Clinical manifestations and treatment outcomes of rare genera fungal keratitis in China

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Abstract

Background: Fungal keratitis is a common cause of blindness, and although major fungal genera in China have been widely reported, little is known regarding rare genera of fungal keratitis. This study is aimed at investigating the clinical manifestations and treatment outcomes of rare genera of fungal keratitis and improving clinicians' understanding of these genera in China.

Methods: A retrospective analysis was performed at Shandong Eye Hospital from October 2008 to December 2017. The clinical manifestations, risk factors, confocal microscopic images, histopathological features, in vitro drug sensitivity tests, and treatment outcomes of 16 patients with 8 rare fungal genera were evaluated.

Results: Slit lamps were used to observe *Rhizopus, Pythium insidiosum,* and *Purpureocillium lilacinum,* which presented with endothelial plaque and hypopyon. *Curvularia crescentulum* keratitis mainly presents with feathered edges. Cases of *Colletotrichum*, *Exserohilum,* and *Bipolaris* also showed these feathered edges extending to the periphery, while *Sarocladium* keratitis only showed obvious edema and infiltration. The mean age was 54.75±12.70 years with an increase in male preponderance. Plant trauma and foreign body scratching caused ten cases (62.5%), and six cases (37.5%) were caused without inducement. In 2/3 cases, *Curvularia crescentulum* keratitis was cured with antifungal medication, while in 14/16 cases (87.5%), surgery was required. An exception occurred in three cases of *Pythium insidiosum*, where patients with fungal recurrence and final evisceration was performed; no recurrence of infection occurred in the rest of the patients. The best-corrected visual acuity (BCVA, LogMAR) of 13 cases was 1.48 ±0.85 before treatment and 0.60 ±0.45 after treatment, and the difference was found to be statistically significant [(95 % CI, 0.33–1.43) (*P*=0.003)].

Conclusions: We report on the rare fungal genera collected in China in order to improve the knowledge of clinicians. In our study, C*urvularia crescentulum* seems to respond well to antifungal drugs. Patients affected by *Pythium insidiosum* often need long-term recovery and multiple keratoplasty, and postoperative fungal recurrence rate is high. For fungal keratitis, the combination of early sensitive antifungal drugs and necessary surgical treatment may still be a beneficial factor in treatment.

Background

Fungal keratitis can result in serious visual impairment and mainly concentrated in tropical and subtropical regions in developing countries, such as India and Brazil [1-3], as it accounts for 40% to 50% of all isolated keratitis cases [4, 5]. Currently, there are more than 70 pathogens that can cause fungal keratitis [6], but the clinical isolates of these pathogens are mainly concentrated in a few genera. The fungal isolates vary from country to country due to climate and environmental impacts. *Fusarium* and *Candida* are the most common pathogens in developed countries (such as the United States and the United Kingdom) [7]. Rare pathogens, such as *Rhizopus, Sarocladium, Colletotrichum, Exserohilum, Bipolaris, Curvularia crescentulum,Purpureocillium lilacinum,* and *Pythium insidiosum* have been reported in dozens of cases among both white and black populations. *Fusarium*, *Aspergillus,* and *Alternaria* are the main pathogens in China [8], but the rare fungal genera mentioned above have not been reported in China.

Different clinical manifestations of fungal keratitis and different sensitivities to antifungal drugs makes it very difficult to treat fungal keratitis. The clinical features and drug sensitivity of common fungal genera have been widely reported, but treatment guidelines for rare genera are lacking. Therefore, the purpose of this study is to gather cases of rare fungal genera treated in Shandong Eye Hospital and analyse the relevant factors predisposing their occurrence, the clinical manifestations, the confocal microscopic images, the drug sensitivity tests, and the treatment outcomes.

Methods

Subjects

The study isolated and identified fungal pathogens of 1608 patients with fungal keratitis from Shandong Eye Hospital between October 2008 and December 2017. Sixteen cases caused by eight rare fungal genera were analysed from these patients. This study was approved by the Ethics Committee of Shandong Eye Hospital. The tenets of the Declaration of Helsinki were adhered to in the conduct of this study, and all surgical patients signed written informed consent forms.

Diagnostic methods

Confocal microscopy, anterior segment coherence optical tomography, corneal scraping cultures, and slit lamp microscopy examinations were performed on all patients. After admission, corneal lesions were examined using cytology smearing, and patients' scraped tissue was inoculated into Sabouraud dextrose agar (SDA) and blood agar plates and were cultured in incubators. The growth characteristics of the colonies were recorded. Internal Transcribed Spacer (ITS) gene sequencing was used to identify those whose morphology could not be identified.

Amphotericin B, fluconazole, itraconazole, and voriconazole (provided by Shandong Boke Biological Co., Ltd.) were tested for vitro antifungal susceptibility, and the minimum inhibitory concentration (MIC) values were reported. Corneal buttons excised from surgical patients were submitted for histopathology evaluation.

Drugs and surgical procedures

Sixteen patients were hospitalized and received topically polyene (5% natamycin eye drops or 0.25% amphotericin eye drops) and imidazole (0.5% fluconazole eye drops or 10mg/ml voriconazole eye drops). When their corneal ulcers became worse, that is, increased infiltrate size or increasing hypopyon, a 0.2 mg/ml intravenous drip was utilized once a day, or 200 mg itraconazole orally once a day were added to the therapy.

Surgical treatment was used when drug therapy was shown to be ineffective after approximately one week. keratectomy was performed when the lesion was located in the paracentral or periphery where the infection size was less than 5 mm with a depth of less than 1/2 the corneal stroma, when the depth reaches 1/2, conjunctival flap can be combined. Lamellar keratoplasty (LKP) was performed when the lesion was located in the optic axis and reached the deep corneal stroma but not the corneal endothelium. Therapeutic penetrating keratoplasty (TPK) was performed when the fungal infection had reached the corneal endothelium, and evisceration was performed when the fungal recurrence after keratoplasty was uncontrollable.

Statistical Analysis Method

Microsoft Excel 2010 was used for data input and management. To analyze the changes of best-corrected visual acuity (BCVA) before and after operation with the Wilcoxon signed-rank test. All statistical analyses were performed using Predictive Analytics Software (PASW) version 18.0.

Results

Microbiology

Among the 1608 fungal keratitis cases, the isolates of *Fusarium* were the most common (53.3%), followed by *Aspergillus* (23.4%), *Alternaria* (12.6%), and other genera (10.7%) such as *Candida*, *Scedosporium, Acremonium, Trichosporon*, and rare genera. The 16 patients of rare fungal keratitis consisted of 1 case of Rhizopus (0.06%), 2 cases of Sarocladium (0.12%), 4 cases of Pythium insidiosum (0.25%), 1 case of Exserohilum (0.06%), 1 case of Bipolaris (0.06%), 2 cases of Colletotrichum (0.12%), 2 cases of Purpureocillium lilacinum (0.12%), and 3 of cases Curvularia crescentulum (0.19%).

Demographics and clinical presentation

Among the 16 patients, 13 were males and 3 were females; most occupations are farmers. The average age was 54.75±12.70 years. Three cases (18.75%) were caused by plant trauma, seven cases (43.75%) were due to foreign body scratching, and six cases (37.5%) were without inducement. None of the 16 cases had a history of topical steroid use. The onset time was 13.81 ±7.20 days. *Pythium insidiosum* keratitis developed quickly, and the onset time was 10.5±5.20 days. The duration of hospitalization was 17.81 ± 8.83 days, *Exserohilum* keratitis was the shortest (<15 days) and *Pythium insidiosum* keratitis was the longest (>30 days).

When medicated for about one week, *Rhizopus, Pythium insidiosum,* and *Purpureocillium lilacinum* had endothelial plaques and hypopyon was observed via slit lamp. *Curvularia crescentulum* keratitis was mainly presented with feathered edges. *Cases of Colletotrichum*, *Exserohilum,* and *Bipolaris* cases also showed feathered edges extending to the periphery, but were not associated with endothelial plaque, satellite lesions, or hypopyon in our study. *Sarocladium* cases only showed obvious edema and infiltration without typical fungal clinical characteristics (Figure 1, and Table 1).

Confocal microscopy images

Hyphae were found in 16 cases (85.25%) upon examination using confocal microscopy, and generally changed as follows: the hyphae were highly reflective; they were partially branched and segregated; their diameter was about 2.3 – 4.4 μm; their shape was dendritic, linear, or short and rod-like; they had irregular hyphal morphology; the average branching angle was 39.03 – 46.85 degrees; and the spore detection rate is very low. The confocal manifestations of different genera are shown in Figure 2.

Histopathological section results

In the 16 patients, 14 cases had corneal pathological sections, and hyphae structures were found in 8 cases (57.14%). A small number of hyphae and inflammatory cells were observed in *Sarocladium* and *Exserohilum* keratitis. When conducting periodic acid–Schiff (PAS) staining, *Pythium insidiosum* keratitis was not stained, only vacuolar hyphae-like structures were found in the whole stroma, and the inflammatory cells were not obvious. Furthermore, immunofluorescence staining yielded positive results. More hyphae and a few spores were observed in *Purpureocillium lilacinum* and *Curvularia crescentulum* keratitis (Figure 3).

In vitro drug sensitivity tests

In addition to *Pythium insidiosum*, the MIC values of amphotericin B, voriconazole, and itraconazole in seven genera were low (0.016 to >16), indicating that they were all susceptible to the three antifungal agents. Among them, *Rhizopus* was found to be more sensitive to amphotericin B. *Sarocladium* was shown to be equally sensitive to three antifungal agents. *Colletotrichum, Exserohilum*, *Bipolaris,* and *Purpureocillium lilacinum* were more sensitive to voriconazole, while *Curvularia crescentulum* was more sensitive to itraconazole and fluconazole was highly resistant in all genera studied (MIC 4 to>256) (Table 2).

Therapeutic results

Among the collected patients, 111 cases (13.07%) of *Fusarium* keratitis were cured by antifungal medication and surgery was performed on 746 (86.93%) cases, including 362 cases (48.53%) of LKP, 237 cases (31.77%) of TPK, 137 cases (18.36%) of keratectomy, and 9 cases (1.21%) of evisceration. The postoperative fungal recurrence rate was 7.5%, and all patients were successfully cured by TPK after recurrence. The rare fungal genera patients' ulcers gradually healed in 2/3 cases (66.67%) of *Curvularia crescentulum* keratitis after antifungal drug treatment. After one week of medication, 14/16 cases (87.5%) underwent surgical treatment after persistent progression or a lack of improvement in symptoms. Among the 14 cases , keratectomies were performed in 7 (50%) cases , the average healing time of the corneal epithelium was 5.17±0.79 days post-operation. Two cases (14.29%) were treated with LKP, one case of *Pythium insidiosum* keratitis recurred after operation, and fungal recurrence was detected by confocal microscopy after corneal transplantation. Five cases (35.71%) were treated with TPK, and two cases of *Pythium insidiosum* keratitis recurred after operation. Evisceration was performed in three recurrent cases with an uncontrollable infection, and the average time of first recurrence was 3.33 days (Figure 4, Table 1).

Visual acuities

The best-corrected visual acuity (BCVA, LogMAR) of 13 cases was 1.48 ±0.85 before treatment and 0.60 ±0.45 after treatment, and the difference was statistically significant [(95 % CI, 0.33–1.43) (P=0.003)], with an average increase of 4.55±2.62 lines.

Discussion

There are different growth patterns among fungal species, so the clinical manifestations, pathogenic invasiveness, and microbial characteristics also differ. There is a challenge presented in the clinical diagnosis of infectious keratitis based on slit lamp examination, and microbial detection is still the gold standard [9]. However, early empirical treatment based on lesion manifestations is also necessary. Many studies have reported that *Fusarium* keratitis more commonly shows feathery edges due to horizontal growth, while *Aspergillus* keratitis more commonly shows immune rings and hypopyon due to vertical growth [10, 11]. In this study, we observed the clinical manifestations of *Rhizopus* keratitis showing endothelial plaque and hypopyon. Warner et al. [12], and Azari et al. [13] reported that *Rhizopus* belongs to the Mucorales order of fungi, and is rarely seen in human eyes. Such an infection can rapidly destroy the tissue structure and accelerate the progression of ocular diseases because of its strong pathogenic invasiveness [14]. Fewer than five cases have been reported, and there are still no reports in China.

Pythium insidiosum classified as an oomycete, which is a fungal-like organism that is seen as branching, sparsely, septate or aseptate filaments; its host animals are mammals, but eye infections are rare in China. The main predisposing factors are water exposure, wearing contact lenses, and trauma. Although this study discussed *Pythium insidiosum* with fungi, its microbial species still requires further discussion. Its clinical manifestations are mainly endothelial plaque, hypopyon, and peripheral reticular infiltration, which indicate that the genus causes strong enzymatic hydrolysis in surrounding and deep tissues, probably because of the lack of ergosterol drug targets in the cytoplasmic membrane leading to insensitivity to antifungal drugs and difficulty in controlling diseases [15, 16].

Curvularia crescentulum belongs to the family of dematiaceous fungi. Its clinical manifestations are raised lesions and feathered edges; hypopyon rarely occurs, and the prognosis is quite good. The medication rate of *Curvularia* *crescentulum* keratitis in this study was 2/3 cases (66.67%). As such, *Curvularia crescentulum* keratitis seems to respond well to antifungal drug. *Exserohilum*, *Bipolaris,* and *Colletotrichum* are also dematiaceous fungi where foreign bodies or plant trauma are predisposing factors[17]. Human infections mainly invade the skin and respiratory tract, rarely infecting the cornea [18-22]. Moreover, no cases of these three genera infections have been reported in China. In this study, the most common manifestation of the three fungal genera was feathered edges, which invaded the surrounding corneal tissues in a carpet-like manner. The main reason for this was the melanin in the cell wall, which can affect the host's immune response to infection and reduce the toxicity of pathogens to deep invasion. In addition, the unique temperature sensitivity of *Colletotrichum* also inhibited its progression to deeper levels (≥35°C growth restriction), although the low virulence of these three genera still needs to be verified in future cases.

Chidambaram et al. [23] reported that the average branching angle of *Fusarium* and *Aspergillus* was 59.7° and 63.3°, respectively. In our study, confocal microscopy images showed an average branching angle of 39.03°–46.85° for the eight rare fungal genera, which was close to that of *Fusarium*. However, *Colletotrichum*'s unique cluster-like manifestations and *Pythium insidiosum*'s bead-like manifestations were different from those of *Fusarium* and *Aspergillus's* manifestations[24]. The positive results of histopathology also showed that most hyphae grew horizontally. However, the direction of hyphae growth should be judged by clinical manifestations, confocal microscopy, and pathological findings together, which can provide a reference for the choice of surgical methods in the future.

Polyene and imidazole are still the main antifungal therapies used in China. Natamycin is considered to be the first-line treatment of filamentous fungal keratitis , such as *Fusarium spp* and *Aspergillus spp*[11, 25]. There was no zoospore in the culture of *Pythium insidiosum* in our study, and no results were obtained from drug susceptibility tests. The other seven genera were susceptible to amphotericin B, voriconazole, and itraconazole; among them, voriconazole seemed more effective against fungi, especially for dematiaceous species. It is impossible to test the susceptibility of natamycin due to a lack of test reagents.

Seven patients (43.75%) with *Colletotrichum*, *Exserohilum*, *Bipolaris*, *Purpureocillium lilacinum,* and *Sarocladium* keratitis were treated with keratectomy (one case of *Sarocladium* combined with conjunctival flap). The average healing time of the corneal epithelium was 5.17±0.79 days postoperatively, and there was no evidence of fungal recurrence which effectively shortened the course of the disease. This indicated that if the above genera are ineffective in drug treatment, surgery without delay could be beneficial to control illness. Most of the foreign reports of *Purpureocillium lilacinum* keratitis had a poor prognosis because the role of hydrolase progressed deeper and with more ease [26-28]. Todokoro et al. [29]reported that two patients were cured completely using only antifungal drugs. In our study, the two patients with *Purpureocillium lilacinum* keratitis underwent surgical treatment, and had a good clinical effect. This might be considered as being related to the combination of preoperative antifungal-sensitive drugs and the total eradication of lesions. At present, there are no effective treatment for *Pythium insidiosum* keratitis, antifungal therapy or surgery is still the main treatment method used in other countries, but afflicted patients face prolonged recovery often requiring multiple keratoplasty, and the fungal recurrence rate after surgery is high [24, 30, 31]. In our study, four cases of *Pythium insidiosum* keratitis were ineffective in antifungal therapy, and three cases (75%) showed fungal recurrence after corneal transplantation. For *Pythium insidiosum* keratitis, antifungal medication combined with antimicrobial therapy has been used in other countries and has achieved certain effects [31]. Agarwal et al. reported cryotherapy or absolute alcohol might prove beneficial [30]. These adjunctive measures may be beneficial to the treatment *Pythium insidiosum* keratitis.

Conclusions

In this study, the clinical manifestations, auxiliary examination and treatment outcomes of rare fungal genera in China are discussed. Analysis should be carried out in order to improve clinicians' understanding of these genera and to promote early diagnosis and improve target treatment. Whether it's common or rare fungal genera, the combination of early sensitive antifungal drugs and the necessary surgical treatments may still beneficial factors in treatment.However, the deficiency of this study is that there are few cases of rare genera, and more information is needed to study their clinical characteristics and treatment outcomes.

Declarations

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Authors’ contributions

XW is responsible for the acquisition of clinical data and the writing of manuscripts. XHL participates in the preparation of culture medium and identification of fungal pathogens. SXL, TW and YNJ guided the data analysis. STW, JTW and CXD performed data collection. WYS reviewed and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed to support the findings of this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study followed the tenets of the Helsinki Declaration on ethical principles for medical research involving human subjects and was approved by the ethics committee of Shandong Eye Hospital, Shandong Provincial Key Laboratory of Ophthalmology, China. Written informed consents were obtained from all subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Abbreviations

BCVA: Best-corrected visual acuity

LogMAR: the logarithm of minimal angle of resolution score

SDA: Sabouraud dextrose agar

MIC: minimum inhibitory concentration

H&E: hematoxylin and eosin staining

PAS: periodic acid–Schiff staining

LKP: Lamellar keratoplasty

TPK: Therapeutic penetrating keratoplasty

PASW: Predictive Analytics Software

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Tables

Table 1. Demographic, microbiological, clinical profile and Treatment of patients with rare genera fungal keratitis

Patient No.	Age(yrs)	Gender	Risk Factor	Culture	Clinical manifestation	BCVA (LogMAR)		Treatment	
						At Presentation	Final	Medical	Surgical
1	48	Male	Foreign body scratch	Rhizopus	Endothelial plaque, hypopyon, satellite lesions	3	0.7	V+I	TPK
2	69	Female	plant trauma	Sarocladium	Edema and infiltration	1	0.5	V+N	Keratectomy+Conjunctival flap
3	60	Male	None	Sarocladium		2	1.7	V+N	Keratectomy
4	44	Male	Foreign body scratch	Exserohilum	feathered edges	0.2	0	V+N	Keratectomy
5	57	Male	plant trauma	Bipolaris	feathered edges	0.8	0.2	V+N	Keratectomy
6	65	Male	None	Colletotrichum	feathered edges	1.7	0.4	V+A	Keratectomy
7	44	Male	plant trauma	Colletotrichum		1.7	0.5	V+N	Keratectomy
8	51	Male	Foreign body scratch	Purpureocillium lilacinum	raised lesions	1.1	0.3	V+N	Keratectomy
9	45	Male	Foreign body scratch	Purpureocillium lilacinum	Endothelial plaque, hypopyon	3	0.3	V+N	TPK
10	54	Male	Foreign body scratch	Curvularia crescentulum	Endothelial plaque	1.1	1.1	V+N	LKP
11	23	Male	Foreign body scratch	Curvularia crescentulum	feathered edges	1.7	1	V+N	--
12	68	Male	None	Curvularia crescentulum		0.5	0.4	V+N	--
13	52	Male	None	Pythium insidiosum	Total corneal graft infection	3	--	V+A	TPK(outside hospital)/TPK/ Evisceration
14	72	Female	None	Pythium insidiosum	subepithelial and stromal infiltrations	3	--	V+N	LKP/TPK/ Evisceration
15	69	Female	None	Pythium insidiosum	Endothelial plaque, subepithelial and stromal	4	--	V+A	TPK/ Keratectomy / Evisceration
16	55	Male	Foreign body scratch	Pythium insidiosum	infiltrations, hypopyon	1.4	0.7	V+N	TPK

* BCVA, best corrected visual acuity; LogMAR , Logarithm of Mininal Angle Resolution; I, Itraconazole; V, Voriconazole; N, Natamycin; A, Amphotericin B; TPK, therapeutic penetrating keratoplasty; LKP, Lamellar keratoplasty.

Table 2 Drug sensitivity of different fungi genera (MIC)

	Rhizopus	*Sarocladium*		*Exserohilum*	*Bipolaris*	*Colletotrichum*		*Purpureocillium lilacinum*		*Curvularia crescentulum*		
Amphotericin B（0.002-32）	1	0.25	4	0.25	1.2	0.25	0.25	▯8	2	0.25	0.5	0.5
Fluconazol (0.016-256)	▯256	▯64	▯64	▯25	32	16	24	64	▯256	4	32	64
Voriconazole (0.002-32)	▯8	0.25	1	0.05	0.12	0.016	0.05	0.25	0.05	0.032	1	1
Itraconazole（0.002-32）	▯16	0.25	▯16	0.25	0.125	1.5	1.2	1	4	0.125	0.25	0.25

* The MIC value of the genera are only provided in this experiment. The results are for reference only.

Figures

Figure 1

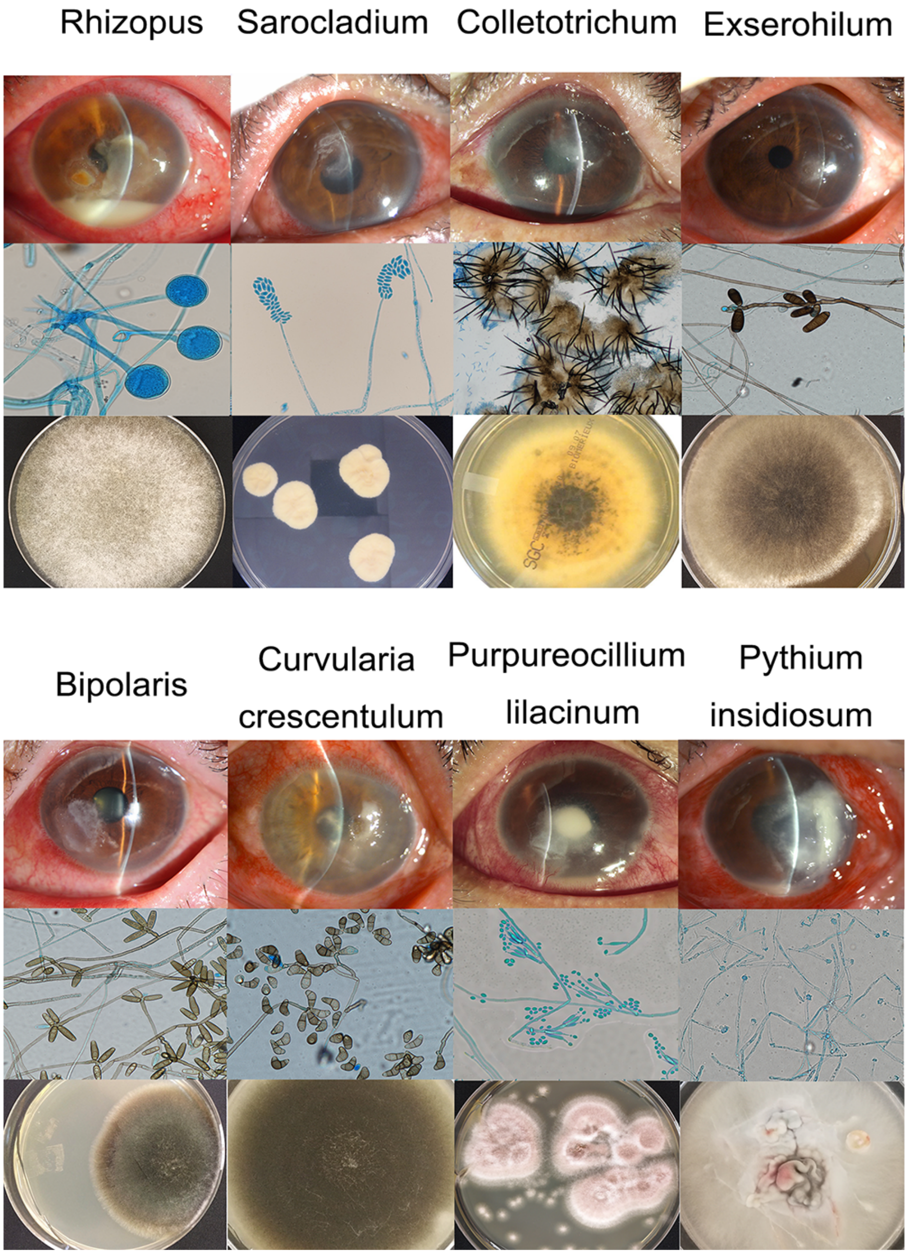

Figure 1

Slit lamp biomicroscopy photography, tape smear, and culture pictures of different genera of fungal keratitis (SDA medium, 28°C, 5–9 days).

Figure 2

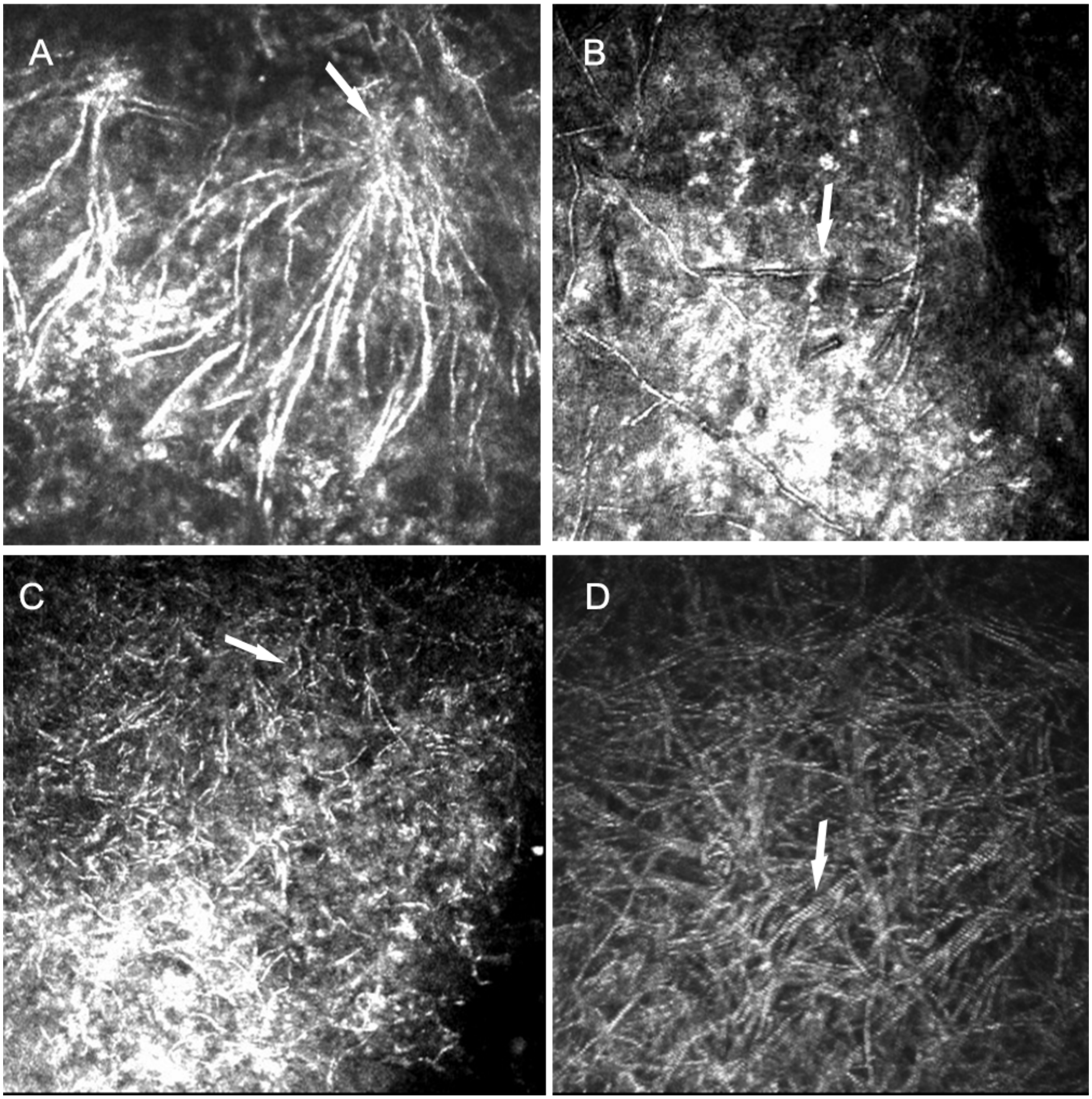

Figure 2

Confocal microscopic images of different fungal genera keratitis. A: Colletotrichum keratitis showed hyphae densely distributed in clusters and scattered inflammatory cells. B: Exserohilum keratitis showed slender hyphae overlap distribution, with sheath wall and segregation, a few spores. C: Purpureocillium lilacinum keratitis showed a large number of curved short rod-like hyphae structures and a small number of spores. D: Pythium insidiosum keratitis showed densely distributed hyphae that were beaded, and few inflammatory cells (arrow point, magnification of 400×400)

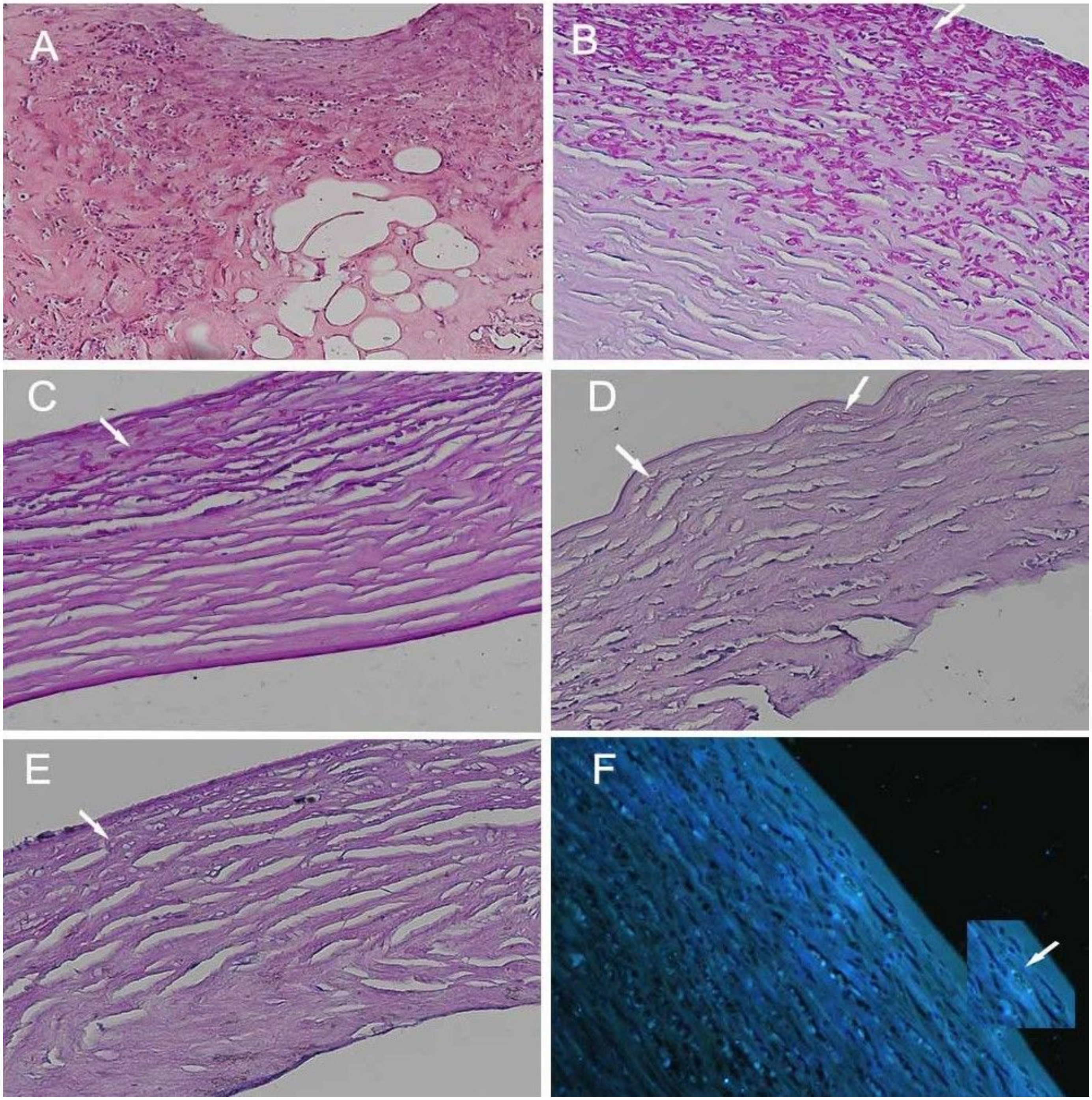

Figure 3

Histopathological manifestations of different fungal genera. A: Sporadic inflammatory cells and no obvious hyphae were observed in Colletotrichum keratitis (H&E staining, 200 × magnification). B,C: Hyphae and a few spores were found in Purpureocillium lilacinum and Curvularia crescentulum keratitis, which invaded the shallow and medium stroma and mainly horizontal growth (PAS staining, 200×magnification). D: A small number of hyphae and inflammatory cells were observed in Exserohilum keratitis, and mainly horizontal growth (PAS staining, 200×magnification). E: A large number of vacuolar hyphae-like structures were found to invade the whole cornea of Pythium insidiosum keratitis, and no obvious inflammatory cells (PAS staining, 200×magnification). F: immunofluorescence staining yielded positive results (200×magnification).

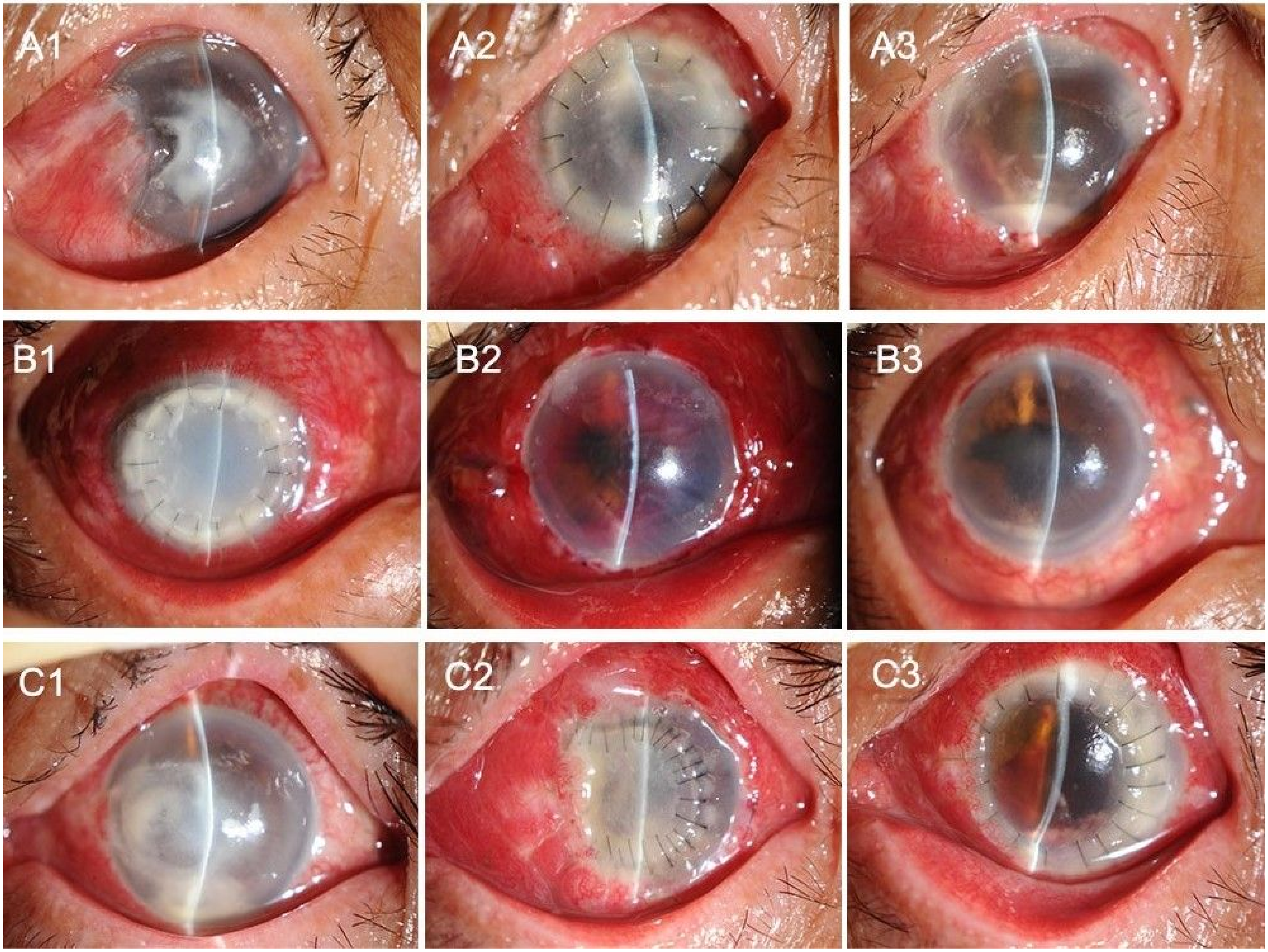

Figure 4

The treatment process of three evisceration cases of Pythium insidiosum keratitis. A1,B1,C1: Slit lamp biomicroscopy photography. A2: Fungal recurrence was observed four days after LKP, and the whole corneal graft was infiltrated. A3: Fungal recurrence was observed two days after TKP, accompanied by hypopyon. B2: Hypopyon occurred five days after TPK. B3: Hypopyon increased half a month after surgery. C2: Corneal graft, implant bed infiltration, and hypopyon were observed three days after TKP. C3: Whole corneal graft infiltration was observed three days after keratectomy.

Supplementary Files

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- STROBEchecklist.docx