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

Hee Jae Huh<sup>1</sup>, Jang Ho Lee<sup>1</sup>, Kyung Sun Park<sup>1</sup>, Tae-Gook Jun<sup>2</sup>, I-Seok Kang<sup>3</sup>, Yae-Jean Kim<sup>3</sup>, Chang-Seok Ki<sup>1</sup>, Nam Yong Lee<sup>1</sup>

Departments of <sup>1</sup>Laboratory Medicine & Genetics, <sup>2</sup>Thoracic and Cardiovascular Surgery, and <sup>3</sup>Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

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,

#### **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.

Received 29 January, 2013, Revised 8 March, 2013

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

### 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/µL with neutrophilia (12,630/µL). Chest X-ray showed small amount of pleural effusion. She continued to have a fever with shorter in-

Accepted 8 April, 2013

Correspondence: Nam Yong Lee, Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-dong, Gangnam-gu, Seoul 135-710, Korea. (Tel) 82-2-3410-2706, (Fax) 82-2-3410-2719, (E-mail) micro.lee@ samsung.com

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  $(16,550/\mu L;$  absolute neutrophil count, 13,410/µL). 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 biseriate 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 Scopulariosis species from A. versicolor complex. Scopulariopsis-specific primers (F, 5'-AATGGGAGGTAAACCCCTTC-3' and R. 5'-ACCATTAC GCCAGCATCCTT-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 number 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 vet 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	f spe	cies o	f Sco	pulariopsis	, Penicillium,	Aspergillus	versicolor	and	А.	sydowii.
----------	-----------------	--------------------	-------	--------	-------	-------------	----------------	-------------	------------	-----	----	----------

Organiam	Soomulariosis ann	Dominillium, ann	Aspergillus spp.			
Organism	scopuariosis spp.	renicultum spp.	A. versicolor	A. sydowii		
Growth Macroscopic morphology	Mature within 5 days Usually powdery light brown with a light tan periphery	Mature within 4 days Powdery and bluish green with white border	Mature within 3 days Usually velvety green	Mature within 3 days Velvety deep blue green		
Microscopic morphology						
Conidiophores	Branched	Branched or unbranched	Smooth and medium length Vesicles 9-16 $\mu$ m	Smooth medium length Vesicle 5-10 $\mu$ m		
Phialides/Annellides	Cylinderical or tenpin shaped annellides	Flask-shaped phialides	Biseriate phiallides Loosely radiate	Biseriate phiallides		
Conidia	Round, thick walled, rough and spiny (4-9 $\mu$ m)	Round (2.5-5 µm)	Round, slightly rough (2-3.5 $\mu$ m)	Subglobose, spinulose (2.5-3 $\mu$ 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 ß-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 hypae, smooth conidiophores, pyriform to spatulate vesicles, and biseriate philides 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.

#### REFERENCES

- Jurjevic Z, Peterson SW, Horn BW. Aspergillus section Versicolores: nine new species and multilocus DNA sequence based phylogeny. IMA Fungus 2012;3:59-79.
- Van Der Linden JW, Warris A, Verweij PE. Aspergillus species intrinsically resistant to antifungal agents. Med Mycol 2011;49 Suppl 1:S82-9.
- Clinical and Laboratory Standards Institute. Interpretive Criteria for Microorganism Identification by DNA Target Sequencing; Approved Guideline. Document MM18-A. Wayne, PA; Clinical and Laboratory Standards Institute, 2008.
- Wang S, Zinderman C, Wise R, Braun M. Infections and human tissue transplants: review of FDA MedWatch reports 2001-2004. Cell Tissue Bank 2007;8:211-9.
- Fan YD, Van Hoeck B, Holovska V, Jashari R. Evaluation of decontamination process of heart valve and artery tissues in European Homograft Bank (EHB): a retrospective study of 1,055 cases. Cell Tissue Bank 2012;13:297-304.
- Villalba R, Mirabet V, Rendal E, González AI, Solves P, Andión C, et al. Microbiological analysis of cryopreserved human heart valves after storage: a survey of 3 banks in Spain. Cell Tissue Bank 2009;10:345-9.
- Soo A, Healy DG, El-Bashier H, Shaw S, Wood AE. Quality control in homograft valve processing: when to screen for microbiological contamination? Cell Tissue Bank 2011;12:185-90.
- Alastruey-Izquierdo A, Mellado E, Cuenca-Estrella M. Current section and species complex concepts in *Aspergillus*: recommendations for routine daily practice. Ann N Y Acad Sci 2012;1273:18-24.
- Larone DH. Medically important fungi. a guide to identification. 5th ed. Washington DC: ASM Press; 2011.

=국문초록=

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

성균관대학교 의과대학 삼성서울병원 <sup>1</sup>진단검사의학교실, <sup>2</sup>흉부외과학교실, <sup>3</sup>소아청소년과학교실 허희재<sup>1</sup>, 이장호<sup>1</sup>, 박경선<sup>1</sup>, 전태국<sup>2</sup>, 강이석<sup>3</sup>, 김예진<sup>3</sup>, 기창석<sup>1</sup>, 이남용<sup>1</sup>

저자들은 현미경적 소견이 유사하여 Scopulariopsis 종으로 오인하였던 Aspergillus versicolor complex를 폐동맥 동종판막 에서 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 직전 의 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 가시돌 기 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 동 정한 A. versicolor complex의 증례를 경험하였으며, 임상 미생물 검사실에서 A. versicolor complex의 동정에 주의가 필요 할 것으로 판단된다. [Ann Clin Microbiol 2013;16:105-109]

교신저자 : 이남용, 135-710, 서울시 강남구 일원동 50번지 삼성서울병원 진단검사의학과 Tel: 02-3410-2706, Fax: 02-3410-2719 E-mail: micro.lee@samsung.com