First case of *Arthrographis kalrae* pulmonary infection in a patient with AIDS

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Summary

Arthrographis kalrae is a hyaline fungus. It is a saprophyte of the environment, mainly found in soil and compost. It is a rare isolate in clinical specimen. In recent years this pathogen has been attributed to various cases of opportunistic infections. Our patient was a 51-year-old Indian woman, farmer by occupation, who had HIV infection and was under HAART treatment. She presented with complaints of cough with scanty expectoration for 2 months. Her CD_4 count was 75 cells/µl. Induced sputum was collected and sent for detailed microbiological examination. Bacteriological and mycological profile was checked. The causal agent was identified as *Arthrographis kalrae* based on morphological characteristics (culture). Unfortunately, samples could not be preserved and sent for ITS region sequencing due to COVID-19 outbreak. The case is of interest because, to the best of our knowledge, *Arthrographis kalrae* has not been reported so far from our country, India.

Introduction

The human immunodeficiency virus (HIV) causes the acquired immunodeficiency syndrome (AIDS) which is the most important public health problem of the 20th century. A decline in the CD_4 count among PLHA makes patients prone to various opportunistic infections, 70% of which accounts for respiratory infections. A variety of bacteria, yeasts and filamentous fungi has been recovered from the respiratory samples in the HIV infected patients. Fungal pneumonias are often overlooked and underdiagnosed, even though they are an important bulk of opportunistic mycoses in HIV reactive patients. The relevance of fungi in causing opportunistic pneumonia can be further highlighted by the fact that it was the opportunistic fungus *Pneumocystis carinii* (now *Pneumocystis jiroveci*) that heralded the discovery of HIV/AIDS, when reports of pneumonia caused by this agent in previously healthy men, who had sex with other men and/or who were injection drug users, first appeared in 1981 in the United States of America.

Here we report a case of a fungal pulmonary infection by a rare fungus, *Arthrographis kalrae* (*A. kalrae*) in a HIV infected female patient with low CD_4 count, which was isolated from her induced sputum and identified by phenotypic and microscopic characteristics. To the best of our knowledge, this is the first report of lower respiratory tract infection caused by *A. kalrae* in India.

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Case Description

Our patient was a 51-year-old woman, farmer by occupation, with AIDS (CD_4 T-cell count, 75 cells/µl) human immunodeficiency virus (HIV) type 1 (HIV-1) who was receiving highly active antiretroviral therapy. She referred to our hospital, with main complaints of cough with canty expectoration for 2 months. She reported no history of fever or dyspnoea. As pulmonary tuberculosis is the most common co-infection seen with HIV/AIDS, she was sent from the ART Centre to the Cartridge based nucleic acid amplification test (CBNAAT) site, for detection of *Mycobacterium tuberculosis*. However, CBNAAT produced a negative report. In order to collect more suitable sputum, induced sputum sample was obtained. Following universally recognized precautions, the patient was instructed to clean her mouth thoroughly and inhale a solution of 3% saline in water via an ultrasonic nebuliser; then, the induced sputum was collected in a sterile container and sent to the microbiology laboratory.

Procedure and observations

In the microbiology laboratory, a detailed evaluation was performed. Initially Bartlett's scoring system was used for microscopic evaluation of the expectorated sputum to check its quality. Then, Gram staining, Ziehl Neelsen (ZN) staining, Modified Toluidine-Blue O staining and KOH mount were performed. Gram staining revealed the presence of pus cells, but no bacteria or budding yeast were detected. ZN staining was done to rule out

the presence of any atypical mycobacteria, and it was negative. Modified Toluidine-Blue O staining was performed to detect the presence of any cysts of *Pneumocystis jiroveci,* but there were none; 10% KOH mount revealed the presence of few hyaline hyphae. Furthermore, the sample was cultured on Blood agar plate, Chocolate agar plate, MacConkey agar plate, Pleuro-pneumonia like organism (PPLO) enrichment broth and 4 sets of Sabouraud's Dextrose agar with chloramphenicol (0.05mg/ml) (SDA) tubes. Four sets of SDAs were used as two sets were kept at 25°C (identification of moulds) and two sets at 37°C (identification of yeasts). Duplicate tubes were used to confirm isolation of the same pathogen from all cultures. Blood and Chocolate agar plates were incubated in candle jar (5-10% CO_2) at 37°C and MacConkey agar plate in incubator at 37°C overnight. As no growth was seen in all the plates, they were re-incubated for another 24 hours. PPLO enrichment broth showed no change in its colour, indicating the absence of *Mycoplasma* species. The SDA tubes were examined once in two days for the 1st week. On the 4th day of inoculation few creamy moist colonies were seen on the SDA tube kept at 37°C, but no growth was detected on the SDA tubes kept at 25°C. On the 6th day growth was seen in both tubes, kept at 25°C and 37°C, respectively (Figure 1). On microscopic observation, elongated oval budding yeast cells were seen. Colonies were also sub-cultured on SDA plates containing cycloheximide (0.4 gm/ml) and kept at 37°C. The fungus was resistant to cycloheximide and creamy colonies were seen. The colonies were initially cream coloured and glabrous, but by the 10th day they became dry, flat, granular and then velvety (Figure 2). Slide culture was performed on Potato Dextrose agar at 25°C. The same growth pattern was seen. Microscopically, the isolate produced hyaline septate hyphae and conidiophores were sometimes in bundles, either unbranched or branched in tree like fashion in slide culture preparations stained with Lacto-Phenol Cotton Blue (Figure 3). They produced chains of consecutive smooth-walled arthroconidia that were mostly rectangular (2-3 by 4-6 µm); some were oval. Intercalary arthroconidia were also seen. Fungi in which arthroconidia predominate are from genus *Geotrichum*, *Trichosporon*, *Geomyces*, *Malabranchea*, *Arthrographis* and *Coccidiodes*. Conidiophores are only present in genus *Arthrographis* and *Geomyces,* so others were ruled out. As genus *Geomyces* has barrel-shaped and alternating arthroconidia, it was also excluded. On the basis of the characteristics presented above, the isolate was identified to belong to the genus *Arthrographis*. It consists of five species: *A. kalrae*, *A. cuboidea*,

Figure 2. Velvety colonies of *Arthrographis kalrae* after 10 days of incubation.

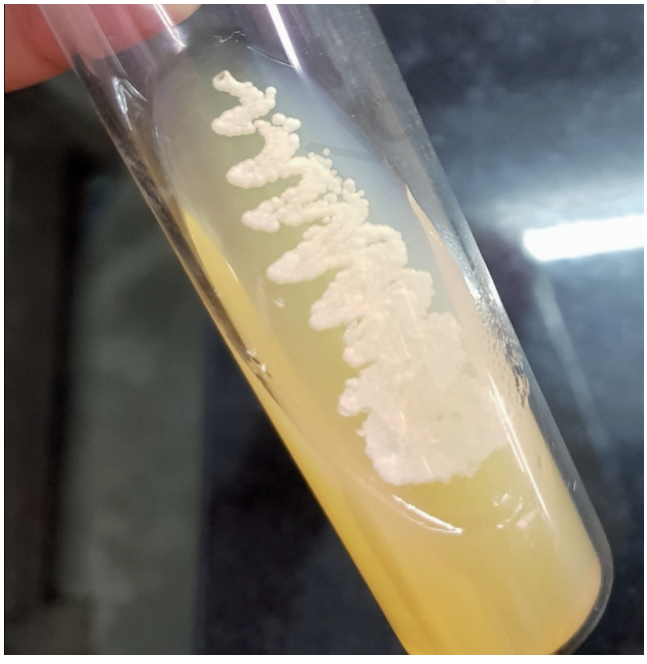

Figure 1. Smooth yeastlike moist colonies of *Arthrographis kalrae.*

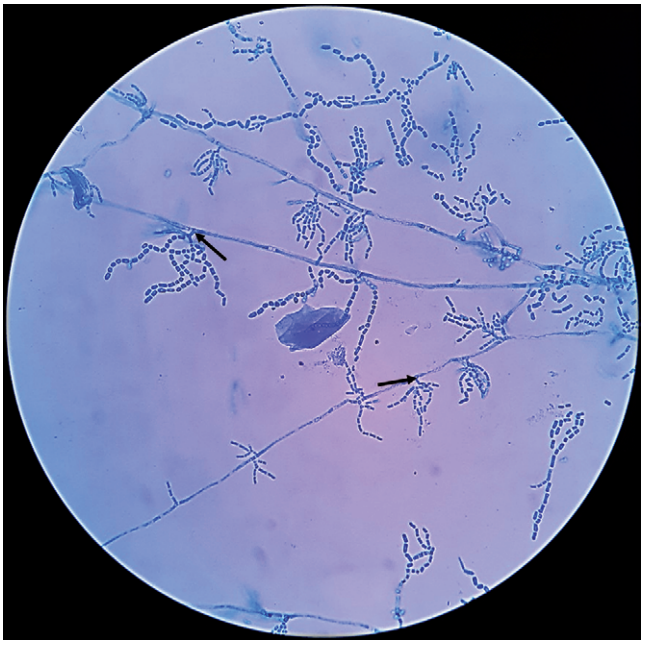

Figure 3. *Arthroraphis kalrae*. A slide culture was stained with Lacto-Phenol Cotton Blue. Black arrow: conidiophore.

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

Reference	Age (yrs)	Sex	Country	Pathology	Risk factor
12	19	F	France	Cystic fibrosis	Malnutrition
1	63	H	Japan	Onychomycosis	ND
2	ND	ND	Slovakia	Onychomycosis	ND
3	80	H	France	Mycetoma	Systemic corticosteroid
5	42	F	Germany	Keratitis	Soft lens wearer
6	52	H	Malaysia	Keratitis	Injury
7	39	M	China	Keratitis and sinusitis	Injury
8	23	F	USA	Keratitis	Soft lens wearer
9	33	H	Australia	Knee joint infection	Injury
10	ND	H	Italy	Knee joint infection	Injury
11	50	F	Spain	Endocarditis	Pericardial patch
13	61	H	Netherlands	Pulmonary infection	Radiotherapy
15	33	H	USA	Meningitis and sinusitis	AIDS
16	39	M	France	Fungal stroke	Malnutrition

A. lignicola, *A. pinicola* and *A. alba*. Among them, *A. alba* fails to grow at 37°C and *A. pinicola* fails to grow on media containing cycloheximide; therefore, they were both ruled out. *A. cuboidea* was distinguished as it is a fast grower and colony matures within 4-5 days; however, in our case a full maturation of the isolated fungi took around 15-20 days. *A. lignicola* was ruled-out as it has broad yellow arthroconidia. Therefore, on the basic of morphological and growth characteristics, the isolated fungi were determined to be *Arthrographis kalrae*.

Discussion

A. kalrae is an uncommon human pathogen commonly found in soil and compost. There has been a rise in the cases of *Arthrographis* infection in the past years. As per 2016 data, one case of infection with *Arthrographis* species and 15 cases of infection with *A. kalrae* have been reported: two onychomycosis (1,2), one mycetoma (3), five keratitis (4-8), one of which associated with sinusitis (7), two knee joint infection (9,10), one endocarditis (11), three pulmonary infections (12-14), one meningitis (15) and one fungal stroke (16)(Table 1).These cases have a worldwide distribution: eight cases in Europe, one in China, one in Japan, three in USA, one in Mexico, one in Malaysia and one in Australia. Ours is the first case identified in India. Three of the five patients with keratitis were soft contact lens wearers; the other two with knee joint infections have had an injury contaminated with soil. The rest of the patients had predisposing infection factors, such as malnutrition, systemic corticosteroids, radiotherapy, AIDS, allogeneic hematopoietic stem cell transplant (12). In our case also the patient was immunocompromised with a low CD4 count of 75 cells/µl. Furthermore, as she was a farmer, was probably exposed to the fungus while working in fields. Sugiura et al. also showed similar findings in Japan.

Diagnoses were always based on phenotypic characteristics and microscopic morphology using de Hoog and Sigler and Carmichael descriptions (17,18). In most cases, identification of the species was confirmed by molecular biology techniques (ITS, D1/D2 sequencing) (12). Our study, in a resource limited setting, has used detailed morphological and cultural characteristics to evaluate the sample and reach its diagnosis (genus level identification and species level identification)(12,19). Furthermore, the samples were preserved for ITS sequencing but unfortunately, due to the tragic pandemic of COVID-19, it was not possible to subculture the isolate. Antifungal susceptibility was not possible in our case study. Moreover, the patient's follow up was also lost.

Limitation of study

Due to COVID-19 pandemic it was very difficult to sub-culture this rare pathogen, perform its antifungal susceptibility testing and send it to the laboratory for ITS region sequencing.

Conclusions

In immunocompromised patients, a variety of opportunistic infections can take place. Therefore, in the presence of immune suppression, malignancy, chronic illness and other risk factors among patients, a careful evaluation of clinical samples is always necessary as it will help clinicians to better treat the patients. Low CD4 count also plays a key role in increasing opportunistic infections in HIV patients.

In conclusion, we report the first case of a pulmonary infection caused by *A.kalrae*. This case illustrates that HIV infected patients are susceptible to opportunistic infections with uncommon pathogens.

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