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Superficial abdominal surgical site infection caused by *Aspergillus welwitschiae*: a case report

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Abstract

Background *Aspergillus* spp. are rare causes of surgical site infections (SSIs). Specifically, *Aspergillus* section *Nigri*, commonly identified as *Aspergillus niger* through morphological findings, has infrequently been reported as an abdominal SSI pathogen.

Case presentation An 86-year-old woman with a history of hypertension, chronic kidney disease, and atrial fibrillation who was taking 6 mg of prednisolone daily for rheumatoid arthritis was admitted to our hospital because of sudden abdominal pain. She was diagnosed with sigmoid colon perforation and underwent an open Hartmann operation on the day of admission. Subsequently, a superficial abdominal SSI was detected. Through analysis of the calmodulin gene, *Aspergillus welwitschiae*, which is classified within the *Aspergillus* section *Nigri*, was identified as the responsible pathogen. The minimum inhibitory concentration of voriconazole (VRCZ) was 2 mg/L. Surgical removal of the infected tissue and VRCZ administration was effectively used to treat the infection.

Conclusions Given the reported low susceptibility of *Nigri* section species to azoles, identification and drug susceptibility testing of these fungi are highly important.

Keywords *Aspergillus Niger*, Black subcutaneous lesions, Aspergillosis in noninvasive postsurgical

Background

The incidence of surgical site infections (SSIs) caused by fungi varies across settings and regions and is generally rare [1, 2]. *Aspergillus welwitschiae* is a species belonging to *Aspergillus* section *Nigri*, which includes species that can cause diseases in humans and animals and is similar to *A. niger* [3]. The presence of low antifungal drug susceptibility has been documented in species of *Aspergillus* section *Nigri*, as well as in other *Aspergillus* species [4, 5]. SSIs caused by *Aspergillus* spp., including *A. fumigatus*, have been previously reported [6], but no reports on SSIs caused by *A. welwitschiae* have been published. Here, we report the case of a superficial abdominal SSI caused by *A. welwitschiae*, which was initially identified as *A. niger*

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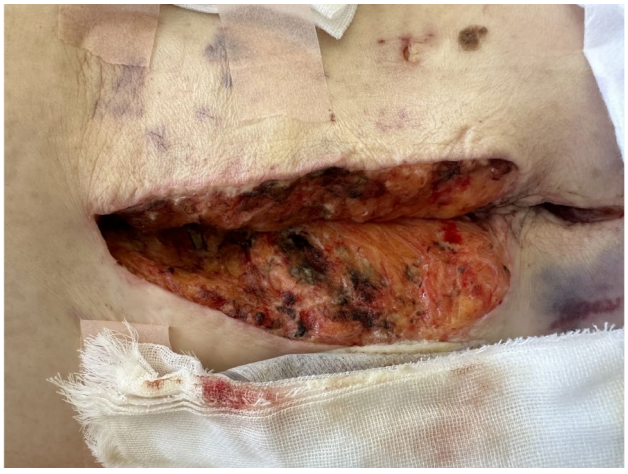

Fig. 1 Appearance of abdominal subcutaneous tissue on Day 8 after surgery. Black pigmented subcutaneous lesions, 2–5 mm in diameter, were observed

according to morphological findings and later confirmed as *A. welwitschiae* through calmodulin gene analysis.

Case presentation

An 86-year-old woman with a history of hypertension, chronic kidney disease, and atrial fibrillation who was taking 6 mg of prednisolone daily for rheumatoid arthritis was admitted to our hospital because of sudden abdominal pain (Day 1). She was diagnosed with sigmoid colon perforation and underwent an open Hartmann operation on the day of admission. Because of the patient's obesity, the wound was not sutured epidermally after surgery, and she remained on a ventilator in the intensive care unit (ICU). On Day 8, black lesions appeared in the subcutaneous tissue of the wound, and therefore, a wound biopsy culture was performed (Fig. 1). On Day 9, the culture results included black colonies on potato dextrose agar and Sabouraud agar (Fig. 2a). No bacterial growth was observed. *A. niger* was suspected on the basis of the typical morphology of the black colonies and the microscopic features of the conidial heads (Fig. 2b). Therefore, voriconazole (VRCZ), at a dosage of 400 mg twice on the first day followed by 200 mg twice daily, was administered through a nasogastric tube, and the subcutaneous tissue of the lesion was surgically removed on Day 9. No gross recurrence was observed after the operation. On Day 12, the serum *Aspergillus* antigen concentration cut-off index (COI) value was 0.6 (Platelia Bio-Rad, normal range: < 0.5 COI). On Days 1 and 17, the serum beta-D-glucan levels (Fungitec G test MK II, Nissui; cut-off value, 20 pg/mL) were 25 and 42 pg/ml, respectively (Table 1). Repeated sputum cultures did not reveal any *Aspergillus* spp. On Days 26 and 38, the serum *Aspergillus* antigen levels were 0.5 and 0.4 COI, respectively. VRCZ was continued until Day 40, with serum trough levels of 4.00, 5.66, and 2.56 μg/ml recorded on Days 15, 22, and 29, respectively. No obvious adverse effects of VRCZ, such as photosensitivity or increased transaminase levels, were observed. The patient continued treatment in the ICU because of unstable circulation and respiration, mainly due to ventilator-associated pneumonia and heart failure, and she died on Day 41. After the patient died, the fungal isolate was identified as *A. welwitschiae* on the basis of the sequence of the calmodulin gene (GenBank/EMBL/DDBJ accession no. LC800242). The neighbour-joining tree generated from the type and authentic strains is shown in Fig. 3. The

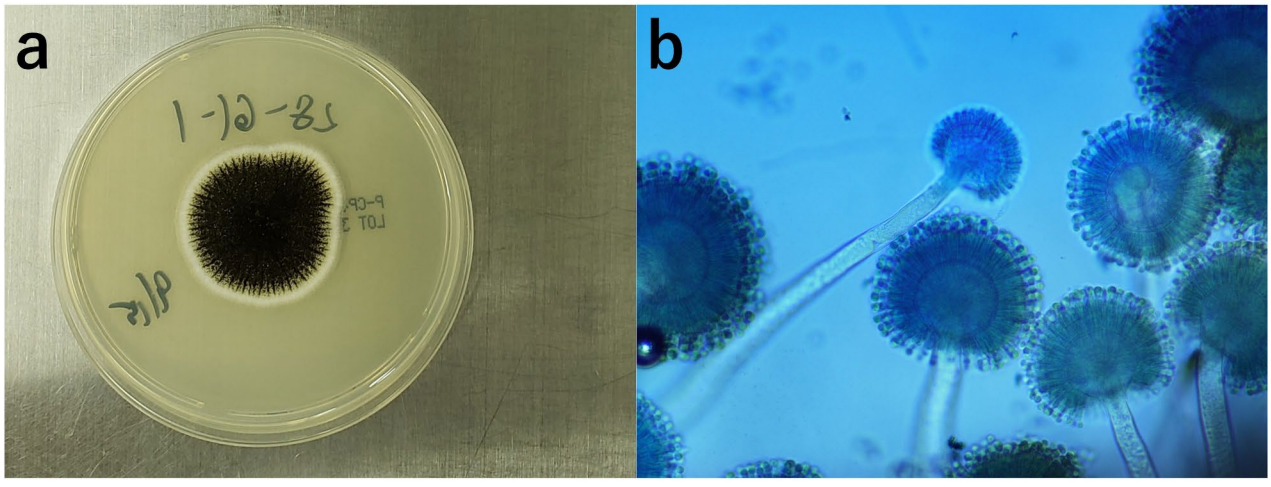

Fig. 2 **(a)** Black colony of a sample from the black subcutaneous lesions cultured on Sabouraud agar. **(b)** Microscopic findings of mycelia from black colonies on Sabouraud agar. This strain was initially suspected to be *A. niger* on the basis of microscopic findings. The strain was subsequently identified as *A. welwitschiae* via calmodulin gene analysis

Table 1 Timeline of disease progression

Day	1	8	9	12	17	26	38	40	41
Event	Operation		VRCZ initiation					VRCZ termination	Patient death
SSI		Documentation	Surgical removal	No gross recurrence					
BDG (pg/ml)	25				42				
AAG (COI)				0.6		0.5	0.4		

VRCZ, voriconazole; SSI, surgical site infection; BDG, beta-D-glucan; AAG, *Aspergillus* galactomannan antigen

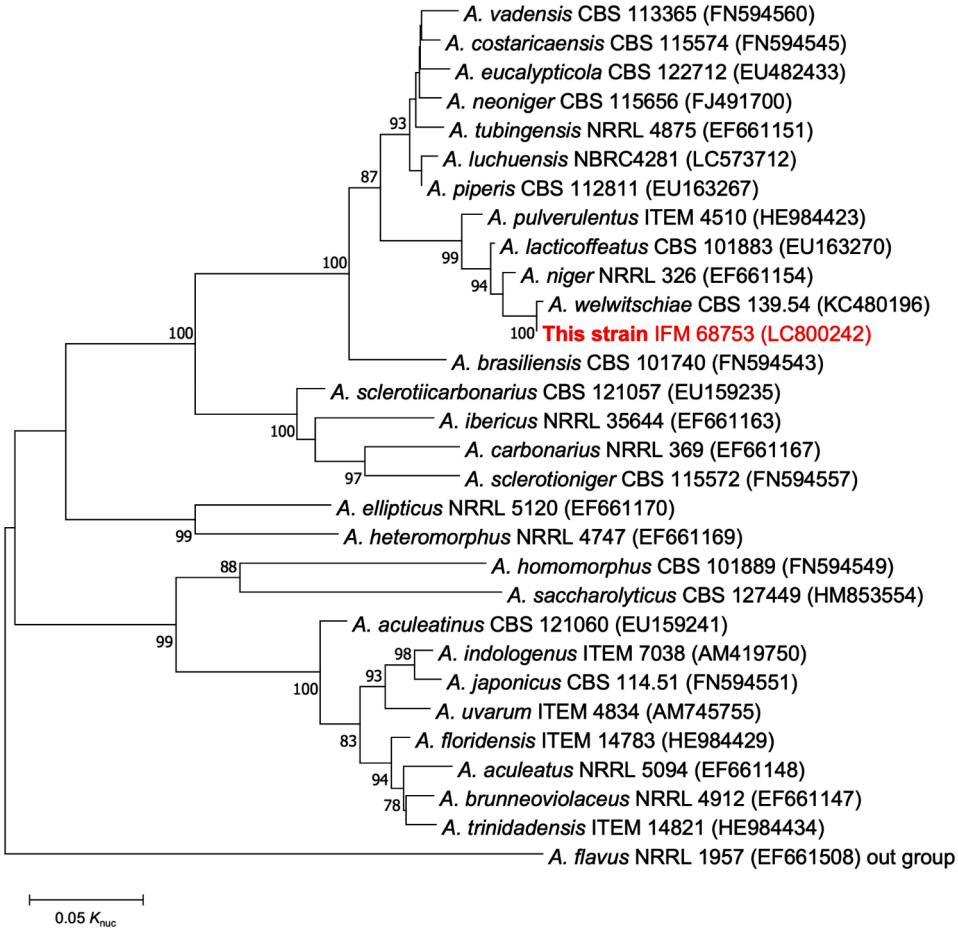

Fig. 3 The phylogenetic position of the isolate IFM 68,753 among *Aspergillus* section *Nigri* was determined according to the calmodulin gene sequence. Evolutionary distances were computed via the Kimura 2-parameter method, with a total alignment of 826 bps for 29 type strains. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown to the left of each node (only values > 70% are shown)

isolate was deposited at the Medical Mycology Research Centre, Chiba University, as IFM 68,753. The minimum effective concentrations (MECs) and minimum inhibitory concentrations (MICs), determined with the CLSI M38 broth microdilution method [7], were as follows: micafungin (MCFG), 0.03 mg/L; caspofungin (CPFG), 0.25 mg/L; amphotericin-B (AMB), 1 mg/L; itraconazole (ITCZ), 1 mg/L; and VRCZ, 2 mg/L (epidemiologic cutoff value [ECV] proposed for *A. niger*: 2 mg/L [8]).

Discussion and conclusions

This report highlights two important clinical issues. First, we report a rare case of abdominal SSI caused by nosocomial *Aspergillus* infection. Second, the identification and drug susceptibility testing of *Aspergillus* section *Nigri* as well as other *Aspergillus* species are important because of the reported low susceptibility to azoles in these species.

To our knowledge, this report is the first recorded case of noninvasive postsurgical aspergillosis caused by *A. welwitschiae*. Abdominal SSIs caused by *A. welwitschiae* have not been previously reported, partly because *A. welwitschia* may have been misidentified as *A. niger*

on the basis of microscopic findings alone. A similar case of abdominal SSI caused by *A. niger* has previously been documented; the fungus was identified via matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) [9]. The species in *Aspergillus* section *Nigri* have been reported to be indistinguishable microscopically and not completely distinguishable even by MALDI-TOF MS [10]. Because calmodulin is known to be the optimal gene for species-level classification and has been used previously for the molecular identification of species in *Aspergillus* section *Nigri* [11], we performed a phylogenetic analysis of the LC800242 calmodulin gene sequence. However, we did not analyse the β-tubulin gene, as this evaluation provides insufficient information for identifying species in *Aspergillus* section *Nigri* [11].

The incidence of SSIs caused by *Aspergillus* spp. is unknown. The most common organisms causing SSIs are the patient's endogenous flora, such as *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, and *Escherichia coli*, which account for approximately 70–95% of all SSIs [12, 13]. Fungal SSIs are rare, with a reported incidence of 4/1000 admissions in intensive care unit (ICU) settings [2]. Fungal causative agents include endogenous fungi such as *Candida* spp. and environmental moulds such as *Aspergillus flavus* [2]. Abdominal SSIs caused by *Aspergillus* spp. are uncommon, representing approximately 2% of all cases of postoperative aspergillosis [6]. Invasive mould infections are known to occur in patients in the burn ICU, and *Aspergillus* has been reported to be one of the most common causative organisms [14]. Notably, *A. niger* accounted for 43.7% of *Aspergillus* strains detected in the environment of a university hospital in Iran [15], suggesting that nosocomial transmission of *Aspergillus* section *Nigri* is theoretically not rare.

The subcutaneous *A. welwitschiae* infection in the present case was recognized on Day 8, suggesting a nosocomial infection. Although we did not obtain environmental culture samples from the ICU in this study, there have been no documented cases of black colony-forming *Aspergillus* in our ICU patients over the past three years. Although *A. fumigatus* has been reported to cause post-surgical invasive aspergillosis [16], in this case, *A. welwitschiae* was thought to have caused only a superficial SSI and was not considered related to the patient's death. Indeed, several factors have been shown to be associated with SSIs [17]. Although the risk factors for postoperative aspergillosis are unknown, the risk factors in this case were believed to be age, diabetes, steroid use, obesity, and potential skin barrier disruption given similar reports of invasive fungal diseases in the ICU [14].

The *Aspergillus* section *Nigri* organisms isolated from clinical samples in the UK were found to comprise 17.8% *A. tubingensis*, 55.6% *A. welwitschiae*, 13.3% *A. niger*, 6.7% *A. acidus*, and 6.7% unknown species on the basis of beta-tubulin/calmodulin sequences [18]. These cryptic species are commonly found to have low susceptibility to ITCZ, and the prevalence of low susceptibility to azoles in *A. fumigatus*, which is considered the most pathogenic species of the *Aspergillus* genus for humans, has been documented globally [19]. Additionally, low susceptibility to azoles has been reported in these species in Japan [4, 5]. According to the previously proposed epidemiologic cut-off values for *A. niger*, 30–55% of *A. niger*, 79.5–89.7% of *A. tubingensis*, and 6.8–18.6% of *A. welwitschiae* strains have been found to have low susceptibility to ITCZ or VRCZ [4]. Despite the low susceptibility of *A. welwitschiae* to VRCZ in our patient, surgical removal of the infected tissue and continued VRCZ administration were considered effective. Although *A. welwitschiae* infection was not considered a direct cause of this patient's death, low susceptibility to azoles may influence the choice of antifungal agents, especially in patients with a poor clinical course. Therefore, we believe that identification and drug susceptibility testing of *Aspergillus* section *Nigri* as well as other *Aspergillus* species is important. AMB is considered a potential treatment option because of its MIC values. The MIC of AMB for *A. welwitschiae* was 0.5 mg/L, which was within the general MIC range for *Aspergillus niger* (0.03–0.5 mg/L) [20]. With an MIC of 1 mg/L, ITCZ was an alternative option given the ECV of 4 mg/L provided by the Clinical & Laboratory Standards Institute (CLSI) for *A. niger* [20].

Our study has several limitations. On Day 9, there was no indication of a rare fungal infection, and we failed to collect a tissue biopsy to confirm the presence of a subcutaneous fungal infection, as the lesion did not reappear following its removal. Although no histological examination was performed and false-positive results of the serum Aspergillus antigen and beta-D-glucan tests, which suggested that the subcutaneous tissue may have been contaminated only by *A. welwitschiae*, are possible, we nevertheless believe that the presence of rare black subcutaneous lesions in this patient and a positive biopsy culture result indicated an *A. welwitschiae* subcutaneous infection.

Here, we report the first case of a superficial abdominal SSI caused by *A. welwitschiae* identified via calmodulin gene analysis. In the past, cryptic species of *A. niger* might have been mistakenly identified as *A. niger* on the basis of microscopic findings or MALDI–TOF MS. Identification and drug susceptibility testing of *Aspergillus* section *Nigri* as well as other *Aspergillus* species are important because these results affect antifungal drug selection.

Abbreviations

SSI	Surgical site infection
MIC	Minimum inhibitory concentration

VRCZ	Voriconazole
ICU	Intensive care unit
MEC	Minimum effective concentration
MCFG	Micafungin
CPFG	Caspofungin
AMB	Amphotericin-B
ITCZ	Itraconazole
MALDI-TOF	MAS matrix-assisted laser desorption ionization time-of-flight mass spectrometry
BLAST	Basic Local Alignment Search Tool

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Author contributions
HH wrote the article. AW, TO, HM and RF supervised and edited the manuscript. AW, TY, and SB performed the genomic analyses. All the authors have read and approved the final manuscript.

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Data availability
The data will be made available upon request.

Declarations

Ethics approval and consent to participate
The Institutional Review Board of the National Hospital Organization Yokohama Medical Center waived the ethical approval and consent requirements for this case report because of its retrospective nature. This study was conducted in accordance with the Declaration of Helsinki guidelines.

Consent for publication
Written informed consent was obtained from the patient's family for publication of this case report.

Competing interests
The authors declare no competing interests.

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