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Emerging *Rhizopus microsporus* Infections in India

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Mucormycosis is being increasingly reported all over the world. The high incidence of mucormycosis in India is due to a large population with uncontrolled diabetes and other immunocompromised states. *Rhizopus oryzae* had been reported as the most common etiological agent associated with human infections, followed by *Rhizopus microsporus*, in some studies in the Western literature (1, 2). However, in contrast to this, the second most common cause in India is *Apophysomyces elegans* (3). The first case of *R. microsporus* infection in India was reported by Bhansali et al. in 2004 (4). Since then, very few reports have been published (Table 1) (5–11). We report here an increase in the number of cases of infection due to *R. microsporus* in India.

In the present study, culture-positive cases of invasive mucormycosis from January 2015 to December 2017 were included. Before 2015, no case of infection due to *R. microsporus* was reported from our institute. The demographic details, findings of laboratory investigations, clinical course, and outcome were recorded. The outcome was considered improved when the patient was symptom free on discharge or when a marked reduction in lesions was seen either on radiological imaging or on clinical examination. Identification of *R. microsporus* was confirmed by sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) (12). The phylogenetic relatedness of our isolates was evaluated with global isolates using the maximum likelihood method, with 500 bootstrap replicates implemented in MEGA v6.00. *In vitro* antifungal susceptibility testing was performed on these isolates as per Clinical and Laboratory Standards Institute (CLSI) document M38-A2 (13).

From 58 total culture-positive cases, 17 isolates were identified as *R. microsporus*. The most common variety isolated in our study was *R. microsporus* var. *rhizopodiformis* (16/17), followed by *R. microsporus* var. *oligosporus* (1/17). Among these patients, 11 were male and 6 were female. Based on the site of involvement, 11 cases were categorized as rhino-orbitocerebral (ROC), 5 as pulmonary, and 1 as cutaneous mucormycosis. Among 11 patients with ROC mucormycosis, 2 had sinonasal disease, and additional orbital extension was seen in 9 cases. Uncontrolled diabetes mellitus was the most common underlying predisposing condition (13/17), with comorbidities such as chronic kidney disease and renal transplantation observed in 2 cases each. Hematological malignancy was present in three cases and abdominal surgery in one case. Liposomal amphotericin B with or without surgical debridement was the mainstay of treatment in all the cases, except for two patients who succumbed before the initiation of antifungals. A fatal outcome was recorded in 47% of the cases (8/17). Nine patients improved after aggressive treatment. However, morbidity in the form of loss of vision (2/9) or orbital exenteration (2/9)

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TABLE 1 Demographic and clinical details of cases included in the study

S. no.[a]	Age[b]/sex	Residence in India/ place of reporting case	Yr	Predisposing factor(s)	Clinical diagnosis[c]	Sample	Treatment	Outcome/morbidity	Identification	Source or reference
1	20/F	Rajasthan	2015	Uncontrolled diabetes type I	ROCM	Nasal scraping	Surgical debridement, liposomal amphotericin B	Improved with loss of vision in left eye	*R. microsporus* var. *rhizopodiformis*	Present study
2	36/F	Haryana	2015	Uncontrolled diabetes type II with diabetic ketoacidosis	ROCM	Nasal crust	Surgical debridement, liposomal amphotericin B	Expired after 1 day of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
3	40/M	Delhi	2015	Uncontrolled diabetes type II	ROCM	Paranasal sinus biopsy	Surgical debridement, liposomal amphotericin B	Expired after 28 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
4	55/M	Uttar Pradesh	2016	Uncontrolled diabetes, diabetic nephropathy with chronic kidney disease	ROCM	Orbital biopsy	Surgical debridement, liposomal amphotericin B, posaconazole	Improved with left orbital exenteration	*R. microsporus* var. *oligosporus*	Present study
5	18/M	Uttar Pradesh	2016	Acute myeloid leukemia	ROCM	Nasal tissue	Surgical debridement, liposomal amphotericin B	Improved with right orbital exenteration	*R. microsporus* var. *rhizopodiformis*	Present study
6	42/M	Uttar Pradesh	2016	Uncontrolled diabetes type II	ROCM	Nasal tissue	Surgical debridement, liposomal amphotericin B	Expired after 20 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
7	50/M	Haryana	2016	Uncontrolled diabetes, chronic kidney disease	PM©	Sputum	Liposomal amphotericin B	Improved after 25 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
8	45/M	Jharkhand	2016	Chronic myeloid leukemia	ROCM	Nasal tissue	Surgical debridement, liposomal amphotericin B	Improved after 17 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
9	50/F	Uttar Pradesh	2016	Uncontrolled diabetes type II	ROCM	Nasal biopsy	Surgical debridement, liposomal amphotericin B	Expired after 13 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
10	55/F	Uttar Pradesh	2016	Uncontrolled diabetes type II	ROCM	Nasal crust	Surgical debridement, liposomal amphotericin B, posaconazole	Improved with loss of vision in right eye	*R. microsporus* var. *rhizopodiformis*	Present study
11	44/M	Bihar	2017	Uncontrolled diabetes, renal transplant recipient	PM	Sputum	Liposomal amphotericin, posaconazole	Expired after 15 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
12	52/M	Bihar	2017	Uncontrolled diabetes type II	PM	Lung biopsy	Amphotericin B, posaconazole	Improved after 15 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
13	65/F	Uttar Pradesh	2017	Uncontrolled diabetes type II	PM	Bronchoalveolar lavage	Liposomal amphotericin B	Improved after 25 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
14	30/M	Madhya Pradesh	2017	Uncontrolled diabetes, renal transplant recipient	ROCM	Nasal tissue	Surgical debridement, liposomal amphotericin B	Improved after 18 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study

(Continued on next page)

TABLE 1 (Continued)

S. no.[a]	Age[b]/sex	Residence in India/ place of reporting case	Yr	Predisposing factor(s)	Clinical diagnosis[c]	Sample	Treatment	Outcome/morbidity	Identification	Source or reference
15	53/F	Delhi	2017	Uncontrolled diabetes type II	ROCM	Orbital and nasal tissue	Surgical debridement, liposomal amphotericin B	Expired after 21 days of therapy	*R. microsporus* var. *rhizopodiformis*	Present study
16	2 mo/M	Uttar Pradesh	2017	Post-abdominal surgery	CM*	Tissue from abdomen	No	Expired before initiation of treatment	*R. microsporus* var. *rhizopodiformis*	Present study
17	28/M	Delhi	2017	Acute lymphoblastic leukemia	PM	Endotracheal aspirate	No	Expired before initiation of treatment	*R. microsporus* var. *rhizopodiformis*	Present study
18	NA	Chandigarh	2004	Diabetes mellitus	ROCM	NA	NA	NA	*R. microsporus* var. *rhizopodiformis*	4
19	65/M	Delhi	2006	Uncontrolled diabetes mellitus, hypertension	ROCM	Maxillary sinus tissue	Surgical debridement, amphotericin B	Cure	*R. microsporus*	5
20	NA	Chandigarh	2009	NA	ROCM	NA	NA	NA	*R. microsporus* var. *rhizopodiformis*	6
21	56/M	Delhi	2010	None	CM	Ulcer biopsy	Surgical excision, amphotericin B	Cure	*R. microsporus*	7
22	40/F	Madhya Pradesh	2012	None	ROCM	Right maxillary sinus tissue	Surgical resection, liposomal amphotericin B	Cure	*R. microsporus*	8
23	39/M	Pune	2014	Trauma	CM	Pus from hand	Liposomal amphotericin B, posaconazole	Cure	*R. microsporus*	9
24 ($n = 13$)[d]	NA	Delhi	2014	NA	ROCM ($n = 7$), PM ($n = 6$)	Lung tissue ($n = 3$), bronchoalveolar lavage ($n = 3$), endotracheal aspirate ($n = 2$), nasal mass ($n = 4$), lung fine-needle aspiration biopsy ($n = 2$), sinus aspirate ($n = 2$), maxillary sinus aspirate ($n = 1$)	NA	NA	*R. microsporus* ($n = 1$), *R. microsporus* var. *rhizopodiformis* ($n = 10$), *R. microsporus* var. *oligosporus* ($n = 5$), *R. microsporus* var. *chinensis* ($n = 1$)	10
25	NA	Chandigarh	2015	NA	ROCM	NA	NA	Expired	*R. microsporus*	11

[a]S. no., serial number.
[b]Age is given in years except where otherwise noted. NA, not available.
[c]ROCM, rhino-orbitocerebral mucormycosis; PM, pulmonary mucormycosis; CM, cutaneous mucormycosis.
[d]In this study, a total of 17 samples were collected from 13 patients.

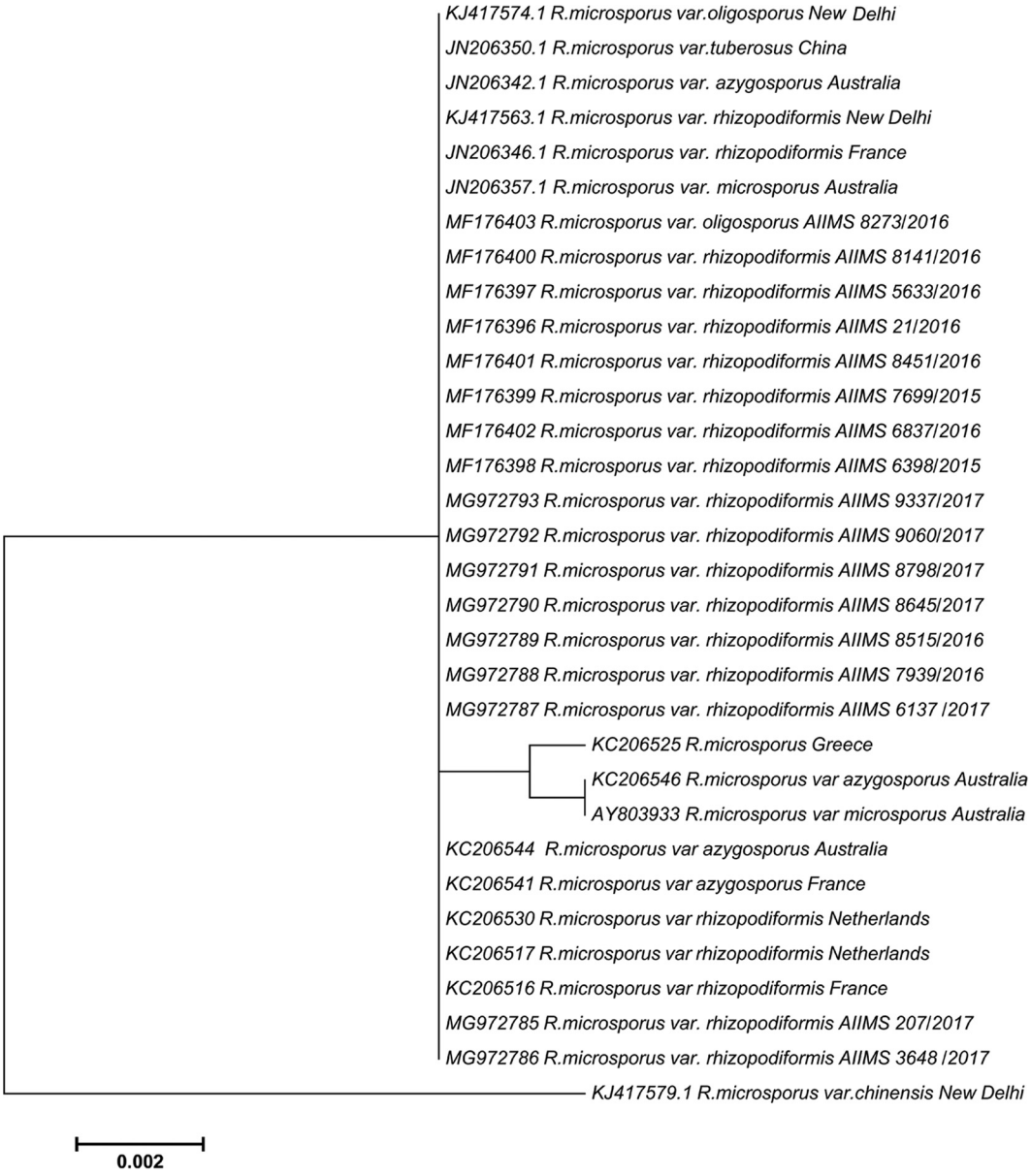

FIG 1 Maximum likelihood phylogenetic analysis of *Rhizopus microsporus*.

was observed (Table 1). The MICs for the antifungals tested, *viz*., amphotericin B, itraconazole, and posaconazole, ranged from ≤0.03 to 0.5, 0.25 to 2, and ≤0.03 to 0.5 μg/ml, respectively. The ITS phylogenetic tree revealed that our isolates were clustered together in a group along with other type strains (Fig. 1).

The present study shows an increasing number of infections due to *R. microsporus* in India over a short span of time. However, these cases just demonstrate the tip of the iceberg. The true incidence in India is still unknown, since many facilities lack mycological expertise, and identification using sequencing is not routinely performed due to financial constraints. Identification of Mucorales to species level holds significance for better understanding of current epidemiology. In addition, more studies on the environmental niches of *R. microsporus* in India are warranted.

Accession number(s). All sequences determined in this study were submitted to GenBank under accession numbers MF176396 to MF176403 and MG972785 to MG972793.

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We declare no conflict of interest.

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