

An Unusual Infection in a Child with Congenital Heart Disease – *Trichosporon asahii* Infection with Rapid Diagnosis by 18s Ribonucleic Acid (RNA)

Dear Editor,

We report a case of a 12-year-old boy with cyanotic heart disease and Klippel-Feil syndrome, palliated with a left-sided Blalock-Taussig (BT) shunt in infancy, and complicated by multiple revisions secondary to thrombosis and subsequent right-sided BT shunt insertion at 4 years of age. At 11 years, he underwent staged insertion of a right ventricle to pulmonary artery (RV-PA) valved conduit (12 mm Hancock), and augmentation of the branch pulmonary arteries.

He, however, presented 6 days after discharge with fever and a purulent sternal wound discharge. He was empirically managed for presumed wound infection with ceftriaxone and vancomycin. Wound and blood cultures were unremarkable and he recovered well. Subsequently, he was admitted a further 5 times within the first year after discharge for suspected infection, managed with several courses of antibiotics including intravenous ceftriaxone, vancomycin and cloxacillin and antifungals including liposomal amphotericin. Wound surface swabs and blood cultures for bacteria and fungi during each episode were consistently negative with no significant findings on computed tomography (CT) thorax, blood biochemistry or immunology, except during the last episode, when coagulase-negative *Staphylococcus aureus* (CONS) was identified from a swab of a chest wall sinus.

The patient presented again after 14 months with fever and purulent wound discharge. Although his fever settled with intravenous vancomycin, ceftriaxone and voriconazole, repeat CT thorax revealed a large, progressive thrombus in the supra-valvular region of the RV-PA conduit, with a wedge-shaped infarct of the right lower lobe (Fig. 1). In view of the progressive nature of the thrombus, and failure to respond to antimicrobial treatment, decision was made for surgical intervention to remove the infected thrombus.

The RV-PA conduit was changed at surgery, and intraoperative findings revealed pustular debris with a large thrombus within the conduit, and friable, infected tissue in the surrounding mediastinum. Histology of the tissue revealed a granuloma with spore-forming fungus (no hyphae).

In view of multiple inconclusive previous cultures, the tissue samples were subjected to a next-generation

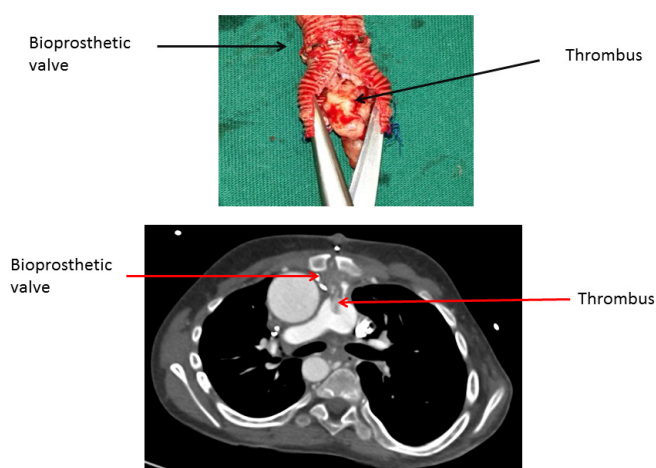


Fig. 1. Computed tomography of thorax.

sequencing (NGS), pipeline-based approach using primers targeting the 18S ribosomal ribonucleic acid (rRNA) gene, details of the primers¹ and the NGS data obtained are provided in Table 1. This molecular analysis revealed fungal deoxyribonucleic acid (DNA), matching *Hortaea werneckii*, *Hypocreales* or *Trichosporon* species (Figs. 2 and 3). From the 18S reads shown in Figure 2, excluding the human reads, the remaining reads were from fungi and not from bacteria, indicating that contamination from skin or commensals would be unlikely. The phylogenetic tree diagram in Figure 3 showed that the majority of fungi reads were from *Hortaea werneckii*, *Aspergillus*, *Hypocreales* or *Trichosporon spp.* Three contigs assembled from the *Trichosporon* reads showed the closest match to *Trichosporon asahii*, at the species level. When compared, the contigs were 99% similar, which suggests that there was only one species of *Trichosporon*, i.e., *T. asahii* in the tissue samples.

The results from the molecular NGS studies preceded subsequent positive cultures from tissue samples of the pericardium, conduit and thrombus which all grew *Trichosporon asahii*. This was most sensitive to voriconazole, and a lesser degree to fluconazole and amphotericin. In addition, cultures from the explanted pacing wire and sternal wound tissue demonstrated CONS resistant to methicillin. He was therefore managed with a combination therapy including intravenous voriconazole,

Table 1. 18S and Fungi ITS-Specific Primer Pairs Used for Next-Generation Sequencing of Patient Tissue Samples and Observed Results

Primer Pairs	Expected PCR Band (bp)	Observed	Sequencing Successful
Forward primer: ITS1F -CTTGGTCATTAGAGGAAGTAA Reverse primer: ITS2 -GCTGCGTCTCTCATCGATGC	250	250, 350 and 400 bp bands	Yes
Forward primer: 18S_0067a_deg -AAGCCATGCATGYCTAAGTATMA Reverse primer: NSR 399 -TCTCAGGCTCCYTCTCCGG	350	350 bp band	Yes
• Total number of reads = 56,692 -Number of reads mapped to human = 4433 (7.8%) -Number of reads mapped to fungi = 38,270 (67.5%) -Number of unmapped reads = 13,989 (24.7%) • Top 20 BLAST hits to fungi -Accounted for 23,825 reads (42%) -<i>Hortaea werneckii</i>, <i>Aspergillus caesiellus</i>, <i>Trichosporon spp*</i>, <i>Hypocreales spp.</i> identified			

BLAST: Basic Local Alignment Search Tool

*The reads for *Trichosporon species* accounted for 31.0% of the 42% BLAST hits for fungi.

ceftriaxone and vancomycin.

Initial treatment for the *Trichosporon asahii* infection comprised intravenous voriconazole; however, he developed hepatotoxicity on day 12 and was switched to liposomal amphotericin with fluconazole. Because of amphotericin nephropathy, treatment was changed back to voriconazole on day 33, which henceforth was tolerated well. The patient

completed 4 months of intravenous antifungal therapy, of which the last 3 months were on intravenous voriconazole, and has since been continued on oral voriconazole. His deep-seated CONS infection of the sternal wound and pacing wire was managed with 3 months of intravenous vancomycin and 6 weeks of oral co-trimoxazole and minocycline, with no further evidence of sternal wound infection.

- Analysis Method:
 - Align sequences using SINA
 - Pruesse, E., Peplies, J. and Glöckner, F.O. (2012) SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. [Bioinformatics, 28, 1823-1829](#)
 - Classify taxonomy using MEGAN4, (choose only very high alignment score)
 - minSupport=5 minScore=80.0 topPercent=5.0 winScore=0.0 minComplexity=0.2
 - Huson, DH, Mitra, S, Weber, N, Ruscheweyh, H, and Schuster, SC (2011). [Integrative analysis of environmental sequences using MEGAN4. Genome Research, 21:1552-1560.](#)
- Library generated 61104 sequences
 - Human has highest number of matches, followed by fungi.

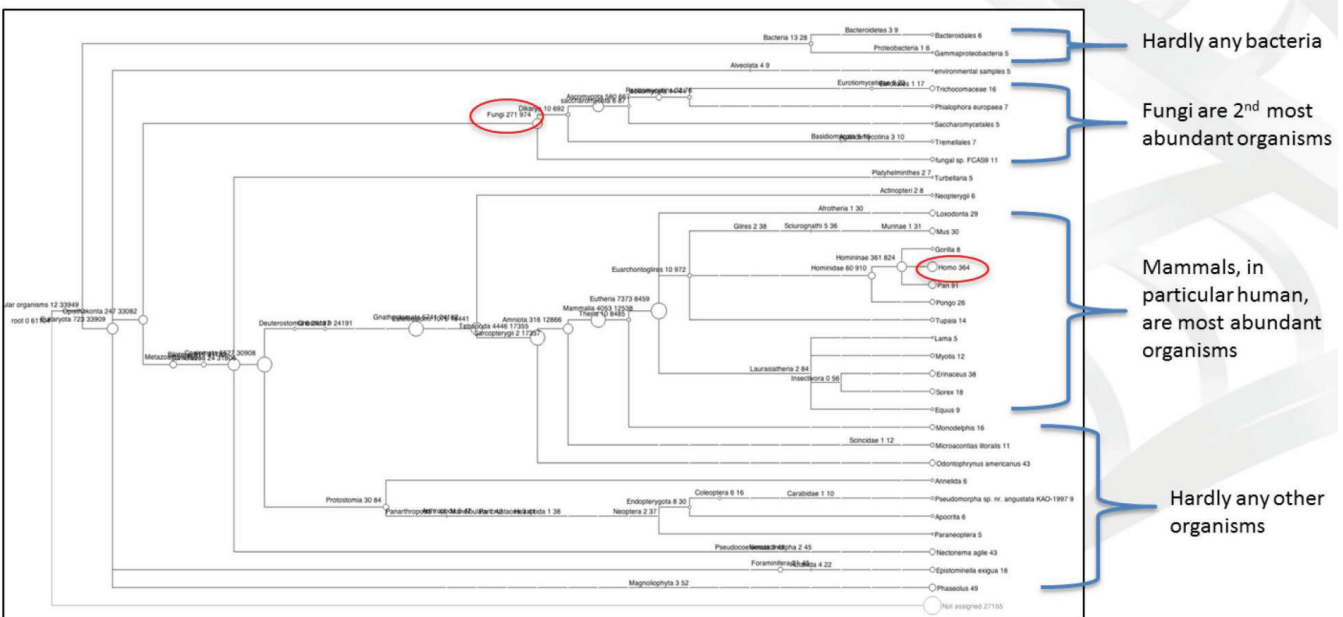


Fig. 2. 18S sequencing results (04V-9, IonXpress010).

Taxonomy tree of BLAST results of 21 contigs, generated using MEGAN4

- Parameters: Score >300, Top 5% for Lowest Common Ancestor determination
- *Hortaea werneckii*, *Aspergillus*, *Hypocreales* and *Trichosporon spp* were the most likely pathogens

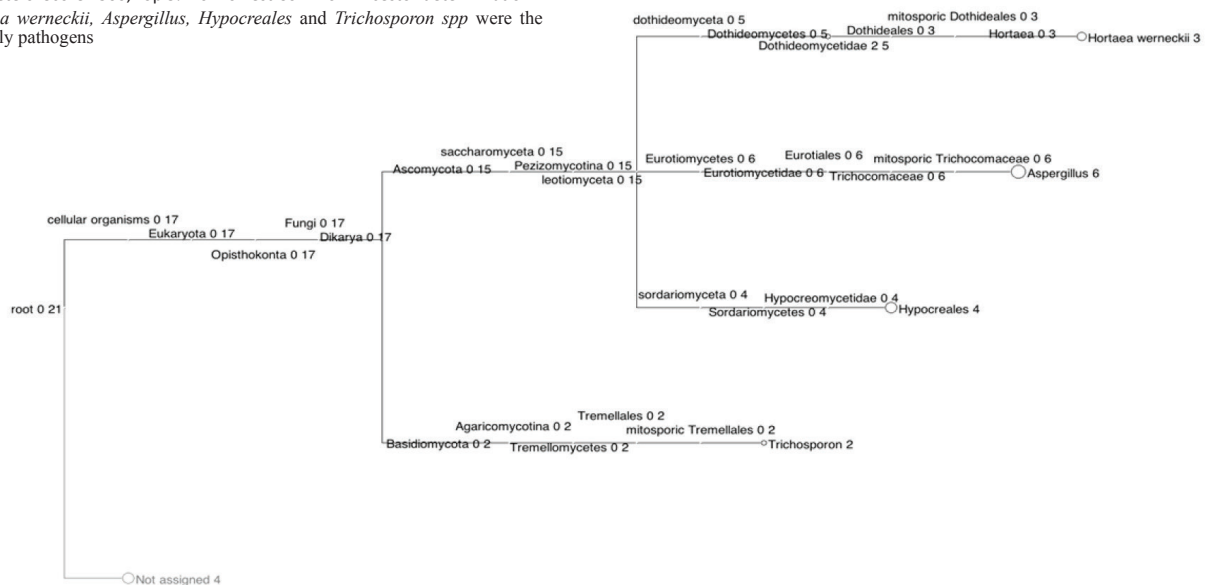


Fig. 3. Fungi ITS sequencing results (02V-20, IonXpress013).

The patient has since kept well and all repeat cultures were negative. Repeat cardiac CT has revealed a patent RV-PA conduit with no evidence of thrombus or collection.

Discussion

Trichosporon asahii has been recognised as a re-emerging pathogen. A recent systematic review² showed that infection occurs in 28% of non-immunocompromised patients, often postoperatively, following broad spectrum antibiotic therapy or in association with catheters and implants. Mortality was very high in earlier cases (64%–83%),^{3,4} but has improved to 44.3%² since azoles are used more commonly. The association of trichosporonosis with cardiac patients is limited to transplanted patients,⁵ although there are case reports of *Trichosporon asahii* and *Trichosporon beigelii* infective endocarditis.^{6,7} Of 11 cases of endocarditis secondary to *Trichosporon spp*,⁷ 9 had infected prosthetic valves. Management included surgical and antifungal therapy, but with poor prognosis and high mortality rate of 82%. However, all but one of these patients were treated with amphotericin B, an antifungal agent now known to have poor in-vitro activity towards *Trichosporon beigelii*.

We describe this case of postoperative *Trichosporon asahii* infection involving the patient's endocardium, prosthetic material with thrombus, and surrounding mediastinal tissue to highlight the importance of a re-emerging pathogen, and propose a rapid NGS molecular test as an alternative screening strategy for those with suspected deep-seated culture-negative infections, which could save vital time in initiating the correct treatment, given the still very high

mortality of infections with this organism. The tissue samples in our case showed hyphae and spores, indicative of proliferation in the tissues rather than contamination. Good communication among surgeons, cardiologists, infectious disease specialists and microbiologists was crucial in managing the patient's infection effectively. Surgical removal of as much infected tissue was imperative, as there is a potential of failure to clear and recurrence of infection. The NGS-based molecular diagnosis of fungal infection allowed for a targeted antimicrobial treatment before the culture result was available.

There are no controlled trials about the duration of antifungal treatment in such cases. Once trichosporonosis has established in tissue, recurrence is common even with prolonged antifungal treatment of up to 2 years.^{8,9} In this case, duration of intravenous antimicrobial treatment was guided by clinical improvement and monitoring of inflammatory markers; we switched intravenous to oral treatment approximately 1 month after all inflammatory markers (C-reactive protein [CRP], procalcitonin, erythrocyte sedimentation rate [ESR]) in our patient had normalised. Treatment in this case was complicated by initial intolerance to voriconazole, requiring a switch to a regimen that was potentially less effective (amphotericin and fluconazole). We did not see signs of recurrence; reasons that could be hypothesised include this patient never having blood stream infection, tissue growth of *Trichosporon* is slow, and the combination of amphotericin with fluconazole may have had the effect of at least keeping fungal growth at bay.⁴ As prevention of any recurrence is essential, the

plan is to continue oral voriconazole indefinitely. The question remains as to duration of management and ultimate long-term outcome, as we are unable to confidently document clearance.

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