

A Case of Misidentification of *Aspergillus versicolor* Complex as *Scopulariopsis* Species Isolated from a Homograft

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We report a case of the isolation of the *Aspergillus versicolor* complex, initially misidentified by morphological characteristics as the *Scopulariopsis* species, from a homograft with a bicuspidalized pulmonary valve. An eighteen-month-old female, who had critical pulmonary stenosis, underwent pulmonary valve replacement. On postoperative day 8, she developed a fever, which did not respond to empiric broad-spectrum antibiotics. While no definitive source was identified, a filamentous fungus was isolated from the thawed homograft tissue culture prior to implantation on the operation day. The colonies were powdery green with white edges on Sabouraud dextrose agar. Microscopic examination showed septate hyphae with branched conidiophores and chains of spiny conidia,

which suggested *Scopulariopsis* species. After direct sequencing of the internal transcribed spacer (ITS) regions, the fungus was identified as the *A. versicolor* complex. To our knowledge, the isolation of the *A. versicolor* complex from a homograft valve has not been previously described. This case shows that laboratory staff should be aware that microscopic morphology of the *A. versicolor* complex can resemble that of a number of other genera, including *Scopulariopsis* species. (**Ann Clin Microbiol 2013;16: 105-109**)

Key Words: *Aspergillus versicolor* complex, Homograft, *Scopulariopsis*

INTRODUCTION

Aspergillus versicolor is the most widely reported and studied species in section *Versicolores*. It has been isolated from soil, plants, and buildings contaminated by molds. It rarely causes human infections and exhibits reduced susceptibility to amphotericin B and variable susceptibility to azoles [1,2]. *A. versicolor* complex grows rapidly and produces both metulae and phialides from small vesicles. Fungal isolations of homograft heart valves have been very rarely reported, and the isolation of *A. versicolor* complex from a homograft transplant with adverse effects has not been previously described. Here, the authors report a case of the isolation of *A. versicolor* complex, initially misidentified as *Scopulariopsis* species.

CASE REPORT

An 18-month-old female with critical pulmonary stenosis with dysplastic valve and who had received a pulmonary valvectomy in infancy was admitted for cardiac valve surgery. Tricuspid and pulmonary valves regurgitation with dysplastic valves was demonstrated by echocardiography. The patient underwent tricuspid valvuloplasty, right ventricle-pulmonary artery conduit connection, and pulmonary valve reconstruction with a homograft. On post-operative day 8, she developed an intermittent fever without focal symptoms including respiratory symptoms or diarrhea after being given empirical antibiotic treatment with intravenous cefuroxime for 7 days. Her temperature was 38.3°C, blood pressure was 97/47 mmHg, pulse rate was at 160 beats per min, and respiratory rate was 48 breaths per min. Her C-reactive protein (CRP) was 2.97 mg/dL (reference interval, 0-0.3), erythrocyte sedimentation rate (ESR) was 36 mm/hr (reference interval, 0-27), and leukocyte count was 15,690/ μ L with neutrophilia (12,630/ μ L). Chest X-ray showed small amount of pleural effusion. She continued to have a fever with shorter in-

Received 29 January, 2013, Revised 8 March, 2013

Accepted 8 April, 2013

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tervals and her antibiotic regimen was modified to vancomycin and cefotaxime on post-operative day 10. She had a clear surgical site and no specific findings were observed on her evaluations, including blood, urine, and pleural fluid cultures, a multiplex respiratory virus polymerase chain reaction (PCR) test, and an echocardiogram. While no definitive infection source was identified with persistent fever, the filamentous form of a fungus, which morphologically suggested the *Scopulariopsis* species, was isolated on post-operative day 11 from the thawed homograft tissue culture performed prior to implant. By this time, despite the administration of broad-spectrum antibiotics, the patient exhibited increases of CRP (9.58 mg/dL) and ESR (64 mm/hr) with leukocytosis (16,550/ μ L; absolute neutrophil count, 13,410/ μ L). Consequently, empirical caspofungin and voriconazole were started after discontinuing vancomycin and cefotaxime on postoperative day 14. The patient became afebrile

after 3 days of antifungal agents and the homograft was subsequently removed on postoperative day 18. The patient was discharged 3 weeks after homograft removal. Oral voriconazole was continued for an additional 5 weeks, constituting a total treatment duration of 8 weeks.

Colonies of our isolate grew after 5 days of incubation at 25 and 30°C on Sabouraud dextrose agar (SDA). The isolate failed to grow at 35 and 40°C and was partially inhibited on cycloheximide-containing media. The colonies were initially colored white, though they gradually turned grayish-green with white edges (Fig. 1). A microscopic examination showed septate hyphae bearing branched conidiophores with chains of spiny conidia (Fig. 2). No vesicles, as seen in typical *Aspergillus* species, were observed. These microscopic characteristics were thought to resemble *Scopulariopsis* species. After 10 days of incubation, slide cultures showed the microscopic features of the isolate

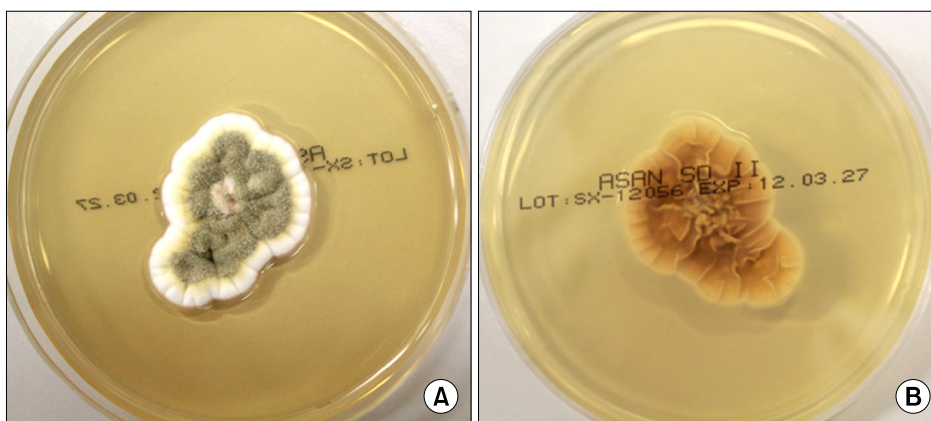


Fig. 1. *A. versicolor* colonies on a SDA plate. (A) The grayish green surface with white edges (B) Reverse of colony.

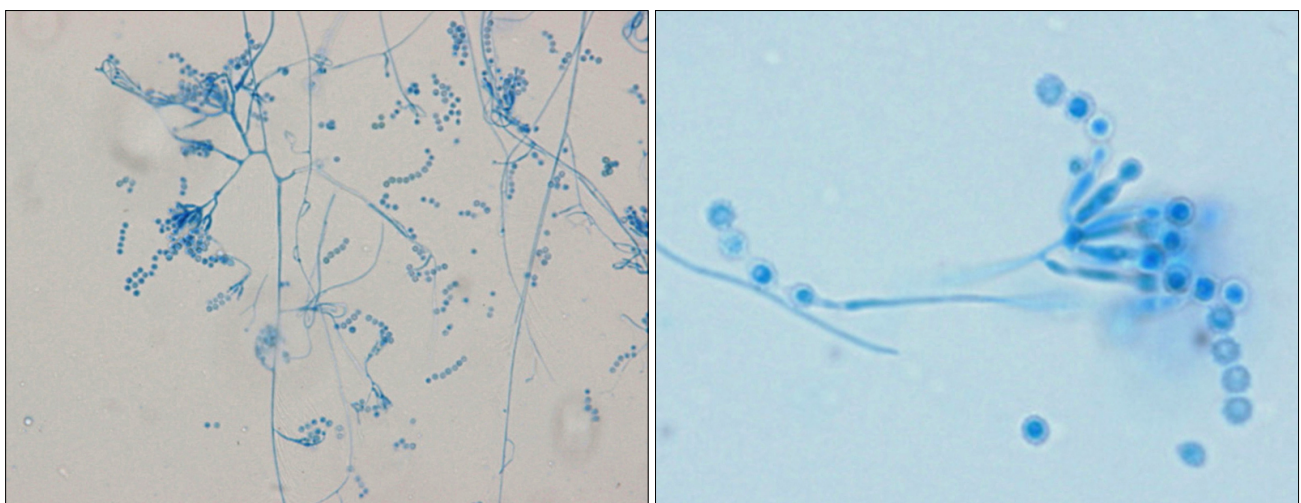


Fig. 2. Microscopic morphology of *A. versicolor* isolated from this case resembling *Scopulariopsis* spp. (lactophenol cotton blue stain, $\times 400$ and $\times 1,000$, respectively).

mixed with those typical of the genus *Aspergillus*, with conidia arising from biserial phialides. It was difficult to discriminate if there was contamination from *Aspergillus* species based on the morphological changes of the isolate. We performed rDNA target sequencing for the isolate: (i) sequences of the internal transcribed spacers (ITS) region covering ITS1, 5.8S, and ITS2 were amplified using ITS-1/ITS-4 and ITS-5/ITS-4 primer sets; (ii) sequences of the large-subunit RNA gene (D1/D2) region were amplified using NL-1/NL-4 primers [3]. The amplified sequences were compared with the GenBank (NCBI) database using the basic local alignment search tool (BLAST) algorithm. The ITS sequence of our isolate exhibited 100% identity with the corresponding sequences from a strain (GenBank accession no. AJ937750.1 and AJ937749.1) of *A. versicolor*. The D1/D2 region sequence was also 100% identical to that of *A. versicolor* (GenBank accession no. AJ937751.1). To resolve the possibility of the contamination of *Aspergillus* species given the discrepancies in the morphological and molecular studies, target-specific PCR was performed to differentiate *Scopulariopsis* species from *A. versicolor* complex. *Scopulariopsis*-specific primers (F, 5'-AATGGGAGGTAAACCCCTTC-3' and R, 5'-ACCATTACGCCAGCATCCTT-3') were designed from the 28S rDNA sequences of *Scopulariopsis* species and *A. versicolor* that were downloaded from GenBank and aligned. Since DNA from the isolate was not amplified by the *Scopulariopsis*-specific primer, its identity was confirmed as *A. versicolor* complex.

DISCUSSION

Human heart valves are used today to correct complex congenital heart diseases and valve defects. The transmission of infection from tissue to recipients has been documented on a num-

ber of occasions, despite the fact that infection following the implantation of homografts is rarely reported [4]. To prevent the transmission of microbes to recipients, the microbiological evaluation and decontamination of tissues is commonly practiced in tissue banks around the world [5]. Although special guidelines have not yet been made for microbiological analysis after a storage period prior to implantation, some tissue banks, including our institution, perform systematic microbiological cultures after thawing the valves according to recent recommendations [6,7]. Low levels of microbial isolation from homografts at thawing and prior to implantation have been reported [6,7]. The majority of the isolates were bacteria, with fungi rarely reported. Villalba et al. showed that six of 304 (2%) homograft valves were microbial culture-positive at thawing. Microbiological isolates consisted in *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Rhodotorula*, *Bacillus* sp. including two cases of *A. fumigates*. The follow-up of all six patients did not reveal any adverse effects under immediate antibiotic treatment [6]. Likewise, Soo et al. reported that three out of 312 (1%) homograft heart valves were culture-positive, and none developed clinical evidence of infection [7]. Unlike other studies, our patient had a clinical spectrum of an active infection with a fever that did not respond to the usual antibacterial agents. She had persistent fever and negative blood cultures for bacteria, responding only to antifungal treatment and the surgical removal of the homograft. To our knowledge, the isolation of *A. versicolor* complex from a homograft with bicuspidalized pulmonary valve has not been previously described. In the case described here, a homograft-related fungal infection was suspected based on the clinical course of the patient.

The isolate presented microscopic morphology, which was more akin to *Scopulariopsis* species than *Aspergillus* species,

Table 1. Differentiating characteristics of species of *Scopulariopsis*, *Penicillium*, *Aspergillus versicolor* and *A. sydowii*.

Organism	<i>Scopulariopsis</i> spp.	<i>Penicillium</i> spp.	<i>Aspergillus</i> spp.	
			<i>A. versicolor</i>	<i>A. sydowii</i>
Growth	Mature within 5 days	Mature within 4 days	Mature within 3 days	Mature within 3 days
Macroscopic morphology	Usually powdery light brown with a light tan periphery	Powdery and bluish green with white border	Usually velvety green	Velvety deep blue green
Microscopic morphology				
Conidiophores	Branched	Branched or unbranched	Smooth and medium length	Smooth medium length
Phialides/Anellides	Cylindrical or tenpin shaped annellides	Flask-shaped phialides	Vesicles 9-16 μ m Biseriate phialides Loosely radiate	Vesicle 5-10 μ m Biseriate phialides
Conidia	Round, thick walled, rough and spiny (4-9 μ m)	Round (2.5-5 μ m)	Round, slightly rough (2-3.5 μ m)	Subglobose, spinulose (2.5-3 μ m)

thus requiring molecular identification. The sequencing using the ribosomal ITS region identify only to the *Aspergillus* species “complex level”, that is, species that are morphologically or biochemically similar and otherwise indistinguishable by classical methods. Thus, other targets, such as β -tubulin region, can be used for the identification of species within the complex [3,8]. The members of the genus *Aspergillus* commonly reproduce via asexual spores called conidia. *A. versicolor* complex usually shows septate hyphae, smooth conidiophores, pyriform to spatulate vesicles, and biserial phialides with metulae covering half to entire vesicle [9]. It is noteworthy that fragmentary rough heads resembling fructifications of *Penicillium* or *Scopulariopsis* species occasionally present (Table 1) [1,9]. Also, *A. versicolor* can show small vesicle which may be missed by the clinical mycologist.

Identification at the species level is achieved by the recognition of these morphologically characteristic structures in the clinical laboratory. We misidentified *A. versicolor* as *Scopulariopsis* species due to microscopical morphologic similarity. Clinical mycologists should be aware that *A. versicolor* can morphologically resemble a number of other genera, including *Scopulariopsis* species. The present case required rapid molecular genetic confirmation since the homograft-related infection was suspicious. DNA-based methods should be considered as a means to obtain an identification of such isolates.

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=국문초록=

동종판막에서 분리된 *Aspergillus versicolor* Complex가 *Scopulariopsis* 종으로 잘못 동정된 1예

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저자들은 현미경적 소견이 유사하여 *Scopulariopsis* 종으로 오인하였던 *Aspergillus versicolor* complex를 폐동맥 동종판막에서 분리하여, 이를 보고하고자 한다. 18개월된 여아가 임계 폐동맥 협착으로 동종판막을 이용한 폐동맥 판막 치환술을 받은 후 수술 후 8일째, 광범위 항생제에 반응하지 않는 열이 발생하였다. 발열의 원인을 찾지 못하던 가운데, 이식 직전의 동종판막으로 시행한 진균 배양에서 사상균이 배양되었다. 균주는 Sabouraud's dextrose 배지에서 솜털같은 양상으로 흰 테두리를 가지는 녹색의 집락으로 관찰되었다. 현미경 관찰 결과 균사는 격벽을 가지고 분지된 분생자병 끝에 가시돌기 모양의 거친 분생자가 사슬을 이루고 있어 *Scopulariopsis* 종이 의심되었다. Internal transcribed spacers (ITS) 유전자에 대한 직접염기서열 결과, 균주는 *A. versicolor* complex로 확인되었다. 본 증례는 동종판막에서 *A. versicolor* complex를 분리한 첫 증례보고이다. 저자들은 형태학적 동정에서 현미경적 소견의 유사성으로 인해 *Scopulariopsis* 종으로 잘못 동정한 *A. versicolor* complex의 증례를 경험하였으며, 임상 미생물 검사실에서 *A. versicolor* complex의 동정에 주의가 필요할 것으로 판단된다. [Ann Clin Microbiol 2013;16:105-109]

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