

Microbiological Profiles of Ocular Fungal Infection at an Ophthalmic Referral Hospital in Southern China: A Ten-Year Retrospective Study

Yinhui Pei, Xiaoling Chen, Yiwei Tan, Xiuping Liu, Fang Duan, Kaili Wu

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangdong Provincial Clinical Research Center for Ocular Diseases, Guangzhou, People's Republic of China

Correspondence: Kaili Wu, State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangdong Provincial Clinical Research Center for Ocular Diseases, Guangzhou, People's Republic of China, Email wukaili@mail.sysu.edu.cn; Fang Duan, State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangdong Provincial Clinical Research Center for Ocular Diseases, Guangzhou, People's Republic of China, Email duanfangg@126.com

Purpose: This study aims to assess the testing methods used to detect fungal pathogens as well as the pathogenic profiles and drug susceptibility among fungal isolates from ocular sources collected in a tertiary eye hospital.

Methods: The laboratory test records of patients with suspected ocular fungal infection from January 2010 to December 2019 at a tertiary eye care center in southern China were retrospectively evaluated. The isolated pathogens identified by smear examination and culture combined with microscopy were analyzed. For 2017–2019 fungal isolates, their antifungal susceptibilities to four antifungal agents were evaluated.

Results: A total of 22,233 specimens (16,315 for culture and 5918 for direct smear examination) from 16,209 individuals were assessed, and 3317 specimens (2169 for culture and 1148 for smear examination) from 2395 patients had confirmed fungal infection. The mean age of patients was 50.6 ± 14.2 years and 63.4% were male. The positive rate of smear examination was 19.4% and significantly higher than 13.3% of fungal culture ($p < 0.001$). Of 5812 patients who were simultaneously examined for culture and smear staining, 897 (15.43%) had positive findings for fungi. Among the 2420 positive findings, 2138 (88.3%) were from the cornea, and 133 (5.5%) were from intraocular samples. *Fusarium* spp. (40.5%) and *Aspergillus* spp. (22.0%) were the major fungal genera among the molds, whereas *Candida* spp. (4.6%) constituted the major yeast. *Fusarium* spp. had a lower antifungal susceptibility than *Aspergillus* spp. against voriconazole and amphotericin. And yeast spp. had the highest susceptibility to caspofungin.

Conclusion: This study provided a 10-year assessment of fungi in ocular infections in southern China, giving support to epidemiological understanding and guiding empiric antimicrobial therapy.

Keywords: fungal infection, eye, corneal scrape, fungal culture, keratomycosis, antifungal susceptibility, endophthalmitis

Introduction

Oculomycosis is a severe and refractory infection that may result in the permanent loss of vision, due to the rapid progression of fungal infections and limited availability of antifungal agents. The epidemiological patterns of causative agents of ophthalmic mycosis vary significantly from region to region. Mycotic ophthalmitis is relatively uncommon in temperate, developed countries such as the UK,¹ USA^{2,3} and Switzerland.⁴ A two-year (2003–2005) national surveillance study in the UK showed the average incidence of fungal keratitis at 0.32% cases per million individuals per year.⁵ In contrast, fungal ophthalmitis has a relatively high incidence in tropical or subtropical, low-income countries, accounting for up to 37.6–72.0% of keratitis in Ghana, India and China.^{6–10} The distribution of fungi not only varies in different countries but also changes with time. Ong et al concluded that the constituent ratio of organismal agents of fungal keratitis switched between filamentous fungi and yeast spp. since 2007.¹ Durand pointed out that yeast spp. were the most common cause of endogenous fungal endophthalmitis, while *Fusarium* spp. and *Aspergillus* spp. were the most frequent fungal etiologies in exogenous

endophthalmitis.¹¹ Thus, for the early application of appropriate antifungal agents in the empiric management of potentially blinding ocular fungal infections, updating our knowledge of specific fungi and drug susceptibility profiles is very important.

Methods of sampling and testing are crucial for early diagnosis. During recent years, MALDI-TOF-MS, PCR and DNA sequencing have been used to identify fungi in ocular infections.^{12–17} However, in the clinical laboratory setting, microscopic identification of fungi is still the most common technique in the diagnosis of eye disorders, especially for quickly identifying pathogens in keratitis and endophthalmitis.^{18,19} Thus, in the process of smearing and culturing of specimens followed by microscopic identification to the genus or species in clinical laboratories, the accuracy of fungal identification depends on having a sufficient amount of scraped material, the staining dye (KOH, Calcofluor white, Gram and Giemsa stain), the size of the corneal ulcer and the clinical laboratory physician's experience.^{18–20}

Moreover, the development of antifungal drug resistance highlights the need for *in vitro* susceptibility testing.²¹ There have been various methods for *in vitro* antifungal susceptibility testing used, including macro- and micro-dilution, E-tests, and disk diffusion, based on the standards of the Clinical and Laboratory Standards Institute (CLSI) or The European Committee on Antimicrobial Susceptibility Testing (EUCAST).^{18,22,23} Different fungal isolates have different susceptibilities for antifungal agents. Although clinical therapeutic approaches cannot be routinely based on the results of antifungal susceptibility testing, *in vitro* determination of susceptibility profiles could provide helpful information in cases of clinically resistant fungal infection and uncommon pathogens as well as for long-term antifungal therapy.^{14,24}

Thus, the understanding of the etiological and epidemiological characteristics of fungal ocular infection in different geographical regions, social economic statuses, climate statuses, ocular anatomical positions, etc. are significant for better prevention and treatment of this disease.^{13,18} This study was undertaken to collect comprehensive information about ocular specimens for laboratory-proven positive fungal infection in the last ten years at a tertiary eye hospital in Guangzhou, South China. The previous studies reported the fungal isolates of keratitis and endophthalmitis, respectively, in same institution,^{25,26} however, the susceptibility of antifungal medicines were not available. Our study updated the spectra of ocular fungal isolates and analyzed the susceptibility of common fungal pathogens against voriconazole amphotericin B, caspofungin and fluconazole, with the goal to provide a reference to clinicians.

Subjects and Methods

This study was designed as a retrospective review of microbiological records of all samples with suspected fungal infection based on patients' clinical manifestations. All tests were undertaken for microbiological evaluation at the Zhongshan Ophthalmic Center between January 2010 and December 2019. The data of antifungal susceptibility by the E-test was reviewed for two years (2017–2019). This study was performed in compliance with the principles of the Helsinki agreement and was approved by the Institutional Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-Sen University. The requirement for patient consent was waived given the retrospective nature of the study. In this article, we analyzed the legal components without disclosing confidential patients' private information.

Organismic Isolation and Identification

Specimens were taken in the microbiological lab in our hospital under topical anesthesia (0.5%, proparacaine hydrochloride) or during operation. Protocols were conducted following our established methods published previously.²⁷ Secretions of the conjunctival sac were collected by sterile cotton swabs; cornea specimens were sampled by scraping the base and edges of the pathological lesions; and anterior chamber fluids and vitreous tissue were obtained by syringe aspiration or vitrectomy in an operating room. Most specimens were simultaneously tested for bacteria and fungi. This study only evaluated the ocular fungal infection over ten years.

The collected specimens were tested by direct smears and culturing by established methods.^{27,28} Fungal culture was conducted using potato dextrose agar medium for 7 days or more at 28°C. Positive fungal findings were evaluated based on colony morphology, growth characteristics and microscopic features with staining following a lactophenol cotton blue mount preparation.^{29,30}

Bacterial cultures were made in nutrient broth for up to 7 days at 37°C and after in sheep blood agar for 24 hours at 37°C.³¹ Then, bacterial isolates were identified by an automated microbiology system (Vitek 2 Compact, BioMerieux, Inc. 100 Rodolphe Street, Durham, USA) or by a MALDI-TOF-MS system (BioMerieux).

Antifungal Susceptibility Testing

Data from the antifungal susceptibility testing from January 2017 to December 2019 were available and retrospectively evaluated. After the identification of fungal isolates using conventional techniques, E-test susceptibility testing for voriconazole, amphotericin B, caspofungin and fluconazole against fungal isolates was performed according to the guidelines of the CLSI method using commercial kits (Autobio Diagnostics Co., Ltd, Zhengzhou, China) certificated by the China Food and Drug Administration. Pure colonies were selected for susceptibility testing. Plates were inoculated in three directions with a nontoxic cotton swab dipped in the diluted stock inoculum suspensions. Then, the E-test strips were applied to the plate. The plates were incubated at 28°C, and MICs were read around 48 h at the lowest drug concentration.

Statistical Analysis

The statistical analysis was performed with SPSS 16.0 (Chicago, IL, USA). The Chi-squared test was applied for the comparison of categorical variables. To determine the temporal trends in fungal proportions, the Pearson correlation coefficient was used. Differences were considered to be significant at $P < 0.05$.

Results

Demographics and Epidemiological Data

A total of 16,209 individuals who were suspected to have ocular bacteria or fungal infection over a ten-year-period (2010–2019) were reviewed. A total of 22,233 specimens were analyzed, including 16,315 specimens for fungal culture and 5918 specimens for smear examination, and specimens from 5812 patients were tested with both culture and the smear tests. Of them, there were 2420 specimens (2395 patients, including 25 patients examined twice on different days) with positive fungal findings detected through different methods. Among 2395 patients, males accounted for 63.3%, and the 50-year-old group had the highest incidence of fungal infection, followed by the 40- and 60-year-old groups (Figure 1A). The number of yearly positive findings of the 2395 patients were similar among the ten years reviewed in this study (Figure 1B). The fungal infections were unequally distributed in different months of the whole year (Figure 1C). October to December had a higher incidence than others, especially compared to the months of April and May.

Features of Sampling and Testing

Overall, there were 3317 (2169 for culture and 1148 for smear examination) positive fungal findings among 22,233 (16,315 for culture and 5918 for smear examination) specimens (14.9%), including 897 positive findings (15.4%) in 5812 patients who were sampled by two examination methods (Table 1). The positive rate was 19.4% (1148/5918) for smear examination, while it is 13.3% (2169/16,315) for culturing. Of 2420 specimens with positive fungal findings, 89.6% (2169 specimens) of fungal isolates were found through culture assays, and 47.4% (1148 specimens) were found through smear staining.

Among the 2420 positive specimens from 2395 individuals, 2138 (1887 from culturing and 251 from smear examination) were from keratitis, and 133 (5.5%) were from intraocular samples. The majority of corneal sampling, accounting for 88.3% of our positive findings, was from outpatients. Among 133 intraocular specimens of endophthalmitis as the result of fungal infection, 26 cases were detected from aqueous fluid, 93 cases from vitreous body, 11 cases from intraocular tissues, 2 cases from intraocular foreign bodies and 1 case from an intraocular lens. In addition, there were 40 specimens (1.65%) obtained from the conjunctiva and 22 cases (0.91%) from the lacrimal duct and its secretions.

Fungal Assay Results

The cumulative distribution of various isolated fungi is shown in Table 2. Among 2169 positive findings from culturing, fungi were identified to the genus level. Of them, *Fusarium* spp. were the most common fungi isolated (879, 40.5%), followed by *Aspergillus* spp. (477, 22.0%) and *Mucor* spp. (219, 10.1%). The other two listed genera, *Penicillium* spp. and *Curvularia* spp. account for 4.2% and 5.5%, respectively. There were 133 (6.1%) isolates belonging to yeast,

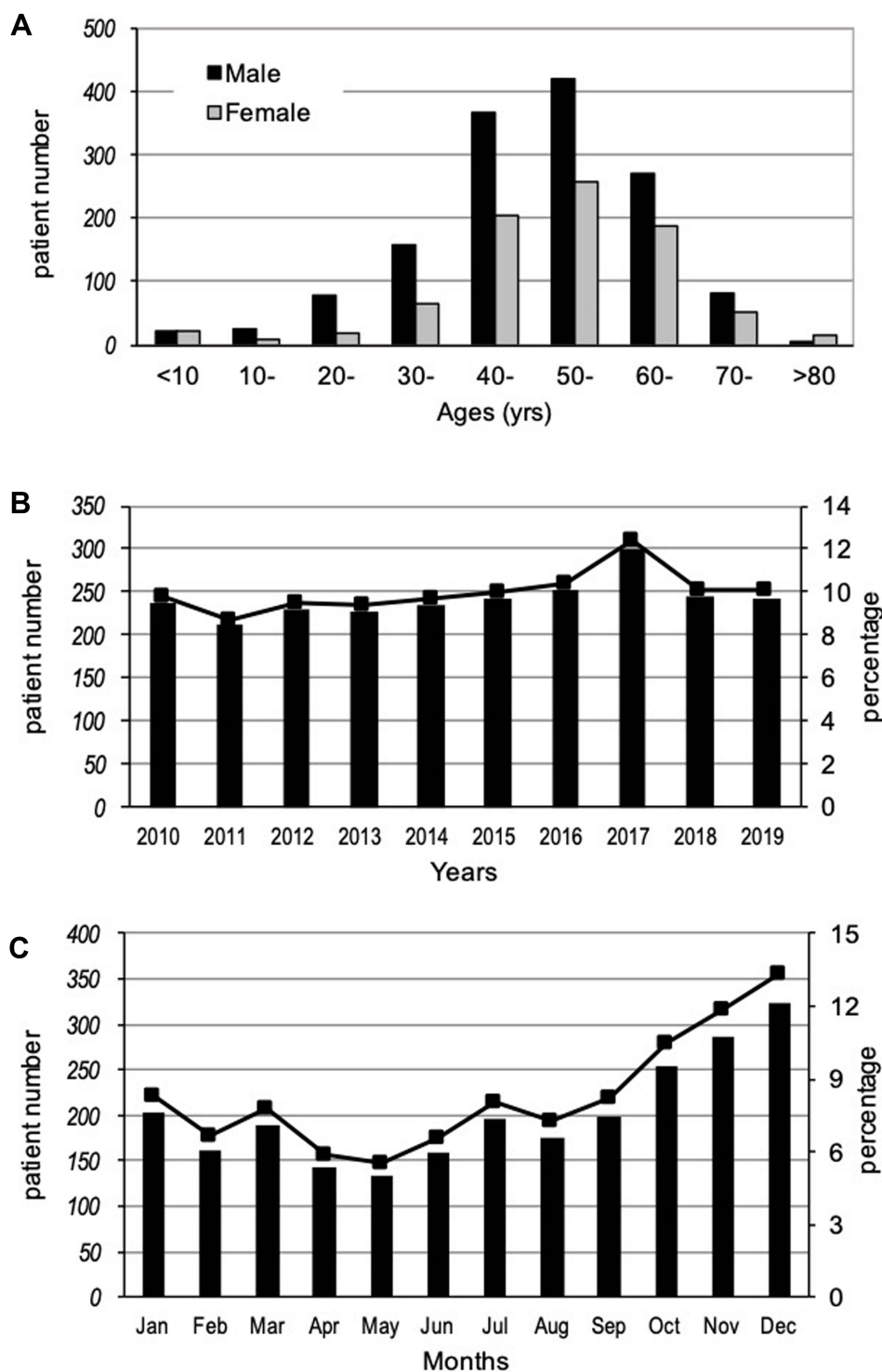


Figure 1 The distributions of patients ages (A), years (B) and months of a year (C) for ocular fungal infection. There were 2395 patients with positive fungal findings over ten years (2010–2019). For B and C, the left ordinate for bars is the number of definite cases in each year or month. The right ordinate for the solid lines shows the percentages of each observed period among the total positive cases.

including 99 *Candida* spp. (4.6%). Of the *Fusarium* spp. isolates, *F. solani*, found in 278 cases (31.6%), was the most common. Among 477 isolates of *Aspergillus* spp., *A. fumigatus* (167 cases, 35.0%), *A. flavus* (149, 31.2%) and *A. niger* (101, 21.2%) were the most common.

Table 1 Fungal Examination Results by Two Methods Over 10 Years

		Positive (%)	Negative (%)
Culture	16,315	2169 (13.3)	14,146 (86.7)
Smear	5918	1148 (19.4)	4770 (80.6)
Culture&smear	5812	897 (15.4)	4915 (84.6)

Table 2 Isolated Organisms from Ocular Fungal Infections, 2010–2019

Category	Isolate Number	%
Fusarium	879	40.5
Aspergillus	477	22.0
Mucor	219	10.1
Candida	133	6.1
Bipolaris	119	5.5
Penicillium	91	4.2
Curvularia	65	3.0
Other filamentous fungi	186	8.6
Total	2169	100

The temporal trends for three major fungal isolates from 2010 to 2019 are shown in Figure 2. There was a significant increasing trend in the isolates of *Fusarium* spp. ($r=0.785$, $p=0.007$). Similarly, *Candida* spp. ($r=0.887$, $p=0.001$), also presented a significant increasing trend. Meanwhile, the proportion of *Aspergillus* spp. presented a decreasing trend ($r=-0.614$, $p=0.059$).

In this study, among 2169 culturing positive fungal isolates, 1887 (87.0%) isolates were identified from keratitis. There were 133 fungal-positive findings from patients with endophthalmitis (Table 3). In intraocular ocular mycosis, the predominant genus was *Aspergillus* spp. and *Candida* spp., followed by *Fusarium* spp., while the predominant pathogenic genus in corneal mycosis was *Fusarium* spp., followed by *Aspergillus* spp.. Although the major categories of fungi resulting in both keratitis and endophthalmitis were the same, the proportions of the fungal genera revealed striking differences. *Fusarium* spp. accounted for 44.8% in mycotic keratitis and 14.3% in fungal endophthalmitis ($P<0.001$); *Candida* spp. accounted for 23.3% in internal ophthalmitis and 2.8% in keratomycosis samples ($P<0.001$). In addition, *Aspergillus* spp. exhibited similar a prevalence in endophthalmitis and keratitis.

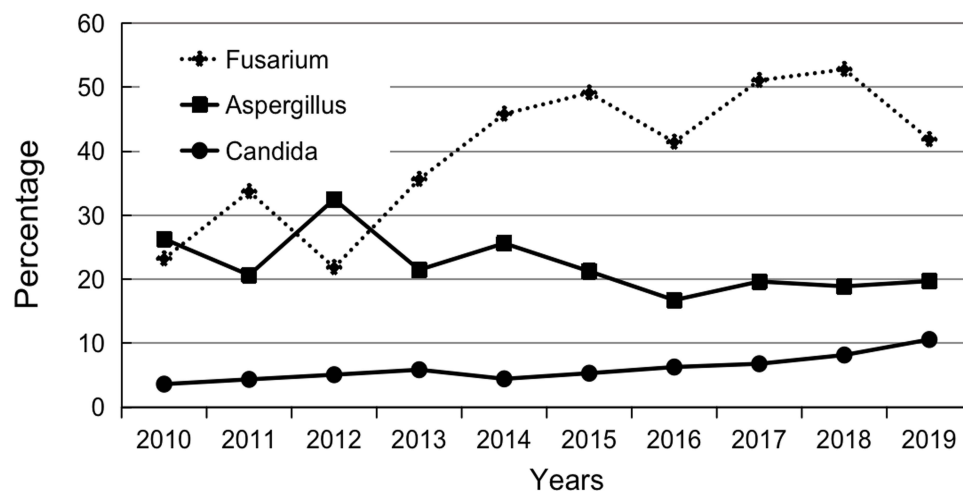


Figure 2 Temporal trends of fungal isolates from 2010 to 2019. There were significant increasing trends in the isolates of *Fusarium* and *Candida* ($p=0.007$ and $p=0.001$ respectively).

Table 3 Comparison of the Major Fungal Categories Between Corneal and Intraocular Isolates

	Intraocular N (%)	Cornea N (%)
Fusarium*	19 (14.3)	845 (44.8)
Aspergillus	31 (23.3)	407 (21.6)
Other filamentous	43 (32.3)	258 (13.7)
Candida*	31 (23.3)	53 (2.8)
Unidentified	9 (6.8)	324 (17.2)
Total	133 (100.0)	1887 (100.0)

Note: * $p < 0.001$: comparison between keratitis and endophthalmitis.

There were 136 fungal-positive specimens that appeared with co-infections of bacteria, of which, 95 (69.85%) were Gram-positive bacteria, including 89 *Staphylococcus* spp. (with 44 specimens with *S. epidermidis*). Of these specimens, 115 were from corneal ulcers, 10 were from patients with endophthalmitis and 11 were from other samples.

Antifungal Susceptibility Testing Results

Table 4 summarizes the in vitro activities of voriconazole, amphotericin B, caspofungin and fluconazole against 559 fungal isolates that were identified during 2017 and 2019, with only 42 from intraocular specimens. A total of 341 *Fusarium* spp., 131 *Aspergillus* spp., 55 Yeast spp., 17 penicillium spp. and 15 *Mucor* spp. isolates were included to determine the MIC50 and MIC90. The isolates of *Fusarium* spp. required higher concentrations of voriconazole than *Aspergillus* spp.. Most of the *Aspergillus* spp. isolates were inhibited by voriconazole at a concentration of ≥ 0.5 $\mu\text{g/mL}$, while most *Fusarium* spp. isolates were inhibited at a concentration of ≥ 4 $\mu\text{g/mL}$. The MIC90 of amphotericin-B was ≥ 32 $\mu\text{g/mL}$ for all fungi, while the MIC50 was 32 $\mu\text{g/mL}$ for *Fusarium* spp., 16 $\mu\text{g/mL}$ for *Penicillium* spp., 4 $\mu\text{g/mL}$ for *Aspergillus* spp., 0.75 $\mu\text{g/mL}$ for Yeast spp. and *Mucor* spp. The MIC50 and MIC90 of caspofungin were ≥ 32 $\mu\text{g/mL}$ for

Table 4 Minimum Inhibitory Concentration ($\mu\text{g/mL}$) of Antifungal Agents in 2017–2019

Species	Drug	MIC Range	MIC50	MIC90	<MIC50 (N, %)	\geq MIC50 (N, %)
<i>Fusarium</i> spp. (n=341)	VCZ	0.012–32	1	4	140 (41.1)	201 (58.9)
	AMB	0.002–32	32	32	81 (23.8)	260 (76.3)
<i>Aspergillus</i> spp. (n=131)	VCZ	0.012–32	0.094	0.5	45 (34.4)	86 (65.7)
	AMB	0.19–32	4	32	57 (43.5)	74 (56.5)
	CSP [#]	0.064–32	32	32	23 (18.3)	103 (81.8)
Yeast spp. (n=55)	VCZ	0.002–32	0.5	32	25 (45.5)	30 (54.6)
	AMB	0.125–32	0.75	32	17 (30.9)	38 (69.1)
	CSP [#]	0.064–32	0.19	32	23 (45.1)	28 (54.9)
	FCA	0.25–256	256	256	25 (45.5)	30 (54.6)
<i>Penicillium</i> spp. (n=17)	VCZ	0.048–32	0.5	32	7 (41.2)	10 (58.8)
	AMB	0.125–32	16	32	8 (47.1)	9 (52.9)
	CSP [#]	0.032–32	32	32	2 (15.4)	11 (84.6)
<i>Mucor</i> spp. (n=15)	VCZ	0.002–32	0.25	16	7 (46.7)	8 (53.3)
	AMB	0.002–32	0.75	32	5 (33.3)	10 (66.7)
	CSP [#]	0.5–32	32	32	4 (40.0)	6 (60.0)

Note: [#]Several patients were not been tested.

Abbreviations: VCZ, Voriconazole; AMB, Amphotericin B; CSP, Caspofungin; FCA, Fluconazole.

Aspergillus spp., Penicillium spp. and Mucor spp., while the MIC₅₀ was 0.19 µg/mL for Yeast spp. Fluconazole were inactive against Yeast spp., showing MICs ≥ 256 µg/mL.

Discussion

Fungal infective disorders of the eye are sight-threatening diseases and cases of ocular mycosis have soared worldwide during the past few decades, particularly in developing countries and tropical and subtropical regions.¹³ Several studies have reported the spectrum of ocular mycosis in the central and northern parts of the Chinese mainland.^{10,32–34} However, limited information of antifungal drug sensitivity is available. In the present study, we reviewed records of 2395 patients with 2420 positive fungal findings among 16,209 patients with 22,233 specimens over 10 years in the tertiary eye hospital in Guangzhou [Northern latitude 22°12′, East longitude 113°15′] in the southern part of China. We found that *Fusarium* and *Aspergillus* were the major fungal genera among molds, whereas *Candida* was the major genus among the yeast group. In addition, the isolates of filamentous fungi, especially *Fusarium* spp. and *Candida* spp. had increasing trends over time. Meanwhile, *Aspergillus* spp. presented a decreasing trend. Like previous reports,^{12,14,35} men aged between 40 and 60 suffered more ocular fungal infections than other people; and a higher incidence of fungal infection occurred from October to January in contrast to other months. Additionally, the results of antibiotic resistance of these pathogens provide reference information for use in clinical practice. We found that molds had the highest sensitivity to voriconazole, followed by amphotericin B, and yeast spp. were most sensitive to caspofungin, followed by amphotericin B, which provides reference information for use in clinical practice.

Currently, the clinical diagnosis of ocular fungal infection is dependent on fungal culturing that takes a long time (often 1 to 2 weeks or more) and often delays early diagnosis and treatment.^{18,20} Thus, direct microscopic examination of ocular specimens has been recommended as a rapid and effective measure of finding fungal infection.^{10,19,36} The demonstration of fungal elements in microscopic examination besides the isolation of fungi in culture is the gold standard for laboratory diagnosis.^{18,37} In our present study, the overall rate of positive fungal findings was 14.9% (excluding bacterial findings), with 19.4% for smear examination and 13.3% for culturing. Since there were 88.3% positive specimens from fungal keratitis, the diagnosis of the fungal keratitis by microscopy with smearing of specimens from corneal ulcers is a truly valuable method. In addition, the positive rate of culture and smear testing for fungi in identified infected patients is up to 70% or more in some published observations.^{1,34} However, fungal culture has a low sensitivity of less than 50%,^{18,38} and it usually takes weeks for fungal organisms to grow on culture media.^{14,39} Our institute is a tertiary center, and thus, a high number of patients with a drug treatment history are referred/transferred to our hospital. In addition, the shift from empirical treatment to evidence-based treatment has increased the prevalence of sampling. Both may be responsible for the relatively lower fungal detection rates in our hospital.

Generally, *Aspergillus* spp. and *Fusarium* spp. are common in tropical and subtropical regions, and *Candida* spp. are dominant in temperate areas.¹⁸ In our research, *Fusarium* spp. were the leading isolate, followed by *Aspergillus* spp. This was comparable to the findings reported in Tunisia,^{40,41} North India,⁴² Addis Ababa,³⁹ Ghana,⁹ South India,⁹ Saudi Arabia,¹² and different parts of China.^{6,10,32,34,43} Xie et al reported that two dominant pathogenic groups were the genus *Fusarium* (77.6%) and *Aspergillus* (10.8%) in 549 positive cultures of fungal keratitis.⁶ In contrast, *Candida* spp. were predominant in South Korea and the USA.^{44,45} In addition, our results revealed that, different from other fungi, *Fusarium* spp. increased during the last ten years, which was not found in several previous studies. Thus, reviewing local cases periodically will provide novel information for clinical treatment.

Drug sensitivity testing was performed against four drugs, ie, voriconazole, amphotericin B, caspofungin and fluconazole, which are frequently used in ophthalmic clinics in China. Although antifungal drug resistance was examined for isolates from specimens collected over two years, these isolates might reflect the latest native biological variability. Filamentous fungi, which account for the majority of fungal isolates, were the most sensitive to voriconazole, followed by amphotericin B, which is consistent with previous reports that suggested that voriconazole is the first choice for treatment of ocular mycosis caused by *Fusarium* and *Aspergillus* isolates.^{46–48} Voriconazole, with a broad spectrum of activity against *Candida* spp., *Aspergillus* spp. and *Fusarium* spp., was reported to have broad-spectrum antifungal activity, greater bioavailability, and higher aqueous and vitreous levels in the eye.^{49–51} Thus, it has become the first-line antifungal therapy against ocular infection, especially in unknown cases, during the last decade.^{13,47,52} However, there are studies that revealed that voriconazole was less effective for the in vitro inhibition of *Fusarium* spp. than AMB^{15,53} and more effective than natamycin for in vivo clinical outcomes of

filamentous fungal keratitis,⁵⁴ although the addition of oral voriconazole to topical natamycin could be beneficial for *Fusarium* keratitis.⁵⁵ In the case AMB, Xie et al⁶ and Alastruey-Izquierdo et al⁵⁶ reported that AMB was more effective against *Fusaria* spp. than *Aspergillus* spp. In our study, both *Fusaria* spp. and *Aspergillus* spp. had less sensitivity to AMB than to voriconazole. Different from molds, yeast spp. had the highest susceptibility to caspofungin, followed by amphotericin B (Table 4). That is, for ocular mycosis caused by yeast spp., we prefer caspofungin to amphotericin B, followed by voriconazole. Overall, the variation in the activity of the antifungal drugs depends on the fungal species and the nature and concentration of the vitreous antifungal agent.

Our study had some limitations, including the retrospective nature of the review of records from a microbiological lab, in which we could not reference the detailed medical history, and the study being performed at a tertiary hospital, where a significant number of patients are referrals after initial treatment. In addition, as the majority of specimens were from the cornea (1887 specimens with culturing positive findings), the relatively lower number of intraocular specimens make the findings to be less comparable to data for the ocular surface and to be hard to evaluate the resistant prevalence of each antifungal drug. When using our data as a reference for treating patients with endophthalmitis, especially for empiric antimicrobial therapy, clinicians should be cautious. Additionally, we also note that that previous studies suggested that the susceptibility levels of fungi to various antifungal compounds may be variable among different species.^{53,57,58} Further evaluation of the susceptibility for filamentous fungi at the species level, even to specific strains, is required in the future.

Conclusion

Above all, our study revealed the etiological and demographic characteristics of ocular fungal infection in a tertiary hospital in southern China from 2010 to 2019 by smear and culture examination. Since the majority of ocular mycosis was fungal keratitis, microscopy with smearing of specimens is a very valuable method for diagnosis. In addition, the isolates of filamentous fungi, especially *Fusarium* spp. and *Candida* spp. had increasing trends over time. Meanwhile, *Aspergillus* spp. presented a decreasing trend. Filamentous fungi, accounting for 90% or more of fungal pathogens, had the highest susceptibility to voriconazole, and yeast spp. had the highest susceptibility to caspofungin in the in vitro drug susceptibility testing. In short, we hope our study will be helpful in generating management strategies for ocular fungal infections and for future studies.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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