Scedosporium spp.: Chronicle of an emerging pathogen

Carolina E. Luna-Rodríguez, Rogelio de J. Treviño-Rangel, Alexandra M. Montoya, Miguel A. Becerril-García, Ángel Andrade and Gloria M. González*

Department of Microbiology, School of Medicine, Autonomous University of Nuevo Leon, Monterrey, Nuevo Leon, Mexico

Abstract

Scedosporium spp. are environmental fungi that have acquired clinical importance over the last decades. Currently, these fungi are considered emerging pathogens that affect both immunocompromised and immunocompetent people. The most serious conditions within scedosporiosis are infections with dissemination to the central nervous system, which have a mortality rate of up to 75%. These fungi present intrinsic resistance to several antifungals, making options in the treatment of scedosporiosis difficult. The frequency of these infections is thought to be underestimated since the signs and symptoms are nonspecific. This leads to the suspicion of other fungal etiological agents that occur more frequently in human pathologies, such as *Aspergillus* spp. Currently, these five species are recognized as causing important diseases in humans: *Scedosporium apiospermum*, *Scedosporium boydii*, *Scedosporium aurantiacum*, *Scedosporium minutisporum*, and *Scedosporium dehoogii*.

Key words: Scedosporiosis. Emerging pathogen. Clinical mycology. Antifungal resistance.

Introduction

Over the past decades, reports of new cases of infections caused by species of the genus *Scedosporium* have increased at an alarming rate worldwide. The group of diseases caused by this fungus is known as scedosporiosis and may affect different parts of the human body, producing a broad spectrum of clinical manifestations ranging from a superficial localized infection to a disseminated invasive infection. The populations at greater risk are those with cystic fibrosis, organ transplant recipients, and individuals with HIV/AIDS. Localized superficial infections generally occur in immunocompetent individuals.

Environmental factors such as natural disasters have increased the number of reports of disseminated invasive infections after the aspiration of contaminated waters in healthy individuals.

For the classification of fungi, the traditional methods involve the characteristics of the fungal colony, some physiological tests (thermotolerance, growth in cycloheximide, etc.), and microscopic characteristics of the fungal structure (especially, conidia or spores) developed in standard cultures used in mycology laboratories. Under these criteria, two species of the *Scedosporium* genus were first described - *Scedosporium apiospermum* and *Scedosporium inflatum* (as an anamorphous or asexual state) and *Pseudallescheria boydii* (as a teleomorph or sexual state)¹.

The taxonomy of this genus has presented major changes since its first description in Italy in 1911. Moreover, a significant number of new species have been described recently, as well as a reclassification of others (Table 1). Currently, five species are recognized as the main agents responsible for scedosporiosis:

Correspondence:

*Gloria M. González

E-mail: gmglez@yahoo.com.mx

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Table 1. Changes in the taxonomy of the genus *Scedosporium* throughout history

Year	Investigator	Descriptions and proposals
1911	Saccardo PA	Described a new fungus from a sample of mycetoma: <i>Monosporium apiospermum</i> (anamorphic phase) Suggested the name <i>Scedosporium</i> for the same fungus
1919	Castellani A and Chalmers AJ	Validated the name <i>Scedosporium</i>
1922	Shear CL	Described a new ascomycete <i>Allescheria boydii</i> (anamorphic and teleomorphic phase)
1944	Emmons CW	Showed that <i>Monosporium apiospermum</i> was an anamorph of <i>Allescheria boydii</i>
1970	Malloch D	Reclassified the teleomorphic phase as <i>Petriellidium boydii</i> recognized <i>Petriellidium</i> as synonymous with <i>Pseudallescheria</i>
	Hughes	Recognized <i>Scedosporium apiospermum</i> as an anamorph of <i>Pseudallescheria boydii</i>
1984	Malloch D and Salkin IA	Described a new species of <i>Scedosporium</i> : <i>Scedosporium inflatum</i>
1991	Gueho E and De Hoog GS	Proposed that <i>Scedosporium inflatum</i> and <i>Lomentospora prolificans</i> were synonyms
1994	Lennon PA	Proposed that the name <i>Scedosporium prolificans</i> should include isolates of <i>Scedosporium inflatum</i> and <i>Lomentospora prolificans</i>
2005	Gilgado F, Cano J, Gené J and Guarro J	Described new species: <i>Pseudallescheria minutispora</i> and <i>Scedosporium aurantiacum</i> Described the complex <i>Pseudallescheria boydii</i> : <i>Pseudallescheria angusta</i> , <i>Pseudallescheria ellipsoidea</i> , and <i>Pseudallescheria fusioidea</i>
2006	Rainer J and De Hoog S	Demonstrated that <i>Scedosporium prolificans</i> is a different species from the <i>Pseudallescheria boydii</i> complex
2008	Gilgado F, Cano J, Gené J, Sutton DA and Guarro J	Described a new species: <i>Scedosporium dehoogii</i> . Showed that <i>Scedosporium apiospermum</i> is different from <i>Pseudallescheria boydii</i> Proposed the name of <i>Scedosporium boydii</i> for the anamorph of <i>Pseudallescheria boydii</i> Modified the name of the <i>Pseudallescheria boydii</i> complex by <i>Pseudallescheria/Scedosporium</i> complex
2010	Gilgado F, Cano J, Gené J and Guarro J	Described the teleomorphic phase of <i>Scedosporium apiospermum</i> : <i>Pseudallescheria apiosperma</i>
	Rainer and Kaltseis	Described a new species: <i>Scedosporium deficiens</i>
2014	Members of the ECMM/ISHAM	Reclassified <i>Scedosporium prolificans</i> as <i>Lomentospora prolificans</i> . Proposed the <i>Scedosporium apiospermum</i> complex: <i>Scedosporium apiospermum</i> , <i>Scedosporium aurantiacum</i> , <i>Scedosporium dehoogii</i> , <i>Scedosporium boydii</i> , <i>Scedosporium minutisporum</i> , and <i>Scedosporium desertorum</i>
2015	ISHAM	Proposed rearrangement of the <i>Scedosporium apiospermum</i> complex: <i>Scedosporium apiospermum</i> , <i>Scedosporium aurantiacum</i> , <i>Scedosporium dehoogii</i> , <i>Scedosporium boydii</i> , <i>Scedosporium minutisporum</i> , <i>Scedosporium ellipsoideum</i> , and <i>Scedosporium angustum</i>
2016	Chen et al.	Proposed <i>Scedosporium apiospermum</i> , <i>Scedosporium boydii</i> , and <i>Pseudallescheria angusta</i> as members of the <i>Scedosporium apiospermum</i> complex
2016	Rougeron, Stchigel, S. Giraud, Bouchara and Cano	Described a new species: <i>Scedosporium cereisporum</i>
2018	Ramirez-Garcia, et al.	Proposed as members of the <i>Scedosporium apiospermum</i> complex: <i>Scedosporium angustum</i> , <i>Scedosporium apiospermum</i> , <i>Scedosporium boydii</i> , <i>Scedosporium ellipsoideum</i> , and <i>Scedosporium fusarium</i>

ISHAM: International Society of Human and Animal Mycology, ECMM: European Confederation of Medical Mycology.

S. apiospermum, *Scedosporium boydii*, *Scedosporium aurantiacum*, *Scedosporium dehoogii*, and *Scedosporium minutisporum*.

Species of the *Scedosporium* genus have macroscopic and microscopic characteristics which are very similar among them. These fungi have the ability to grow under

Figure 1. Reproductive structures of the genus *Scedosporium*. **(A)** Initial asexual phase: the presence of septate hyaline hyphae with oval conidia. **(B)** Intermediate sexual phase (in some cases): production of closed ascomata with ascospores inside. **(C)** Delayed asexual phase: presence of sinemas with a large number of conidia in the apex.

Table 2. Morphological characteristics of the pathogenic species of the genus *Scedosporium*

Species	Thermotolerance 45°C present (+) absent (-)	Microscopic morphology		
		Conidia	Cleistothecia	Sinema
<i>Scedosporium apiospermum</i>	+	Group	+	+
<i>Scedosporium boydii</i>	+	Solitary or group	+	+
<i>Scedosporium aurantiacum</i>	+	Solitary	-	+
<i>Scedosporium dehoogii</i>	-	Solitary	-	-
<i>Scedosporium minutisporum</i>	+	Solitary	+	+

anaerobic culture conditions, are thermotolerant (45°C), and also present a tolerance to sodium chloride and magnesium chloride at 5% concentrations in liquid culture medium². Table 2 shows some characteristics of the different species which confirm this genus.

Macroscopically, in Sabouraud Dextrose Agar culture medium, these fungi develop flat cotton-like or velvety-looking colonies with defined or irregular edges. Initially, the colonies are white, but as they mature, they acquire an olive green to dark gray color. *S. aurantiacum* is characterized by its ability to produce a diffusible yellow pigment in the conventional culture mediums used in mycology laboratories. Microscopically, *Scedosporium* spp. produces septated hyaline hyphae with low or clustered short thin conidiophores (Fig. 1A). The conidia are unicellular, with a claviform to globose form and olive or hyaline color.

In a culture medium with low nutritional content such as the potato dextrose agar medium (PDA), they may develop a closed ascoma or cleistothecia immersed in the culture medium in a time lapse of 2-3 weeks of incubation. Ascoma is spherical structures with a yellowish or black color and a diameter of approximately

140-200 µm formed by a single layer of fine flat cells (Fig. 1B). Inside the ascoma, gold unicellular ellipsoid ascospores are developed, which are released once the ascoma is mature². The development of ascoma has been described in *S. minutisporum*, *S. apiospermum*, and *S. boydii*.

On the other hand, another type of structure called sinema develops usually in the border of the colony when cultures are incubated for a period of time >3 weeks. The sinemas are bundles of erect hyphae, yellow or gray, with conidia at the apex (Fig. 1C). These structures have been observed in pathogenic strains of the genus *Scedosporium*, with the exception of *S. dehoogii*².

Ecology

The *Scedosporium* genus includes saprophyte and cosmopolitan fungi, which are found in the soils of regions with temperate and tropical weather. Its isolation is more frequent in soils with high human activity, i.e., agricultural soil, urban gardens, playgrounds, sports gardens, industrial areas, and domestic gardens. This could

be due to organic pollution and a high amount of nitrogen components in these environments³.

In recent years, different research groups have been directed toward establishing whether or not there is a specific ecological niche for each one of the species of this genus. Kalteis et al. conducted a study in Austria and the Netherlands, in which they took samples from soils with high human activity, as well as in natural areas such as the woods, wetlands, and sludge (sea and mud). Interestingly, none of the *Scedosporium* species were isolated in the samples taken from natural areas. *S. apiospermum* was the most abundant species in areas with high human activity, present in 58.7%-77.7% of samples, followed by *S. dehoogii* with a proportion of 13.8%-28.8%. On the other hand, *S. aurantiacum* was only found in 4.8-7.7% of the samples and *S. minutisporum* was only recovered in 5.8%. In a revision of the clinical cases of scedosporiosis occurred in these countries, a total of 179 reports were found. However, *S. apiospermum* was the main agent isolated in 57% of the cases, followed by *S. boydii* in 33.5%, *S. aurantiacum* in 6.1%, and *S. minutisporum* in 2.2% of the cases. *S. dehoogii* was only isolated in two cases of superficial infections³.

In France, patients with cystic fibrosis are the population most affected by this fungus⁴. *S. boydii* was the microorganism most frequently isolated in 62% of samples of sputum and bronchial aspiration, followed by *S. apiospermum* in 24%, *S. aurantiacum* in 10%, and *S. minutisporum* in 4%⁵.

Due to the high incidence of this fungus among these patients, a study was conducted on the distribution of this fungus in different soils with high human activity. Findings show that *S. dehoogii* was the most abundant species with 43.6% of isolates, followed by *S. aurantiacum* with 22.3%, *S. boydii* with 20.3%, and *S. apiospermum* with 19.5%. *S. minutisporum* was the least isolated one with 0.3%⁶. Interestingly, *S. dehoogii* was not recovered from clinical samples despite being the most frequently isolated species in the soils of this country⁵.

In Australia, a similar situation to the one mentioned in France occurs. One in every six patients with cystic fibrosis suffered from a colonization/infection by this fungal group, having *S. aurantiacum* as the most predominant species⁷. Harun et al. researched the prevalence of these fungi in the soils of an urban zone in Sydney. Interestingly, *S. aurantiacum* was the predominant species with 54.6%, followed by *Lomentospora prolificans* with 43%, *S. boydii* with 2.1%, and *S. dehoogii* with 0.3%⁸.

In Thailand, after the tsunami in 2004, two infection cases caused by *S. apiospermum* were reported⁹. In 2016, Luplertlop et al. conducted an environmental study in soil and water samples from public parks isolating *S. apiospermum* as the most frequent species with 72.4%, followed by *S. aurantiacum* with 6.1% and *S. dehoogii* with 5.1%¹⁰.

In Chile, clinical cases related to these fungi are rare. There are only reports of two clinical cases of rhinosinusitis where *S. boydii* and *S. apiospermum* were recovered. However, when a study was conducted on the prevalence of these fungi in soil samples of urban areas in the city of Arica, Valparaíso, and Puerto Varas, only four isolates of *S. dehoogii* were obtained¹¹.

In Mexico, the reports of clinical cases are very similar to those published in Chile. Currently, there are only three published cases of mycetoma¹² and one case of endoftalmitis¹³ caused by *S. boydii*. In 2017, Elizondo-Zertuche et al. conducted a study to discover the prevalence of these fungi in soils from different country regions. Soils with high human activity were analyzed obtaining a total of 100 isolates, from which *S. apiospermum* was the most predominant species with 69%, followed by *S. boydii* with 16%, *S. aurantiacum* with 7%, *S. dehoogii* with 5%, and *S. angustum* with 3%¹⁴.

Epidemiology

The epidemiology of these infections is still unknown; nevertheless, in recent years, the number of multicentered studies related to scedosporiosis in the different populations linked to risk factors has increased. Alarming, in the past decade, reports of scedosporiosis cases have increased in Europe, America, Asia, and Oceania¹⁵, as shown in Figure 2.

In 2015, a multicenter study took place in Australia in 15 third-level hospitals to determine the epidemiology of the different invasive infections due to filamentous fungi other than *Aspergillus*. Of a total of 162 patients, 165 isolates of filamentous fungi were identified with mucoromycetes and *Scedosporium* spp. being the most common at 45.7% and 33.3%, respectively¹⁶.

Patients with cystic fibrosis present frequent colonization in the lungs with *Scedosporium* spp. with a prevalence between 3.4% and 17.4% in Europe¹⁷, US¹⁸, and Australia⁸.

The species of *Scedosporium* are recognized as significant pathogenic agents in solid organ transplants and hematopoietic stem cell transplant. In Spain, Husain et al. conducted a review of cases of organ

Figure 2. Global distribution of environmental isolations and clinical cases of *Scedosporium* spp.

receptors with a report of infection by *Scedosporium* sp. finding a total of 80 cases, with 57 cases of solid organ transplants and 23 cases of hematopoietic stem cell transplant. The organs involved in the infections of the solid organ receptors were the kidney (35%), heart (28%), liver (18%), lungs (14%), and small intestine (5%). Of these patients, 18% received prophylactic antifungal treatment. *S. apiospermum* was the pathogenic agent in 83% of the receptors of solid organs and *L. prolificans* was the pathogenic agent in 19% of these cases. In the cases involving hematopoietic stem cell receptors, 64% received prophylactic antifungal treatment and *S. apiospermum* was the pathogenic agent in 39% of these cases¹⁹.

In the US, a prospective surveillance study for invasive fungal infections was carried out in 23 transplant centers during the period of 2001-2006. There were 169 identified cases of infection by filamentous fungi: 105 by mucorales, 37 by *Fusarium* sp., and 27 by *Scedosporium* sp. Of these infections, 73.4% occurred in hematopoietic stem cell recipients and 26.4% in solid organ receptors²⁰.

In a literature review conducted in Japan, they found 28 cases with scedosporiosis that presented skin manifestations (pustules and nodules), of which only eight patients had a history of trauma. The pathogen in 22 of the 28 cases was *S. apiospermum*²¹.

Outbreaks caused by a pathogenic species of this genus had not been identified although it has been known that these fungi have a predilection for soils with high human activity. In 2015 in Germany, Bernhardt et al. published the first report of a group of infections associated with *S. boydii* in five of six patients who had undergone cardiac surgery in the same surgery unit²².

In natural disasters, a situation known as "near-drowning" may occur. This condition is defined as a severe episode of involuntary immersion and aspiration of fluids that justifies hospitalization. It is estimated that this occurrence is 2-20 times more frequent than drowning (according to the WHO, about 372,000 people drown annually around the world). One of the complications of this condition is infection, which can have a fatal outcome. In this regard, the morbidity and mortality associated with infections in victims who have been close to drowning are unknown. Some microorganisms considered as harmless saprophytes have been reported with some frequency as a cause of severe or lethal infection, even in immunocompetent individuals.

Katragkou et al., in a retrospective review of the period from 1950 to 2006, highlighted the importance of scedosporiosis after a near-drowning event. In this study, they found 23 clinical cases of scedosporiosis derived from natural disasters that occurred in 13 countries, such as floods or tsunamis. *S. apiospermum* was the pathogen

in 21 of the 23 reported cases. *S. aurantiacum* and *S. boydii* were the pathogens isolated in the two remaining cases. The reported mortality was 70%²³.

In Brazil, Oliveira et al. conducted a search of reported clinical cases of scedosporiosis from 1982 to 2013. In this revision, 15 cases of subcutaneous, pulmonary, and in the central nervous system scedosporiosis were found, linked to *S. apiospermum*. The most frequent clinical manifestation was the presence of a fungus ball (33.3%), mainly in patients previously recovered from tuberculosis. On the other hand, there was a report in the same study of the first case of infection of the central nervous system secondary to a near-drowning²⁴.

Clinical presentations

Transmission of these infections is mainly through trauma, surgeries, and aspiration of contaminated water. The anatomic places where the different species of the *Scedosporium* genus have been isolated are diverse. To this day, there is no proof that this microorganism has a predilection for a specific anatomic site (Fig. 3).

Localized infections, mainly mycetomas, are among the most frequent cases of reported scedosporiosis. Mycetoma is a chronic suppurative infection of the skin and subcutaneous tissue, which affects the lower limbs in over 80% of the cases. The condition is characterized by a symptomatic triad consisting of swelling of the affected zone, fistula formation, and purulent discharge containing grains. These infections are produced as a result of the inoculation of the microorganism through skin trauma. The eumycotic mycetomas are caused mainly by *Madurella mycetomatis*, *Trematosphaeria grisea*, and *S. apiospermum*²⁵.

The acquisition of an infection by *Scedosporium* spp. after the introduction of a contaminated foreign body may occur in the eyes, developing keratitis or even endophthalmitis. Although uncommon, there have been reports of otitis caused mainly by *S. apiospermum*²⁶.

Scedosporiosis is considered to be a major cause of death in solid organ and hematopoietic stem cell receptors, making it the third most common transplant-related pathogen filamentous fungi after mucorales and *Fusarium* spp. The most frequent etiologic agent isolated among this population of patients is *S. apiospermum*¹⁹.

Colonization of the airways, paranasal sinuses, and pulmonary infections has been linked to *Scedosporium* species. These infections occur, especially, in patients with preexisting pulmonary cavities (bronchiectasis, tuberculosis, sarcoidosis, cystic fibrosis, etc.), developing “fungus balls” in some cases, which are radiologically

Figure 3. Anatomical sites where the pathogenic species of the genus *Scedosporium* have been isolated: *Scedosporium apiospermum*, *Scedosporium boydii*, *Scedosporium aurantiacum*, *Scedosporium dehoogii*, and *Scedosporium minutisporum*.

indistinguishable from an aspergilloma²⁷. In patients with cystic fibrosis, these fungi are placed as the second filamentous fungi after *Aspergillus* to colonize the lungs of these individuals, causing allergic bronchopulmonary mycosis as the mildest manifestation, up to a disseminated pulmonary invasive infection²⁸.

In individuals with a debilitated immune system such as patients with HIV/AIDS, it is acknowledged that a CD4⁺ cell count of <100/μl is a predisposing factor of an invasive scedosporiosis and that patients with dissemination to the central nervous system usually show a CD4⁺ cell count of <50/μl. These findings were reported in the exhaustive revision conducted by Tammer et al. in relation to the cases of pseudallescheriosis/scedosporiosis in patients with HIV during the period of 1990-2010. In this study, there were 14 reports of infection by *P. boydii* or *S. apiospermum* in this HIV-positive population²⁹.

In healthy patients who suffered an event of near-drowning, severe clinical conditions are often reported. Aspiration of contaminated water through

the respiratory tract, with significant amounts of conidia, initially offsets a pulmonary infection. In these situations, clinical symptoms can occur from a few days to several weeks after the incident. Fever is frequently reported, along with productive cough, hemoptysis, wheezing, and rhonchi. Radiographic findings of the thorax can vary, ranging from unilateral focal infiltrates to diffuse bilateral infiltrates and from nodes to bronchopneumonia³⁰. Hematogenous dissemination starting in the lungs may manifest on the skin with a maculopapular eruption or as nodular lesions, which may enlarge and become necrotic²⁵. An invasion of the central nervous system can manifest as a cerebral abscess (in approximately 77% of cases), meningitis, encephalitis, ventriculitis, or vascular affection, which produces sinus thrombosis and stroke²³.

There are several isolated clinical cases of scedosporiosis in patients with diverse diseases and under varied therapeutic regimes. The case of a patient with a vulgar psoriasis diagnosis has been reported, a patient who, years before, had been diagnosed with erythrodermic psoriasis. This patient was treated with prednisone and cyclosporine, developing nodes and abscesses in both the knees with an absence of fistulae and granules. Mycological laboratory tests were positive. The fungus was identified as *S. dehoogii* using the β -tubulin gene sequence. The patient rejected undergoing antifungal therapy; however, the lesions disappeared after the removal of cyclosporine and a decrease of the prednisone dose, and there were no relapses during the next 64 months of follow-up. In this paper, the authors conclude that the spontaneous recovery may have come as a result of the immune reconstitution after the immunosuppression was discontinued³¹.

Another interesting case was the report of a patient with a painful swelling and sensitivity in the back of the hand and left wrist, with an evolution of 2 months. Physical examination revealed a fluctuating erythematous weal, poorly defined, with pustules and scabs. Aspiration of the lesion and biopsy was taken to conduct mycological and histological studies. A fungal colony initially identified as *Alternaria* spp. was obtained. The sequence analyses of the internal transcribed spacer (ITS) and D1/D2 ribosomal regions were performed, identifying the fungus as *S. apiospermum*. The patient was treated with debridement and fluconazole for 4 weeks, and at the last follow-up appointment 4 months after treatment, the patient had recovered and did not present recurrences³².

Diagnosis

Clinical diagnosis of scedosporiosis continues to be a challenge, since in most cases, signs and symptoms are non-specific, resulting in the suggestion of other fungal etiological agents which occur with more frequency in human pathology.

Direct microscopy and histopathology

In most cases, direct microscopic and histopathological studies are very useful since they may serve as evidence of a presumptive diagnosis of a fungal infection. However, these studies may not precisely identify the etiological agent of a fungal disease. It is important to remember that a negative result should not rule out the possibility of a mycotic infection.

The direct microscopic examination involves the use of potassium hydroxide at 10% or the use of fluorescent substances such as Blankophor, Uvitex 2B, or Calcofluor White Stain²⁷. In tissue, *Scedosporium* sp. presents hyaline septate hyphae, which cannot be differentiated from other hyaline fungi such as *Aspergillus* spp. or *Fusarium* spp. The hyphae have dichotomic ramifications, which may present intercalated chlamydospores. Rarely, pigmented oval conidia may be observed in tissue²⁵. A positive result should always be considered significant, even if it is not possible to obtain a culture from the samples.

In biopsy histopathological examinations, initial staining is hematoxylin and eosin. This stain is used to observe tissue morphology, yet it is of limited usefulness for the visualization of fungal structures, since they may be confused with artifacts, fibrin, or necrotic masses. Thus, there are histochemical techniques which facilitate the visualization of hyphae in tissue, such as periodic acid-Schiff stain, Gridley stain, or the silver methenamine technique (Gomori-Grocott)³³.

Thornton et al. developed two monoclonal antibodies (HG12 and GA3) to distinguish *Scedosporium* from other hyaline fungi through immunofluorescence. These antibodies recognize a carbohydrate epitope of an unidentified antigen present in the cell wall of conidia and hyphae, which are specific to *S. apiospermum*, *S. boydii*, and *S. aurantiacum*. While some cross reactions were observed with antigens of *Pseudallescheria africana* and *Lophotrichous fineti*, these monoclonal antibodies may be used to differentiate scedosporiosis from an infection by other hyaline fungi³⁴.

Culture

Scedosporium grows well in conventional culture media for bacteria and fungi (blood-agar, chocolate-agar, Sabouraud dextrose agar, PDA, and yeast extract peptone dextrose). Optimal incubation temperature for the growth of these fungi is between 25°C and 35°C. The growth of these fungi is easily overshadowed by other fast-growing fungi such as *Aspergillus*; therefore, several semi-selective culture media have been developed for *Scedosporium* spp.: YPD complemented with cycloheximide, SceSel+, and Scedo-Select III³⁵. These media greatly facilitate the growth and recovery of the etiologic agent in clinical samples, especially of respiratory secretions. Although these culture media facilitate the isolation of these fungi, they do not allow their precise identification.

Proteomics

Mass spectrometry (MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) is a technique recently used in the identification of microorganisms through the creation of a spectrum based on protein profiles, which is unique for a species in particular. In the last decade, this technique has been utilized with more frequency in the identification of clinical species. Protein profiles for the identification of the most common pathogenic filamentous fungi are available but not routinely performed in many hospital centers. Even though commercial systems easily identify filamentous fungi frequently isolated from in clinical samples, they are not equipped to properly differentiate closely related fungi groups. Therefore, research has been oriented to create individual databases for specific taxonomic groups of interest. Nevertheless, to date, there is no universal repository for said databases, and they have to be obtained from the authors of their respective publications and evaluated locally.

Recently, MALDI-TOF MS has been tested for the identification of *Scedosporium* spp. According to the new proposed nomenclature, a clear distinction can be made between two groups: those which cannot be distinguished using this technique (*S. apiospermum*, *S. boydii*, *S. ellipsoideum*, and *S. angustum*) and the species which present a clear distinction among each other (*S. aurantiacum* and *L. prolificans*)³⁶. The lack of a trustworthy database for the species of this complex has hampered the application of this technique for the identification of *Scedosporium* spp.

Molecular biology techniques

A wide range of molecular techniques based on DNA amplification by the polymerase chain reaction (PCR) technique has been used in the identification of the *Scedosporium* spp. species. Examples of these are the PCR reverse-based line hybridization and restriction fragment length polymorphism³⁷. Despite being able to identify the most frequent pathogen agents (*S. apiospermum*, *S. aurantiacum*, and *L. prolificans*), they present drawbacks as they are unable to precisely distinguish between the species of the *S. apiospermum* complex (*S. apiospermum*, *S. boydii*, *S. angustum*, *S. ellipsoideum*, and *S. fusioidea*).

On the other hand, techniques such as the loop-mediated isothermal amplification³⁸, amplified fragment length polymorphism³⁷, and the rolling circle amplification³⁹ are techniques which have accurately accomplished the identification of the *Scedosporium* sp. species. The use of these techniques is not yet standardized for routinely diagnostic use.

In 2015, the International Society of Human and Animal Mycology established a DNA barcoding for the identification of fungi of medical importance. A DNA barcoding is a short (500-800 pb) genetically diverse sequence, which is flanked by a known preserved region, allowing for the design of universal oligonucleotides. The sequence of the ITS region of the ribosomal DNA allows for the identification of organisms in a quick, effective, universal, and accessible way, without the need for experts. In the case of closely related groups, an additional gene may be required to discern between the species of the group. For the identification of *S. aurantiacum* and *S. minutisporum*, sequencing of the ITS region is enough; however, for the identification of *S. apiospermum*, *S. boydii*, *S. ellipsoideum*, *S. angustum*, and *S. dehoogii*, an additional sequencing of the β -tubulin (BT2) is required⁴⁰.

Antifungal susceptibility

This fungus presents variable behavior against the different antifungals tested *in vitro* since certain strains present an intrinsic resistance to a wide variety of antifungals. In a bibliographic review on *in vitro* susceptibility of the species of the *Scedosporium* genus where 521 isolations of clinical and environmental origins were analyzed, *in vitro* susceptibility against itraconazole, posaconazole, and voriconazole was highly variable, with minimum inhibitory concentrations (MIC) ranging from 0.016 to >16 mg/L. The lowest values of

MIC were frequently observed for voriconazole. *P. elipsoidea*, *S. minutisporum*, *S. aurantiacum*, *S. boydii*, and *S. dehoogii* presented less susceptibility for posaconazole, with a MIC range of 0.5->16 mg/L⁴¹. Voriconazole was the only triazole showing antifungal activity against *S. aurantiacum*, with a MIC range of 0.5-1 µg/mL, while amphotericin B, itraconazole, caspofungin, micafungin, isavuconazole, and anidulafungin did not display antifungal activity against this species. *S. dehoogii* was the most resistant against voriconazole, with a MIC value of >16 µg/mL, while *S. angustum* presented a resistance to isavuconazole, itraconazole, and anidulafungin⁴².

S. apiospermum isolates have been classified as susceptible to miconazole, voriconazole, and posaconazole and resistant to fluconazole and flucytosine. A variable susceptibility to ketoconazole, itraconazole, and amphotericin B was observed. Micafungin is the most active echinocandin against this fungus⁴³.

Treatment

Most international guidelines recommend voriconazole as the first-line therapy in a scedosporiosis. In the retrospective revision of Troke et al., a successful therapeutic response was accomplished in 57% of 107 patients with scedosporiosis. All patients received an initial dose of voriconazole of 6 mg/kg intravenously, twice a day on day 1, followed by 4 mg/kg intravenously twice a day, and later a change to oral therapy of 200 mg twice a day. The total duration of the therapy with voriconazole varied from 1 to 802 days. The best therapeutic responses were observed for cutaneous/subcutaneous infections (91%) or bone infections (79%) and the lowest for infections of the central nervous system (43%)⁴⁴.

Antifungal combined therapy has emerged as a promising option. Goldman et al. described a case of an immunocompromised patient with a cutaneous infection by *S. apiospermum*, which disseminated despite treatment with voriconazole. Addition of micafungin and the granulocyte macrophage colony-stimulating factor provided a partial recovery, which indicates a possible synergic role for the combined therapy and use of immunotherapeutic agents⁴⁵.

Conclusion

Emergent fungal infections have increased over the years. Changes in mycologic taxonomy as well as the onset of new species responsible for infections in

humans have increased the number of fungi of medical significance.

Today, the role of the species of the *Scedosporium* genus in the development of invasive infections with a less than favorable prognosis may be underestimated, due to the restrictions of the diagnostic techniques routinely employed.

Conflicts of interest

The authors have no conflicts of interest to declare.

Financing

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Ethical disclosures

Protection of human beings and animals. The authors declare that there were no experiments conducted on humans or animals for this study.

Data confidentiality. The authors declare that there are no patient data in this article.

Right to privacy and informed consent. The authors declare that there are no patient data within this article.

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