Fungal Infections Caused by Kazachstania spp., Strasbourg, France, 2007–2020

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Learning Objectives

Upon completion of this activity, participants will be able to:

• Distinguish mammals that can be infected with Kazachstania bovina
• Assess clinical characteristics of persons with positive testing for Kazachstania spp.
• Analyze the antifungal resistance pattern of Kazachstania spp.
• Evaluate potential risk factors for a positive test for Kazachstania spp.

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Rare fungal pathogens are emerging as agents of invasive fungal infections. We analyzed 13 cases of fungal infections caused by *Kazachstania (Arxiozyma)* spp. in Strasbourg University Hospital, Strasbourg, France. Among the cases, 4 patients had proven fungal disease (3 cases of invasive fungal disease and 1 mucocutaneous infection) and 9 were colonized by *Kazachstania (Arxiozyma)* spp. *Candida albicans* was also isolated from 11 of the 13 patients. None of the patients with proven invasive fungal disease met host criteria, but most had underlying diseases. All strains were identified as *K. telluris* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and 3 were confirmed as *K. bovina* by internal transcribed spacer sequencing. For all tested strains, the MICs for fluconazole were ≥2 µg/mL. Emergence of this rare fungal infection might be explained by the increasing number of patients with immunocompromised conditions and gastroesophageal diseases.

Incidence of invasive fungal infections (IFI) has increased over the past 2 decades, mostly associated with candidemia (1). Rare fungal pathogens have also emerged as agents causing IFI, notably in immunocompromised persons (2).

*Kazachstania (Arxiozyma)* spp. are ubiquitous yeasts belonging to the *Saccharomycetaceae* family. *Kazachstania bovina* was described as *Saccharomycetes telluistris* in 1957, as *Candida bovina* in 1958, as *Torulopsis bovina* in 1970, and finally as *K. bovina* in 2005 on the basis of multigene phylogenetic analyses (3–5). *K. bovina* belongs to the *K. telluris* species complex, which also includes *K. pintolopesii*, *K. sloofi ae*, *K. heterogenica*, and *K. telluris* (5). Recently, a case of IFI caused by *C. bovina* (the former name of *K. bovina* in humans) was described (6). We report a case series of fungal infections caused by *Kazachstania (Arxiozyma)* spp. and classify them as invasive infections, mucocutaneous infections, or colonizations. We also describe the antifungal susceptibility testing and the methods used to identify the species.

This analysis is part of a study of opportunistic infections approved by the institutional ethics committee of the Hôpitaux Universitaires de Strasbourg. According to regulations in France, the database was declared to the Commission Nationale de l’Informatique et des Libertés. The study was registered at ClinicalTrials.gov (no. NCT03920735).

**Methods**

To conduct a retrospective observational study of *Kazachstania* spp. infections, we identified all patients in the Strasbourg University Hospital, a 2000-bed tertiary-care hospital, who had *Kazachstania (Arxiozyma)* spp.–positive samples during 2007–2020. We collected data on demographics, underlying diseases, clinical and radiologic aspects, mycologic results, treatments, and outcomes.

We classified *Kazachstania* spp. diseases as proven IFI according to the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium updated consensus or as mucocutaneous infections (7). We defined colonization as isolation of *Kazachstania* spp. from a nonsterile site and absence of associated clinical and radiologic signs.

We incubated blood cultures by using Bactec Mycosis IC/F Plus Aerobic/F media (Becton Dickinson, https://www.bd.com) at 37°C. Other samples were incubated at 35°C on Sabouraud chloramphenicol agar or on chromogenic media chromID Candida (bioMérieux, https://www.biomerieux.fr) before December 2019 and CHROMagar *Candida* (Becton Dickinson) thereafter. We used a slide culture on potato carrot bile medium (Bio-Rad Laboratories, https://www.bio-rad.com) to microscopically observe *K. bovina*.

We identified the strains by using matrix-assisted laser desorption/ionization time-of-flight (MALDITOF) mass spectrometry on a MicroFlex spectrometer and using BioTyper software (Brüker Daltonics, https://www.bruker.com). We confirmed species identification by sequencing the internal transcribed spacer (ITS) region of the ribosomal DNA with primers ITS1 and ITS4 (Eurofins Genomics GmbH, https://eurofinsgenomics.eu) (8). We compared sequences with those in GenBank by BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and with those in the Westerdijk Fungal Biodiversity Institute database (https://www.wi.knaw.nl). We performed antifungal susceptibility testing by using Etest or ATB Fungus 3 methods (bioMérieux). The French National Reference Center for Mycoses and Antifungals tested 1 isolate by using the microdilution method according to the European Committee on AntimicrobialSusceptibility Testing (EUCAST) guidelines (https://eucast.org) (RESSIF no. 20319).

**Results**

We identified 13 patients with *Kazachstania (Arxiozyma)* spp.–positive samples. We found no temporal or spatial hospital associations between cases. Median patient age was 63 (range 40–77) years, and 7 (53.8%) patients were male (Table 1). Of the 13 patients, 4 had a proven fungal disease, of which 3 were classified as IFI: 1 case of fungemia and pyelonephritis, 1 mediastinitis (Figure 1), and 1 angiocholitis. The
fourth patient had a mucocutaneous infection with biopsy-proven esophageal infection. Presence of *Kazachstania* spp. in the other 9 patients was classified as colonization. Most patients had underlying diseases that might have favored the infection or colonization, but none of the patients with proven IFI met the European Organisation for Research and Treatment of Cancer/Mycoses Study Group Education and Research Consortium host criteria (7). Of note, underlying esophageal pathology was reported for 4 of the 13 patients. Among the 4 patients with proven infections, treatment was caspofungin for 2 and surgery for the other 2 (in addition to proton pump inhibitor for 1 patient with esophageal infection) without any effective antifungal treatment against *Kazachstania* spp. The outcome was favorable for all 4 patients with proven infection.

The colonies appeared white on Sabouraud chloramphenicol agar and the chromID *Candida* medium and pink on the CHROMagar *Candida* medium. Growth was slower on the chromID *Candida* medium than on the other 2 media (Figure 2). A slide culture of *K. bovina* incubated for 72 h at 27°C on potato carrot bile medium showed spherical to ellipsoidal yeast cells with multilateral budding, without filamentation. Some asci containing ascospores were visible.

MALDI-TOF mass spectrometry identified all strains in this study as *K. telluris* (Table 2). Because mass spectrometry cannot distinguish between the species of the *K. telluris* complex, we identified the strains involved in IFIs, and therefore stored in our laboratory, by ITS sequencing and confirmed them as *K. bovina* (GenBank accession nos. MZ435268, MZ435269, and MZ435270). The sequences from the strains in this study were 100% similar to 2 other *K. bovina* isolates from 2 different centers (GenBank accession nos. KY103626.1 and NR_144228.1).

For all strains tested, MICs for fluconazole were 2 µg/mL to >256 µg/mL (Table 2). For 11 of the 13 patients, including all with proven fungal infection, we identified another *Candida* species (most often *C. albicans*) (Table 2).

Two patients with invasive infection reported exposure to pigeons. Moreover, culture of a sample of pigeon droppings from the aviary of patient 1 enabled identification of *K. bovina* by ITS sequencing (GenBank accession no. OK037112).

### Table 1. Clinical characteristics of *Kazachstania* spp. infections and colonizations, Strasbourg, France, 2007–2020*

| Patient | Age, y/sex | Underlying condition | Exposure | Type of infection | Therapy | Outcome |
|---------|------------|----------------------|----------|------------------|---------|---------|
| 1       | 67/F       | Diabetes, endometrial cancer (remission) | Pigeon | Fungemia + UTI | FLC + CAS | Survived |
| 2       | 63/F       | Esophagus squamous cell carcinoma | Pigeon | Mediastinitis after gastric ulceration | CAS | Survived |
| 3       | 66/M       | CDP (endocrine carcinoma), recurrent angiocholitis | NA | Angiocholitis | FLC, surgery† | Survived |
| 4       | 84/F       | Esophageal achalasia | None | Esophagitis | PPI, surgery‡ | Survived |
| 5       | 68/F       | CVID, gastro–jejunal anastomotic stenosis | NA | Colonization | None | Died (cardiogenic shock) Survived |
| 6       | 46/M       | Caustic esophageal stenosis, pneumonia | NA | Colonization | None | Survived |
| 7       | 59/F       | Systemic scleroderma | NA | Colonization | None | Survived |
| 8       | 40/M       | Former smoker, *Staphylococcus* ventrilot-associated pneumonia | NA | Colonization | None | Died | Survived |
| 9       | 51/F       | AutoHSCT for oculo-cerebral NHL | NA | Colonization | FLC | Survived |
| 10      | 60/M       | Proven *Mycobacterium fortuitum* infection | NA | Colonization | None | Survived |
| 11      | 59/M       | COPD, emphysema, denutrition | NA | Colonization | None | Died | Survived |
| 12      | 77/M       | Congestive heart failure, ischemic cardiomyopathy, smoker | NA | Colonization | None | Died | Survived |
| 13      | 66/M       | Angioimmunoblastic T-cell lymphoma, neutropenia, pulmonary tuberculosis | NA | Colonization | None | Died | Survived |

*AutoHSCT, autologous hematopoietic stem cell transplantation; CDP, cephalic duodenopancreatectomy; CAS, caspofungin; COPD, chronic obstructive pulmonary disease; CVID, common variable immunodeficiency; FLC, fluconazole; NHL, non-Hodgkin lymphoma; NA, not applicable; PPI, proton pump inhibitor; UTI, urinary tract infection.

†Degastrogastrectomy and hepatico–jejunal and gastrointestinal anastomosis.

‡Peroral endoscopic myotomy to treat achalasia.

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**SYNOPSIS**
Discussion

Among the K. telluris species complex, host specificity for K. bovina seems to be low because it has been isolated only from pigeons, a cow, and humans (4,5). To date, only 2 cases of invasive human infection caused by K. telluris complex have been described (6,9). One case was a K. bovina bloodstream infection, and the other was mediastinitis caused by K. slooffiae. An article about extremely rare invasive fungal infections collected in the FungiScope registry did not include any cases of Kazachstania infection (10).

The isolation of K. bovina from 2 blood cultures from patient 1 in this study, as well as from a patient by Brunet et al. (6), clearly suggests pathogenicity of this fungus. Nevertheless, we identified another Candida species (most often C. albicans) in 11 of the 13 patients in our study, including all with proven fungal infection.

Most patients had an underlying condition that might have favored the infection, including a gastroesophageal pathologic condition in 4 of the 13 patients in our study, similar to the cases reported by Brunet et al. and Mercier et al., suggesting a possible portal of entry (6,9). Moreover, 2 patients with IFI in our study reported exposure to pigeons; for 1 patient, we also isolated K. bovina from the pigeon droppings. Even if K. telluris complex in pigeons had been previously identified, to our knowledge, no cases of zoonotic transmission have been reported (5).

MALDI-TOF mass spectrometry identification of all strains as K. telluris, and further identification of 3 strains involved in IFI by ITS sequencing as K. bovina show the value of sequencing emerging pathogens for proper identification and epidemiology. Misidentification or incomplete identification has been noticed in a previous report of human infection with K. bovina (6).

No specific breakpoints have been established for the antifungal susceptibility of Kazachstania spp.
MICs for more isolates.

The microdilution method should be used to determine sensitivity of Kazachstania spp. to fluconazole, the EUCAST breakpoints showing a susceptible pattern. More recent testing of 5 Kazachstania isolates showed fluconazole MICs to range from 2 to 8 μg/mL (11). To determine the susceptibility of Kazachstania spp. to fluconazole, the EUCAST microdilution method should be used to determine MICs for more isolates.

Table 2. Mycologic characteristics of Kazachstania spp. infections and colonizations, Strasbourg, France, 2007–2020*

| Patient | Sample               | Fungus species                  | Identification technique | Antifungal susceptibility, μg/mL | GenBank accession no. |
|---------|----------------------|---------------------------------|--------------------------|---------------------------------|-----------------------|
| 1       | 2 blood cultures, urine | K. bovina + C. albicans        | Sequencing               | Etest 24 0.125 0.012 0.047 0.25 | MZ435268              |
|         |                      |                                 |                          | EUCAST 2 <0.016 <0.125 0.015 0.015 |                      |
| 2       | Mediastinal collection; false membranes; pleural fluid† | K. bovina + C. albicans, + C. glabrata | Sequencing               | Etest 8 0.125 NA 0.5 0.19 | MZ435270              |
| 3       | Bile (surgical sample) | K. telluris SC + C. albicans | MALDI-TOF                | Etest >256 0.19 NA 0.047 0.25 | Not stored            |
| 4       | Esophageal biopsy; fibroscopy: white plaques of the mucosa‡ | K. bovina + C. albicans | Sequencing               | Etest 6 0.032 NA 0.125 0.25 | MZ435269              |
| 5       | Gastric liquid        | K. telluris SC + C. albicans   | MALDI-TOF                | NA NA NA NA NA | Not stored            |
| 6       | BAL fluid             | K. telluris SC + C. albicans   | MALDI-TOF                | NA NA NA NA NA | Not stored            |
| 7       | Stool                 | K. telluris SC + C. lusitaniae | MALDI-TOF                | NA NA NA NA NA | Not stored            |
| 8       | BAL fluid             | K. telluris SC                  | MALDI-TOF                | NA NA NA NA NA | Not stored            |
| 9       | Urine, stool          | K. telluris SC + C. albicans   | MALDI-TOF                | NA NA NA NA NA | Not stored            |
| 10      | BAL fluid             | K. telluris SC + C. albicans   | MALDI-TOF                | NA NA NA NA NA | Not stored            |
| 11      | Sputum                | K. telluris SC + C. albicans, C. dubliniensis A. niger | MALDI-TOF AMB-fungus 4 <0.125 <0.125 <0.5 NA | Not stored |
| 12      | Stool                 | K. telluris SC + C. albicans   | MALDI-TOF                | NA NA NA NA NA | Not stored            |
| 13      | BAL fluid, stool      | K. telluris SC + C. kefyr       | MALDI-TOF AMB-fungus 8 <0.125 <0.125 <0.5 NA | Not stored |

*AMB, amphotericin B; AMB; BAL, bronchoalveolar lavage; CAS, caspofungin; Etest (bioMérieux, https://www.biomerieux.fr); EUCAST, European Committee on Antimicrobial Susceptibility Testing; FLC, flucytosine; FLR, fluconazole; ITC, itraconazole; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NA, not applicable; SC, species complex; VRC, voriconazole.
†Anatomopathologic examination of the esophageal biopsy showed necrosis and inflammation.
‡Anatomopathologic examination of the gastric perforation showed necrosis and inflammation.

EUCAST non–species-related breakpoints for Candida are ≤2 μg/mL (susceptible) and >4 μg/mL (resistant). Our finding of fluconazole MICs between 2 μg/mL and >256 μg/mL for tested strains in our study suggests decreased susceptibility to this molecule in 5 of 7 isolates. MICs for the other antifungals tested were low for all strains. Our MIC results should be considered with caution because for 1 isolate, Etest and EUCAST indicated different fluconazole MICs. The antifungal susceptibility testing performed by the National Reference Center on Invasive Mycoses and Antifungals, using the EUCAST method on 31 strains of Kazachstania spp., showed high azole MICs for 55% of isolates and high caspofungin MICs for 6.5% (11). More recent testing of 5 K. bovina isolates showed fluconazole MICs to range from 2 to 8 μg/mL (12). To determine the susceptibility of Kazachstania spp. to fluconazole, the EUCAST microdilution method should be used to determine MICs for more isolates.

No specific antigens have been designed for the diagnosis of Kazachstania infections. We would like to have assessed the (1→3)-β-D-glucan test was not available in our laboratory before 2020. However, because we identified another species of Candida in 11 of the 13 patients in our study, the contribution of the (1→3)-β-D-glucan test might be debatable.

The retrospective design of our study led to some limitations. We retrospectively classified cases of invasive infection or colonization, but we might have missed some information (7). Sequencing and antifungal susceptibility testing were not performed on all strains, notably those that were not involved in invasive fungal infections and were not stored.

The emergence of this very rare fungal infection might be explained by the increasing number of...
patients with immunocompromised conditions and gastroesophageal diseases. The use of MALDI-TOF mass spectrometry and ITS sequencing to identify yeasts might also contribute to increased documentation of these fungal infections.

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Dr. Kaeuffer is a medical doctor who works in infectious diseases in the University Hospital of Strasbourg, France, with a special interest in medical mycology.

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