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Original article

Molecular identification, phylogeny and antifungal susceptibilities of dematiaceous fungi isolated from human keratomycosis

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ABSTRACT

Purpose: To investigate the dematiaceous fungal profile of patients with ocular mycoses attending a tertiary eye care hospital in Coimbatore, India

Methods: The identification of dematiaceous fungus based on their morphology, their genotypes, and the measurement of the minimum inhibitory concentrations (MICs) using microdilution method of routinely used antifungal drugs were all compared.

Results: A total of 148 dematiaceous fungi were isolated during a study period of 27 months. Isolates were confirmed as *Curvularia* spp. (n = 98), *Exserohilum* spp. (n = 32), *Alternaria* spp. (n = 14), *Exophiala* spp. (n = 2), *Cladosporium* sp. (n = 1) and *Aureobasidium* sp. (n = 1). Out of 50 well grown isolates characterized genotypically based on the amplification and sequencing of the ITS region of the ribosomal RNA gene cluster and subsequent BLAST analysis, *Curvularia lunata* (n = 24), *C. aeria* (n = 1), *C. spicifera* (n = 8), *C. hawaiiensis* (n = 1), *C. maydis* (n = 2), *C. papendorfii* (n = 2), *C. geniculata* (n = 3), *C. tetramera* (n = 2) and *Exs. rostratum* (n = 7) were identified. *In vitro* antifungal susceptibilities of the most tested dematiaceous isolates showed that voriconazole had a MIC_{50} of 0.25 μg ml^{-1}, while amphotericin B had a MIC_{50} of 0.25 μg ml^{-1} for *Curvularia* spp. and *Alternaria* spp.

Conclusion: Voriconazole proved to be the most effective drug against the pigmented filamentous fungi, followed by amphotericin B, itraconazole and econazole.

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1. Introduction

Mycotic keratitis/keratomycosis is a general term for fungal infection of the cornea; it is one of the major causes of ocular infectious fungal disease that leads to vision loss and morbidity. In countries having temperate climate such as the United Kingdom and northern parts of the United States of America, the incidence of mycotic keratitis is very low. However, in tropical and sub-tropical countries it is more than 50% of all the culture-proven cases of keratitis [1,2]. Especially in India, the incidence of dematiaceous fungal keratitis is common due to the tropical climate and a large agrarian population [3,4]. Trauma, occupation, age, weather conditions, pre-existing ocular diseases, systemic diseases, use of contact lens and chronic use of corticosteroids are reported to be the major risk factors [4,5].

Dematiaceous fungi are deeply pigmented fungi which contain melanin or melanin-like pigment within their hyphae and/or spores [6,7]. They are ubiquitous among soil, spread via airborne spores and are common phytopathogens [8,9]. More than 100 species of dematiaceous fungi have been reported to cause human infections including phaeohyphomycosis, chromoblastomycosis and eumycotic diseases [6,10,11]. The most frequently implicated genera in ocular mycoses are *Curvularia, Alternaria, Exserohilum* and *Cladosporium*, and this group has emerged as the third most common cause of keratomycosis after *Fusarium* and *Aspergillus* species [8,12]. *Curvularia* has been reported as the most prevalent causative agent of dematiaceous fungal keratitis [13–15] followed by endophthalmitis [13,16,17] and conjunctivitis [18]. Dematiaceous fungi are primarily identified based on their morphological characteristics, i.e., pigmentation, septation, length and width of the conidia, size of the hyphae and conidiophores [11,19,20]. Conventional identification of these pigmented molds based on morphological features is often difficult and may lead to misidentification of the correct species. Also, the morphological patterns may not be clear due to factors like the absence of spores, prior antifungal therapy and slow growth of the fungi. The taxonomy of *Bipolaris*, *Curvularia, Exserohilum* and related genera is confusing due to the nomenclatural conflicts of the teleomorphic genus *Cochliobolus* and their anamorphs in *Curvularia* and *Bipolaris* [21,22]. Under such circumstances, molecular methods that are available today offer an alternative approach for the appropriate identification of dematiaceous fungi [23,24]. DNA amplification followed by sequencing of the internal transcribed spacer (ITS) region of the ribosomal RNA gene cluster and comparison of the sequence data with the entries in GenBank by nucleotide BLAST analysis has become a highly useful and alternative diagnostic tool for the accurate identification of dematiaceous fungi [25–28].

Rapid identification and initiation of appropriate antifungal therapy are essential for the successful treatment of fungal corneal infections [1,29–31]. The amount of antifungal data which are available today for dematiaceous molds is inadequate. Antifungal susceptibility testing is essential for the surveillance of resistance and for the comparison of the *in vitro* efficacy of new and existing antifungal agents. Based on the available susceptibility data, voriconazole, itraconazole and amphotericin B are considered to be important drugs in the treatment of dematiaceous fungal infections [8,20,32–35]. However, amphotericin B resistant *Curvularia* spp. have also been reported from Tamilnadu [36]. Therefore, the present study explores the dematiaceous fungal profile of patients with ocular mycoses attending a tertiary eye care hospital in Coimbatore, Tamilnadu, India by the comparative analysis of the morphological identification and genotypic identification of dematiaceous fungi and the determination of the minimum inhibitory concentrations (MICs) of commonly used antifungal agents in order to collect antifungal susceptibility data for dematiaceous fungi from this part of the world.

2. Materials and methods

2.1. Isolates

Corneal scrapings were collected between October 2012 and December 2014 from keratitis patients attending Aravind Eye Hospital and Postgraduate Institute of Ophthalmology, Coimbatore, India. The samples were processed microbiologically for the isolation of the causative agents as described earlier [37]. Dematiaceous fungi were identified based on their deep pigmentation on potato dextrose agar (PDA - 250 g of potato slices, 15 g agar, 10 g dextrose and 1000 ml distilled water) plates. Microscopic observation after lactophenol cotton blue mount preparation of the fungal cultures employing the cellophane tape flag method [38] was performed for the initial identification of the isolates. All the identified fungal isolates were maintained in 0.85 % saline solution and refrigerated at 4 ºC for further studies.

2.2. Morphological study

The fungal isolates were grown on Sabouraud's dextrose agar (SDA - 10 g of mycological peptone, 40 g dextrose, 15 g agar and 1000 ml distilled water) medium for 14 days at 28 °C. Spores on the surface of each plate after incubation were harvested by adding 5 ml of sterile distilled water. Number of spores as well as the size and number of septa in conidia per isolate were measured using a hemocytometer fitted on a light microscope (Carl-Zeiss, Germany) with an appropriate objective lens (20×). The number of spores per ml was calculated using the formula [A + B + C + D + E (represents 4 corner areas plus one central area on Pertoff -Hausser counting chamber)] × 2000 and sizes were calculated, and mean values were taken [39].

2.3. Molecular identification

Morphologically confirmed *Curvularia* isolates (n = 50) were grown in potato dextrose broth (PDB; Difco Laboratories, Inc., USA) for 5 days at 28 °C on a rotary shaker at 200 rpm. Only 50 (51 %) *Curvularia* isolates grew well in subculture and hence those 50 isolates subjected for molecular identification. The fungal mycelium was harvested by filtration through Whatman filter paper and DNA was extracted by using MasterPure Yeast DNA purification kit (Epicenter, Madison, Wisconsin, USA) according to the instructions of the manufacturer. The complete ITS (including ITS1–5.8S rRNA-ITS2) region was amplified by PCR using the ITS1 (5′-TCCGTAGGTG AACC-3′) forward and ITS4 (5′- TCCTCCGCTTATTGATATGA-3′) reverse primers [40]. The reaction mixture contained 1 mM Taq polymerase buffer (Zenon, Szeged, Hungary), 200 mM dNTPs, 1 mM of each primer, 5U Taq polymerase (Zenon, Szeged, Hungary) and 50 ng of genomic DNA in a final volume of 25 µl. Amplification conditions for the complete ITS region with the primers ITS1 and ITS4 were as follows: denaturation at 94 °C for 3 min, 35 cycles of 94 °C for 30 s, 48 °C for 40 s and 72 °C for 40 s, and a final extension at 72 °C for 2 min

The ITS amplicons were subjected to DNA sequencing at LGC Genomics (Germany) and the individual sequence readings were assembled to contigs by using the PREGAP and GAP4 programs of the STADEN Package [41]. A BLAST search of these sequences against the GenBank nucleotide databases (www.ncbi.nlm.nih.gov) was performed to identify the isolates at the species level. All the sequence data were deposited in GenBank (accession numbers KU221450 to KU221499). The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model [42]. Evolutionary analyses were performed in MEGA7 software with 1000 bootstrap replicates [43].

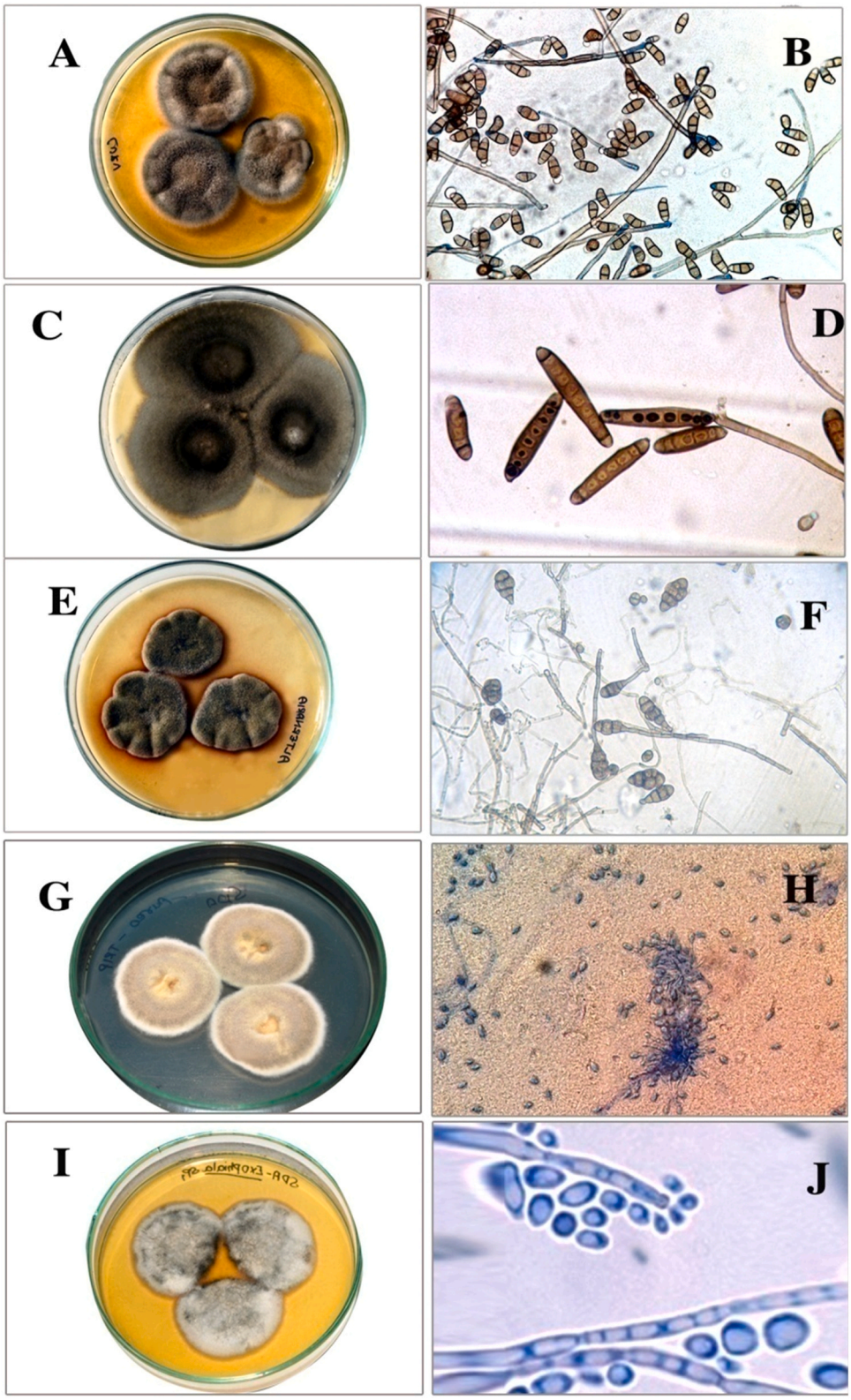

Fig. 1. Macroscopic and microscopic appearance of *Curvularia* sp. (A & B), *Exserohilum* sp. (C & D), *Alternaria* spp. (E & F), *Aureobasidium* sp. (G & H), *Exophiala* sp. (I & J) under 45 × objective of light microscopy.

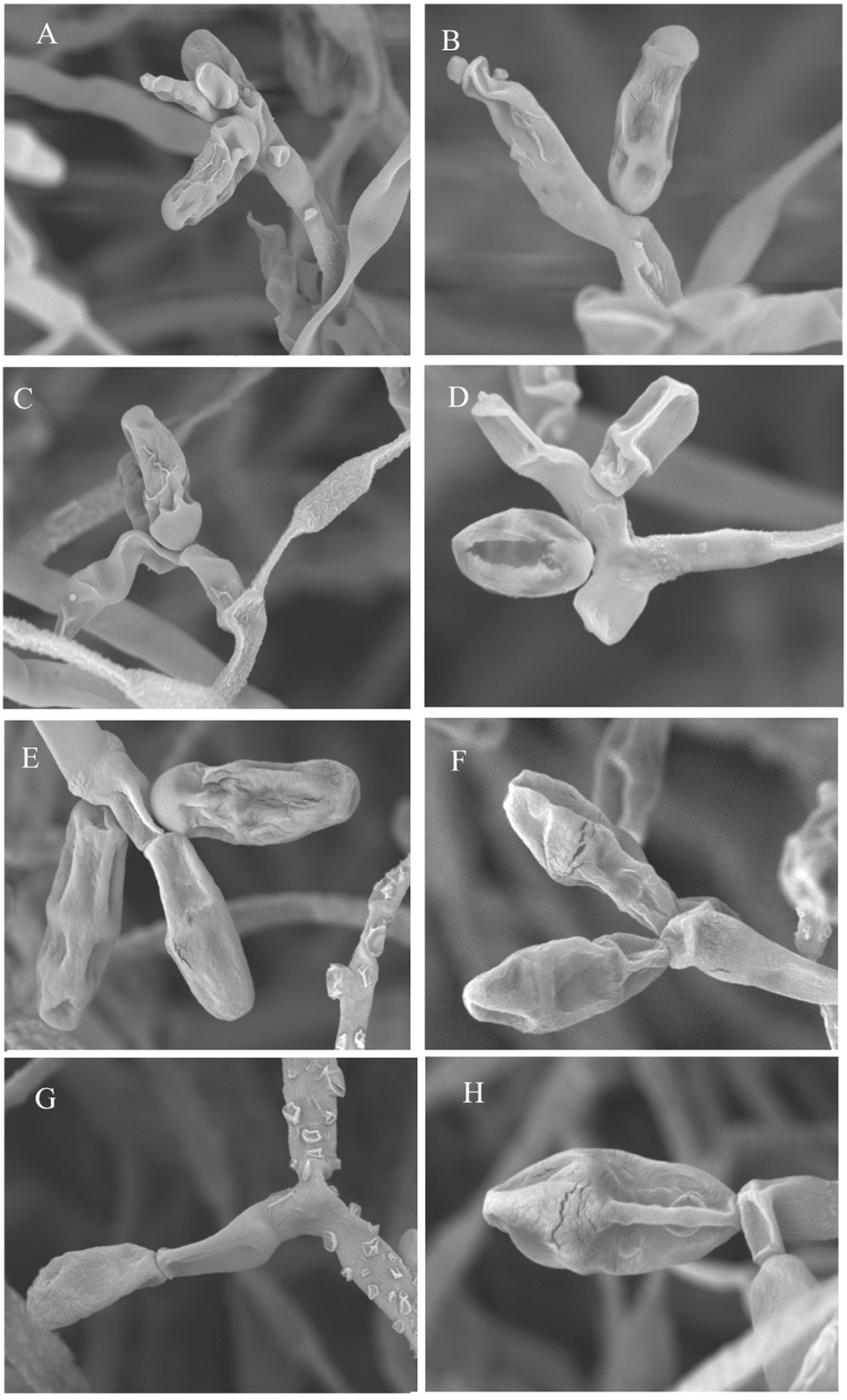

Fig. 2. Scanning Electron Microscopic images of 21-days old *C. lunata* culture showing septate sympodial geniculate conidiophores with multicellular conidia on 4th day of incubation; at 2000 × (A-B), 3000 × (C), 4000 × (D); 21st day of incubation; at 4000 × (E-G), 5000 × (H).

Table 1
Dematiaceous molds from keratomycosis.

Morphological identification by lactophenol cotton blue staining at 40 × magnification	Number of isolates subjected to molecular identification	Molecular identification by complete ITS sequencing	% agreement between morphological identification and molecular identification
Curvularia spp. (n = 112)	50	*Curvularia lunata* (n = 24) *Curvularia aeria* (n = 1) *Curvularia hawaiiensis* (n = 1) *Curvularia spicifera* (n = 8) *Curvularia papendorfii* (n = 2) *Curvularia geniculata* (n = 3) *Curvularia maydis* (n = 2) *Curvularia tetramera* (n = 2) *Exserohilum rostratum* (n = 7)	72

2.4. In vitro antifungal susceptibilities

Antifungal susceptibility testing was performed, and the data were interpreted as outlined in CLSI document M38-A2 [44]. The commonly used and commercially available drugs such as amphotericin B (AMB), nystatin (NYT), ketoconazole (KTZ), miconazole (MCZ) and fluconazole (FLZ) (HiMedia, Mumbai, India), natamycin (NAT) and itraconazole (ITZ) (Sigma-Aldrich, St. Louis, MO, USA), voriconazole (VRZ), clotrimazole (CLZ) and econazole (ECZ) (Aurolab, Madurai, India) were selected for the study.

Inoculum (0.4×10^4 to 5×10^4 CFU/ml) was diluted in the ratio of 1:50 with RPMI-1640 media (Sigma-Aldrich, St. Louis, MO, USA). *A. flavus* ATCC 204304 was included in all batches of experiments for quality control. For the broth microdilution method, 100 µl of each drug dilution and 100 µl of the prepared spore suspension were added into U-bottomed microtiter plate wells. The plates were incubated at 28 °C until growth was visible in the control well. MIC results were observed visually and were defined as the lowest drug concentration that caused 80 % inhibition of the growth in comparison to the growth control [44]. The MIC_{50} was taken as the MIC that was the median value and similarly, the MIC_{90} was the 90^{th} percentile value and represented the concentration of the drug that would inhibit 90 % of the isolates tested [45].

3. Results

A total of 9131 ocular samples were collected during the 27 months of the study period and the incidence of culture-proven cases was noted to be 2129 (23.3 %). Among these, 1309 (61.48 %), 792 (37.20 %) and 28 (1.31 %) were found to be caused by fungi, bacteria and parasites, respectively. Of all the fungal isolates obtained, it was found that 186 (14.20 %) cases were due to dematiaceous fungi. Among these isolates, *Curvularia* spp. (n = 105), *Exserohilum* spp. (n = 25), *Alternaria* spp. (n = 14), *Exophiala* spp. (n = 2), *Cladosporium* sp. (n = 1) and *Aureobasidium* sp. (n = 1) were identified based on macroscopic and microscopic features (Fig. 1 & 2). A total of 38 isolates did not sporulate even after 21 days of incubation and were considered as unidentified dematiaceous fungi (UID), which were excluded from the current study. Based on amplification of the ITS region and comparison of the sequences with those available in the GenBank nucleotide database, the isolates were identified as *C. lunata* (n = 24), *C. aeria* (n = 1), *C. spicifera* (n = 8), *C. hawaiiensis* (n = 1), *C. maydis* (n = 2), *C. papendorfii* (n = 2), *C. geniculata* (n = 3), *C. tetramera* (n = 2) and *Exs. rostratum* (n = 7).(Fig. 2).

The ITS sequence analysis of the isolates revealed that the morphologically characterized '*Curvularia* spp.' isolates also included of *Exserohilum rostratum* (n = 7). Percentage of correlation between morphological and molecular identification of *Curvularia* spp. was noted as 72% (Table 1).

A maximum likelihood phylogenetic tree (Fig. 3) was generated with a total of 50 sequences for ITS1–5.8S rRNA-ITS2 with 9 reference strains *viz*., *C. spicifera* SZMC 13068, *E. rostratum* CATAS-ER01, *S. rostrata* CBS 128062, *C. hawaiiensis* CBS 103.97, *C. lunata* UFMGCB4427, *C. aeria* CNRMA4.1002, *C. papendrofii* MAL 1162, *C. geniculata* UPM1190 and *B. tetramera* IARI 3446 as out group. Approximately, 4 distinct clades were revealed that included *C. spicifera*, *C. lunata*, *C. geniculata* and *C. tetramera*. Clade 1 grouped *E. rostrata* and *C. spicifera* along with the standard sequences of SZMC 13068, CATAS-ER01 and CBS 128062; clade 2 grouped *C. lunata*, *C. geniculata* and *C. aeria* along with standard sequences such as UFMCGB 4427, CNRMA4.1002. Also, *E. rostrata* were grouped with *C. spicifera*, *C. lunata* with *C. aeria* (*B. aeria*).

Conidial characteristics of all the isolates tested revealed that *Exserohilum* spp. exhibited a maximum mean conidial length of 212.9 µm, followed by *Alternaria* spp. (143.2 µm) and *Curvularia* spp. (75.2 µm). The recorded mean maximum width of *Curvularia* spp. conidia was 17.8 µm, followed by *Alternaria* spp. (16.1 µm) and *Exserohilum* spp. (6.3 µm). *Curvularia* spp. had the lowest number of septa (1–8), but they produced the highest number of spores (68,000/ml) when compared to other dematiaceous isolates (Table 2).

3.1. In vitro antifungal susceptibilities of dematiaceous molds from fungal keratitis

The MIC_{50} and MIC_{90} values of the tested antifungal agents are shown in Table 3· The MIC_{50} of VRZ was recorded as 0.25 µg ml^{-1} for all the tested dematiaceous isolates, except for *Alternaria* spp. which showed MIC_{50} of 0.5 µg ml^{-1}. AMB was found to be the next effective antifungal agent with MIC_{50} of 0.25 µg ml^{-1} for *Curvularia* spp. and *Alternaria* spp., followed by 0.5 µg ml^{-1} for *Exserohilum* spp., *Exophiala* spp., *Cladosporium* spp. and *Aureobasidium* spp. The minimum MIC_{50} of ITZ was 0.5 µg ml^{1} for *Curvularia* spp., *Exserohilum* spp., *Exophiala* spp., *Cladosporium* spp. and *Aureobasidium* spp. Exactly, 91 % of *Curvularia* spp. and *Exserohilum* spp. were susceptible to ≤ 1 µg ml^{-1} of ECZ. It was also noted that FLZ exhibited MIC_{90} of ≥ 32 µg ml^{-1} for all the isolates. The MIC of NTM and NYT ranged between 1 µg ml^{-1} and 128 µg ml^{-1} for the isolates tested. From these results the most effective antifungal agent was noted to be VRZ,

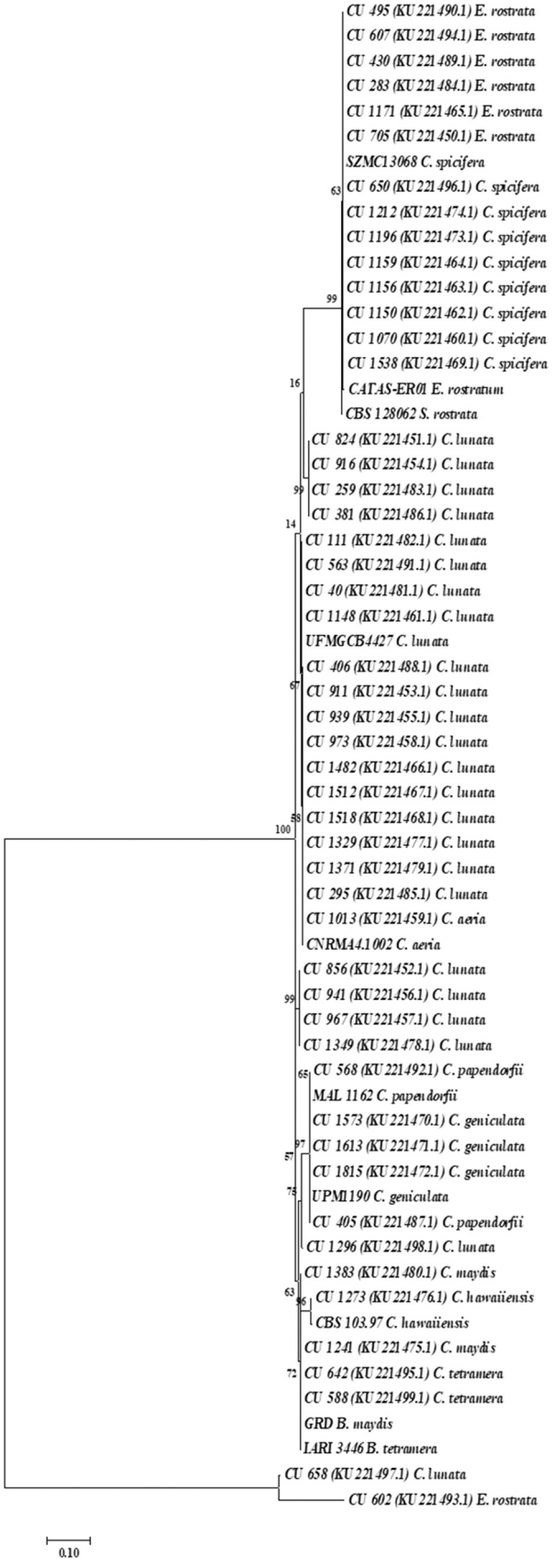

Fig. 3. Phylogenetic tree using maximum likelihood evaluation method with 1000 bootstrap replications showing the genetic diversity of *Curvularia* spp. (n = 50) isolates and reference strains (SZMC13068, CATAS ER01, CBS 128062, UFMGCB 4427, CNRMA4.1002, MAL 1162, UPM 1190, CBS 103.97, IARI 3446) based on the sequence analysis of internal transcribed spacer (ITS) sequences. Scale bar represents genetic distance.

followed by AMB, ITZ & ECZ. FLZ and NTM, while NYT was ineffective against all the isolates tested.

4. Discussion

Keratitis due to dematiaceous fungi is becoming more common in recent years [10,11,16,46]. In India, filamentous fungi such as *Fusarium* spp. and *Aspergillus* spp. are the most predominant in corneal infection, while the third most common cause of fungal keratitis is the group of dematiaceous fungi [8,12]. In the present study, a total of 1309 fungi were obtained after processing the 9131 ocular samples. Among these, a total of 186 isolates were identified as dematiaceous fungi. In the present study, *Curvularia* spp. was found to be the most predominant among the dematiaceous group, which is in accordance with previous reports [13–18,47].

ITS has become the main molecular marker sequence for the identification of fungi, because it is usually able to resolve inter- and sometimes also intraspecific differences, it is present in all fungal genera in several copies which makes the amplification efficient, and it is uploaded in the highest numbers among the different markers to sequence databases [16,25,48]. Though the phylogenetic analyses resulted in an unresolved tree, it revealed approximately 4 distinct clades that include *C. spicifera*, *C. lunata*, *C. geniculata* and *C. tetramera*. Clade 1 grouped *E. rostrata* and *C. spicifera* along with the standard sequences of SZMC 13068, CATAS-ER01 and CBS 128062; clade 2 grouped *C. lunata*, *C. geniculata* and *C. aeria* along with standard sequences such as UFMCGB 4427, CNRMA4.1002. Also, the analysis revealed that *E. rostrata* are grouped with *C. spicifera*, *C. lunata* with *C. aeria* (*B. aeria*). *C. australiensis, C. hawaiiensis* and *C. spicifera* have atypical, straight, short conidia and were originally classified as *Bipolaris* species. However, they have recently been transferred to the genus *Curvularia* based on molecular phylogenetic studies [21,49], therefore these former *Bipolaris* species are reported in the present study as *Curvularia* species. Conidial characteristics of the examined isolates were in agreement with the observations of previous studies [21,35,50,51]. Sporulation rate on SDA was lower for most of the tested isolates, therefore other general-purpose media (Czapek Dox agar, corn meal agar and oatmeal agar) may be employed for better sporulation.

While analyzing the antifungal susceptibilities, it was revealed that VRZ was the most effective drug, followed by AMB, ITZ and ECZ. All the tested isolates were inhibited at the concentration of ≤ 0.5 μg ml^{-1} of VRZ, except from a few isolates of *Alternaria* spp. The *in vitro* efficacy of VRZ against dematiaceous fungi was also reported by Espinel-ingroff [32,33,52], Sabatelli *et al.* [34], Durkin *et al.* [53], Da Cunha *et al.* [20,54] and Alex *et al.* [16].

AMB (0.0625 μg ml^{-1}–1 μg ml^{-1}) recorded effective MICs next to VRZ, similar to the findings of other studies [16,21,33,34,52,53] where MIC values ranged between 0.03 μg ml^{-1} and 1 μg ml^{-1}. However, a MIC value of 2 μg ml^{-1} for AMB was also noted in the case of two isolates of each *Curvularia* spp. and *Cochliobolus* spp. Even though AMB showed good *in vitro* activity, it has been reported that its ocular penetration is low and hence higher doses may be required which may also lead to severe systemic toxicity [55–57].

In this study, very low concentrations of ITZ and ECZ inhibited most of the tested dematiaceous fungal isolates. However, a few isolates of *Curvularia* spp. and *Alternaria* spp. required higher concentration of ITZ and ECZ. Lower MICs of ITZ were also reported by Guarro *et al.* [51], Wilhelmus and Jones [8], Pfaller [33,52], Sabatelli *et al.* [34], Espinel-Ingroff [32], Durkin *et al.* [53], Alex *et al.* [16], and Da Cunha *et al.* [20,54]. The current antifungal susceptibility data of KTZ, CLZ and MCZ (MIC ≤ 32 μg ml^{-1}) are also comparable with previous studies [8,16,21,53,58].

We report NTM, NYT and FLZ as the least effective drugs for dematiaceous fungi from keratomycosis. NTM and NYT could inhibit all the isolates only at the concentration of 64 μg ml^{-1}. None of the

Table 2
Conidial characteristics recorded in the case of the studied dematiaceous fungi.

Strains	Length (µm)	Width (µm)	Number of septa	Number of spores/ml
Curvularia spp. (n = 98)	75.2 (12.8–127.9)	17.8 (5.1–19.3)	5 (1–8)	68,000 (2000–1,50,000)
Exserohilum spp. (n = 32)	212.9 (178.4–245.1)	6.3 (5.2–8.3)	8 (7–11)	27,000 (2000–67,000)
Alternaria spp. (n = 14)	143.2 (116.8–189.8)	16.1 (14.2–23.7)	6 (3–9)	35,000 (4000–71,000)

*Ranges are shown in parentheses

Table 3
In vitro minimal inhibitory concentration (MIC) values (µg/ml) of selected antifungal agents against the dematiaceous fungal isolates.

Species (No of isolates tested)	Antifungal agent	MIC (µg/ml)		MIC range (µg/ml)	% Total at ≤ 1 µg ml^{-1}
		50 %	90 %		
Curvularia spp. *(n = 98)*	AMB	0.25	0.5	0.0625–2	98
[Curvularia lunata (n = 24)	NTM	8	16	1–32	0
Curvularia spicifera (n = 8)	NYT	4	8	1–8	6
Curvularia aeria (n = 1)	VRZ	0. 25	0.5	0.0625–0.5	100
Curvularia hawaiiensis (n = 1)	ITZ	0.5	2	0.0625–4	78
Curvularia maydis (n = 2)	KTZ	1	16	0.125–32	58
Curvularia tetramera (n = 2)	CLZ	1	4	0.125–8	63
Curvularia papendorfii (n = 2)	FLZ	16	32	4–64	0
Curvularia geniculata (n = 3)	MCZ	2	4	0.5–4	31
Curvularia spp. *(n = 55)]*	ECZ	0.5	1	0.0625–4	91
Exserohilum spp. (n = 32)	AMB	0.5	0.5	0.0625–2	94
[*Exserohilum rostratum* (n = 7)	NTM	8	16	4–16	0
Exserohilum spp. (n = 25]	NYT	4	8	1–8	3
	VRZ	0.25	0.5	0.0625–0.5	100
	ITZ	0.5	2	0.0625–4	88
	KTZ	1	8	0.125–16	53
	CLZ	2	4	0.25–4	50
	FLZ	16	32	4–64	0
	MCZ	2	4	0.5–4	50
	ECZ	0.5	2	0.125–2	91
Alternaria spp. (n = 14)	AMB	0.25	0.5	0.25–1	100
	NTM	32	64	8–128	0
	NYT	32	128	16–128	0
	VRZ	0.5	1	0.25–2	93
	ITZ	1	4	0.25–8	57
	KTZ	4	16	2–32	0
	CLZ	4	8	1–8	7
	FLZ	32	64	8–64	0
	MCZ	4	8	1–8	7
	ECZ	2	4	0.25–8	43
Others (n = 4)	AMB	0.5	0.5	-	100
[*Exophiala* spp. (n = 2)	NTM	8	64	8–16	0
Cladosporium spp. (n = 1)	NYT	64	64	-	0
Aureobasidium spp. (n = 1)]	VRZ	0.25	0.5	0.25–0.5	100
	ITZ	0.5	1	0.125–1	100
	KTZ	8	16	4–16	0
	CLZ	2	4	1–2	0
	FLZ	16	64	16–64	0
	MCZ	2	8	2–8	0
	ECZ	2	4	2–4	0

AMB, amphotericin B; NTM, natamycin; NYT, nystatin; VRZ, voriconazole; ITZ, itraconazole; KTZ, ketoconazole; CLZ, clotrimazole; FLZ, fluconazole; MCZ, miconazole, ECZ, econazole;
MIC_{50} and MIC_{90} were defined as the lowest concentrations inhibiting the growth of 50% and 90% of the isolates of a species, respectively.

isolates were inhibited below 16 µg ml^{-1}of FLZ, similarly to the results of Durkin *et al.* [53], Da Cunha *et al.* [54] and Krizsán *et al.* [21]. Although NTM is the drug of choice for fungal keratitis in developed countries [55,59,60], in the present study NTM showed lesser activity against the tested molds. Similar to our findings, the inefficacy of NTM was reported by Pradhan *et al.* [61] and Krizsán *et al.* [21].

5. Conclusion

In conclusion, *Curvularia* spp. are the foremost pathogens in dematiaceous fungal infections of the eye in Tamilnadu, South India. Discrimination of the dematiaceous molds by morphological characteristics is inadequate; and therefore, genotypic characterization is recommended for an appropriate diagnosis and choice of therapy. Based on the observed MIC data in the present study we report that VRZ - followed by AMB, ITZ and ECZ - are the potential antifungal agents for the treatment of dematiaceous fungal eye infections in humans.

Declaration of interest

The authors declare no conflicts of interest.

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