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A Multifaceted and Multi-Institutional Analysis of the COVID19-Associated Mucormycosis Outbreak in the Delhi Area Indicates the Simultaneous Convergence of Multiple Risk Factors

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Background

A major outbreak of COVID-19-associated mucormycosis (CAM) in India in spring 2021 aggravated the death toll of COVID-19. As the causes of that CAM outbreak remain unclear, we performed a multi-faceted study of environmental, host-, pathogen-, and healthcare-related factors in CAM patients in the metropolitan New Delhi area.

Methods

Case/control design: We reviewed all adult patients (≥18 years of age, n = 50) who were diagnosed with culture- or biopsy-proven mucormycosis at 7 participating public and private hospitals in the New Delhi area between April 1 and June 30, 2021 and within 60 days of a prior COVID-19 infection. Prior Covid-19 infection was defined as COVID-19 symptoms and at least one positive SARS-CoV-2 PCR. Sixty-nine contemporary adult patients admitted to the same hospitals for treatment of PCR-confirmed COVID-19 infection served as the control cohort. We performed two distinct case/control analyses, as summarized in **Table 1**.

Table 1: Summary of case/control analyses performed.

	Analysis 1	Analysis 2
Inclusion criteria for the CAM cohort	Patients hospitalized for CAM, regardless of their hospitalization status for COVID-19 (n = 50)	Patients developing CAM who had already been hospitalized for COVID-19 (n = 31)
CAM cohort includes patients with	Severe COVID-19 Moderate COVID-19 Mild COVID-19	Severe COVID-19 Moderate COVID-19
Control cohort for both analyses	Contemporary patients (n = 69) hospitalized at the participating hospitals for treatment of COVID-19	
Control cohort includes patients with	Severe COVID-19 Moderate COVID-19	

Statistical analyses: Univariate comparisons of continuous variables were performed using the Wilcoxon rank-sum test. Categorial variables were compared using Chi-square or Fisher's exact test, as appropriate. A logistic regression model with backward elimination was used to identify independent predictors of CAM development. All tests were 2-sided with a significance level of $p < 0.05$.

Collection of meteorological data: Temperature, relative humidity, and evaporation at the Agrometeorological Observatory New Delhi (28°38'23"N, 77°09'27"E, altitude: 229 m above sea level) were recorded daily by the Indian Agricultural Research Institute.

Quantification of fungal spore concentrations in outdoor air: Fungal spore concentrations in environmental air were determined by the Vallabhbhai Patel Chest Institute using a 24-hour volumetric trap air sampler (Burkard Manufacturing Co Ltd) with an air flow of 10 L/min. Total mold particle recovery per day was determined by microscopic examination of slides fitted in the lid assembly of the air sampler.

Microbiological analyses: Tissue specimens were processed for direct microscopic examination by 10% KOH-Blankophor staining. Additionally, tissue samples and hospital fomite swabs were cultured on Sabouraud dextrose agar. Patient isolates were identified by MALDI-TOF mass spectrometry and ITS sequencing.

Whole genome sequencing (WGS): Fungal DNA from selected patient isolates was sequenced with a NOVASEQ6000 sequencer (Illumina). Paired-end reads were aligned to reference isolates and additional species-typed Sequence Read Archive isolates (NCBI).

Results

Table 2: Univariate comparison of patients with CAM and controls.

	Analysis 1			Analysis 2		
Unless indicated otherwise, number of patients and percentages are given	**CAM cases n = 50**	**Controls n = 69**	**P-value**	**CAM cases n = 31**	**Controls n = 69**	**P-value**
Age (years), median (range)	57 (34 – 77)	50 (19 – 86)	0.07	58 (34 – 77)	50 (19 – 86)	0.09
Gender, male	35 (70)	47 (68)	0.83	21 (68)	47 (68)	0.97
Living conditions			**0.04**			**0.04**
Rural	7/42 (17)	3/68 (4)		5/28 (18)	3/68 (4)	
Urban	35/42 (83)	65/68 (96)		23/28 (82)	65/68 (96)	
Unknown	8	1		3	1	
Underlying conditions						
Arterial Hypertension	15 (30)	27 (39)	0.30	9 (29)	27 (39)	0.33
Chronic kidney disease	1 (2)	2 (3)	> 0.99	0 (0)	2 (3)	> 0.99
Chronic liver disease	2 (4)	0 (0)	0.17	2 (6)	0 (0)	0.09
Heart failure or coronary artery disease	1 (2)	4 (6)	0.40	1 (3)	4 (6)	> 0.99
Cancer (any malignancy)	5 (10)	0 (0)	**0.01**	4 (13)	0 (0)	**< 0.01**
Hematological malignancy	3 (6)	0 (0)	0.07	3 (10)	0 (0)	**0.03**
Solid tumor	2 (4)	0 (0)	0.17	1 (3)	0 (0)	0.31
Chronic lung disease	2 (4)	7 (10)	0.30	1 (3)	7 (10)	0.43
Asthma	2 (4)	5 (7)	0.70	1 (3)	5 (7)	0.66
COPD	0 (0)	2 (3)	0.51	0 (0)	2 (3)	> 0.99
Surgery within last 14 days	1 (2)	0 (0)	0.42	1 (3)	0 (0)	0.31
Immunosuppressive therapy [a]	4 (8)	0 (0)	**0.03**	3 (10)	0 (0)	**0.03**
Cytopenia						
Neutropenia (ANC < 1000)	1/42 (2)	0/68 (0)	0.38	1/27 (4)	0/68 (0)	0.28
Lymphopenia (ALC < 1000)	20/43 (47)	20/68 (29)	0.07	13/27 (48)	20/68 (29)	0.08
COVID-19 severity			**< 0.001**			**< 0.01**
Mild	19 (38)	N/A		N/A	N/A	
Moderate	18 (36)	57 (83)		18 (58)	57 (83)	
Severe	13 (26)	12 (17)		13 (42)	12 (17)	
Hospitalization for COVID-19	31 (62)	69 (100)	**< 0.001**	50 (100)	69 (100)	N/A
ICU admission for COVID-19	14 (28)	34 (49)	**0.02**	14 (45)	34 (49)	0.70
Lowest SO_2 at room air, median (range)	90% (60% – 98%)	85% (65% – 98%)	**< 0.01**	85% (60% – 98%)	85% (65% – 98%)	0.24
Supplemental oxygen for COVID-19	30 (60)	66 (96)	**< 0.001**	22 (71)	66 (96)	**< 0.01**
Highest level of oxygen support			**0.01**			**< 0.01**
Nasal cannula	12/30 (40)	23/66 (35)		5/22 (23)	23/66 (35)	
Face mask/non-rebreathing mask	5/30 (17)	31/66 (47)		4/22 (18)	31/66 (47)	
BiPap/non-invasive ventilation	11/30 (37)	9/66 (14)		11/22 (50)	9/66 (14)	
Invasive ventilation	2/30 (7)	3/66 (5)		2/22 (9)	3/66 (5)	
Glucocorticosteroids for COVID-19	40 (80)	67 (97)	**< 0.01**	26 (84)	67 (97)	**0.03**
High dose glucocorticosteroids	24/34 (55)	58/68 (85)	**< 0.001**	19/30 (63)	58/68 (85)	**0.02**
Unknown	6	1		1	1	
Days of steroids, median (range)	11 (5 – 20)	15 (3 – 45)	0.05	11 (5 – 20)	15 (3 – 45)	0.12
Monoclonal antibodies	2 (4)	4 (6)	> 0.99	2 (6)	4 (6)	> 0.99
Tocilizumab	2 (4)	0 (0)		2 (4)	0 (0)	
Itolizumab	0 (0)	1 (1)		0 (0)	1 (1)	
Bevacizumab	0 (0)	3 (4)		0 (0)	3 (4)	
Other COVID-19 therapeutics						
Remdesivir	10 (20)	30 (43)	**< 0.01**	10 (32)	30 (43)	0.29
Intravenous immunoglobulins	2 (4)	0 (0)	0.17	2 (6)	0 (0)	0.09
Baricitinib	0 (0)	8 (12)	**0.02**	0 (0)	8 (12)	0.06
Diabetes mellitus (DM)						
Previously diagnosed DM	33 (66)	19 (28)	**< 0.001**	21 (68)	19 (28)	**< 0.001**
Newly diagnosed DM	12/17 (71)	7/50 (14)	**< 0.001**	6/10 (60)	7/50 (14)	**< 0.01**
Diabetic ketoacidosis	4/45 (9)	0/25 (0)	0.29	2/27 (7)	0/25 (0)	0.49
Latest HbA1c, median (range)	8.0 (4.2 – 16.6)	6.7 (3.2 – 12.4)	**< 0.001**	7.6 (4.2 – 14.8)	6.7 (3.2 – 12.4)	**< 0.001**
Latest HbA1c ≥ 8.0	25 (50)	7/65 (11)	**< 0.001**	12 (39)	7/65 (11)	**< 0.01**
Highest glucose level [b]	385 (180 – 600)	303 (110 – 588)	**0.04**	373 (180 – 570)	303 (110 – 588)	0.16

Table 3: Multivariate comparison of CAM patients and controls.

Analysis 1	Odds Ratio	95% CI	P-value
Associated with higher risk of CAM			
Previously or newly diagnosed diabetes mellitus	5.67	2.16 to 37.36	**< 0.001**
Cancer (any malignancy)	5.68	1.91 to infinite	**0.006**
Associated with lower risk of CAM			
Supplemental oxygen	0.17	0.02 to 0.53	**< 0.001**

Analysis 2	Odds Ratio	95% CI	P-value
Associated with higher risk of CAM			
Severe COVID-19 (versus mild or moderate)	4.09	1.42 to 15.45	**0.004**
Cancer (any malignancy)	5.98	1.79 to infinite	**0.012**
Previously or newly diagnosed diabetes mellitus	8.26	4.08 to 59.63	**< 0.001**
Associated with lower risk of CAM			
Supplemental oxygen	0.13	0.02 to 0.42	**< 0.001**
Remdesivir	0.40	0.12 to 0.97	**0.039**
ICU admission for COVID-19	0.41	0.16 to 0.93	**0.030**

Table 4: Presentation, treatment, and outcome of CAM patients.

Time of CAM diagnosis, median (range)	
Days after onset of COVID-19 symptoms	17 (0 – 37
Days after positive COVID-19 PCR	16 (-2 – 55)
Days after start of glucocorticosteroids for COVID-19	13 (4 – 30)
Site(s), n (%)	
Sinusitis	5 (10)
Orbital	3 (6)
Rhino-cerebral	1 (2)
Rhino-(sinu-)orbital	25 (50)
Rhino-(sinu-)orbito-cerebral	9 (18)
(Sinu-)Pulmonary	3 (6)
Others	4 (8)
Diagnosis, n (%)	
Suspicious CT/MRI	38 (76)
Mucorales-positive histopathology/KOH	50 (100)
Cavernous sinus thrombosis, n (%)	4/38 (11) [c]
Genus cultured, n (%)	
Lichtheimia	2 (4)
Rhizopus	32 (64)
No growth	16 (32)
Treatment, n (%)	
Liposomal amphotericin B	45 (90)
Posaconazole	6 (12)
Surgical debridement	35 (70)
Outcome, n (%)	
Discharged	36 (72)
Died in hospital	14 (28)

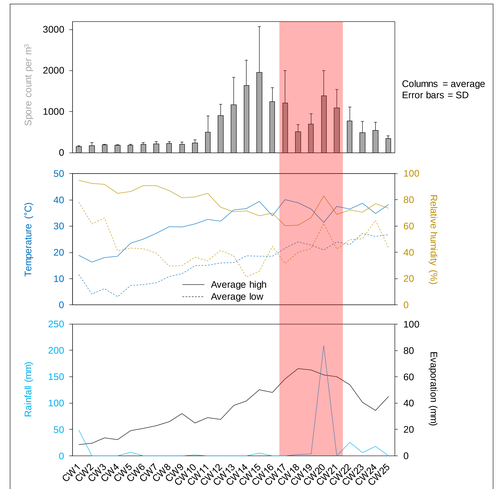
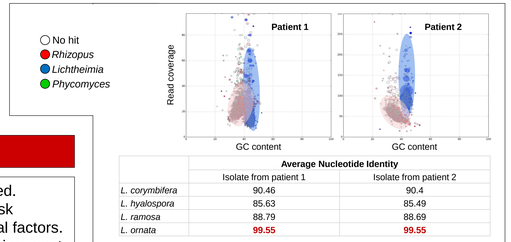


Figure 1: Temporal correlation of CAM cases, spore burden in ambient air, and key meteorological data in spring 2021.
96% of the CAM cases in this study were seen in the red-shaded period (CW 17-21). CW = calendar week, SD = standard deviation.



	Average Nucleotide Identity	
	Isolate from patient 1	Isolate from patient 2
L. corymbifera	90.46	90.4
L. hyalospora	85.63	85.49
L. ramosa	88.79	88.69
L. ornata	**99.55**	**99.55**

Figure 2: WGS identified the rare Mucorales pathogen *Lichtheimia ornata* in two isolates from CAM patients.

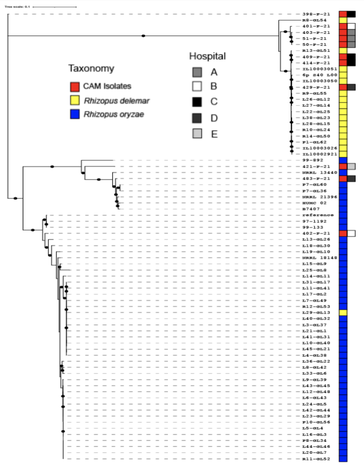


Figure 3: Phylogenetic analysis of *R. oryzae/delemar* isolates showed no clonal population of isolates from the same hospital.

Conclusions

- Consistent with prior studies,[1,2] previously or newly diagnosed diabetes mellitus was a key predictor of CAM risk, especially when poorly controlled.
- Surrogates of access to advanced treatment of COVID-19 (ICU admission, remdesivir, supplemental oxygen) were associated with lower CAM risk
- The CAM incidence peak was preceded by a significant uptick in environmental spore concentrations but was not linked to specific meteorological factors.
- Fomite cultures were negative (data not shown) and WGS showed no clonal population of patient isolates → no link of CAM cases to hospital environment.
- *Rhizopus* was the predominant causative genus (64%), but two cases of the rare pathogen *Lichtheimia ornata* were detected by WGS.
- Altogether, our data suggest that an intersection of host, environmental, pathogen and healthcare-related factors contributed to the emergence of CAM.

Footnotes: [a] any (systemic) immunosuppressive agent received prior to the diagnosis of COVID-19; [b] highest glucose level recorded during the treatment of COVID-19; [c] denominator = patients who had a suspicious CT or MRI. **References:** [1] Hoenigl et al, 2022, Lancet Microbe; [2] Arora et al., 2022, J Infection.