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SHORT COMMUNICATION

Catheter-related blood stream infection caused by *Millerozyma farinosa* in an immunocompetent patient: a case report and a brief review of the literature

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SUMMARY

Millerozyma farinose is a halotolerant yeast that has recently been described as an emerging human pathogen, especially in immunocompromised patients. Both the diagnostic process and treatment options are still unclear.

Here, we report a case of an immunocompetent oncological patient who developed a catheter-related bloodstream infection (CRBSI) with a concomitant respiratory tract infection caused by *M. farinosa*.

In this report, we discuss how prompt microbiological identification and attentive evaluation of the patient's clinical status can play a significant role in the appropriate management of infections caused by uncommon fungi. MALDI-TOF technology has also substantially improved the timely diagnosis of rare fungi. Furthermore, our diagnosis was subsequently confirmed by 5.8S rRNA sequencing. In our patient, the rapid diagnosis of fungaemia was crucial, together with catheter removal and the initiation of antifungal treatment, for the patient's clinical improvement.

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INTRODUCTION

Millerozyma farinosa, formerly known as *Pichia farinosa*, belongs to the Saccharomycetaceae family (Dujon, 2010). Ubiquitous in the environment, it is found mainly in foods such as fermented alcoholic beverages, soybean paste, and miso. Additionally, it has been used for food production and fermentation (Mallet *et al.*, 2012). While considered a rare opportunistic pathogen, it has recently emerged as a cause of fungaemia, mainly in immunocompromised patients. *M. farinosa* is a diploid yeast that can produce high amounts of glycerol and xylitol. It can adjust osmotic pressure by accumulating glycerol, which endows tolerance to 3 M NaCl. The so-called osmotolerant yeasts and, among them, the halotolerant ones, constitute a heterogeneous group of yeasts belonging to very different genera and sharing the capacity to resist low water-activity environments (Lages *et al.*, 1999).

This report describes a case of catheter-related blood stream infection (CRBSI) caused by *M. farinosa* identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) in a 68-year-old immunocompetent patient.

Key words:
Millerozyma farinosa, Bloodstream infection, DNA sequencing, MALDI-TOF MS.

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CASE REPORT

A 68-year-old male patient, living in a rural area, with a history of infiltrating esophageal carcinoma treated in 2019 with chemo- and radio-therapy, underwent pharyngo-laryngeal-esophagectomy and pharyngo-gastroplasty, associated with tracheostomy packaging in 2020. Four months later, due to dysphagia and a sub-stenosis of the pharyngo-gastric anastomosis refractory to pneumatic dilation, a Port-a-cath was placed for total parenteral nutrition.

In the following days, there was a rapid worsening of the clinical picture with the onset of dyspnea and fever. The patient was admitted to the Emergency room and later transferred to the Intensive Care Unit where

Figure 1 - *Millerozyma farinosa colonies isolated from a BAL sample on Sabouraud dextrose agar (SDA) after 2 days of incubation at 37°C, showing morphological similarity to other more common yeast species when grown on SDA.*

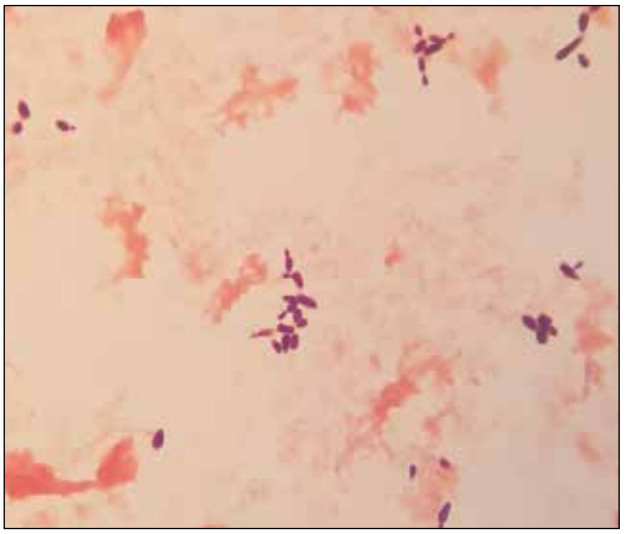

Figure 2 - *Microscopy characterization (100×) of M. farinosa directly from a positive blood culture bottle observed with Gram staining: Gram-positive cells, oval or elongated.*

he was placed on mechanical respiration and cardiovascular support with norepinephrine. On day 1, two sets of blood culture were collected and were later reported positive for *Enterococcus faecium* and *Klebsiella pneumoniae ESBL*; an antibiotic treatment was started. A bronchoalveolar lavage (BAL) collected on day 2 resulted negative for a concomitant SARS-COV2 infection. Upon cardio-circulatory stabilization, he was transferred to the Internal Medicine Department, where he continued the antibiotic treatment.

On day 6, *M. farinosa* was first isolated in our patient on Sabouraud dextrose agar (*Figure 1*) from a BAL sample (load: 1000000 CFU/ml) along with *Candida albicans* (load: 300 CFU/ml). Complete blood counts on day 7 revealed that leucocyte and neutrophil counts were slightly above the normal range: 10.88x10^3/µL and 8.92x10^3/µL, respectively (normal range: 4.00-10.00 x10^3/µL for leucocytes and 2.00-8,00 x10^3/µL for neutrophils). Urine culture from a urinary tract catheter was reported positive for *Candida glabrata* (load: 10,000 CFU/ml) with negative urinalysis for leucocytes on day 8.

Two sets of blood culture bottles were collected (one from the Port-a-cath and the other from a peripheral vein) during a febrile episode on day 8 and incubated in a BD BACTEC FX™ automated detection system. Twenty-four and forty hours following incubation, the aerobic blood culture bottle from the Port-a-cath and the peripheral vein were reported positive, respectively. Yeast-like structures were revealed upon microscopic evaluation of a Gram-stained slide prepared directly from the positive blood culture bottles (*Figure 2*). As a result, following our diagnostic process to obtain a quicker identification of the yeast in question, we used a blood culture panel of a multiplex polymerase chain reaction (PCR) system (FilmArray) which resulted negative. That result excluded *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis* as causative agents since they were the only yeast species included in our panel. Leucocyte and neutrophil counts returned to their normal range on day 9.

The yeast from blood culture was identified as *M. farinosa* on day 10 using MALDI-TOF-MS (Bruker Daltonics, Bremen, Germany; score: 2.294); the same identification technique was used on the BAL sample. The technique entails using the surface of a target plate to mix some colonies of the fungus (analyte) with a matrix compound. When the plate is loaded into the instrument, a laser beam is applied, resulting in an ablation process causing ionization of the analyte molecules with the matrix molecules. The generated ions are then separated, depending on their mass and charge, to be analyzed on a TOF analyzer followed by the MS software, generating an MS profile.

On day 11, *in vitro* antifungal susceptibility was tested using Sensititre YeastOne broth microdilution (TREK Diagnostic Systems, East Grinstead, West Sussex, UK). Results are shown in *Table 1*.

Given the few cases in which *M. farinosa* has been reported as a disease-causing agent in humans, we performed 5.8S rRNA sequencing to further confirm our diagnosis. Genomic DNA was extracted from the cultured isolate using the PrepMan TM Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, CA, USA) and amplified using the universal fungal-specific primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) for

Table 1 - *Minimum Inhibitory Concentration (MIC) of the antifungal agents tested using YeastOne Sensititre (TREK Diagnostic Systems) against M. farinosa clinical isolate.*

Antifungal drug	*MIC (mcg/ml)*
Anidulafungin	0.015
Micafungin	0.015
Caspofungin	0.03
Posaconazole	0.03
Voriconazole	0.06
Itraconazole	0.12
Fluconazole	8
Amphotericin B	0.25

internal transcribed spacer regions of fungal ribosomal DNA (White *et al.*, 1990). Polymerase chain reaction (PCR) was performed in a 2700 thermal cycler (Applera, Foster City, CA, USA) set to the following conditions: denaturation at 94°C for 5 min; 40 cycles of 94°C for 30 sec, 58°C for 30 sec, 7°C for 1 min, and a final extension at 72°C for 5 min. PCR products were visualized on a 2% agarose gel stained with ethidium bromide. Amplicons were sequenced using BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, USA) in a 310 ABI PRISM1 sequencer (Applied Biosystems, Foster City, USA). Nucleotide sequences were analyzed using Finch TV software Version 1.4.0 (www.geospizia.com). The consensus obtained using the EMBOSS explorer (http://www.bioinformatics.nl/emboss-explorer) was used for the GenBank BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence showed a query cover of 99% and a 100% identity with *M. farinosa* (accession number KY104281.1), confirming our previous identification via mass spectrometry. The sequence originated from this case was deposited in GenBank under accession number MW757340.1.

The central venous line was removed and antifungal treatment was started with intravenous caspofungin; a loading dose of 70 mg on the first day followed by 50 mg/day. The patient's clinical conditions gradually improved during antifungal therapy. Control blood cultures were collected on day 14 and returned negative and antifungal therapy was stopped. A new pneumatic dilation procedure was performed with improvement of dysphagia and resumption of spontaneous feeding with a pureed diet. The patient was discharged on day 23 and underwent a follow-up visit 2 months later. He has since remained afebrile.

DISCUSSION

Most invasive fungal infections (IFI) are caused by *Candida* spp., while fungaemia caused by uncommon fungi, such as *Saprochaete capitata/Magnusiomyces capitatus* (previously named *Geotrichum capitatum, Trichosporon capitatum* or *Blastoschizomyces capitatum*), and *Pichia* species is extremely rare (Cavanna *et al.*, 2017; Fernández-Ruiz *et al.*, 2017).

To the best of our knowledge, this is the fourth pub-

Table 2 - *A summary of clinical features of the three cases of human infections caused by Millerozyma farinosa described in the literature.*

Reference	*Age*	*Underlying conditions*	*Infectious agent identified*	*Immunity profile*	*Source of infection*	*Identification method*	*AST*	*Antifungal treatment*	*Outcome*
Anaissie et al., 1989	12	Teratoma	*Millerozyma farinosa*	Normal neutrophil count	CVC (later removed)	-	-	No antifungal treatment, only removal of CVC	Survived
Adler *et al.*, 2007	13	Anaplastic large-cell lymphoma, PVC positioned (later removed during hospital stay), chemotherapy, bacterial sepsis	*Candida boidinii*[1]	Severe neutropenia	Blood (withdrawn from PVC)	Biochemical	-	-	Survived
			Pichia farinosa[2]			Biochemical (second attempt with a different test), later confirmed with molecular ID.	E-test system and broth microdilution	Oral fluconazole	
Hong *et al.*, 2017	71	Progressive bladder cancer, chemotherapy through CVC (later removed)	Millerozyma farinosa	Neutropenia	Blood and CVC tip	Biochemical confirmed by molecular ID	Broth microdilution	Micafungin IV	Survived

AST, antimicrobial susceptibility testing; CVC, central venous catheter; PVC, peripheral venous catheter; ID, identification; IV, intravenous.
[1]A yeast belonging to the Saccharomycetes family.
[2]Teleomorph of Candida cacaoi, currently known as Millerozyma farinosa. Belongs to the Saccharomycetaceae family.

lished case of human infection caused by *M. farinosa* (Anaissie *et al.*, 1989; Adler *et al.*, 2007; Hong *et al.*, 2018) and the first to be reported in Italy. Clinical features of the previously reported cases are summarized in *Table 2*. Currently there are no guidelines for treatment of infections caused by *M. farinosa* and no evidence-based data available on its antifungal susceptibilities.

Furthermore, in *Table 3*, we present 11 reported cases of uncommon fungi and *Pichia* species causing infections in adult patients (Shin *et al.*, 2003; Ostronoff *et al.*, 2006; Yang *et al.*, 2009; Shaaban *et al.*, 2010; Kanno *et al.*, 2017; Hamal *et al.*, 2008; Gabriel *et al.*, 2012; Yun *et al.*, 2013; Chan *et al.*, 2013; Valenza *et al.*, 2006; Cavanna *et al.*, 2017). Five of these eleven cases were catheter-related and the majority were described as immunocompetent patients.

Identification of those uncommon fungi and *Pichia* species has frequently been challenging; at times misidentified using both mass spectrometry and bio-

Table 3 - *A summary from the literature of some reported cases of human infections caused by Pichia species and uncommon fungi.*

Reference	*Age*	*Underlying conditions*	*Infectious agent identified*	*Immunity profile*	*Source of infection*	*Identification and AST methods*	*Antifungal treatment*	*Outcome*
Chan *et al.*, 2013	21	Sickle cell disease, permanent intravenous access device (removed during the first admission), recent acute chest syndrome, history of priapism, avascular necrosis of the right hip	*Candida pelliculosa (reported as Candida non-albicans)*	Immunocompetent	Blood (CVC)	**ID:** Mass spectrometry, confirmed by an outside laboratory.	Fluconazole	Survived
			Pichia anomala[1]		Blood (during Fluconazole treatment)	**ID:** Mass spectrometry, biochemical; **AST:** Broth microdilution	Micafungin	
Valenza *et al.*, 2006	46	Morbid obesity, alcohol and nicotine abuse, pneumonia, cholecystectomy, CVVHD	*Candida utilis*	Immunodeficient following a severe pneumococcal septicaemia	Blood and BAL	**ID:** Biochemical; **AST:** Broth microdilution	Fluconazole	Died
			Candida fabianii			**ID:** Molecular.		
Hamal *et al.*, 2008	40	Congenital combined aortic incompetence of the mitral valve, craniectomy	Choices of 3 isolates: *Candida utilis, Candida pelliculosa, Candida fabianii*	Not mentioned	Blood	**ID:** Biochemical; **AST:** E-test strips agar diffusion. Positive for Biofilm on microtiter plate.	Fluconazole then Voriconazole (resistance developed to both)	Not mentioned
			Pichia fabianii[2]		Infected mitral valve post-surgery, endocarditis	**ID:** Molecular	Amphotericin B	
Gabriel *et al.*, 2012	53	Severe rhabdomyolysis with major renal failure, mesenteric ischemia, paraplegia, continuous hemofiltration	*Pichia fabianii*	Immunocompetent	Blood	**ID:** Biochemical (failed), molecular identification; **AST:** E-test strips agar diffusion.	Caspofungin	Survived
							Fluconazole	
Yun *et al.*, 2013	47	Plasma cell myeloma (ASCT and chemotherapy)	*Candida utilis*	Neutropenic	Blood and CVC tip	**ID:** Biochemical; **AST:** Broth microdilution.	Intravenous amphotericin B	Died
			Lindnera (Pichia) fabianii[2]		Blood (with ongoing amphotericin B treatment)	**ID:** Molecular identification; **AST:** Broth microdilution.	Caspofungin	

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Reference	*Age*	*Underlying conditions*	*Infectious agent identified*	*Immunity profile*	*Source of infection*	*Identification and AST methods*	*Antifungal treatment*	*Outcome*
Kanno *et al.*, 2017	81	Mild cognitive disorders	*Candida albicans* *Kodamaea ohmeri*[3]	Immunocompetent	Blood and BAL	**ID:** Biochemical; **AST:** E-test strips agar diffusion.	Caspofungin Voriconazole	Survived
Shaaban *et al.*, 2010	34	Asthma (on inhaler therapy), heavy alcohol use, thrombophlebitis, transesophageal fistula	*Kodamaea ohmeri*	Immunocompetent	Blood	**ID:** Biochemical confirmed by molecular ID; **AST:** Broth microdilution (due to lack of resources, echinocandins were not tested)	Micafungin (empirically)	Survived
Yang *et al.*, 2009	71	T2DM, tinea pedis, cellulitis	*Kodamaea ohmeri*	Immunocompetent	Blood	**ID:** Biochemical confirmed by molecular ID; **AST:** Strips in microbroth.	Fluconazole (empirically) Amphotericin B	Survived
Ostronoff *et al.*, 2006	58	CML in accelerated phase undergoing chemotherapy	*Pichia ohmeri*[4]	Immunocompetent	Blood and CVC tip	**ID:** Biochemical; **AST:** Microdilution assay.	LAB	Survived
Shin *et al.*, 2003	59	VP shunt infection, pneumonia	*Pichia ohmeri*	Not mentioned	Blood (PVC) and phlebitis skin site	**ID:** Biochemical.	Amphotericin B	Survived
Cavanna *et al.*, 2017	80	Recent aortic valve replacement, recent bacterial pneumonitis, septic shock	*Saprochaete capitata*[5]	Immunocompetent	Blood (CVC)	**ID:** Mass spectrometry; **AST:** Broth microdilution.	Fluconazole (empirically)	Died

AST, Antimicrobial susceptibility testing; CVC, central venous catheter; ID, identification; CVVHD, continuous venovenous hemodiafiltration; BAL, broncoalveolar lavage; ASCT, autologous stem cell transplantation; T2DM, type 2 diabetes mellitus; CML, chronic myelogenous leukemia; LAB, libosomal amphotericin B; VP, ventriculoperitoneal; PVC, peripheral venous catheter.
[1]Candida pelliculosa teleomorph, renamed to Wickerhamomyces anomalus.
[2]Candida fabianii teleomorph.
[3]Formerly known as Pichia ohmeri which belongs to Saccharomycetes family.
[4]Currently known as Kodamaea ohmeri.
[5]Anamorph of Magnusiomyces capitatus and formerly known as Trichosporon capitatum.

chemical methods (Valenza *et al.*, 2006; Hamal *et al.*, 2008; Gabriel *et al.*, 2012; Yun *et al.*, 2013; Adler *et al.*, 2007). This highlights the importance of continuously updating reference libraries regarding mass spectrometry for an accurate identification of the various *Pichia* species.

In our case, the diagnosis of *M. farinosa* in the BAL sample using MALDI-TOF MS and, excluding *Candida* species tested for in our multiplex PCR panel, substantially aided the subsequent prompt identification of *M. farinosa* at the species level in blood culture. Additionally, the sequence analysis of ribosomal DNA (rDNA) ITS regions confirmed the mass spectrometry result, avoiding misidentification of that rare *Pichia* species.

We believe that the blood stream infection was catheter-related, since the worsening of the patient's conditions was reported following the positioning of the Port-a-cath and then later improved upon its removal and initiation of antifungal therapy. Risk factors including the use of broad-spectrum antibiotics, total parenteral nutrition, malignancy, intensive care, urinary tract catheterization and repeated surgical procedures contributed to the concomitant infection of *M. farinosa* in the respiratory tract and the patient's high susceptibility to colonization by *Candida* species (Hofmeyr *et al.*, 2006). Despite these risk factors, our patient was immunocompetent, making him the second reported case of human disease caused by *M. farinosa* in an immunocompetent patient.

In our opinion, the use of modern and up-to-date techniques coupled with a confirmation based on nucleic acid sequence information has substantially improved the timely diagnosis of these rare fungi. With the growing implementation of highly sensitive molecular analysis in laboratory diagnostic techniques, we hypothesize that those species defined as "emerging" have always been active pathogens previously misidentified as more common yeast species.

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Disclosure of Interest

The authors declare that they have no conflicts of interest.

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