, if any, highlighted in blue and green colours, respectively. The size of each dot is proportional to the number of isolates included in the analysis for each ST.

Table 1. Clinical and epidemiological data of the seven patients with *Talaromyces marneffei* infections

Case no.	Year	Sex/Age	Ethnicity	Hometown	Purpose of staying in Shenzhen	HIV status	Other underlying diseases	Clinical specimens positive for *T. marneffei*	Outcome
1	2014	M/33	Han	Shenzhen	Hometown	Positive	–	Blood	Survived
2	2015	M/73	Han	Shenzhen	Hometown	Indeterminate	–	Blood, sputum, tracheal aspirate	Succumbed
3	2016	F/75	Han	Shenzhen	Hometown	Negative	SLE[a] and Sjogren's syndrome on methylprednisolone, mycophenolate mofetil and hydroxychloroquine	Blood	Survived
4	2018	M/30	Han	Heyuan, Guangdong	Working	Positive	–	Blood, nasal swab	Survived
5	2018	M/44	Han	Chongqing	Working	Positive	–	Blood	Survived
6	2018	M/41	Han	Guangxi	Working	Positive	–	Blood, BAL[b],	Survived

7	2018	M/59	Han	Xuzhou, Jiangsu	Working	Negative	Myelodysplastic syndrome on prednisone, blastic transformation	skin abscess BAL[b]	Succumbed

[a]SLE, systemic lupus erythematosus; [b]BAL, bronchoalveolar lavage

Table 2. Allelic profiles and sequence types for the seven Shenzhen *Talaromyces marneffei* clinical strains and ex-type strain ATCC 18224^T

Strain	Allelic profile (*MP1*, *MPLP4*, *MPLP7*, *MPLP10*, *MPLP13*)[a]	Sequence type
PM52	4,1,2,2,1	ST-36
PM53	3,1,1,13,1	ST-37
PM60	3,2,2,4,1	ST-38
PM61	4,2,1,10,**6**	ST-39
PM62	6,2,2,2,1	ST-40
PM63	3,2,1,6,3	ST-41
PM64	6,2,2,2,1	ST-40
ATCC 18224^T	**12**,3,2,**16**,**7**	ST-42

[a]Novel alleles identified in the present study are highlighted in bold

Table 3. Minimum inhibitory concentrations of different antifungal agents against the *Talaromyces marneffei* strains isolated in this study

Strain	Minimum inhibitory concentration (μg/mL)											
	Amphotericin B		Anidulafungin		Isavuconazole		Itraconazole		Posaconazole		Voriconazole	
	EUCAST[a]	CLSI[b]	EUCAST[a]	CLSI[b]	EUCAST[a]	CLSI[b]	EUCAST[a]	CLSI[b]	EUCAST[a]	CLSI[b]	EUCAST[a]	CLSI[b]
PM52	0.25	1	>4	>4	≤0.008	≤0.008	≤0.008	0.016	≤0.008	0.016	≤0.008	≤0.008
PM53	0.25	2	>4	2	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008
PM60	0.25	1	>4	0.5	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	0.016	≤0.008	≤0.008
PM61	0.25	1	>4	>4	≤0.008	≤0.008	≤0.008	0.03	≤0.008	0.03	≤0.008	≤0.008
PM62	0.25	1	>4	0.5	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008
PM63	1	0.5	>4	>4	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008	≤0.008
PM64	0.25	0.5	>4	>4	0.015	0.03	≤0.008	0.015	≤0.008	0.015	0.015	0.25

[a]European Committee on Antimicrobial Susceptibility Testing and [b]Clinical and Laboratory Standard Institute