A Rare Fungal Species, *Quambalaria cyanescens*, Isolated from a Patient after Augmentation Mammoplasty – Environmental Contaminant or Pathogen?

Xin Fan1*, Meng Xiao1*, Fanrong Kong2, Timothy Kudinha2,3, He Wang1, Ying-Chun Xu1*

1 Department of Clinical Laboratory, Peking Union Medical College Hospital, and Graduate School, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China, 2 Centre for Infectious Diseases and Microbiology Laboratory Services, Westmead Hospital, Westmead, New South Wales, Australia, 3 Charles Sturt University, Orange, New South Wales, Australia

Abstract

Some emerging but less common human fungal pathogens are known environmental species and could be of low virulence. Meanwhile, some species have natural antifungal drug resistance, which may pose significant clinical diagnosis and treatment challenges. Implant breast augmentation is one of the most frequently performed surgical procedures in China, and fungal infection of breast implants is considered rare. Here we report the isolation of a rare human fungal species, *Quambalaria cyanescens*, from a female patient in China. The patient had undergone bilateral augmentation mammoplasty 11 years ago and was admitted to Peking Union Medical College Hospital on 15 September 2011 with primary diagnosis of breast infection. She underwent surgery to remove the implant and fully recovered thereafter. During surgery, implants and surrounding tissues were removed and sent for histopathology and microbiology examination. Our careful review showed that there was no solid histopathologic evidence of infection apart from inflammation. However, a fungal strain, which was initially misidentified as "Candida tropicalis" because of the similar appearance on CHROMagar *Candida*, was recovered. The organism was later on re-identified as *Q. cyanescens*, based on sequencing of the rDNA internal transcribed spacer region rather than the D1/D2 domain of 26S rDNA. It exhibited high MICs to 5-flucytosine and all echinocandins, but appeared more susceptible to amphotericin B and azoles tested. The possible pathogenic role of *Q. cyanescens* in breast implants is discussed in this case, and the increased potential for misidentification of the isolate is a cause for concern as it may lead to inappropriate antifungal treatment.

Introduction

Human beings live in a fungal-rich and fungal-diverse environment. Some emerging less common fungal pathogens are known environmental species (from soil, plants, insects, medical facilities, wastes or other outdoor or indoor environments). These fungal organisms are generally of low virulence and some may exhibit natural antifungal drug resistance [1–3], which presents clinical diagnosis and treatment challenges [4–6]. While some of the fungal infections can be diagnosed easily, in particular if isolated from blood, cerebrospinal fluid, etc., and with clear infection clinical signs, others present challenges in understanding their role in certain infections. Here we report an interesting case to highlight the challenges clinical pathologists and medical doctors faced in the decision making process of a case involving a rare fungus.

The *Quambalariaceae* is a family of fungi in the class *Exobasidiomycetes*. The family contains the single genus *Quambalaria*, which contains five species, including *Quambalaria cyanescens*, *Q. coyrecup*, *Q. eucalypti*, *Q. pitereka* and *Q. simpsonii* [7–9]. The first *Q. cyanescens* strain was isolated from human skin (strain no. CBS 357.73, type strain of the species) and reported as *Sporothrix cyanescens* by de Hoog et al. in 1973 (Table 1) [10]. In 1987, Moore et al. erected the genus *Cerinosterus*, reset the previous *S. cyanescens* into this new genus and renamed it as *Cerinosterus cyanescens* [8]. However, a later study by analysis of partial large subunit (LSU)-rDNA sequences and the nutritional profile revealed that *C. cyanescens* was a close relative of *Microstroma juglandis*, but differed from other species within...
Table 1. Summary of Q. cyanescens isolates from this study, published literatures or GenBank, and genetic comparison within Q. cyanescens species and to selected strains of other Quambalaria species.

| Strain          | Country     | Origin        | ITS GenBank accession no. | Identity (%) | D1/D2 GenBank accession no. | Identity (%) | Reference                  |
|-----------------|-------------|---------------|---------------------------|--------------|-----------------------------|--------------|----------------------------|
| **Q. cyanescens** Type strain |             |               |                           |              |                             |              |                            |
| CBS 357.73      | Netherlands | Human skin    | DQ119135.1; DQ317622.1    | Reference    | DQ317615.1; AM261925.1      | Reference    | [8,10,12]                  |
| **Q. cyanescens** Human source isolate |             |               |                           |              |                             |              |                            |
| 11PU348         | China       | Implants      | KF953496.1                | 99.3         | KF953497.1                  | 100.0        | This study                  |
| **Q. cyanescens** Environmental isolates |             |               |                           |              |                             |              |                            |
| IM298177        | Australia   | Plant         | AJ535500.1                | 100.0        | NA                          | NA           | Unpublished                 |
| IM178848        | Australia   | Plant         | AJ536610.1                | 99.7         | NA                          | NA           | Unpublished                 |
| MK742           | Turkey      | Beetle        | AM261920.1                | 99.8         | AM261920.1                  | 100.0        | [12]                        |
| MK808           | Syria       | Beetle        | AM261921.2                | 100.0        | NA                          | NA           | [12]                        |
| MK1710          | Bulgaria    | Beetle        | AM261922.2                | 100.0        | NA                          | NA           | [12]                        |
| MK1617          | Spain       | Beetle        | AM261924.2                | 99.8         | NA                          | NA           | [12]                        |
| SW326           | Unknown     | Unknown       | NA                        |              | NA                          | 100.0        | [38]                        |
| CFF326          | Czech       | Beetle        | DQ119134.1                | 100.0        | DQ119136.1                  | 99.6         | [12,39]                     |
| CBS 876.73      | Australia   | Plant         | DQ317623.1                | 99.8         | DQ317616.1                  | 100.0        | [8]                         |
| WAC12952        | Australia   | Beetle        | DQ823419.1                | 100.0        | DQ823440.1                  | 100.0        | [9]                         |
| WAC12954        | Australia   | Beetle        | DQ823420.1                | 100.0        | DQ823442.1                  | 100.0        | [9]                         |
| WAC12955        | Australia   | Beetle        | DQ823421.1                | 99.9         | DQ823441.1                  | 100.0        | [9]                         |
| WAC12953        | Australia   | Beetle        | DQ823422.1                | 99.0         | DQ823443.1                  | 99.8         | [9]                         |
| BRIP48396       | Australia   | Beetle        | EF444874.1                | 99.8         | NA                          | NA           | [39]                        |
| BRIP48398       | Australia   | Beetle        | EF444875.1                | 99.9         | NA                          | NA           | [39]                        |
| BRIP48403       | Australia   | Beetle        | EF444876.1                | 100.0        | NA                          | NA           | [39]                        |
| U16             | USA         | Beetle        | HF569147.1                | 100.0        | NA                          | NA           | Unpublished                 |
| U105            | USA         | Beetle        | HF569150.1                | 100.0        | HF569150.1                  | 100.0        | Unpublished                 |
| U110            | USA         | Beetle        | HF569153.1                | 100.0        | HF569153.1                  | 100.0        | Unpublished                 |
| U121            | USA         | Beetle        | HF569155.1                | 100.0        | NA                          | NA           | Unpublished                 |
| U161            | USA         | Beetle        | HG421947.1                | 99.5         | HG421947.1                  | 100.0        | Unpublished                 |
| U163            | USA         | Beetle        | HG421948.1                | 99.5         | HG421948.1                  | 100.0        | Unpublished                 |
| U182            | USA         | Beetle        | HG421949.1                | 99.5         | HG421949.1                  | 100.0        | Unpublished                 |
| CFF4578         | USA         | Beetle        | HG421950.1                | 100.0        | HG421950.1                  | 100.0        | Unpublished                 |
| U144a           | USA         | Beetle        | HG421951.1                | 100.0        | HG421951.1                  | 100.0        | Unpublished                 |
| U100            | USA         | Beetle        | HG421952.1                | 100.0        | HG421952.1                  | 100.0        | Unpublished                 |
| CFF4580         | USA         | Beetle        | HG421953.1                | 100.0        | HG421953.1                  | 100.0        | Unpublished                 |
To resolve this problem, Sigler et al. established the new genus *Fugomyces*, and designated *C. cyanescens* as *Fugomyces cyanescens* [11,12]. Recently, phyloge-netic studies conducted by de Beer et al. have reassigned this species in the family *Quambalariaeaceae* as *Q. cyanescens*, based on the analysis of internal transcribed spacer (ITS) region and LSU sequences combined with ultrastructural characteristics [8].

*Q. cyanescens* is one of the rare clinical basidiomycetous pathogens. Most of *Q. cyanescens* isolated from the humans were reported in the 1990s, including pseudoepidemic nosocomial pneumonia cases reported in a US hospital [13], a possible pulmonary case in a heart transplant patient [14] and potential fungemia in lymphoma patients [11]. However, none of these published human-related cases deposited convincing molecular data.

In this case study, we report the mycology and molecular characteristics of a *Q. cyanescens* isolate from a 43 year-old female who previously received injected augmentation mammoplasty, and discuss the possible pathogenic role of the organism.

### Methods

1. **Ethics statement**

The present case was from China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study. Study protocol was approved by the Human Research Ethics Committee of Peking Union Medical College Hospital (No. S-263), and written consent was obtained from the patient.

2. **Clinical case**

A 43-year old woman was admitted to the Plastic Surgical Department of Peking Union Medical College Hospital on 15 September 2011 because of left breast pain, with symptoms of redness and swelling. She had previously undergone bilateral injected augmentation mammoplasty around 11 years ago in Fujian Province, China.

The woman was in good health status except for the inflammation of the breast and did not report any other major disease in her clinical history. The blood test results were all within normal values. Clinical examination showed that she was afebrile and no ulceration was present in her left breast. Primary diagnosis was made as left breast infection. Surgical operation was performed to take out the bilateral implants as per patient’s request. However, no microbiological examination was done before surgery.

During surgery to remove the implants, it was noted that the yellow-brown semisolid implant had spilled and was mixed with unknown granule, and also there was damage in the mammary tissues. Partial implants and surrounding tissue were sent for histopathologic and microbiological laboratory examination. After surgery, cefmetazole (IV, 1 g bid) was given, combined with metronidazole (IV, 0.915 g, q12h) for 7 days. The patient fully-recovered and was subsequently discharged on 24 September 2011 before the microbiology laboratory results were finalized. She didn’t receive any antimicrobial or antifungal treatment since then, nor were any relapses reported at the 12- and 24-month follow-up visits.

3. **Initial laboratory examinations**

Microbiology and histopathology examinations were immediately performed on the partial implants and surrounding tissue from the left breast (16 September 2011). No other specimens were sent for microbiological testing. On histopathology examination, breast implants were found to be surrounded by fibrous capsules...
and infiltrated with inflammation cells and phagocytosis by giant cells and capillary hypertrophy was also observed, which indicated foreign-body reaction. However, no solid evidence of bacterial or fungal infection was found.

In the meantime, bacterial culture was performed on the partial implants and tissue by inoculating them on Columbia agar supplemented with 5% sheep blood, China-blue lactose agar and chocolate agar. However, no fungal culture was performed initially as per surgeon’s instructions. No bacteria were recovered. However, a notable amount (from the first to the second sector of the streaked plate) of yeast-like colonies were observed on Columbia blood agar on day 4 of incubation. Preliminary microscopic examination of the colonies showed yeast-like cells with a sympodial conidiogenesis. One pure colony of the isolate was then inoculated onto a chromogenic medium (CHROMagar Candida, CHROMagar Company, Paris, France) for identification, and was assigned as ‘Candida tropicalis’ on day 8 based on the production of dark blue pigments. However, the patient had been discharged before the microbiology results were finalized.

4. Sequence-based identification

The above ‘C. tropicalis’ strain was included in the CHIFNET surveillance study (strain ID no. 11PU348). Genomic DNA was extracted by beating a fungal suspension with glass beads as described before [15]. Amplification of the fungal internal transcribed spacer (ITS) region and the D1/D2 domain of the 26S rDNA gene was performed as previously described with primer pairs ITS1/ITS4 and F63/R635, respectively [15–17]. The PCR products were sequenced in both directions using corresponding PCR amplification primer pairs at Ruibiotech Co. Ltd. (Beijing, China) using the DNA analyzer ABI 3730XL system (Applied Biosystems, Foster City, CA). Species identification was performed by comparing the obtained ITS and D1/D2 sequences against those in the Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Center database and GenBank using the BioloMICSNet and BLASTn software, respectively. A sequence similarity of 97% and 99% was applied as species identification ‘cut-off’ value for the ITS region and D1/D2 domain, respectively [18].

5. Phylogenetic analysis

All Q. cyanescens ITS and D1/D2 nucleotide sequences available in GenBank till 15 November 2013 (34 and 20 sequences for the ITS region and D1/D2 domain, respectively, Table 1) were compiled. Phylogenetic analysis was performed with software MEGA (Molecular Evolutionary Genetic Analysis software, version 6.0) using the Neighbor-Joining (NJ) method [19,20], with all positions containing gaps and missing data eliminated from the data set. The significance of the cluster nodes was determined by bootstrapping with 1,000 randomizations. The evolutionary distances were computed using the Maximum Composite Likelihood method [21] and were in the units of the number of base substitutions per site. In addition, the ITS and D1/D2 sequences of Q. coryneformis WAC12947 (GenBank accession no. DQ923444.1 and DQ923451.1) [9], Q. eucalypti CMW1101 (DQ317625.1 and DQ317618.1) [8], Q. pteroeke CMW6707 (DQ317627.1 and DQ317620.1) [8], Q. simpsonii CBS124772 (GQ092990.1 and GQ093221.1) [7] and M. juglandis KR0015442 (EU069490.1 and EU069497.1) [22] were downloaded for phylogenetic comparison (Table 1).

6. Antifungal susceptibility testing

Minimum inhibitory concentrations (MICs) of Q. cyanescens 11PU348 to fluconazole, voriconazole, itraconazole, posaconazole, caspofungin, micafungin, anidulafungin, 5-flucytosine and amphotericin B were determined in vitro by broth microdilution methods as per Clinical and Laboratory Standards Institute (CLSI) M38-A2 guidelines [23]. Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 were used as the quality control strains for the test [23].

7. Nucleotide sequence accession numbers

The ITS region and D1/D2 domain sequences of strain 11PU348 were deposited in GenBank with accession numbers KF953496 and KF953497, respectively.

Results

1. Sequence-based identification

By querying ITS region and D1/D2 domain sequences against those in the CBS database, the ITS region and D1/D2 domain sequences of Q. cyanescens 11PU348 showed 99.3% (576/580 bp) and 100% (600/600 bp) similarity to the ITS and D1/D2 sequences of Q. cyanescens type strain CBS 357.73 (GenBank accession number DQ119135.1 and DQ317615.1, respectively).

2. Phylogenetic analysis

The nucleotide sequence alignments within Q. cyanescens, using sequences of Q. cyanescens type strain CBS 357.73 as references, showed this species with little inter-species variation within both the ITS region (99.0% to 100%) and D1/D2 domain (99.6% to 100%) (Table 1). Of note, the ITS region can clearly discriminate Q. cyanescens and other four Quambalaria species, with highest sequence similarity of less than 97.0%. However, the D1/D2 domain was not able to identify the five species within Quambalaria genus (sequence similarity >99.0%). The NJ analysis of the ITS region and D1/D2 domain yielded similar results (Figure 1).

3. Phenotypic characteristics on agar

Q. cyanescens isolate 11PU348 grew well at 28°C and 37°C, but failed to grow at 42°C on Sabouraud dextrose agar. By three-sector streaking on Sabouraud dextrose agar, the strain exhibited yeast-like colonies which were initially moist, smooth, of various sizes and white colored within 48 h at 28°C (Figure 2a), but creamy, butyrous and exuding dark-orange pigment after 72 h incubation (Figure 2b). However, the strain grew slower when incubated at 37°C, and tended to be mold-like, especially in the first sector of the streaked plates (Figure 2d and 2e).

After more than 2 weeks’ incubation at either 28°C or 37°C, a pure culture of the organism yielded a typical filamentous fungi phenotype that appeared to be restricted, velvety, furrowed, compact and cerebriform, accompanied by a red pigment and a burgundy reverse color. The production of pigments was more obvious at 28°C than at 37°C (Figure 2c and 2f).

On CHROMagar Candida, the colonies of Q. cyanescens 11PU348 were dark blue hybridizing with white, which was very similar to the phenotype of C. tropicalis when incubated at 37°C for 48 h (Figure 2h), but generating dark-orange pigment when incubated at 28°C (Figure 2g).

4. Microscopic morphology

Yeast-form of Q. cyanescens 11PU348 showed the typical sympodial conidiogenesis, and had smooth-walled, obovoidal, solitary or bearing secondary conidia. The filamentous form of the strain showed hyphae which were regular, hyaline, smooth-walled, branched and suberect. The conidia formed by sympodial growth.
of the conidiogenous cells (primary conidia) mostly give rise to several secondary conidia.

5. Antifungal susceptibilities

*Q. cyanescens* isolate 11PU348 exhibited high MICs to 5-flucytosine (MIC >64 μg/ml) and all echinocandins tested, including anidulafungin (MIC >8 μg/ml), micafungin (MIC = 8 μg/ml) and caspofungin (MIC = 8 μg/ml). However, the isolate appeared more susceptible to amphotericin B (MIC = 0.5 μg/ml) and azoles (MICs of fluconazole, voriconazole, itraconazole and posaconazole were 0.5 μg/ml, <0.008 μg/ml, 0.015 μg/ml and 0.015 μg/ml, respectively).

Discussion

*Q. cyanescens* is rarely identified in the clinical microbiology laboratory, and its pathogenic role is still uncertain. A review of literature shows that this fungus was recovered primarily from individuals who were immunocompromised or debilitated [11,14], including a possible pulmonary case in a heart transplant patient [14], fungemia in lymphoma patients [11]. However, none of the above studies provided unequivocal clinical evidence of infection. In addition, Jackson *et al.* reported a pseudo-epidemic of *Q. cyanescens* pneumonia in a US hospital introduced by contamination of bronchoscopy suites [13], which suggests that the species may be an environmental contaminant in human patients.

Furthermore, fungal infections due to augmentation mammoplasties are rare. To date, only 15 out of 21 cases of breast implant fungal infections have been reported [24–37] (Table 2). *Aspergillus*, *Candida*, *Curvularia*, *Paecilomyces*, *Penicillium*, and *Trichosporon* spp. were potential causative agents. Most of the cases were efficaciously managed with implant removal, but some patients recovered after intravenous antifungal therapies (Table 2).

We note that the pathogenic role of *Q. cyanescens* in this clinical case is questionable. There was no corroborating direct microscopic, histopathologic or serological evidence of fungal infection. Although the isolation was obtained from a specimen which showed histological signs of acute inflammation, this could be due to either real infection or foreign-body reaction. In addition, no samples (except routine bloods) other than the implant and the surrounding tissue removed during surgery were sent for laboratory examination, nor was repeat isolation attempted, as the present study was done retrospectively. The patient fully recovered after removal of implants, without any antifungal therapy administered. Although no other micro-organisms were isolated from this patient, and no fungal organisms were isolated from other patients who underwent plastic surgery during the same time-period, the possibility of environmental contamination cannot be excluded.

If this described case was due to a real infection, the slow progression of the inflammation, and the fact that the patient was both afebrile and asymptomatic with all blood test results within normal values, is consistent with an infection caused by a low
A previous experimental study in a murine model demonstrated that *Q. cyanescens* does have a low virulence potential [11]. Misidentification of *Q. cyanescens* 11PU348 was noted during confirmative identification process in CHIF-NET study [17].

Table 2. Fungal infections in patients after augmentation mammoplasty previously reported.

| Species             | No. of cases | Country reported | Duration (mammoplasty to infection) | Implant removal | Antifungal therapy | Reference |
|---------------------|--------------|------------------|-------------------------------------|-----------------|---------------------|-----------|
| *Candida albicans*  | 1            | Italy            | 3 years                             | No              | Caspofungin         | [25]      |
| *Candida albicans*  | 1            | Turkey           | 5 years                             | Yes             | Not specified       | [32]      |
| *Candida albicans*  | 1            | US               | 4 years                             | Yes             | Fluconazole         | [29]      |
| *Candida albicans*  | 1            | US               | 10 months                           | Yes             | Not specified       | [34]      |
| *Candida parapsilosis* | 1            | US               | 16 days                             | Yes             | Fluconazole         | [26]      |
| *Trichosporon beigelii* | 1            | US               | 16 months                           | No              | Fluconazole         | [30]      |
| *Trichosporon spp.* | 1            | Singapore        | 17 months                           | No              | Fluconazole         | [33]      |
| *Aspergillus flavus* | 1            | UK               | 18 months                           | Yes             | Not specified       | [36]      |
| *Aspergillus flavus* | 1            | US               | 4 years                             | Yes             | Not specified       | [31]      |
| *Aspergillus niger* | 1            | UK               | 5 years                             | Yes             | Not specified       | [24]      |
| *Aspergillus niger* | 1            | US               | Several months                      | Yes             | Not specified       | [35]      |
| *Curvularia spp.* | 5            | US               | 4–12 months                         | Not specified   | Not specified       | [27]      |
| *Curvularia spp.* | 1            | US               | 6 months                            | Yes             | Not specified       | [34]      |
| *Paecilomyces variotii* | 1            | US               | 14 months                           | Yes             | Not specified       | [37]      |
| *Penicillium* | 3            | US               | Not stated                          | Not specified   | Not specified       | [28]      |
l’Etoile, France; Bruker Biotyper, Bruker Daltonics, Bremen, Germany) failed to identify strain 11PUC340. Subsequent ITS sequencing identified the strain as Q. cyanescens. The main reason for the misidentification in the initial identification (that reported to clinic) was the yeast-like colonies with dark blue appearance at 45 °C in conventional RIDMAgur Candida at 37 °C, which was very similar to the appearance of C. tropicalis (Figure 2h). Although the patient in this case was cured by removal of the breast implant, the high MICs to 5-flucytosine and all echinocandins of Q. cyanescens were notable. Therefore, accurate identification of Q. cyanescens is important to avoid ineffective antifungal treatment. Mass spectroma data of Q. cyanescens were neither represented in Vitek MS nor in Bruker Biotyper identification databases. Hence both MALDI-TOF MS systems assigned “no identification” to this isolate and importantly, did not misidentify the strain to another species. Although we were not able to identify Q. cyanescens against the current commercially available library of spectra, our result will nevertheless contribute to the existing spectral building.

In the most recent study, the ITS and D1/D2 sequences were used to cluster the Quambalaria genus and replaced Q. cyanescens species from another genus [8]. But in the present study, we found that the D1/D2 domain was not able to distinguish the different species within Quambalaria genus (Figure 1; Table 1). Compared with the D1/D2 domain, the ITS region was accurate in the identification of Q. cyanescens and other species within this genus (Figure 1; Table 1).

Conclusions

In conclusion, Q. cyanescens is a rare clinical basidiomycetous pathogen. Here we report a Q. cyanescens strain isolated from a patient after augmentation mammoplasty in China. The possibility of its real pathogenic role was discussed. The high MICs to 5-flucytosine and all echinocandins highlight the importance of accurate identification so that appropriate therapy can be prescribed. To date, ITS sequencing remains the only available method to obtain an accurate identification result on this organism, while the pathogen is potentially misidentified as C. tropicalis by CHROMOmagur Candida.

Author Contributions

Conceived and designed the experiments: XF MX FK XCY. Performed the experiments: XF MX. Analyzed the data: XF MX FK. Contributed reagents/materials/analysis tools: HW. Wrote the paper: TK.

References

1. Fleming RV, Walsh TJ. Anassie EJ (2002) Emerging and less common fungal pathogens. Infect Control Clin North Am 16: 915–933, vii–viii.
2. Huprikar S, Shoham S, Practice ASTIDCo (2013) Emerging fungal infections in solid organ transplantation. Am J Transplant 13 Suppl 4: 262–271.
3. Shoham S (2013) Emerging fungal infections in solid organ transplant recipients. Infect Dis Clin North Am 27: 305–316.
4. Hauser PR, Trng LR, Hsu JJH, Liao WS, Chen YC, et al. (2001) Nosocomial Exophiala jeanesi i pseudoinfection after sonography-guided aspiration of thoracic lesions. J Formos Med Assoc 100: 615–619.
5. Pfaller MA, Diekema DJ, Crook DW, Denning DW, Hauser IA (2011) Mixed mould species in laboratory cultures of respiratory specimens: how should they be reported, and what are the indications for susceptibility testing? J Clin Pathol 64: 543–545.
6. Xiao M, Wang H, Lu J, Chen SC, Kong F, et al. (2014) Candida quercitrusa and Candida valida: investigation of three clustered cases and mycological characteristics of this novel species. J Clin Microbiol. In press.
7. Cheewangkoon K, Groenewald JZ, Summerell BA, Hyde KD, To-Anun C, et al. (2009) Myriaceae, a cache of fungal biodiversity. Persoonia 23: 53–65.
8. de Beer ZW, Berghofer D, Bauer R, Pegg GN, Coomans PA, et al. (2000) Phylogeny of the Quambalariaeaceae fam. nov., including important Excyphalous pathogens in South Africa and Australia. Stud Mycol 55: 289–298.
9. Paap T, Burgess TI, McComb JA, Shearer BL, Hardy GESJ (2008) Quambalaria species, including Q. cyanescens sp. nov., implicated in canker and shoot blight diseases causing decline of Corymbia species in the southwest of Western Australia. Mycol Res 112: 57–69.
10. de Hoog GS, de Vries GA (1973) Two new species of Sporothrix and their relation to Blastobotrys: nov. Antonie Van Leeuwenhoek 39: 515–520.
11. Sigler I, Harris JM, Dixon DM, FIn AL, Salink IF, et al. (1990) Microbiology and potential virulence of Sporothrix cyanescens, a fungus rarely isolated from blood and skin. J Clin Microbiol 28: 1009–1013.
12. Kohoutik M, Sklovská E, Paloušová S (2006) The taxonomic and ecological characterisation of the clinically important heterobasidiomycete Fumigomyces cyanescens and its association with bark beetles. Czech Mycol 38: 81–98.
13. Jackson L, Klote SA, Normand RE (1990) A pseudoeupenic of Sporothrix cyanescens pneumonia occurring during renovation of a bronchoscopy suite. J Med Vet Mycol 28: 425–439.
14. Tambini R, Farina C, Fiocchi R, Dupont B, Guelho E, et al. (1996) Possible pathogenic role for Sporothrix cyanescens isolated from a lung lesion in a heart transplant patient. J Med Vet Mycol 34: 195–198.
15. Wang H, Xiao M, Chen SC, Kong F, Sun ZY, et al. (2012) In vitro susceptibilities of yeast species to fluconazole and voriconazole as determined by the 2010 National China Hospital Invasive Fungal Surveillance Net (CHIF-NEt) study. J Clin Microbiol 50: 3952–3959.
16. Amburg DC, Burke DJ, Strathern JN (2002) Methods in yeast genetics: a Cold Spring Harbor laboratory course manual.
17. Zhang L, Xiao M, Wang H, Gao R, Fan X, et al. (2014) Yeast identification algorithm based on use of the Vitek MS system selectively supplemented with ribosomal DNA reagents/materials/analysis tools: HW. Wrote the paper: TK.
38. Hall L, Wohlfel S, Roberts GD (2003) Experience with the MicroSeq D2 large-
subunit ribosomal DNA sequencing kit for identification of commonly
encountered, clinically important yeast species. J Clin Microbiol 41: 5099–5102.
39. Pegg GS, O'Dwyer C, Carnegie AJ, Burgess TI, Wingfield MJ, et al. (2008) Quambalaria
species associated with plantation and native eucalypts in
Australia. Plant Pathology 57: 702–714.
40. Zhang Z, Wang C, Yao Z, Zhao J, Lu F, et al. (2011) Isolation and identification
of a fungal strain QY229 producing milk-clotting enzyme. European Food
Research and Technology 232: 861–866.