Mycopathologia
DOI 10.1007/s11046-017-0137-5

Comparative Pathogenicity of *Lomentospora prolificans* (*Scedosporium prolificans*) Isolates from Mexican Patients

Mariana Elizondo-Zertuche · Alexandra M. Montoya · Efrén Robledo-Leal · Idalia Garza-Veloz · Ana L. Sánchez-Núñez · Raquel Ballesteros-Elizondo · Gloria M. González

Received: 6 December 2016 / Accepted: 20 April 2017

Abstract We identified 11 *Lomentospora prolificans* isolates recovered from Mexican patients using phenotypic and molecular characteristics. The identification of isolates was assessed by internal transcribed spacer (ITS rDNA) sequencing. In vitro susceptibility to amphotericin B, fluconazole, voriconazole, posaconazole, caspofungin, anidulafungin and micafungin was determined according to Clinical and Laboratory Standards Institute (CLSI) procedures. Three isolates (07-2239, 11-2242 and 04-2673) were used to induce systemic infection in immunocompetent ICR mice. Survival and tissue burden studies were used as markers of pathogenicity. All of the strains were resistant to every antifungal tested with MIC's for AmB (8–>8 μg/ml), VRC (16–>16 μg/ml), PSC (16–>16 μg/ml), FLC (64–>64 μg/ml) and echinocandins with MICs ≥8 μg/ml. One hundred, ninety and sixty percent of the infected mice with the strains 07-2239, 11-2242 and 04-2673 died during the study, respectively. Regarding tissue burden, the highest fungal load of the infected mice was detected in brain followed by spleen and kidney, regardless of the strain.

Keywords Pathogenicity · In vitro susceptibility · Survival study · Tissue burden · *Lomentospora prolificans*

M. Elizondo-Zertuche · A. M. Montoya · A. L. Sánchez-Núñez · G. M. González (✉)
Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Madero y Dr. Eduardo A. Pequeño s/n, Colonia Mitras Centro, 64460 Monterrey, Mexico
e-mail: gmglez@yahoo.com.mx

E. Robledo-Leal
Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Mexico

I. Garza-Veloz
Laboratorio de Medicina Molecular, Unidad Académica de Medicina Humana y Ciencias de la Salud, Universidad Autónoma de Zacatecas, Zacatecas, Mexico

R. Ballesteros-Elizondo
Departamento de Histología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Mexico

Introduction

Lomentospora prolificans (formerly *Scedosporium prolificans*) [1] is an opportunistic filamentous mold; it has a worldwide distribution and is frequently present in soil, sewage and polluted waters. It is responsible for serious infections due to its high virulence, propensity for invasion, dissemination and multidrug resistance to antifungals [2, 3]. Infections caused by this organism can be localized, extended to the surrounding tissues or disseminated to distant organs depending of the infection route and immunological status. When *L. prolificans* infects immunocompetent individuals, usually having a prior trauma in the form of puncture wounds,

Published online: 29 April 2017

skin ulcers or surgery, it produces localized infections that involve skin, bone and joints [4, 5]. In immunocompromised patients with cystic fibrosis, where the most important risk factor for the acquisition of this pathogen is inhalation and especially in those suffering hematologic malignancies or organ-transplant recipients receiving immunosuppressive treatment, lesions spread and they are usually fatal in less than a month. Johnson et al. reported in 2014 that Scedosporiosis (*Scedosporium apiospermum*/*Lomentospora prolificans*) followed lung transplantation in 55% of the cases as well as other organ transplants (multivisceral, 18%; heart, liver and small intestine in 45%). Scedosporiosis was preceded by colonization in 36% of the reports. Diseases included pneumonia (64%), mediastinitis (18%) and fungemia/disseminated infections (18%) [5, 6]. It is well documented that an altered immune status of the host seems to be the main cause of invasion by this mold, which mimics most of the pathologic aspects of other opportunistic fungi such as *Fusarium* spp., [7] *Acremonium* spp., [8] or *Aspergillus fumigatus* [9]. In these cases, the infection is probably acquired by inhalation of conidia and the fungus is able to develop in practically any body organ. On the other hand, *L. prolificans* shows universal in vitro and in vivo resistance to available antifungal drugs [5, 10–14] and recovery from neutropenia has been considered a mandatory prerequisite for resolving the infection irrespective of the antifungal treatment used [10].

In this study, we identified 11 clinical isolates of *L. prolificans* by morphological and molecular criteria. Furthermore, we evaluated the pathogenicity of three isolates of *L. prolificans* in an immunocompetent murine model of disseminated infection.

Materials and Methods

Strains Identification

A total of 11 isolates collected over a period of 10 years (2003–2013) of *L. prolificans* were recovered from a broad spectrum of clinical presentations and identified based on their macroscopic and microscopic morphologies and ITS rDNA sequencing. Regarding the phenotypic approach, clinical isolates were grown at 30 °C on potato dextrose agar (PDA) for 2 weeks. For microscopic observation, slide cultures were made and incubated at 30 °C for 7 days. Carbohydrates assimilation was determined for sucrose, L-arabinose, lactose, D-ribose and maltose according to a method previously described [15]. Hydrolysis of urea was determined using Christensen's urea agar which was incubated at 30 °C for 4 days, cycloheximide tolerance was determined by the isolates' development on Mycosel agar incubated at 30 °C for 10 days, and the gelatinase activity was assessed in gelatin agar slants after 20 days at 30 °C [16]. Aspartyl proteinase activity was evaluated using bovine serum albumin medium [17]. Phospholipase activity was carried out on egg yolk medium [18]. Hemolytic activity was assayed using Sabouraud blood medium [19]. In these three last tests, plates were incubated at 30 °C for 7 days and activity was expressed in terms of presence or absence. Thermotolerance was studied by incubating the isolates on PDA for two weeks at 30, 37 and 40 °C [16]. *Candida albicans* ATCC 90028 was used as the quality control for all enzymatic activity determinations.

In vitro susceptibility testing was performed by the broth microdilution method for filamentous fungi according to the CLSI document M38-A2 [20]. The assayed antifungal agents were: amphotericin B (AmB) (Bristol-Myers Squibb, Princeton, NJ), fluconazole (FLC) (Pfizer, Inc., New York, NY), posaconazole (PSC) (Merck, Rahway, NJ) and voriconazole (VRC) (Pfizer, Inc., New York, NY), which were obtained as pure reagent-grade powders; caspofungin (CSF), anidulafungin (ANF) and micafungin (MCF) were purchased as Cancidas (Merck Sharp & Dohme), Eraxis (Pfizer, Inc.) and Mycamine (Astellas Pharma, Inc.), respectively. The final concentrations of the drugs ranged from 0.125 to 64 μg/ml for FLC, from 0.03 to 16 μg/ml for VRC, PSC and AmB and from 0.015 to 8 μg/ml for echinocandins. The microplates were incubated at 35 °C for 72 h, and the MICs for all antifungals were read visually. Amphotericin B, voriconazole and posaconazole end points were determined as the first clear well showing no growth, whereas fluconazole was read at ≥50% inhibition compared to the control well. For echinocandins, MECs were determined microscopically as the lowest concentration of drug promoting the growth of small, round, compact hyphae relative to the appearance of the filamentous forms seen in the control wells. Assays were done in duplicate using *Candida parapsilosis* ATCC 22019 and *Paecilomyces variotii* MYA 3630

as quality control organisms. For in vitro susceptibility testing, we determined the geometric mean and range of MICs and MECs.

For ITS rDNA sequencing, the DNA of the isolates was extracted using the method described by González et al. [21]. Ribosomal DNA ITS domains were amplified using the forward primer ITS-5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and reverse primer ITS-4 (5′-TCCTCCGCTTATTGATATGC-3′) [22]. Amplifications were performed in a final volume of 30 µl containing 1× GoTaq Colorless Master Mix (Promega, Madison, WI), 200 nM of each primer and 20 ng of DNA. The thermocycling conditions were: 94 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 53 °C for 30 s and 72 °C for 30 s, with final extension at 72 °C for 3 min. The final products were electrophoresed in 1.5% agarose gels and stained with SYBR® Green I Gel Nucleic Acid Gel Stain (Invitrogen™, Eugene, OR). HyperLadder I (Bioline USA Inc.) was used as a molecular weight marker for size determinations. The pattern of amplified bands was photographed and analyzed with the UVP Bioimaging System, EpiChemi III Darkroom. PCR products were purified using the commercial Wizard SV Gel and PCR Clean-Up system (Promega, Madison, WI). Sequencing was performed utilizing the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster city, CA) following the manufacturer's directions. Reactions were run and analyzed in an Avant 3100 Genetic Analyzer (Applied Biosystems, Foster city, CA). For confirmation of phenotypic identification, DNA sequence fragments were compared to ISHAM-ITS database (http://its.mycologylab.org/), Mycobank (http://mycobank.org/) and NCBI GenBank, sequence entries using BLAST. Identifications were made when BLAST searches yielded ≥99% identity. The obtained sequences are submitted to GenBank with the accession numbers cited in Table 1.

Experimental Disseminated Infection

We established two different inocula based on our own previous in vivo studies with the strain 07-2239 of *L. prolificans*, comparing the mortality rate caused by several inocula ranging from 10^3 to 10^6 conidia/animal and where we observed that concentrations of 1×10^5 conidia/animal and 5×10^3 conidia/animal, caused a

Table 1 Demographic and clinical characteristics of 11 patients infected or colonized by *L. prolificans*

Patient	Strain	GenBank	Age (years)	Gender	Underlying disease	Site of isolates	Antifungal treatment	Outcome after diagnosis
1	03-1714	KJ176696	48	F	CML, BMT	BAL, urine, blood	ITC, CAS	Died d 4
2	05-835	KJ176698	57	M	AIDS	BAL	AmB	Died d 2
3	05-2190	KJ176699	61	M	AIDS	Blood, LCR	FLC	Died d 2
4	06-1220	KJ176700 KJ176697	55	M	AML	BAL	AmB	Died d 2
5	04-2673	KJ176697	14	F	Sinusitis maxillaries	Sinus maxillaries	Surgery	Cured
6	09-1125	KJ176702	47	F	CML, sepsis	BAL, vitreous body		Died d 23
7	10-1167	KJ176703	12	F	Fibrosis cystic	Sputum	None	Colonization
8	11-2242	KJ176704	57	F	RT	Blood	AmB	Died d 1
9	07-2239	KJ176701	67	M	AML, sepsis	Peritoneal fluid, blood	FLC, AmB	Died d 1
10	12-261	KJ176705	40	M	AML	Blood	AmB	Died d 2
11	13-196	KJ176706	52	M	AML, sepsis	Skin biopsy	FLC, AmB	Died d 3

AIDS acquired immunodeficiency syndrome, *CML* chronic myelogenous leukemia, *BMT* bone marrow transplantation, *BAL* bronchoalveolar lavage, *AML* acute myelogenous leukemia, *RT* renal transplantation

100 and 50% mortality in 2 weeks, respectively (data not shown).

The strains from different origins selected were 04-2673, 11-2242 and 07-2239 (sinusitis maxillaries colonization, renal transplantation and acute myelogenous leukemia) were cultured at least twice on PDA plates to check the cultures purity and viability. After 7 days of incubation at 30 °C, conidial cells were harvested and washed twice in sterile saline, and the number of conidia in the suspensions was counted with a hemocytometer and adjusted to the desired concentration. To corroborate the conidial counts, serial dilutions were cultured on PDA plates at 30 °C. Male ICR mice aged 4 weeks (weighing 20–22 g; purchased from Harlan, Mexico) were used for the in vivo studies. The animals were housed in cages of five mice each. All mice were given food and water ad libitum and were monitored daily. Care, maintenance and handling of the animals were in accordance with the Mexican government's license conditions for animal experimentation and the Guidelines for the Care and Use of Laboratory Animals. Experiments were conducted with the approval of the Ethics and Research Committee of Facultad de Medicina, UANL in Monterrey, Nuevo León, Mexico (registration code MB11-008). No immunosuppressive scheme was used.

For the survival study, we used ten mice for each strain. Mice received an intravenous injection of 1×10^5 conidia/animal. Deaths were registered through day 30 post-infection. Moribund mice were killed, and deaths were recorded as occurring on the next day. Animals that survived to day 30 were killed by inhalation of metofane, followed by cervical dislocation. The spleens and brains were removed aseptically, homogenized separately in 2 ml of sterile phosphate-buffered saline solution (138 mM NaCl, 3 mM KCl, 8.1 mM Na_2HPO_4 and 1.5 mM KH_2PO_4), and the entire organs were plated onto PDA and incubated at 30 °C for 7 days to corroborate the presence of fungi as the cause of death of the animals.

For the tissue burden study, 12 mice for each strain received an intravenous injection of 5×10^3 conidia/animal and groups of 4 mice per strain were sacrificed on day 4, 7 and 10 post-infection. Half of kidneys, spleens and brains of mice were assessed for fungal burden by means of quantitative cultures, and the other half of the organs were removed aseptically, weighed and transferred to sterile glass homogenizers containing 2 ml of sterile phosphate-buffered saline solution (138 mM NaCl, 3 mM KCl, 8.1 mM Na_2HPO_4 and 1.5 mM KH_2PO_4). The organs were mechanically homogenized (Polytron-Aggregate, Kinematic), and serial tenfold dilutions of the suspensions were plated onto PDA and incubated at 30 °C for 7 days to determine the number of viable CFU in each organ. Five uninfected mice were used as controls per experimental day. The entire in vivo experiments were performed twice at different times.

Histopathology

For the tissue burden study, after mice were killed, tissues (half of each organ) were immediately removed and fixed with 10% buffered formalin. Samples were dehydrated, paraffin embedded and sliced into 5-μm sections. The sections were stained with Grocott methenamine silver for light microscopy observations.

Statistics

Mean survival time (MST) was estimated by the Kaplan–Meier method and compared among groups by the log-rank test. The fungal tissue burdens of the tested organs in the different experimental groups were analyzed using the Kruskal–Wallis test in SPSS (version 17.0 for Windows; Chicago, IL, USA) and plotted using GraphPad Prism version 6.01 (Graph Prism Software Inc., USA). $P \leq 0.05$ was statistically significant.

Results

Demographic and Clinical Data

Eleven isolates of *L. prolificans* were registered at Departamento de Microbiología, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Mexico, from 2003 to 2013. The demographic data of the patients are shown in Table 1. The median age of the patients was 52 years with a range of 12–67 years. There were a total of six males (55%) and five females (45%). Seven out of the eleven patients had a hematologic malignancy and/or underwent bone marrow or solid organ transplantation or received long-term corticosteroids; this pathology was

the most common underlying disease (64%) followed by AIDS (18%). Three of these seven patients developed fungemia. Nine out of 11 (82%) patients died as a result of their underlying diseases associated with their disseminated fungal infection. Colonization of the respiratory tract was noted on one CF (fibrosis cystic) patient and one with chronic sinusitis.

Strain Identification

The 11 isolates had colonial and microscopic characteristics compatible with the genus *Lomentospora*. The isolates grew on PDA plates at 30 °C, reaching 55–65 mm after 2 weeks; the colonies were flat, with short mycelium and olive gray to blackish in color. Microscopic examination of slide cultures revealed hyaline, septate hyphae. Conidiogenous cells were flask shaped with single or multiple oval, smooth and brown conidia at their tips. Results of physiologic testing demonstrated that 100% of the isolates assimilated L-arabinose, lactose and maltose, but none assimilated sucrose and D-ribose. All isolates liquefied gelatin, hydrolyzed urea and were producers of phospholipase but none produced aspartyl proteinases or hemolysins. Their growth was inhibited by the presence of cycloheximide on Mycosel agar. All isolates grew at 30, 37, and 40 °C. Based upon these features, isolates were identified as *L. prolificans* [2] and further confirmed by ITS (≥99% ISHAM-ITS database) [22].

Antifungal Susceptibility

According to the MICs obtained, all the strains were resistant to AmB (8> 8 μg/ml), VRC (16–>16 μg/ml), PSC (16–>16 μg/ml), FLC (64–>64 μg/ml). However, echinocandins exhibited a moderate in vitro activity against all strains when the minimum effective concentration (MEC) was used as the end point for antifungal susceptibility testing (MEC geometric mean 3.36, 4 and 4.36 μg/ml for ANF, MCF and CSF, respectively). The MICs of the control strains were within the acceptable range for the tested drugs (Table 2).

Experimental Disseminated Infection

The results confirmed the high virulence exhibited by clinical strains of *L. prolificans*, since most of the strains tested in the present study produced lethal infections in all the mice (Fig. 1). One hundred

Table 2 In vitro susceptibilities of 11 strains of *L. prolificans* against seven antifungals

Strain/antifungal	MIC/MEC 72 h (μg/mL)			
	Range	GM[a]	50%	90%
L. prolificans (11)				
Amphotericin B	16–>16	16	16	>16
Anidulafungin	2–8	3.36	2	8
Micafungin	1–8	4	4	8
Caspofungin	1–8	4.36	4	8
Voriconazole	16–>16	16	16	16
Posaconazole	16–>16	16	>16	>16
Fluconazole	64–>64	64	>64	>64

MIC minimum inhibitory concentration, *MEC* minimum effective concentration

[a] Geometric mean

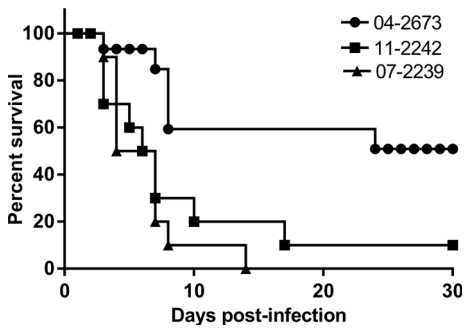

Fig. 1 Survival of mice infected with *L. prolificans* by intravenous inoculation through the lateral vein with (1×10^5 conidia/mouse)

percent of the mice infected with strain 07-2239 and 90% of those infected with strain 11-2242 died between 3–14 and 3–17 days, respectively. However, a slight reduction in virulence was observed in the mice infected with strain 04-2673, where animals died between days 3 and 24 post-infection, with a 40% survival at the end of the study. The mean survival time (MST) in days for *L. prolificans* strains 07-2239, 11-2242 and 04-2673 with 95% confidence interval (95% CI) was: 6.2 (4.18–8.22), 9.1 (4.11–14.08) and 17.8 (10.85–24.75), respectively ($P < 0.015$). The animals manifested a behavior consisting of running in circles or gyrating by means of continuous lateral rolling, ataxia, weight loss, ruffled hair and stiff neck 3 days before their death.

The fungal tissue burden results are summarized in Table 3 and Fig. 2. In general, the highest fungal load of all three strains was detected in brain followed by

Table 3 Fungal tissue burden results in mice intravenously infected with inocula of 5×10^3 CFU/mouse

Strain (GenBank accession number)	Organ	Log CFU/g tissue [median (range)]		
		Days post-infection		
		4	7	10
L. prolificans	Brain	4.70 (4.25–5.08)	3.65 (3.58–3.68)	3.81 (3.71–3.86)
04-2673	Spleen	3.59 (3.49–3.88)	3.31 (3.12–3.46)	2.28 (2.22–2.44)
(KJ176697)	Kidney	2.70 (2.63–3.11)	2.59 (2.48–2.67)	2.11 (1.96–2.26)
07-2239	Brain	5.02 (4.73–5.07)	4.96 (5.40–4.60)	4.79 (4.67–5.95)
(KJ176701)	Spleen	3.75 (3.48–4.01)	3.51 (3.45–3.60)	3.22 (3.12–3.31)
	Kidney	3.05 (2.99–3.14)	2.90 (2.85–2.95)	2.56 (2.45–2.82)
11-2242	Brain	4.88 (4.74–5.09)	4.73 (4.56–4.94)	4.71 (4.64–5.23)
(KJ176704)	Spleen	3.85 (3.68–3.95)	3.65 (3.55–3.66)	3.34 (3.08–3.54)
	Kidney	3.10 (3.04–3.16)	2.98 (2.89–3.00)	2.55 (2.49–2.87)

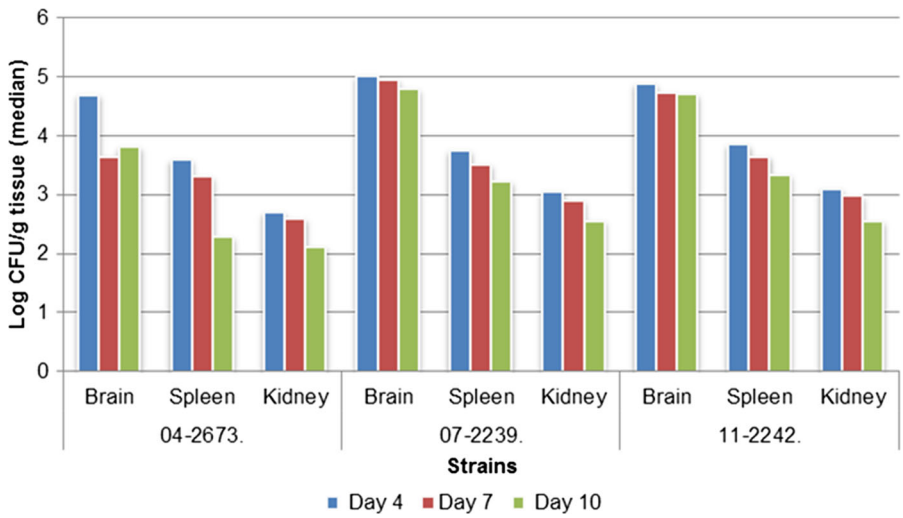

Fig. 2 Graph of fungal tissue burden results in mice intravenously infected with inocula of 5×10^3 CFU/mouse of the strains tested by day 4, 7 and day 10 post-challenges

spleen and kidney tested on the three experimental days ($P = 0.4283$). No significant difference was found between different strains or separate days. There was a slight decrease in tissue burden during the days 7–10 of the experiments ($P = 0.4156$), and clearance of the fungal elements was never observed in any studied organs. Histopathological examination revealed the presence polymorphonuclear cells infiltration, neurons presented morphological signs of necrosis characterized by a hyperchromic and pycnotic nucleus, and abundant presence of hyphae and conidia in all the brain tissue samples was taken from the dissected mice during each of the three experimental days (Fig. 3).

Discussion

Lomentospora prolificans can be considered as a truly emerging dangerous pathogen; previous studies have reported that it is associated with more severe infections compared to those caused by other *Scedosporium* species and the members of the *Scedosporium apiospermum* species complex. Support for these clinical finding has been demonstrated in previous studies [26–30]. Rodríguez-Tudela et al. reported patients with malignancies, disseminated infection with *L. prolificans* was the most frequent (81.9%), and it was associated with a high mortality rate 64%. This is consistent with our results.

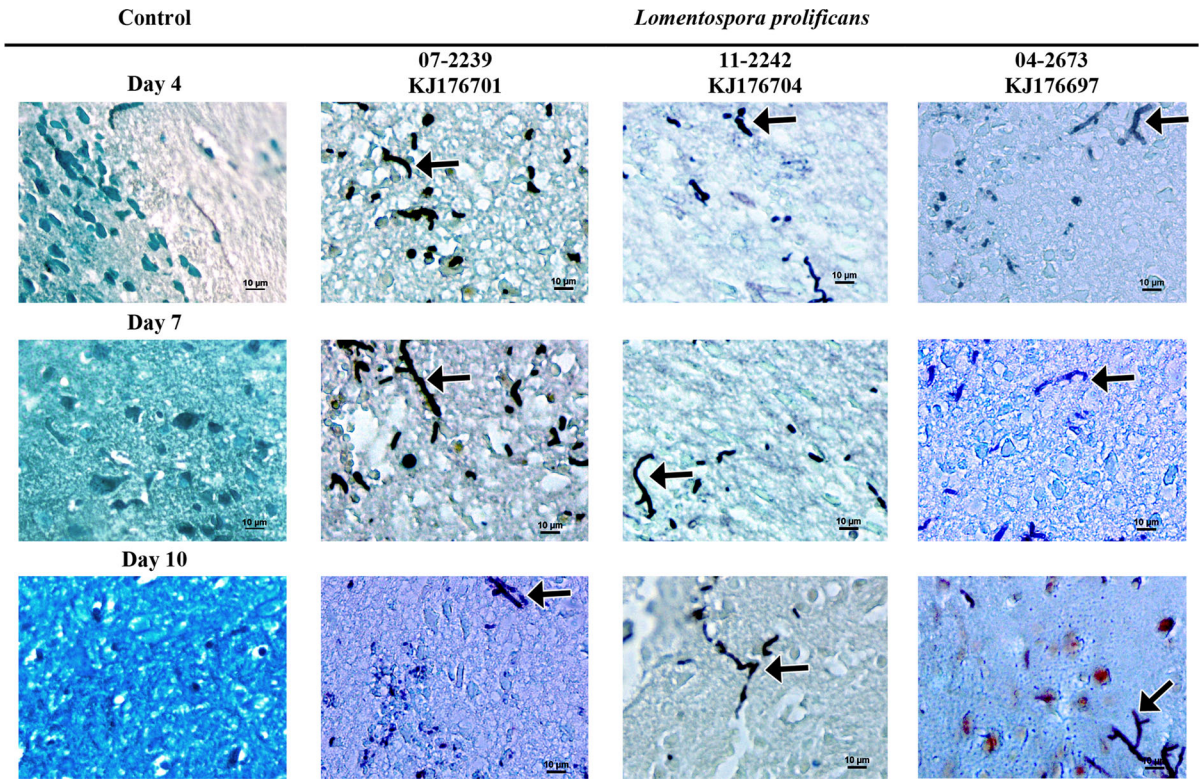

Fig. 3 Representative histopathological Grocott methenamine silver-stained sections of brain from ICR mice intravenously infected with 5×10^3 CFU/mouse of the strains tested by days 4, 7 and day 10 post-challenge showing hyphal elements, polymorphonuclear cell infiltration and signs of necrosis characterized by a hyperchromic and pycnotic nucleus

Most of the patients receiving immunosuppressive therapy developed fatal disseminated infection, and nearly all of them underwent antifungal treatment, however; the short survival period of most of the patients with disseminated infections is alarming. One of the most remarkable characteristics of the infections caused by this mold is the high rate of positive blood cultures, as reported previously especially in patients with disseminated infections [26]. However, the interpretation of these positive cultures is very difficult and may lead to misinterpretation, and many of the blood cultures didn't turn positive until few days before the death of the patient, thus limiting their diagnostic utility [5]. Despite this mold being easily identified once isolated, the mortality of disseminated infections is so high that new procedures allowing a more rapid diagnosis are required.

Breakthrough *L. prolificans* infections are frequently found in patients with neutropenia under antifungal prophylaxis [27]. This reflects the extreme resistance of this species to these drugs in contrast to other opportunistic hyphomycetes, e.g., *P. boydii* and *S. apiospermum*. *L. prolificans* is known to be multidrug resistant, especially to amphotericin B, 5-flucytosine, echinocandins and most azoles. While there are limited data on the successful treatment of patients with deep seated *L. prolificans* infection, the combination of voriconazole and terbinafine appears to be the most effective [28–30]. The resistance of *L. prolificans* isolates to all antifungal in this study suggests that there is a need for further studies on susceptibility and resistance mechanisms of *L. prolificans*. In general, our results correlate with clinical data because infections caused by these molds are usually nonresponsive to antifungals and their outcomes are usually fatal compared to *S. apiospermum* [10, 13, 30].

According to provisions in the guidelines of the International Society for Human and Animal Mycology (ISHAM)-ITS database, sequencing of this region it is sufficient to reach an accurate identification of these microorganisms [22]. The sequencing results presented here confirm the high similarity within *L.*

prolificans, which is known to be highly conserved at the ITS region [31, 32].

A remarkable aspect of this study is that three of the eleven strains tested exhibited virulence characteristics which could be related to the pathology from which they were isolated; the mildly virulent strain was isolated from a sinusitis maxillaries colonization, and the highly virulent strains were isolated from fatal disseminated infections in patients with acute myelogenous leukemia and renal transplantation, all in Nuevo León, México. This could indicate that strains with different virulence patterns can exist in the same region, in agreement with Ortoneda et al. [23]. All *L. prolificans* strains studied caused similar mortality rates and tissues infiltration as shown by histopathology examinations in the animals' brains, spleens and kidneys. These findings are consistent with results from other authors [23–25], which confirms the predilection of these fungi for brain tissues, being more often reported in patients with disseminated infections. The ability of *L. prolificans* to cause invasive infection is also supported by its ability to attack all visceral organs, particularly when it initiates infection through intravenous route.

In conclusion, *L. prolificans* is a highly virulent opportunistic fungus, as the clinical and experimental data demonstrate. Due to the resistance of this fungus to all available antifungal drugs, it is of enormous importance to develop preventive strategies focusing on reducing both environmental and host risk factors, including decreasing the exposure of the airways and reducing the risk associated with neutropenia.

Acknowledgements This research was made possible through support from Conacyt-INFRA-2015-01 (code 251142).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest. The authors alone are responsible for the content and the writing of the paper.

References

1. Lackner M, de Hoog GS. Proposed nomenclature for *Pseudallescheria*, *Scedosporium* and related genera. Fungal Divers. 2014;67:1–10.
2. De Hoog GS, Guarro J, Gene J, Figueras MJ. *Hyphomycetes*. Genus: *Scedosporium*. In: Atlas of clinical fungi, 2nd ed. Utrecht, Centraalbureau voor Schimmelcultures, 2000:899–901.
3. Gosbell IB, Morris ML, Gallo JH, Weeks KS, Neville SA, Rogers AH. Andrews rh, Ellis HD. Clinical, pathologic and epidemiologic features of infection with *Scedosporium prolificans*: four cases and review. Clin Microbiol Infect. 1999;5:672–86.
4. Panackal AA, Marr KA. *Scedosporium/Pseudallescheria* infections. Semin Respir Crit Care Med. 2004;25(2):171–81.
5. Rodriguez-Tudela JL, Berenguer J, Guarro J, Kantarcioglu AS, Horre R, de Hoog GS, Cuenca-Estrella M. Epidemiology and outcome of *Scedosporium prolificans* infection, a review of 162 cases. Med Mycol. 2009;47(4):359–70.
6. Johnson LS, Shields RK, Clancy CJ. Epidemiology, clinical manifestations, and outcomes of *Scedosporium* infections among solid organ transplant recipients. Transpl Infect Dis. 2014;16:578–87.
7. Guarro J, Gene J. Opportunistic fusarial infections in humans. Eur J Clin Microbiol Infect Dis. 1995;14:741–54.
8. Guarro J, Gams W, Pujol I, Gene J. *Acremonium* species: new emerging fungal opportunists. *In vitro* antifungal susceptibilities and review. Clin Infect Dis. 1997;25:1222–9.
9. Latge JP. *Aspergillus fumigatus* and aspergillosis. Clin Microbiol Rev. 1999;12:310–50.
10. Ortoneda M, Capilla J, Pujol I, Pastor FJ, Mayayo E, Fernández-Ballart J, Guarro J. Liposomal amphotericin B and granulocyte colony-stimulating factor therapy in a murine model of invasive infection by *Scedosporium prolificans*. J Antimicrob Chemother. 2002;49(3):525–9.
11. Capilla J, Yustes C, Mayayo E, Fernández B, Ortoneda M, Pastor FJ, Guarro J. Efficacy of albaconazole (UR-9825) in treatment of disseminated *Scedosporium prolificans* infection in rabbits. Antimicrob Agents Chemother. 2003;47(6):1948–51.
12. Lackner M, de Hoog GS, Verweij PE, Najafzadeh MJ, Curfs-Breuker I, Klaassen CH, Meis JF. Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species. Antimicrob Agents Chemother. 2012;56(5):2635–42.
13. Wiederhold NP, Lewis RE. Antifungal activity against *Scedosporium* species and novel assays to assess antifungal pharmacodynamics against filamentous fungi. Med Mycol. 2009;47(4):422–32.
14. Tortorano AM, Richardson M, Roilides E, et al. ESCMID and ECMM joint guidelines on diagnosis and management of hyalohyphomycosis: *Fusarium* spp., *Scedosporium* spp. and others. Clin Microbiol Infect. 2014;Suppl 3:27–46.
15. Yarrow D. Methods for the isolation, maintenance, and identification of yeasts. In: Kurtzman CP, Fell JW, editors. The yeasts: a taxonomic study. 4th ed. Amsterdam: Elsevier; 1998.
16. Espinel-Ingroff A, Goldson PR, McGinnis MR, Kerkering TM. Evaluation of proteolytic activity to differentiate some dematiaceous fungi. J Clin Microbiol. 1988;26:301–7.
17. Chakrabarti A, Nayak N, Talwar P. *In vitro* proteinase production by *Candida* species. Mycopathologia. 1991;114:163–8.
18. Price MF, Wilkinson ID, Gentry LO. Plate method for detection of phospholipase activity in *Candida albicans*. Sabouraudia. 1982;20:7–14.
19. Luo G, Samaranayake LP, Yau JY. *Candida* species exhibit differential in vitro hemolytic activities. J Clin Microbiol. 2001;39(8):2971–4.

20. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, 2nd ed. CLSI M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA, 2008.
21. González GM, Rojas OC, Bocanegra-García V, González JG, Garza-González E. Molecular diversity of *Cladophialophora carrionii* in patients with chromoblastomycosis in Venezuela. Med Mycol. 2013;51(2):170–7.
22. Irinyi L, Serena C, García-Hermoso D, et al. International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database-the quality controlled standard tool for routine identification of human and animal pathogenic fungi. Med Mycol. 2015;53(4):313–37.
23. Ortoneda M, Pastor FJ, Mayayo E, Guarro J. Comparison of the virulence of *Scedosporium prolificans* strains from different origins in a murine model. J Med Microbiol. 2002;51(11):924–8.
24. Nweze EI, Okafor JI. Comparative virulence of *Scedosporium* species in animal models. Braz J Infect Dis. 2010; 14(3):271–6.
25. Harun A, Serena C, Gilgado F, Chen SC, Meyer W. *Scedosporium aurantiacum* is as virulent as *S. prolificans*, and shows strain-specific virulence differences, in a mouse model. Med Mycol. 2010;48(1):S45–51.
26. Tintelnot K, Just-Nübling G, Horré R, Graf B, Sobottka I, Seibold M, Haas A, Kaben U, De Hoog GS. A Review of German *Scedosporium prolificans* cases from 1993 to 2007. Med Mycol. 2009;47(4):351–8.
27. Lamaris GA, Chamilos G, Lewis RE, Safdar A, Raad II, Kontoyiannis DP. *Scedosporium* infection in a tertiary care cancer center: a review of 25 cases from 1989–2006. Clin Inf Dis. 2006;43:1580–684.
28. Del Palacio A, Garau M, Amor E, Martinez-Alonso I, Calvo T, Carrillo-Muñoz A, Guarro J. Case reports. Transient colonization with *Scedosporium prolificans*. Report of four cases in Madrid. Mycoses. 2001;44:321–5.
29. Vagefi MR, Kim ET, Alvarado RG, Duncan JL, Howes EL, Crawford JB. Bilateral endogenous *Scedosporium prolificans* endophthalmitis after lung transplantation. Am J Ophthalmol. 2005;139:370–3.
30. Howden BP, Slavin MA, Schwarer AP, Mijch AM. Successful control of disseminated *Scedosporium prolificans* infection with a combination of voriconazole and terbinafine. Eur J Clin Microbiol Infect Dis. 2003;22:111–3.
31. Wedde M, Müller D, Tintelnot K, de Hoog GS, Stahl U. PCR based identification of clinically relevant *Pseudallescheria/Scedosporium* strains. Med Mycol. 1998;36:61–7.
32. Lennon PA, Cooper CR, Salkin IF, Lee SB. Ribosomal DNA internal transcribed spacer analysis supports synonym of *Scedosporium inflatum* and *Lomentospora prolificans*. J Clin Microbiol. 1994;32:2413–6.