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General Overview of A Rare Mold: *Lomentospora prolificans*

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Abstract

There has been a rise in the prevalence and diversity of fungal infections both in Turkey and across the globe. This increase is attributed to a growing number of immunocompromised patients and the use of antifungal prophylaxis. Consequently, there has been a surge in cases of rare mold infections, one of which is caused by Lomentospora prolificans. It is mostly seen in Australia, Spain, and southwestern America. It was also encountered in a patient for the first time in Turkey. L.prolificans mostly causes arthritis or osteomyelitis, and often causes invasive infections in immunocompetent individuals because of trauma, while it causes disseminated and hematogenous infections in immunocompromised individuals. It can be seen in various body parts, especially the lungs, bones, and brain. Direct microscopy, culture, and histological analysis are the main diagnostic methods for L.prolificans. It has several virulence factors such as PRM, glucosylceramides, α-glucan, superoxide dismutase, proteolytic enzymes, catalase, melanin, and siderophores. Generally, it shows resistance to all available antifungals, and disseminated infections frequently result in death. This review was written to provide a general overview of the taxonomy, epidemiology, diagnostic methods, virulence factors, and antifungal susceptibility properties of L.prolificans due to its recent increase in frequency and therefore noteworthiness.

Keywords: ***Lomentospora prolificans,*** **pan-antifungal resistance, rare Mold**

1. Introduction

There has been a rise in both the number and types of fungal infections in Turkey and worldwide (GÜLMEZ KIVANÇ, 2022). Due to an increase in immunosuppressed patients and the use of antifungal prophylaxis, there has also been a spike in rare mold infections caused by fungi such as *Lomentospora, Scedosporium, Paecilomyces, Rasamsonia*, and *Scopulariopsis*, in addition to the commonly observed *Aspergillus* spp and *Mucorales* species (Hoenigl et al., 2021). Treating rare mold infections has proved challenging, but it is possible to manage the process for the patient by correctly identifying the fungi that cause the infection and applying appropriate treatment. Although there is limited information on the identification, diagnosis, and treatment of rare molds, guidelines have been developed to manage the process effectively. The European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and European Confederation of Medical Microbiology (ECMM) have published diagnosis and treatment guidelines for Europe. Later, ECMM, The International Society for Human & Animal Mycology (ISHAM), and American Society for Microbiology (ASM) published more global diagnostic and treatment guidelines, allowing knowledge and experiences to be shared from all over the world (GÜLMEZ KIVANÇ, 2022).

L. prolificans is a seldom-seen mold that primarily resides in the soil as a saprophyte, predominantly found in Australia, Spain, and southwestern America. Naturally, the reported cases from these regions are more frequent (Hoenigl et al., 2021). The first instance of *L. prolificans* in Turkey was documented in a patient with aplastic anaemia, leading to fungemia (Yazgan et al., 2023).

This review aims to present a comprehensive overview of the taxonomy, epidemiology, diagnostic methods, virulence factors, and antifungal susceptibility properties of *L. prolificans*, a rare mold encountered for the first time in Turkey.

2. Taxonomy

In recent years, fungal species of the *Scedosporium/Pseudallesheria* genus have undergone reclassification (Chen, Halliday, Hoenigl, Cornely, & Meyer, 2021).

The molecular phylogeny study of the genus was initially conducted in 2005 by Gilgado et al. (Gilgado, Cano, Gené, & Guarro, 2005). This study analyzed partial sequences of the β-tubulin (two loci) and calmodulin genes, as well as the internal transcribed spacer (ITS) region of the rRNA gene and demonstrated that *P. boydii* is a species complex.

Following the implementation of the ‘’One Fungus = One Name’’ in fungal taxonomy, the genus name *Scedosporium* was favored over *Pseudallesheria* (Chen et al., 2021). It is known that there are currently 10 species in the genus, previously referred to as the *Pseudallesheria/Scedosporium* complex (Buldain et al., 2021). The current *Scedosporium* complex includes *S. aurantiacum, S. cereisporum, S. angustum, S. desertorum, S. minutisporium, S. dehoogii, S. apiospermum, S. fusoideum, S. ellipsoideum, S. boydii* (Chen et al., 2021).

Following molecular phylogenetic studies, it was suggested that *Scedosporium prolificans* is only distantly related to *Scedosporium* and was reclassified as *Lomentospora prolificans*. A new genus, *Lomentospora*, was created for this species (Chen et al., 2021).

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3. Epidemiology

Scedosporium/Lomentospora species are commonly found in various environments, particularly in areas impacted by human activities, such as oily soils, cattle manure, and sewers. Polluted water serves as a reservoir for these species, facilitating their survival. While they have been identified as a cause of infection in near-drowning cases, they are more prevalent in soil than in water, suggesting that soil is their primary habitat. Additionally, they can be found on decaying material (Ramirez-Garcia et al., 2018).

L. prolificans primarily causes arthritis or osteomyelitis and often leads to invasive infections in immunocompetent individuals following trauma. In immunocompromised individuals, it can cause disseminated and hematogenous infections. This mold is commonly found in various parts of the body, particularly the lungs, bones, and brain, and exhibits neurotropism. Infection typically occurs through direct inoculation, inhalation, and direct inoculation of contaminated water in near-drowning cases. In patients with cystic fibrosis, *Scedosporium/Lomentospora* species are prevalent in chronic airway colonization, ranking second after Aspergillus fumigatus among filamentous fungi (Buldain et al., 2021).

L. prolificans generally exhibits resistance to all available antifungals, and disseminated infections often result in death (Walsh, 2018). Due to its ability to infect both immunocompetent and immunocompromised individuals, it can act as both a primary or opportunistic pathogen (Konsoula et al., 2022).

In a systematic review, Konsoula, Agouridis, Markaki, Tsioutis, and Spernovasilis (2023) examined 87 studies describing 142 cases of disseminated *L. prolificans* infection. It was observed that the underlying situations seen in the majority of patients, from most common to least common, include malignancy, hematopoietic stem cell transplantation, solid organ transplantation, and AIDS. The most affected organs include the lungs, central nervous system, skin, eyes, heart, and bones/joints. Additionally, a high mortality rate of 87.3% was noted.

4. Diagnostic features

Direct microscopy, culture, and histological analysis serve as the primary diagnostic methods for identifying this fungus (Ramirez-Garcia et al., 2018). Because identification in rare mold infections primarily relies on conventional methods, diagnosis is time-consuming (GÜLMEZ KIVANÇ, 2022).

Diagnosis involves isolating the agent from the patient's biopsy sample, sterile body fluids, or blood culture. *Lomentospora/Scedosporium* species exhibit various clinical features, ranging from respiratory tract colonization to allergies, invasive, or disseminated infections. These species are the most commonly isolated fungi from respiratory secretion samples of patients with chronic pulmonary diseases such as cystic fibrosis (Ramirez-Garcia et al., 2018). The use of SceSel, a special selective medium, for respiratory tract samples of cystic fibrosis patients provides a better chance of isolation than other media, as this medium prevents overgrowth of Aspergillus species. Additionally, inhibitory mold agar, brain heart infusion agar (BHIA), Sabouraud Dextrose Agar (SDA), or horse blood agar can be used for isolation (Hoenigl et al., 2021). *L. prolificans* exhibits rapid growth, maturing within five days. Its growth is inhibited by cycloheximide (Walsh, 2018).

On the medium, young colonies appear cottony or moist, with colors ranging from light gray to black. As they mature, the color of the colonies changes to dark gray to black. White mycelium may develop as the colonies age. The reverse side of the petri dish appears gray to black (Walsh, 2018).

The sexual stage of *L. prolificans* is not known. It has septate hyphae. The conidiophore of *L. prolificans* is swollen and then has a narrow and long neck at the end of the annelid to which the clusters of conidia are attached. Conidia

are single-celled, ovoid, and smooth (Walsh, 2018). In tissue samples, it can have pigmented hyphae that are visible under direct microscopy. Therefore, it can be an agent of phaeohyphomycosis (Hoenigl et al., 2021).

Histopathological examination of fungal-infected tissue is crucial for confirming invasive infection, yet identifying the causative pathogenic microorganism is impossible without culture. Despite the differences among molds, they can exhibit similar characteristics (Konsoula et al., 2022). Expert intervention is necessary to differentiate molds in histopathological examinations. When stained with GMS (Gomori methenamine silver), lemon-shaped conidia become visible. It is also common to observe thrombosis in blood vessels (Chen et al., 2021).

Scedosporium/Lomentospora molds can be rapidly detected and identified directly from clinical samples using molecular methods. Each method has its strengths, but standardized assays are not commercially available (Chen et al., 2021). Molecular methods are increasingly prevalent, although they should only complement conventional methods (Konsoula et al., 2022).

Broad-range or panfungal PCR (Polymerase Chain Reaction) can identify fungi directly from fresh tissue, formalin-fixed paraffin-embedded tissue, as well as liquid clinical samples such as bronchoalveolar lavage (BAL) or cerebrospinal fluid (CSF). Typically, ITS1 (Internal Transcribed Sequence 1), ITS2, or both, along with the 28S rDNA locus, are amplified using universal fungal primer sequences followed by DNA sequencing (Chen et al., 2021). Additionally, the partial β-tubulin gene is necessary to distinguish closely related species (Ramirez-Garcia et al., 2018). Melting curve analysis can also be utilized with real-time PCR. When employing the broad-range PCR method for non-sterile samples, particularly BAL, interpreting the results presents a challenge. A positive PCR test cannot differentiate lung infection from airway colonization. Furthermore, the sensitivity of the panfungal PCR method for BAL samples is low in patients undergoing mold treatment (Chen et al., 2021).

Scedosporium/Lomentospora species-specific markers and panfungal biomarkers can be employed in serology. The most well-known panfungal biomarker is (1,3)-β-d-Glucan (BDG), found in the fungal cell wall. BDG can be detected in the blood samples of patients with IFD (invasive fungal disease), except for infections caused by *Mucorales* and *Cryptococcus* fungi. Currently, there is not commercially available *Scedosporium/Lomentospora*-specific serological assay (Chen et al., 2021).

5. Virulence Factors

Key virulence factors of *L. prolificans* consist of peptidorhamnomannan (PRM) and other peptidopolysaccharides of the cell wall, such as glucosylceramides (GlcCer) and α-glucan (Buldain et al., 2021; Ramirez-Garcia et al., 2018).

They trigger an immune response upon interacting with TLR2 (Toll-Like Receptor 2), TLR4, and Dectin-1, as well as through other unidentified receptors (Buldain et al., 2021). The innate immune response against fungal infections is initiated by recognizing components in the fungal cell wall known as Pathogen Associated Molecular Patterns (PAMPs). This recognition is facilitated by Pattern Recognition Receptors (PRRs), which in turn activate signaling

pathways leading to the production of pro-inflammatory cytokines, phagocytosis, and the induction of adaptive immunity. While specific recognition of *Scedosporium/Lomentospora* species has not been extensively described, the PRRs most implicated in the recognition of pathogenic fungi are Dectin-1 and Dectin-2, TLRs, C type Lectin Receptors (CLRs), and Mannose Receptor/CD206 (MR) (Buldain et al., 2021).

The rapid spread of *L.prolificans* and the accelerated progression of the disease are attributed to its ability to undergo conidiation in tissue. PRM is expressed in the conidia and hyphal cell wall, and is associated with fungal adhesion. PRM may facilitate the spread and colonization of the fungus. Furthermore, the recognition of PRM by antibodies contributes to improved diagnosis (Ramirez-Garcia et al., 2018). PRM also plays a role in fungal phagocytosis, induces macrophage death, and stimulates the synthesis of nitric oxide, superoxide, and TNF (Buldain et al., 2021).

GlcCer, derived from sphingolipids (Konsoula et al., 2022), stimulates the production of nitric oxide and superoxide, enhances the killing of conidia, and promotes the recruitment of immune system cells (Buldain et al., 2021). The receptors involved in this activation by GlcCer and PRM have not been fully elucidated. α-glucans stimulate TNF release via TLR2, MyD88 (myeloid differentiation primary response 88), and CD14 (Buldain et al., 2021). Additionally, rhamnomannans bind to TLR4, leading to the release of TNF (tumor necrosis factor), IL-10, 6 (Interleukin 10,6), and IP-10/CXCL10 (IFN-gamma-inducible-protein 10) (Buldain et al., 2021).

In addition to these, superoxide dismutase, proteolytic enzymes, catalase, siderophores, and melanin are other important virulence factors (Ramirez-Garcia et al., 2018). Melanin pigment protects the mold against UV radiation and other types of environmental stress, as well as against oxidative damage (Ramirez-Garcia et al., 2018).

6. Antifungal Susceptibility Characteristics

Treating deep infections caused by *L. prolificans* is challenging due to its inherent resistance to many modern antifungals, earning it the label of being pan-antifungal resistant in scientific literature (Konsoula et al., 2022). Given its pan-antifungal resistance, the options for antifungal drugs are limited, making the development of new antifungals crucial for effective treatment (Ramirez-Garcia et al., 2018). This organism is resistant to echinocandins, polyenes, pyrimidines, allylamines, and exhibits limited resistance to azoles (Kirchhoff, Dittmer, Buer, Rath, & Steinmann, 2020). Various factors contribute to antifungal resistance, and while examining resistance mechanisms is necessary, doing so for each fungus individually would be time-consuming. To address this, a study (Pellon et al., 2018) recommends leveraging the multiresistance feature of *L. prolificans* and applying the understood resistance mechanisms from this mold to other fungi. Consequently, it would only require a single microorganism to investigate resistance against different antifungals (Pellon et al., 2018).

Mutations in the FKS1 gene, which encodes the catalytic subunit of β,1-3-glucan synthase and is the target of echinocandins, may explain the reduced susceptibility of *L. prolificans* to echinocandins. Point mutations in the coding sequence of CYP51A orthologs lead to decreased affinity of azole drugs to their targets or overexpression of efflux pumps, which may account for the resistance of *Scedosporium/Lomentospora* species to azole drugs (Ramirez-Garcia et al., 2018). Wu et al. (Wu et al., 2020) conducted in vitro susceptibility testing of 42 clinical isolates of *L. prolificans*, all of which were found to have high MIC (minimum inhibitory concentration) values

(Voriconazole; MIC_{90}>16 µg/ml, Itraconazole; MIC_{90}>16 µg/ml, Posaconazole; MIC_{90}>16 µg/ml, Isavuconazole; MIC_{90}>16 µg/ml, Amphotericin B; MIC_{90}>16 µg/ml, Terbinafine; MIC_{90}>64 µg/ml) and high minimum effective concentration (MEC) value (micafungin; MEC_{90}>8 µg/ml). The only exception was miltefosine, an alkyl-phospholipid analogue, with an MIC_{90} value of 4. Various drug combinations were tested in this research, and the combination of voriconazole and terbinafine was found to have the highest synergistic effect. In this study, the CYP51 and FKS1 genes of *L. prolificans* were also examined, and amino acid residues associated with azole resistance were found in the CYP51 protein and echinocandin resistance in the FKS1 hot spot regions. The results of this study support the notion that this mold is intrinsically resistant to these two antifungals.

The guideline (Hoenigl et al., 2021), provided by the cooperation of ECMM, ISHAM, and ASM with the aid of scientists from 24 countries to offer practical guidance for the diagnosis and treatment of rarely seen mold infections, recommends a combination of voriconazole with another antifungal, especially terbinafine, and suggests adding or removing some other antifungals for the treatment of infections caused by *L. prolificans*. The use of voriconazole as monotherapy is moderately recommended (Hoenigl et al., 2021). Surgical debridement with voriconazole is also advised if possible (Ramirez-Garcia et al., 2018).

Recently, new antifungals like F901318 and N-chlorotaurine (NCT) have displayed potential. F901318 inhibits dihydrooratate dehydrogenase, a crucial player in pyrimidine synthesis (Ramirez-Garcia et al., 2018). In a study by Kirchhoff et al. (2020), F901318 (olorofim) was evaluated for its antifungal activity against various fungal pathogens using the broth microdilution assay. Among these pathogens were 20 clinical isolates of *L.prolificans*, and it was observed that MIC values ranged from 0.032 to 0.5 mg/L.

The N-chloro derivative of the amino acid taurine acts as an oxidant. The chemical synthesis of NCT as its sodium salt was successfully achieved, and it exhibited broad-spectrum antimicrobicidal activity against microbes (Ramirez-Garcia et al., 2018).

Fosmanogepix (APX001), with its active compound manogepix (APX001A), is a new drug option that targets the fungal enzyme Gwt1. This enzyme is responsible for binding mannoproteins to the fungal cell wall, which aids in the adhesion of the fungus to host epithelial and mucosal cell surfaces. Manogepix has broad activity against various fungi including azole-resistant *A.fumigatus, Scedosporium* spp., and *L.prolificans* (Logan, Wolfe, & Williamson, 2022).

In the treatment of *L.prolificans* infections, it is important to use immune modulators, especially in individuals with suppressed immune systems. Cytokines, which play a role in immune response in fungal infections, have been studied for use alone or in combination with other drugs. Granulocyte-colony stimulating factor (G- CSF) has been found to be effective against *L.prolificans* invasion when used with antifungals in neutropenic patients. G-CSF induces the proliferation and differentiation of myeloid progenitor cells, leading to an increase in the number of circulating neutrophils and an enhanced phagocytic response (Konsoula et al., 2022). In a study published in 2002, the co-administration of liposomal amphotericin B (LAMB) and G-CSF was found to significantly increase survival compared to untreated control, showing promise in the treatment of disseminated infections of this mold. However,

further studies are needed to determine the most appropriate doses (Ortoneda et al., 2002). IFN-gamma and granulocyte-macrophage colony-stimulating factor (GM-CSF) are other cytokines studied for therapeutic purposes. IFN-gamma, a cytokine involved in the immune response against invasive fungal infections, is associated with the antifungal activity of neutrophils and/or macrophages, migration, and adhesion. It has been found that IFN-gamma and GM-CSF together accelerate the antifungal activity of neutrophils by causing increased superoxide production (Konsoula et al., 2022).

7. Conclusion

In conclusion, the rise in fungal infections in Turkey and globally is attributed to the growing number of immunosuppressed individuals and increased use of antifungal prophylaxis. There has also been an uptick in uncommon mold infections, with this review focusing on *L.prolificans* and highlighting its diagnostic properties, virulence factors, and antifungal sensitivity.

The surge in infections caused by once rare molds underscores the importance of improved recognition and the development of rapid identification systems to guide effective patient treatment. Disseminated infections from *L.prolificans*, which typically exhibits resistance to all available antifungals, often lead to fatalities. As such, the development of new antifungals targeting this agent is considered a crucial area of study given the increasing frequency of rare molds.

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