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Reduced susceptibility to fluconazole in *Cryptococcus neoformans*: A major concern for emerging drug resistance in North India

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A B S T R A C T

Background and Purpose: Cryptococcal meningitis (CM) remains a serious opportunistic infection, particularly among individuals living with HIV/AIDS. Fluconazole (FLC), favored for its accessibility and cost-effectiveness, faces increasing resistance worldwide, particularly in low-resource settings, such as India, where antifungal susceptibility data are scarce. This study aimed to assess the prevalence of reduced FLC susceptibility among *Cryptococcus neoformans* clinical isolates in North India and also to evaluate its clinical implications in the management of CM.

Materials and Methods: In total, 21 cases of Cryptococcal Meningitis diagnosed between 2022 and 2024 were analyzed. Clinical features, demographic data, laboratory parameters, and antifungal susceptibility profiles were assessed, with subgroup comparisons between HIV-positive and HIV-negative patients. Antifungal susceptibility testing for amphotericin B, fluconazole, itraconazole, and voriconazole was performed using broth microdilution as per Clinical and Laboratory Standards Institute guidelines (M27-A3 document).

Results: A total of 21 patients with CM were included; 12 (57.1%) were HIV-positive, and 9 (42.9%) were HIV-negative. Cases showed seasonal clustering with peaks during May–August, highest in July 2024. Antifungal susceptibility testing was performed on 17 clinical isolates. Fluconazole minimum inhibitory concentrations (MICs) were within the range of 1–16 µg/mL with MIC_{50}/MIC_{90} of 8/8 µg/mL (geometric mean 6.4 µg/mL). Amphotericin B MICs were within the range of 0.25–2 µg/mL (MIC_{50}: 1 µg/mL; MIC_{90}: 2 µg/mL; geometric mean: 1.18 µg/mL). Itraconazole and voriconazole showed low MICs (itraconazole: 0.0625–0.125 µg/mL and voriconazole: 0.03–0.25 µg/mL). Univariate analysis revealed that elevated FLC MIC (≥8 µg/mL) had no significant association with age, gender, HIV status, or mortality.

Conclusion: The findings underscore rising FLC MICs in *C. neoformans* isolates in North India, necessitating enhanced antifungal surveillance and stewardship to optimize CM treatment outcomes.

Keywords: Antifungal susceptibility, Cryptococcal meningitis, *Cryptococcus neoformans*, Fluconazole, HIV

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Introduction

Cryptococcus neoformans is a leading cause of cryptococcal meningitis (CM), particularly among immunocompromised individuals, including those with HIV/AIDS. Despite the rollout of antiretroviral therapy (ART), CM remains a major cause of AIDS-related mortality, contributing to nearly 180,000 deaths annually, with a substantial burden in South Asia and sub-Saharan Africa [1]. In recognition of its continuing public health importance, the World Health Organization (WHO) has listed *C. neoformans* among the highest-priority fungal pathogens, underscoring the urgent need to strengthen diagnostic capacity and antifungal stewardship [2].

Recent WHO recommendations (2022) and the ECMM/ISHAM/ASM global guideline (2024) recommend amphotericin B– based induction therapy, preferably in combination with flucytosine, followed by fluconazole (FLC) for consolidation and maintenance phases. Fluconazole is also widely used for prophylaxis and long-term suppression, particularly in resource-limited settings where flucytosine and lipid formulations of amphotericin B are unavailable or unaffordable [3, 4]. This heavy reliance on azole-based regimens, especially when dosing is suboptimal or prolonged exposure occurs, has raised concern regarding the emergence of antifungal resistance.

In this context, reduced FLC susceptibility and resistance in *Cryptococcus* spp., including heteroresistance, have emerged as clinically important concerns. Interpretation of antifungal susceptibility

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testing in *Cryptococcus* is challenging due to limited clinical breakpoints; therefore, surveillance based on minimum inhibitory concentration (MIC) distributions and epidemiological cut-off values remains essential for monitoring emerging resistance [5]. In North India, where drug availability and cost may influence antifungal choice, region-specific data on FLC susceptibility are needed to guide treatment decisions and antifungal stewardship. This study aimed to evaluate the prevalence of reduced FLC susceptibility among *C. neoformans* clinical isolates in North India and to underscore the implications of emerging antifungal resistance for the management of Cryptococcal disease in this region.

Materials and methods

This study was conducted in the Department of Microbiology at a tertiary care referral center in Lucknow, India, over three years (January 2022 to December 2024). The study protocol was approved by the Institutional Ethics Committee (Reference code: ECR/262/Inst/UP/2013/RR/19). All samples were obtained from patients admitted to our tertiary care center. The study included 21 patients with clinically suspected chronic meningitis, irrespective of age or gender.

Diagnosis of cryptococcal meningoencephalitis was based on at least one of the following criteria: (1) positive cerebrospinal fluid (CSF) culture for *Cryptococcus* species, (2) positive CSF India ink preparation, or (3) positive CSF Cryptococcal antigen test performed using the Cryptococcal Antigen Latex Agglutination System (CALAS; latex agglutination method; Meridian Bioscience, USA).

Study population and data collection

Patients presenting with symptoms suggestive of meningitis were screened for cryptococcal meningoencephalitis. Clinical and demographic data, including age, gender, underlying conditions, presenting symptoms, laboratory findings, fungemia status, and outcomes, were retrieved from electronic medical records. The CSF analysis included cell counts, glucose and protein levels, and microbiological investigations. Patients were stratified into HIV-positive and HIV-negative groups for comparative analysis.

Isolation, identification and antifungal susceptibility of Cryptococcus neoformans

The CSF samples were cultured on Sabouraud's dextrose agar (HiMedia Laboratories, India) with antibiotics (chloramphenicol and gentamicin) to inhibit bacterial contaminants and isolate fungal organisms. Identification of *C. neoformans* was confirmed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS [Vitek MS, bioMérieux, Marcy-l'Étoile, France), with an updated *in vitro* diagnostic database/library capable of differentiating the *Cryptococcus neoformans* and *Cryptococcus gattii species* complexes. Antifungal susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M27-A3 document). Four antifungal agents, namely fluconazole, itraconazole, voriconazole, and amphotericin B, were evaluated. Flucytosine was excluded from evaluation as it is not routinely used in clinical practice at our center, owing to its limited availability and high cost.

Statistical analysis

Categorical variables were reported as frequencies and percentages, and continuous variables as means ± SD or medians with interquartile ranges. Comparisons between groups were performed using the chi-square test for categorical variables and Student's *t*-test for continuous variables. Analyses were performed in SPSS software (version 28.0), and a p value < 0.05 was considered statistically significant.

Results

A total of 21 patients diagnosed with cryptococcal meningitis were included in the study. The demographic and clinical profiles of HIV-positive and HIV-negative patients are presented in Table 1. Median age of the cohort was 35 years (range: 13–65 years), with a mean age of 34.9 ± 10.9 years among HIV-positive patients and 37.7 ± 21.0 years among HIV-negative patients (p=0.727). Regarding gender, the majority of patients (66.6%) were male. The HIV infection was identified in 12 patients (57.1%), whereas 9 patients (42.9%) were HIV-negative. The HIV-positive individuals tended to be younger and predominantly male, compared to HIV-negative individuals.

This study identified several underlying conditions and exposures as potential risk factors for cryptococcal meningitis. Surgical interventions were reported exclusively among HIV-negative patients (n = 2), both of whom had a history of neurosurgical procedures, suggesting possible iatrogenic exposure. Alcohol consumption was reported by 8 patients (38.1%) and was evenly distributed between the HIV-positive and HIV-negative groups. Smoking habit was noted in 3 patients (14.3%), again without significant difference between the groups.

Regarding underlying illnesses, isolated cases of diabetes mellitus (n=1) and diabetes mellitus with hypertension (n=1) were found among HIV-negative individuals. Connective tissue disorders, including systemic lupus erythematosus (SLE), were also confined to the HIV-negative group (n=3). Notably, the majority of the cohort (76.2%) did not report any other significant comorbid illnesses, reinforcing the dominant role of immunosuppression, whether HIV-related or otherwise, in disease pathogenesis.

Intensity of infections increased over the three years, with the highest number of cases (n=7) recorded in July 2024. Cryptococcal infections demonstrated a seasonal trend from 2022 to 2024, peaking between May and August. Least number of infections was observed in 2022, and infections were consistently absent during January–March and October–December. All 21 patients

were from 15 districts in Uttar Pradesh. The highest number of cases (n=3) were observed in Bahraich, followed by Sitapur, Kanpur, Unnao and Deoria (2 each). Sixteen patients (76.2%) were from rural areas, and 5 (23.8%) resided in urban regions. No statistically significant differences were observed between the HIV-positive and HIV-negative groups regarding residential distribution (p=0.338).

Table 1. Demographic and clinical profiles of patients with cryptococcosis.

Characteristics		Total	HIV-positive	HIV-negative	Chi-square (p value)*
Age	0-20 years	5	2	3	6.402 (0.078)
	21-40 years	8	7	1	
	41-60 years	6	3	3	
	61-80 years	2	0	2	
	Age, Mean±SD (range)	21	34.916±10.933 (20-60)	37.667±21.030 (13-65)	0.727
Gender	Male	14	9	5	0.875 (0.397)
	Female	7	3	4	
Residence	Rural	16	8	8	1.400 (0.338)
	Urban	5	4	1	
Risk factors	Surgical intervention	2	0	2	2.947 (0.171)
	Alcohol intake	8	4	4	0.269 (0.673)
	Smoking habit	3	2	1	0.130 (1.000)
Underlying illness	Diabetes Mellitus (n)	1	1	0	3.532 (0.388)
	Diabetes Mellitus and Hypertension (n)	1	0	1	
	Connective tissue disorder (n)	3	0	3	
	None (n)	16	11	5	
ICU admission (n)		13	8	5	0.269 (0.673)
Lung involvement (n)		10	5	5	0.398 (0.670)
Treatment outcome	Mortality (n)	12	6	6	0.151 (1.000)
	Survival (n)	9	6	3	
Opportunistic infections	Tuberculosis (n)	10	5	5	0.992 (1.000)
	CMV infection (n)	1	1	0	
	None (n)	10	6	4	

ICU: intensive care unit, CMV: Cytomegalovirus; *Significant at $p < 0.05$

This study compared hematological and cerebrospinal fluid (CSF) parameters in HIV-positive and HIV-negative patients with cryptococcal meningitis as detailed in Table 2. Median hemoglobin and platelet counts were slightly lower in HIV-positive patients, though not statistically significant. Notably, HIV-negative patients had a significantly higher median total leukocyte count, compared to HIV-positive patients (16,300 vs. 4,650 cells/mm³; p=0.041). The CD4 counts, available for 10 HIV-positive individuals, had a median of 114 cells/µL. Time from symptom onset to hospital presentation was similar between groups (44 vs. 40 days; p=0.754). The CSF analysis showed a higher median leukocyte count in HIV-negative patients, compared to HIV-positive patients (70 vs. 10 cells/mm³); however, the difference was not significant (p=0.114).

Both groups had low CSF glucose levels, consistent with fungal meningitis. The CSF protein levels were elevated in both groups, with higher levels in HIV-negative patients; however, the difference was not statistically significant. Diagnostic testing showed India ink positivity in 60% of tested cases. The CALAS showed significantly higher positivity in HIV-positive patients (p=0.042). The CSF fungal cultures were positive in 62.5% of samples, with no significant difference between the groups (p=0.611).

Inhibitory activities of amphotericin B, fluconazole, itraconazole, and voriconazole against 16 clinical isolates of *C. neoformans* from initial cerebrospinal fluid cultures are presented in Table 3. For fluconazole, the MIC ranged from 1 to 16 µg/ml, while amphotericin B exhibited a MIC range of 0.25–2 µg/ml. All isolates were sensitive to itraconazole and voriconazole, with MIC ranges of 0.0625–0.125 µg/ml and 0.03–0.25 µg/ml, respectively. The MIC_{50} and MIC_{90} values of *C. neoformans* for amphotericin B, fluconazole, itraconazole, and voriconazole are summarized in Table 3.

A univariate analysis was performed on 17 cryptococcal isolates categorized according to their FLC minimum inhibitory concentrations (MICs), comparing clinical and demographic parameters between isolates with MICs <8 µg/mL (n=6) and those with MICs ≥8 µg/mL (n=11) [5], as shown in Table 4. Age and gender distributions showed no statistically significant association with FLC MIC values (p=0.464 and p=0.237, respectively). In terms of HIV status, 11 patients were HIV-positive, and six were HIV-negative. Distribution of MICs between these groups was not statistically significant (p=1.000), indicating no apparent association between HIV serostatus and FLC resistance. Mortality rates did not differ significantly between the two MIC groups (p=1.000), further emphasizing the lack of correlation between FLC MIC and patient outcome in this cohort.

All 21 patients in this study received induction therapy with amphotericin B (1 mg/kg daily) for two weeks, followed by maintenance therapy with FLC (400 mg daily) for eight weeks. In the present study, magnetic resonance imaging (MRI) findings were available for 18 patients. The MRI head showed leptomeningeal enhancement in six patients, followed by lacunar infarct in four patients, inflammatory granulomas (n=3), dilated Virchow-Robin spaces (n=2), cryptococcomas (n=2), and meningeal enhancement in one patient.

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Table 2. Initial laboratory investigations of the patients.

Variables	HIV-positive	HIV-negative	*p* value
Hemoglobin, mg/dl, median (range)	9.45 (7.2-14.2)	9 (6.4-12.5)	0.416
WBC count, cells/cu.mm., median (range)	4650 (1410-17100)	16,300 (3400-20300)	0.041*
Platelets, cells/cu.mm., median (range)	1.655 (1.00-2.80)	2 (1.00-3.60)	0.530
Time from symptom onset to hospital presentation, days, median (range)	44 (8-110)	40 (25-100)	0.754
CD4 count, n=10, median (range)	114 (28-212)	-	
CSF cell count, cells/cu.mm., median (range)	10 (4-180)	70 (5-180)	0.114
CSF glucose, mg/dl, median (range)	35.9 (4.1-106)	24.6 (18-55)	0.794
CSF protein, mg/dl, median (range)	97.2 (41-995)	136 (18.40-154)	0.808
CSF India ink positivity (n=15)	9	6	0.175 (1.000)
CSF CALAS positivity (n=18)	13	5	5.688 (0.042)*
CSF fungal culture positivity (n=16)	10	6	0.788 (0.611)

WBC: white blood cell, CSF: cerebrospinal fluid, CALAS: cryptococcal antigen latex agglutination system; *Significant at $p < 0.05$

Table 3. Minimum inhibitory concentration value, geometric mean, MIC_{50} and MIC_{90} of 17 Cryptococcal isolates.

No.	Drug	Geometric mean	MIC range (µg/ml)	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
1	Amphotericin B	1.18	0.25- 2	1	2
2	Itraconazole	0.073	0.0625- 0.125	0.06	0.125
3	Fluconazole	6.4	1- 16	8	8
4	Voriconazole	0.124	0.03- 0.25	0.125	0.25

Table 4. Univariate analysis of 17 Cryptococcal isolates with different fluconazole minimum inhibitory concentrations.

		Total (N=17)	FLC < 8 µg/ml (N=6)	FLC≥8 µg/ml (N=11)	Chi-square (*p* value)*
Age	0-20 years	4	2	2	2.925 (0.464)
	21-40 years	7	3	4	
	41-60 years	4	0	4	
	61-80 years	2	1	1	
Gender	Male	13	6	7	2.853 (0.237)
	Female	4	0	4	
HIV	Positive	11	4	7	0.016 (1.000)
	Negative	6	2	4	
Outcome	Expired	11	4	7	0.016 (1.000)
	Stable	6	2	4	

FLC: fluconazole

Discussion

The global burden of HIV-associated cryptococcal meningitis has declined in the ART era, likely due to expanded ART coverage and improved HIV program performance [6]. Despite this progress, a growing number of cases are now being reported in non-HIV immunocompromised hosts and even in immunocompetent individuals. This shift is likely attributable to advancements in medical care, including increased use of immunosuppressive therapies and higher rates of solid organ and hematopoietic stem cell transplantation [7]. In the current study, 9 out of 21 patients (42.8%) were HIV-positive, while the remaining 12 patients (57.2%) were HIV-negative, highlighting a changing epidemiological landscape.

In addition to *C. neoformans*, the *Cryptococcus gattii* species complex is increasingly recognized as an important cause of cryptococcosis in India. A recent global review highlighted that reports of the *C. gattii* complex are frequent in India and noted that India, particularly Delhi, accounts for some of the most frequent reports of *C. gattii* sensu stricto, largely from ecological sources [8]. The environmental niche of the *C. gattii* complex is closely linked to trees, especially decayed wood and tree hollows, and ecological studies from north-western India have confirmed its presence, supporting endemic circulation in North India [8].

Notably, molecularly characterized clinical CNS infections caused by the *C. gattii* complex have been documented in India, demonstrating that this pathogen is clinically relevant beyond environmental isolation [9]. Molecular epidemiology data from Indian clinical isolates show circulation of multiple genotypes/species within the *C. gattii* complex, including *C. gattii* sensu stricto (VGI) and *C. tetragattii* (VGIV), with North Indian tertiary centers, such as those in Delhi, contributing to these datasets [10]. Furthermore, case reports of cryptococcal meningitis caused by *C. gattii* from North India emphasize the relevance of this pathogen in the regional epidemiology of cryptococcal meningoencephalitis [11]. In line with previously published data, the present study found that patients with HIV-associated cryptococcal meningitis were generally younger than their non-HIV-infected counterparts. This age disparity reflects the natural history of HIV infection, which tends to affect a younger demographic, and highlights the continued susceptibility of this group to opportunistic fungal infections despite improved access to ART [12]. Consistent with earlier reports [13], a clear male predominance was observed in both HIV-positive and HIV-negative groups. This gender bias may be attributed to testosterone-enhanced fungal virulence and gender-based immune differences, such as more efficient macrophage-mediated fungal clearance in females. These findings suggest that hormonal and immunological factors may contribute to both

susceptibility and severity of cryptococcal disease, warranting further investigation into gender-specific host-pathogen interactions [14, 15].

This study highlighted the diverse array of predisposing factors for cryptococcal meningitis, particularly among HIV-negative individuals. Surgical interventions, especially neurosurgical procedures, were observed exclusively in the HIV-negative group, suggesting that iatrogenic disruption of the blood-brain barrier may serve as a portal for fungal invasion [16]. Chronic alcohol consumption, reported in 38.1% of patients and equally distributed across HIV-positive and HIV-negative groups, supports previous literature identifying alcohol consumption as an independent risk factor, likely due to its immunosuppressive effects on neutrophil function, T-cell activation, and mucosal immunity [17]. Similarly, tobacco use, noted in 14.3% of patients, although not statistically significant, has been implicated in other studies as a contributor to invasive fungal infections due to compromised pulmonary defenses and altered host-pathogen interactions [18]. Comorbidities, such as diabetes mellitus, hypertension, and SLE, were observed only among HIV-negative individuals, underscoring the importance of non-HIV-related immunosuppressive conditions, including those induced by corticosteroid use or immunomodulatory therapy, in predisposing to cryptococcal infection. It is noteworthy that 76.2% of patients had no documented comorbidities, highlighting the contribution of idiopathic or unrecognized immunosuppressive states to disease development. Collectively, these findings emphasize that cryptococcal meningitis should no longer be viewed solely as an HIV-associated infection and must be considered in a wider clinical context, particularly in patients with surgical histories, chronic illnesses, or immunosuppressive therapies [19]. Kajeekul et al. also reported a higher pulmonary involvement among HIV-negative patients (15.6% vs. 0.8%, $p < 0.01$), a pattern that was also noted in our cohort, though not statistically significant (5/9 HIV-negative vs. 5/12 HIV-positive; p=0.670). This supports prior observations that pulmonary cryptococcosis may be more clinically apparent in immunocompetent or immunocompromised hosts [20].

This study revealed a clear seasonal pattern in cryptococcal meningitis cases from 2022 to 2024, peaking between May and August, with the highest number of cases in July 2024. No cases were reported during cooler, drier months, suggesting that monsoon-related increases in humidity and rainfall may enhance environmental persistence and proliferation of *Cryptococcus* in moist niches, such as bird droppings and decaying organic matter. These findings align with those of Indian environmental studies reporting higher recovery of *Cryptococcus* from pigeon droppings during monsoon months, highlighting the role of humidity and rainfall in promoting persistence and proliferation in moist ecological niches [21].

Geographically, cases were widely distributed with no urban clustering. A notable 76.2% of patients resided in rural areas, consistent with previous Indian studies linking rural residence to delayed diagnosis, poor sanitation, and limited HIV awareness [22]. However, no statistically significant association was found between residence and HIV status (p=0.338), indicating cryptococcal meningitis is prevalent across both rural and urban populations.

Although referral bias and lack of environmental sampling limit direct conclusions, the observed distribution provides valuable insight into the regional epidemiology of cryptococcal meningitis in northern India. Laboratory findings of this study in cryptococcal meningitis patients align with existing literature, highlighting key differences between HIV-positive and HIV-negative individuals. Compared to HIV-positive patients, HIV-negative individuals exhibited a stronger inflammatory response, characterized by higher CSF leukocyte counts and protein levels, which is consistent with the findings reported by Kajeekul et al. [20]. In contrast, HIV-positive patients had lower peripheral and CSF WBC counts, and higher cryptococcal antigen positivity, reflecting higher fungal burden and reduced immune clearance. India ink positivity and CSF culture results followed similar trends across studies, being more frequently positive in HIV-positive patients due to greater fungal loads [19].

Antifungal susceptibility profile of *C. neoformans* isolates in the present study aligns with regional and international observations while highlighting concerning MIC shifts, particularly for fluconazole. Amphotericin B MICs ranged from 0.25 to 2 µg/mL (MIC_{50}: 1 µg/mL; MIC_{90}: 2 µg/mL), suggesting an upward MIC drift. This is consistent with Yang et al., who observed that among FLC non-susceptible isolates, most had amphotericin B MICs ≥1 µg/mL, suggesting the possible co-occurrence of reduced susceptibility phenotypes [23]. Compared with earlier Indian data (Gugnani et al.; amphotericin MIC: 0.03–0.5 µg/mL), isolates in the present study showed higher amphotericin MIC distributions, suggesting a regional/temporal shift warranting continued MIC surveillance [24].

Fluconazole MICs extended up to 16 µg/mL, with an MIC_{50}/MIC_{90} of 8/8 µg/Ml (at the ECV), indicating clustering around the non-wild-type threshold and a potential risk of suboptimal response with standard dosing. This mirrors international trends; Naicker et al. reported declining FLC susceptibility over a decade, with MIC_{50} and MIC_{90} rising to 4 and 8 µg/mL, respectively, by 2017, alongside an increasing proportion of isolates with MIC ≥ 16 µg/mL [25]. Similarly, Yang et al. reported substantial non-susceptibility (43.2% with MIC ≥8 µg/mL; 11.1% with MIC ≥16 µg/mL) [23].

In contrast, earlier Indian isolates reported by Gugnani et al. showed lower FLC MICs (2–4 µg/mL), suggesting a possible upward shift in our setting [24]. Environmental data further reinforce this concern; Taha et al. documented non-wild-type isolates with FLC MICs reaching 32–64 µg/mL, suggesting environmental reservoirs may contribute to reduced susceptibility [26].

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Collectively, these findings indicate that FLC susceptibility should not be presumed and support periodic MIC surveillance to guide consolidation/maintenance therapy.

In contrast, extended-spectrum azoles remained excellent *in vitro* activity in the present cohort, with voriconazole MICs ranging from 0.03 to 0.25 µg/mL and itraconazole MICs ranging from 0.0625 to 0.125 µg/mL. This is consistent with surveillance data; Naicker et al. reported that among *C. neoformans* isolates with FLC MIC ≥ 16 µg/mL, extended-spectrum azole Etests (voriconazole/itraconazole/posaconazole) were performed on 16/229 (7%) isolates, and only one isolate showed MICs > 32 µg/mL to all three agents [25]. Similarly, in a Chinese cohort, Yang et al. found voriconazole resistance to be uncommon (6 isolates), while in India, Lahiri et al. reported universal voriconazole susceptibility [23, 27]. Collectively, these findings support voriconazole and itraconazole as potential alternatives when FLC MICs are elevated, while recognizing practical constraints (availability, drug interactions, and cost).

Findings of the present univariate analysis closely align with those of O'Connor et al., who similarly reported no significant correlation between FLC MICs and clinical outcomes, including survival, in patients with AIDS-associated cryptococcal meningitis. Their large randomized controlled trial demonstrated that antifungal susceptibility testing did not predict patient response or mortality, supporting the observation in the present study that higher FLC MICs were not associated with differences in mortality or disease characteristics [28]. Likewise, the retrospective analysis conducted by Nasri et al. also revealed no statistically significant association between elevated FLC MICs and mortality, despite identification of a subset of patients with higher MIC values [29]. This is consistent with the conclusion reached in the present study that mortality was comparable between low and high MIC groups and that HIV status did not significantly influence MIC distribution.

Main limitations of this study include its small sample size and single-center design, which may introduce referral bias and limit generalizability. It lacked environmental sampling and molecular typing of *Cryptococcus* isolates, preventing deeper ecological and strain-level analysis.

Conclusion

This study highlighted the shifting clinical and antifungal susceptibility patterns of cryptococcal meningitis in northern India, showing that while HIV-positive individuals remain a key risk group, an increasing number of cases now occur in HIV-negative patients with non-HIV immunosuppressive conditions. Amphotericin B, itraconazole, and voriconazole retained strong *in vitro* activity, while FLC showed early signs of reduced susceptibility. Seasonal peaks during the monsoon suggest environmental influences. Overall, the findings emphasize the need for heightened clinical awareness, careful antifungal stewardship, and management strategies tailored to immune status and local susceptibility trends.

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Authors' contributions

K. K. contributed to the literature search, data acquisition, methodology, formal analysis, and writing of the original draft. P. G. contributed to the study design, formal analysis, and writing-review and editing. T. S. contributed to data acquisition and writing-review and editing. S. V. contributed to data acquisition and writing review and editing. Vij. K. contributed to the study design and writing-review and editing. Viv. K. contributed to the study design and writing-review and editing. G. B. contributed to the study of design, validation, and writing-review and editing.

Conflicts of interest

Authors declare that no competing interests exist.

Financial disclosure

Authors declare no financial interest concerning the content in this manuscript.

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