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First case of *Arthrographis kalrae* fungemia in a patient with cystic fibrosis

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ABSTRACT

Arthrographis kalrae is a hyalin fungus. It is a saprophyte of the environment, mainly found in soil and compost. In recent years, cases of opportunistic infections attributed to this pathogen have been described. Our patient was a 19-year-old woman with cystic fibrosis. She presented a bacterial and fungal pulmonary colonization with *Aspergillus fumigatus* and *Arthrographis. kalrae*. After her lung transplantation, she developed an *A. kalrae* fungemia, treated with caspofungin 50 mg/day associated to liposomal amphotericin B i.v. 3 mg/kg/day. The patient died 8 months after her transplantation as the result of a bacterial septic shock.

1. Introduction

Cystic fibrosis (CF) is a genetic disease characterized by increased viscosity of the bronchial mucus and impaired mucocilliary clearance which predisposes to microbial colonization and infections of the respiratory tract. Adult patients with CF have a high incidence of fungal colonization (42%) and invasive disease (11%). A variety of yeasts and filamentous fungi have been recovered from their respiratory samples, before and after lung transplantation. *A. fumigatus* and *Candida* spp. are the most common fungal species isolated, responsible of invasive infection after lung transplantation in patients with CF [1]. Recently, invasive pulmonary infections with emerging molds like *Scedosporium* spp., *Rasamsonia argillacea* or *Exophiala dermatitidis* have been described, associated with fungemia for some of them: *Scedosporium apiospermum* and *Fusarium solani* [1]. *Arthrographis kalrae*, an emerging mold is an environmental saprophytic fungus, mainly found in soil and compost. In recent years, cases of opportunistic infections attributed to this pathogen were described: pulmonary infection, endocarditis, sinusitis, meningitis, keratitis, onychomycosis and mycetoma, affecting both immunocompromised and immunocompetent patients.

2. Case

Our patient, a 19-year-old woman, was diagnosed with cystic fibrosis at the age of 4 months. She developed an exocrine pancreatic insufficiency, an insulin-dependent diabetes, a moderate chronic renal failure and a severe malnutrition (BMI 14). She also presented a permanent bacterial pulmonary colonization with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, treated by aerosols of colistin. From 1998 to 2005, *A. fumigatus* has been sporadically isolated from sputa cultures. Since 2005, the lung colonization by *A. fumigatus* became permanent (positive sputa culture, presence of anti-Aspergillus antibodies) for which a treatment with itraconazole 200 mg/day was initiated, increased up to 500 mg/day due to low serum levels. In March 2012, *A. kalrae* and various *Aspergillus* species (*A. fumigatus*, *A. flavus* and *A. nidulans*) were regularly isolated in sputa cultures. Itraconazole was then stopped and replaced by voriconazole 2×200 mg/day, later increased to 2×250 mg/day. A bilateral lung transplantation was performed in November 2013 (day 0). The pre-transplant sputum culture found bacteria colonies (*P. aeruginosa*, *S. aureus*), one colony of *A. terreus*, three of *A. fumigatus* and ten of *A. kalrae*. On day 1 post-transplant, the patient was in septic shock. Blood cultures, broncho-alveolar lavage (BAL) and two tracheal aspirations were made. The patient received a probabilistic antibiotic treatment associating ceftazidime, ciprofloxacin, teicoplanin, aerosols of colistin and voriconazole 2×200 mg twice a day. On day 3, mold colonies were isolated in blood cultures, BAL, tracheal aspirations and in a bronchial smear of the explanted lung cultures. On day 4, molds were identified as *A. kalrae*, associated with one colony of *A. terreus* in the BAL. Given these results, antifungal therapy was modified on day 4 for caspofungin 50 mg/day in combination with liposomal amphotericin B i.v. 3 mg/kg/day. Three colonies of *A. kalrae* and three colonies of *A. fumigatus* were found in another BAL performed on day 5 post-transplant. No *A. kalrae* colony was isolated in the following cultures from respiratory samples. The CT-scans and the chest radiographies performed during

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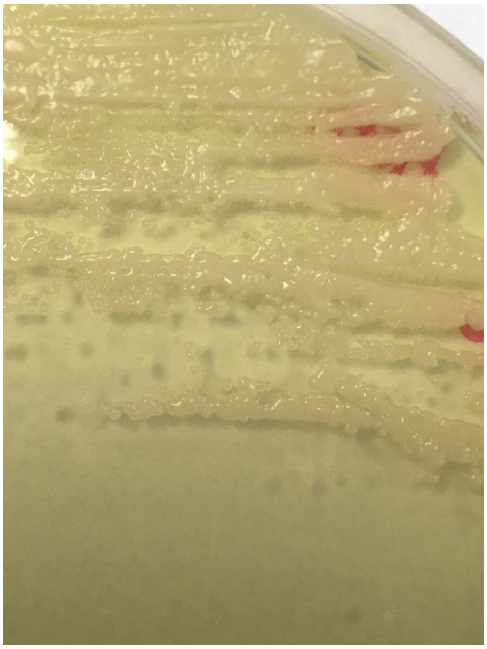

Fig. 1. : Macroscopical aspect of *A. kalrae*: 2 days after growing on chromID®*Candida*, at 37 °C.

this acute episode (day 0, 3 and 21) were not contributory. Only the first CT-scan showed signs of pulmonary infection of the lingula and lower lobe on day 21. On day 33, the liposomal amphotericin B i.v. was replaced by aerosols of liposomal amphotericin B 3×/week, maintained until day 110 post-transplant. Despite the negativation of pulmonary samples and the resolution of this acute fungal infection in the following months, the patient died 8 months after her transplantation because of a bacterial septic shock in a context of humoral rejection and a state of severe malnutrition.

After two days of incubation at 35 °C on chromogenic medium chromID® *Candida* (Biomerieux, France) for the respiratory samples and on Bactec Plus aerobic F® (Beckton Dickinson, France) at 37 °C, there was growth of small yeast-like colonies. Identification was performed based on three methods: a morphological examination, the biochemical characteristics and a mass spectrometry study. Macroscopically, we observed small creamy, beige yeast-like colonies after two days of growth on chromogenic medium chromID® *Candida* (Fig. 1). After 7 days of incubation, the colonies became dry and grainy. The same aspects were observed on Sabouraud chloramphenicol (Biorad, France) and Sabouraud chloramphenicol actidione (Biorad, France) at 37 °C and 27 °C (Figs. 2 and 3). However, growth on Sabouraud chloramphenicol medium with actidione was slower (5 days for yeast-like colonies) than without. A slide culture incubated for 48 h at 27 °C on PCB medium (Biorad, France) showed characteristic hyaline, septate hyphae with one-celled, smooth-walled arthroconidia and irregularly branched hyphae, with dendritic conidiophores (Fig. 4). The mature arthroconidia were elongated. The isolate was also inoculated into wells of an ID 32 plate (Biomerieux, France). After 72 h of incubation, the profile code obtained did not correspond to any taxon scored in the manufacturer's database. In parallel, mass spectrometry was performed using the Microflex LT mass spectrometer (Bruker Daltonics, France), and analyzed using the MALDI Biotyper software (version 3.1 with enlarged database version 3.3.1.0 containing 4613 entries). Mass spectrometry technique allowed to identify *A. kalrae* with logscore values > 2.0 (2.28, 2.24), according to the manufacturer's recommendations. The identification was confirmed by a DNA sequencing (using the internal transcribed spacer, ITS1 and ITS4) and comparison of obtained sequences to GenBank (http://www.ncbi.nlm.nih.gov/genbank, accession number for closest hit

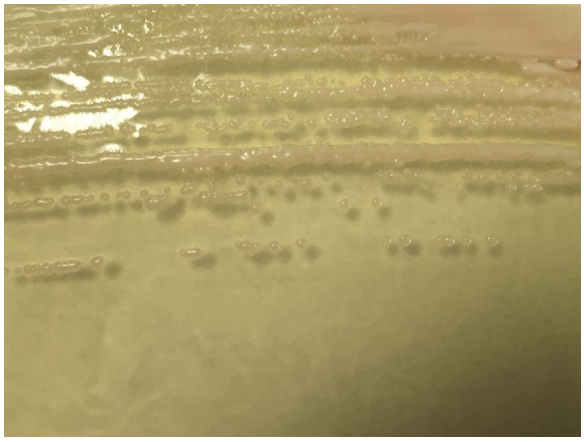

Fig. 2. : Macroscopical aspect of *A. kalrae*: 2 days after growing on Sabouraud chloramphenicol at 37 °C.

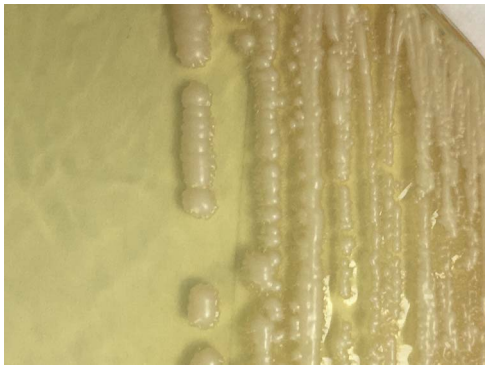

Fig. 3. : Macroscopical aspect of *A. kalrae*: 4 days after growing on Sabouraud chloramphenicol actidione at 37 °C.

KM588309.1) and CBS (http://www.cbs.knaw.nl, accession number for closest hit CBS 10896_ex24366_12125_ITS) databases. *A. kalrae* was identified with an E-value of 0 (GenBank), an overlap of 100% (CBS) and 100% identification concordance. The blood culture strain MICs measured by Etest® gave the following results: amphotericin B 0.75 mg/ml, flucytosine > 32 mg/ml, voriconazole 0.125 mg/ml, posaconazole 0.50 mg/ml, fluconazole > 32 mg/ml, micafungin 0.012 mg/ml, caspofungin 0.047 mg/ml. The strain was sent to the National Reference Center, at Pasteur Institute. There, the MICs values were determined according to the EUCAST method and gave the following results: amphotericin B 0.125 mg/ml, flucytosin > 64 mg/ml, voriconazole 0.125 mg/ml, posaconazole 0.125 mg/ml, fluconazole 64 mg/ml, micafungin 4 mg/ml and caspofungin > 4 mg/ml.

3. Discussion

Arthrographis is a genus containing 5 species: *A. kalrae, A. cuboidea, A. lignicola, A. pinicola* and *A. alba*. *A. kalrae* is a saprophytic fungus with a worldwide distribution, found in soil and compost [1]. In recent years, clinical cases of invasive infections attributed to this pathogen have increased. Currently, one case of infection with *Arthrographis* sp. and 14 cases of infection with *A. kalrae* have been reported (Table 1): two onychomycosis [2,3], one mycetoma [4], five keratitis [5–9] one of which associated with sinusitis [8], two knee joint infection [10,11], one endocarditis [12], two pulmonary infections [13,14], one meningitis [15] and one fungal stroke [16]. These cases have a worldwide distribution: seven cases in Europe, one in China, one in Japan, three in USA, one in Mexico, one in Malaysia and one in Australia. Three of the five patients with keratitis were soft contact lens wearers; the other two and the two

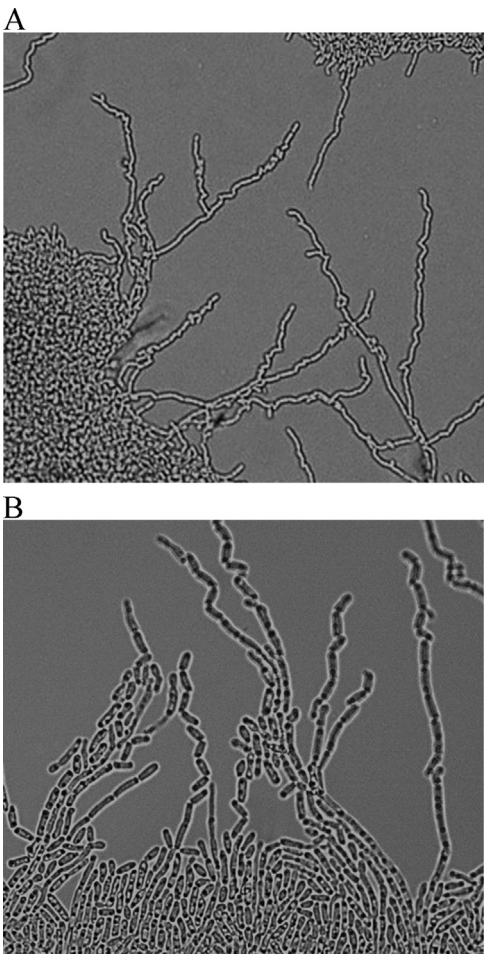

Fig. 4. : Microscopial aspect of *A. kalrae*: slide culture incubated for 48 h at 27 °C on PCB medium (potato, carrot, bile). X100 B: Microscopial aspect of *A. kalrae*: slide culture incubated for 48 h at 27 °C on PCB medium (potato, carrot, bile). X400.

patients with the knee joint infections have had an injury contaminated with soil. The rest of the patients had predisposing infection factors like malnutrition, systemic corticosteroids, radiotherapy, AIDS, allogeneic hematopoietic stem cell transplant. Diagnoses were always based on phenotypic characteristics and microscopic morphology using de Hoog and Sigler [17], and Carmichael descriptions [18]. In most cases, identification of the species was confirmed by molecular biology techniques (ITS, D1/D2 sequencing). No data were available about the most appropriate treatment for this kind of infection. In the previous described cases, when tested the strains were sensitive to azoles, amphotericin B and terbinafin and resistant to flucytosin, but no sensibility results were showed for echinocandins. A recent study tested the susceptibility of 22 *A. kalrae* strains [19]. The azoles showed high activity (mean MIC 0.46 μg/ml), amphotericin B very little activity (mean MIC: 2 μg/ml) and echinocandins showed no *in vitro* activity (mean MEC at 24 h > 8 μg/ml). These results are different from ours but concordant with those of the National Reference Center for Invasive Mycoses and Antifungals, which is using the EUCAST method. All patients described except the one with onychomycosis were treated with azoles associated or not with amphotericin B. Some reported patients also required surgery (keratitis and knee joint infection). Three patients died during their *A. kalrae* infection, one of them because of the fungus (patient with fungal stroke). Four infections became recurrent and needed a chronical treatment. Given the fact that our patient developed a septic shock with a fungemia despite a prophylaxis with voriconazole, the choice was made to treat her with caspofungin in combination with liposomal amphotericin B. The clinical response was favorable with this treatment, regardless the resistance to the caspofungin detected by the National Reference Center.

In the case of our patient, the evolution of the fungal infection was favorable with a rapid negativity of blood cultures and respiratory samples. The combination of caspofungin and liposomal amphotericin B was effective in our patient whose colonization by the fungus could not be eliminated by a long-term treatment with voriconazole. We therefore describe here the first case, to our knowledge, of an *A. kalrae* fungemia in a cystic fibrosis patient with an assumed pulmonary portal of entry.

Ethical form

Please note that this journal requires full disclosure of all sources of funding and potential conflicts of interest. The journal also requires a declaration that the author(s) have obtained written and signed consent to publish the case report from the patient or legal guardian(s).

The statements on funding, conflict of interest and consent need to be submitted via our Ethical Form that can be downloaded from the submission site www.ees.elsevier.com/mmcr. **Please note that your manuscript will not be considered for publication until the signed Ethical Form has been received.**

Table 1
Characteristics of previously published cases of infection with *A. kalrae*.

Reference	Age (Yrs)	Sex	Country	Pathology	Risk Factor	Surgery	Antifungal agent	Outcome
[16]	39	M	France	Fungal stroke	Malnutrition	No	No	Death (2 Days after diagnosis)
[8]	39	M	China	Keratitis and sinusitis	Injury	Yes	AMB+ITZ	Recovery
[13]	61	H	Netherlands	Pulmonary infection	Radiotherapy	Yes	ITZ	Recovery
	ND	ND	Mexico	Pulmonary infection	HSCT	No	ND	ND
[6]	42	F	Germany	Keratitis	Soft lens wearer	yes	VRC	Chronicity
[4]	80	H	France	Mycetoma	Systemic corticosteroid	No	ITZ	Recovery
[7]	52	H	Malaysia	Keratitis	Injury	Yes	AMB+FCZ (eyedrop)	Chronicity
[9]	23	F	USA	Keratitis	Soft lens wearer	Yes	AMB+ITZ (i.v.)	Chronicity
[15]	33	H	USA	Meningitis and sinusitis	AIDS	No	FCZ	Death (pulmonary infection)
[10]	33	H	Australia	Knee joint infection	Injury	Yes	VCZ - FCZ -POS	Chronicity
[11]	ND	H	Italy	Knee joint infection	Injury	Yes	ND	ND
[12]	50	F	Spain	Endocarditis	Pericardial patch	Yes	liposomalAMB+VZC+POS	Death (during surgery)
[20]	ND	ND	Slovakia	Onychomycosis	ND	ND	ND	ND
[2]	63	H	Japan	Onychomycosis	ND	ND	ND	ND
[5]	ND	ND	USA	Keratitis	ND	ND	ND	ND

ND (not determined), AMB Amphotericin B, ITZ Itraconazole, VRC Voriconazole, FCZ Fluconazole, POS Posaconazole.

Conflict of interest

There are none.

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