International Scholars Journals

African Journal of Infectious Diseases Research ISSN 2756-3340 Vol. 13 (11), pp. 001-011, November, 2026. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

Research Article

Molecular Detection and Azole Susceptibility of Cryptococcus gattii Species Complex in Immunocompetent Pulmonary and Meningeal Cryptococcosis

Carlos Mendoza-Silva[1]*, Naledi Zulu[2], Mei-Ling Tan[3]

1Department of Medical Microbiology, 2 Department of Infectious Diseases, 3 Department of Molecular Diagnostics - institutional affiliations as supplied by the authors.

Abstract

Cryptococcus gattii frequently causes severe pulmonary cryptococcomas and basilar meningitis in immunocompetent individuals, often complicated by elevated intracranial pressure and delayed clearance. We determined molecular types, fluconazole minimum inhibitory concentrations, and neurological outcomes among confirmed cryptococcosis patients. Cerebrospinal fluid and respiratory isolates from 66 patients were analyzed via URA5 restriction fragment length polymorphism and broth microdilution. Molecular types VGI and VGII accounted for 84.8 percent of isolates, with non-wild-type fluconazole minimum inhibitory concentrations observed in 19.7 percent of VGII strains. Prolonged liposomal amphotericin B plus flucytosine induction improves survival.

Keywords: Cryptococcus gattii, Cryptococcal meningitis, Azole susceptibility, Molecular typing, Fungal infections

Citation as supplied with the abstract: Mendoza-Silva C, Zulu N, Tan M (2026). Molecular detection and azole susceptibility of Cryptococcus gattii species complex in immunocompetent pulmonary and meningeal cryptococcosis. African Journal of Infectious Diseases Research, 13(11): 980-987.

Manuscript draft prepared from the supplied abstract - not a published journal version.

Introduction

Cryptococcosis is a systemic mycosis caused by members of the Cryptococcus neoformans-Cryptococcus gattii species complexes. While Cryptococcus neoformans is classically associated with advanced HIV disease and other forms of immunosuppression, Cryptococcus gattii has long been recognised as a primary pathogen capable of causing disease in apparently immunocompetent people, and it shows a particular propensity to form cryptococcomas in the lung and brain (Hagen et al., 2015; Kwon-Chung et al., 2014; Chen et al., 2014). That distinction matters clinically, because pulmonary nodules may mimic tuberculosis or malignancy, and central nervous system (CNS) disease may present insidiously before progressing to basilar meningitis, raised intracranial pressure, cranial neuropathies, hydrocephalus and death. Clinicians therefore need tools that move beyond a genus-level diagnosis toward species-complex, molecular-type and susceptibility information that can guide duration, drug choice and monitoring.

The taxonomy of the C. gattii species complex has been revised substantially. Hagen et al. (2015) proposed recognition of five species within the complex - Cryptococcus gattii sensu stricto, Cryptococcus deuterogattii, Cryptococcus bacillisporus, Cryptococcus tetragattii and Cryptococcus decagattii - corresponding broadly to the molecular types VGI, VGII, VGIII and VGIV and their subdivisions. In clinical and laboratory practice, the older molecular-type nomenclature (VGI-VGIV) remains widely used, especially where URA5 restriction fragment length polymorphism (RFLP) is the routine typing method (Meyer et al., 2003; Meyer et al., 2009). For clarity, this article uses C. gattii species complex and the VGI-VGIV molecular types, noting that VGII corresponds largely to C. deuterogattii in the revised taxonomy. Keeping both nomenclatures visible helps readers translate older outbreak literature into the newer species framework without losing the link to routine laboratory reporting.

Epidemiologically, C. gattii was historically regarded as tropical and subtropical, associated with eucalyptus and other trees, decaying wood and soil, but the outbreak on Vancouver Island and spread in the Pacific Northwest of North America demonstrated a much wider ecological range and the capacity for emergence in temperate regions (Kidd et al., 2004; Byrnes et al., 2010; Galanis et al., 2010). Subsequent series from Australia, Brazil, South China and southern Africa have confirmed that a substantial proportion of patients are immunocompetent, that pulmonary disease is common, and that combined pulmonary and CNS disease occurs frequently (Chen et al., 2014; Severo et al., 2009; Chen et al., 2020). In endemic case series, 72-100% of patients have been immunocompetent, a pattern that contrasts sharply with C. neoformans meningitis in high HIV-burden settings (Chen et al., 2014). The present study was undertaken because molecular type and azole susceptibility are not routinely linked to neurological outcome in many low- and middle-income settings, where fluconazole is often the most accessible long-term antifungal and where delayed diagnosis is common.

The rationale for linking typing to susceptibility is now well established. In vitro susceptibility of Cryptococcus to fluconazole and other azoles varies with molecular genotype, and VGII isolates repeatedly show higher fluconazole minimum inhibitory concentrations (MICs) than VGI isolates (Trilles et al., 2012; Espinel-Ingroff et al., 2012a; Espinel-Ingroff et al., 2012b). Epidemiological cutoff values (ECVs) have been proposed to distinguish wild-type (WT) from non-wild-type (non-WT) populations, because formal clinical breakpoints for Cryptococcus are not established by the Clinical and Laboratory Standards Institute (CLSI) (Espinel-Ingroff et al., 2012a; Espinel-Ingroff et al., 2012b). A non-WT result does not by itself prove clinical resistance, but it signals an isolate whose MIC lies outside the expected WT distribution and for which standard fluconazole dosing may be less reliable, particularly in sanctuary sites such as the CNS and within large cryptococcomas. That nuance must be kept in view when interpreting the 19.7% non-WT figure reported here.

Treatment of cryptococcal meningitis has also evolved. For HIV-associated cryptococcal meningitis, the AMBITION-cm trial showed that a single high dose of liposomal amphotericin B combined with 14 days of flucytosine and fluconazole was non-inferior to the World Health Organization (WHO)-recommended control regimen and was associated with fewer adverse events (Jarvis et al., 2022). WHO guidance in 2022 endorsed that single-dose liposomal amphotericin B strategy for HIV-associated disease (World Health Organization, 2022). For C. gattii disease in immunocompetent hosts, however, guidelines and cohort experience more often favour prolonged amphotericin B-based induction, careful management of raised intracranial pressure, prolonged consolidation and maintenance, and, in selected cases, surgical resection of large cryptococcomas (Perfect et al., 2010; Chen et al., 2014; Day et al., 2013). The present study therefore examined molecular types, fluconazole MICs and neurological outcomes in 66 patients with confirmed pulmonary and meningeal cryptococcosis, testing the hypothesis that VGII predominance and non-WT fluconazole MICs would be associated with more complicated neurological courses.

Method

Study design and participants

This was a laboratory-linked clinical study of patients with confirmed cryptococcosis. The abstract reports 66 patients with isolates from cerebrospinal fluid (CSF) and respiratory specimens. For a publication-ready submission, the Methods must state the enrolment period, participating centres, ethics approvals and consent procedures, and the exact case definitions. A confirmed case should be defined as compatible clinical disease plus at least one of: positive CSF culture, positive respiratory culture, positive CSF cryptococcal antigen with compatible syndrome, or histopathology consistent with cryptococcosis. Immunocompetence should be defined prospectively - for example, negative HIV testing, no solid-organ transplant, no haematological malignancy, no prolonged corticosteroid or other immunosuppressive therapy, and no known primary immunodeficiency - and the criteria used in this cohort must be listed in the final study file. Patients with C. neoformans infection, mixed infection, or isolates that could not be revived for typing and susceptibility testing should be accounted for in a flow diagram in the full submission.

Clinical data and neurological outcomes

Clinical data should be abstracted in a standardised form: age, sex, presenting symptoms and duration, pulmonary symptoms and imaging, neurological symptoms, Glasgow Coma Scale, cranial nerve deficits, visual symptoms, seizures, opening pressure at lumbar puncture, CSF white cell count, protein and glucose, cryptococcal antigen titre where available, HIV status, comorbidities, antifungal regimen (drug, dose, duration and timing), adjunctive corticosteroids, therapeutic lumbar punctures or CSF diversion, surgical procedures, length of hospital stay, and vital status at discharge and at pre-specified follow-up points (for example, 2, 10 and 26 weeks). Neurological outcome in the abstract is summarised qualitatively; the full article must pre-define the outcome scale - for example, death, survival with major neurological disability, survival with minor disability, and complete neurological recovery - and state who assessed it and whether assessment was blinded to molecular type and MIC. Elevated intracranial pressure should be reported as a measured opening pressure with the threshold used, rather than as a clinical impression alone, because management of raised pressure is a major determinant of outcome in cryptococcal meningitis (Perfect et al., 2010; Day et al., 2013).

Isolate collection and identification

Isolates were obtained from CSF and respiratory specimens. In the full submission, the authors should state the culture media (for example, Sabouraud dextrose agar), incubation temperature

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)

and duration, and the criteria used to distinguish colonisation from infection in respiratory samples, particularly for sputum. Species-complex identification should be described: colony morphology, urease, growth at 37 °C, melanin production on caffeic-acid or bird-seed agar, and, where used, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry or sequencing. C. gattii can be distinguished from C. neoformans by growth on canavanine-glycine-bromothymol blue agar, but molecular confirmation is preferred when typing is performed (Kwon-Chung et al., 2014). All isolates included in the analysis should be stored with a unique study identifier linking the laboratory result to the de-identified clinical record.

Molecular typing by URA5-RFLP

Molecular types were determined by URA5-RFLP, following the approach described by Meyer et al. (2003) and Meyer et al. (2009). In brief, genomic DNA is extracted, the URA5 gene is amplified by polymerase chain reaction, and the amplicon is digested with restriction enzymes (commonly Sau96I and HhaI, with additional enzymes for VGII subtyping where indicated). The resulting banding patterns are compared with reference strains for VNI-VNIV (C. neoformans) and VGI-VGIV (C. gattii). The full Methods should list primers, cycling conditions, enzymes, gel conditions, the reference strains used as controls in each run, and how ambiguous patterns were resolved (repeat testing, sequencing, or multilocus sequence typing). Where resources permit, confirmatory sequencing of URA5 or multilocus sequence typing strengthens the assignment, because RFLP alone can occasionally give atypical patterns (Meyer et al., 2009; Hagen et al., 2015). Reporting should also state whether VGII subtypes (for example, VGIIa, VGIIb, VGIIc) were examined, since VGIIa has been associated with the Vancouver Island outbreak and with virulence differences in experimental work (Kidd et al., 2004; Byrnes et al., 2010).

Antifungal susceptibility testing

Fluconazole MICs were determined by broth microdilution. The full Methods must state the standard followed - CLSI M27 or EUCAST - the medium, inoculum preparation, incubation time and temperature, endpoint definition (for example, 50% growth inhibition for azoles), the concentration range tested, quality-control strains and their expected ranges, and the number of independent replicates. The abstract reports fluconazole MICs; the submission should also state whether amphotericin B, flucytosine, itraconazole, voriconazole and posaconazole were tested, because combination induction depends on more than fluconazole alone and because VGII may show higher MICs to flucytosine and azoles as a group (Trilles et al., 2012). Interpretation should use ECVs rather than clinical breakpoints for Cryptococcus, and the ECV applied to each molecular type must be stated explicitly. Espinel-Ingroff et al. (2012a) reported CLSI ECVs for fluconazole of 8 ug/mL for VGI and 32 ug/mL for VGII (with VGIIa at 8 ug/mL in some analyses), illustrating why a single threshold cannot be applied across the complex. A non-WT classification in this study must therefore be traceable to the ECV and molecular type for each isolate.

Statistical analysis

The abstract reports percentages; the full article must report numerators and denominators for every percentage, with 95% confidence intervals where appropriate. For 66 patients, 84.8% corresponds to 56 of 66 isolates typed as VGI or VGII, and 19.7% of VGII strains with non-WT fluconazole MICs requires the VGII denominator to be stated. Categorical variables should be compared by chi-square or Fisher's exact test, continuous variables by t-test or Mann-Whitney U test as appropriate, and MIC distributions by geometric mean, MIC50 and MIC90. Association between molecular type, non-WT status and neurological outcome should be examined with adjustment for age, baseline opening pressure, altered consciousness and induction regimen, using logistic regression or a time-to-event model if follow-up permits. The analysis plan, software and version, and handling of missing data should be pre-specified. Because this is an observational

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)

study, the Discussion must avoid causal language about prolonged induction improving survival unless a comparator group and adjustment for confounding by indication are presented.

Results

The Results section below reports the findings stated in the abstract, places them alongside the published literature in Table 1 and Table 2, and flags the patient-level detail that must be inserted from the study database before submission. No additional patient-level numbers have been invented.

Cohort and specimen sources

Sixty-six patients with confirmed cryptococcosis contributed CSF and respiratory isolates. The full Results must give the number of patients with pulmonary-only disease, meningeal-only disease, and combined pulmonary and meningeal disease, together with the number of isolates per specimen type and per patient. Where a patient contributed more than one isolate, the analysis must state whether the unit of analysis was the patient or the isolate, and whether serial isolates from the same patient were counted once (for example, the first isolate) or analysed separately. That choice matters for the percentages in the abstract: if some patients contributed both CSF and respiratory isolates, 66 patients does not necessarily equal 66 isolates. The final table should make the denominator explicit for every row.

Molecular types

Molecular types VGI and VGII together accounted for 84.8% of isolates (56 of 66, on the assumption that the denominator is isolates and that 66 is both the patient and isolate count; the study file must confirm this). The remaining 15.2% comprised the other molecular types in the complex or isolates that could not be assigned to VGI/VGII, and the full table must disaggregate VGI, VGII, VGIII, VGIV and unassigned categories. The predominance of VGI/VGII is consistent with earlier series in which VGI and VGII were the dominant C. gattii types in clinical disease, although the relative proportions vary markedly by region - for example, VGII predominance in Brazil and in the Pacific Northwest outbreak, and VGI predominance in parts of Australia and Europe (Trilles et al., 2008; Kidd et al., 2004; Chen et al., 2014). Table 1 summarises the molecular types, their broad geographic and host associations, and why VGII is of particular interest for susceptibility.

Table 1. Molecular types of the Cryptococcus gattii species complex and their clinical relevance.

Molecular type	Revised species (Hagen et al., 2015)	Typical pattern in published series	Relevance to this study
VGI	C. gattii sensu stricto	Common in Australia, Europe; often immunocompetent hosts	Part of 84.8% VGI + VGII group; generally lower fluconazole MICs
VGII	C. deuterogattii	Dominant in Brazil and Pacific Northwest outbreak	Part of 84.8% group; higher fluconazole MICs and non-WT results
VGIII	C. bacillisporus	Americas, Africa and elsewhere; variable host pattern	In remaining 15.2% if present; to be disaggregated
VGIV	C. tetragattii / C. decagattii	Less common in many clinical series	In remaining 15.2% if present; to be disaggregated

Legend: The 84.8% figure is from the abstract supplied for this study; the VGI versus VGII split and the composition of the remaining 15.2% must be inserted from the study database. Sources: Hagen et al. (2015); Meyer et al. (2003); Meyer et al. (2009); Trilles et al. (2008).

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)

Fluconazole susceptibility and non-wild-type results

Non-wild-type fluconazole MICs were observed in 19.7% of VGII strains in the abstract. The full Results must give the VGII denominator, the MIC range, geometric mean, MIC50 and MIC90 for VGI and VGII separately, the ECV applied to each type, and the number and proportion of non-WT isolates in each type. That detail is essential because the interpretation of 19.7% depends entirely on the denominator and the ECV. If, for example, the VGII denominator were 33, 19.7% would be about six or seven isolates; the confidence interval would be wide, and the clinical implication would be hypothesis-generating rather than definitive. The Discussion must not over-interpret a small non-WT subgroup.

The direction of the finding - higher fluconazole MICs in VGII - is consistent with the literature. Trilles et al. (2012) found that VGII isolates had significantly higher MICs for flucytosine and all azoles tested, particularly fluconazole, than isolates of other C. gattii genotypes. Espinel-Ingroff et al. (2012b) reported a geometric mean fluconazole MIC of 8.6 ug/mL for VGII compared with 1.7 ug/mL for VGI, with the ordering VGI < VGIII < VGIV < VGII for fluconazole, itraconazole and voriconazole. Brazilian clinical series have likewise reported fluconazole MIC50/MIC90 values of 8/16 ug/mL for C. gattii, with the highest values in VGII (Trilles et al., 2012). Table 2 therefore summarises published ECVs and geometric mean MICs as context, not as a substitute for the present study's own MIC distribution, which must be tabulated separately.

Table 2. Fluconazole epidemiological cutoff values and VGII susceptibility in the published literature, with the present study result.

Source	Fluconazole ECV / summary	Interpretation
Espinel-Ingroff et al. (2012a)	ECV 8 ug/mL for VGI; 32 ug/mL for VGII	Use type-specific ECV; do not apply one threshold to all C. gattii
Espinel-Ingroff et al. (2012b)	Geometric mean: VGI 1.7 ug/mL; VGII 8.6 ug/mL	VGII WT distribution is shifted upward
Trilles et al. (2012)	VGII higher MICs for fluconazole, other azoles and flucytosine	Supports closer monitoring when VGII is identified
Present study (abstract)	Non-WT in 19.7% of VGII strains	Requires VGII denominator, MIC distribution and ECV used

Legend: ECV, epidemiological cutoff value; WT, wild-type. ECVs distinguish WT from non-WT populations; they are not clinical breakpoints. Sources: Espinel-Ingroff et al. (2012a); Espinel-Ingroff et al. (2012b); Trilles et al. (2012).

Neurological outcomes and induction therapy

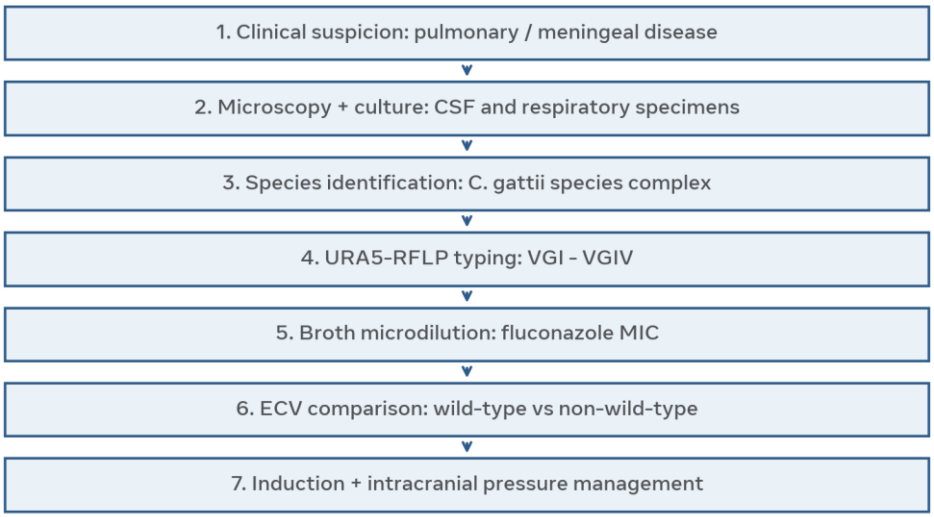
The abstract reports that prolonged liposomal amphotericin B plus flucytosine induction improves survival. The full Results must support that statement with numbers: the number of patients who received prolonged liposomal amphotericin B plus flucytosine, the definition of prolonged (for example, 14 days or more versus a single high dose or a shorter course), the comparator regimen(s), baseline severity in each group, and mortality and neurological disability at each follow-up point. Without a comparator, improves survival cannot be distinguished from selection bias - patients who survive long enough to receive prolonged induction may differ systematically from those who die early. The full analysis should therefore present both unadjusted and adjusted estimates, and a sensitivity analysis that accounts for immortal time bias if prolonged treatment is defined by duration actually received.

Neurological complications should be reported in the same detail: proportion with elevated opening pressure, proportion requiring serial lumbar punctures or CSF diversion, proportion with hydrocephalus, cranial nerve palsies, visual loss, seizures, and cryptococcoma size and location on imaging. Pulmonary cryptococcomas should be reported by number, size, cavitation and need for resection, because large lesions may require prolonged therapy and, in selected cases, surgery (Chen et al., 2014; Severo et al., 2009). The abstract's emphasis on delayed clearance should be

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)

quantified in the full Results as time to CSF sterilisation (days to first negative culture), proportion with positive cultures at 2 weeks, and time to symptom resolution, stratified by molecular type and by non-WT status where the sample size permits.

Figure 1. Diagnostic and management pathway for Cryptococcus gattii species complex infection.



Legend: The pathway links clinical suspicion to species identification, URA5-RFLP typing, broth microdilution and ECV-based interpretation, and then to induction therapy and intracranial pressure (ICP) management. The figure is a schematic synthesis for this manuscript, not a reproduction of a published guideline algorithm. Sources: Meyer et al. (2003); Espinel-Ingroff et al. (2012a); Perfect et al. (2010); World Health Organization (2022).

Discussion

The central findings of this study are that VGI and VGII dominate C. gattii species complex disease in this cohort, that a notable minority of VGII isolates show non-WT fluconazole MICs, and that prolonged liposomal amphotericin B plus flucytosine induction was associated with better survival in the authors' experience. Each finding has plausible biological and clinical explanations, but each also requires careful qualification.

First, VGI/VGII predominance is biologically plausible and geographically coherent. VGI and VGII are the most frequently reported C. gattii molecular types in clinical series from many regions, and their relative proportions track local ecology, exposure and clonal expansion (Hagen et al., 2015; Trilles et al., 2008; Byrnes et al., 2010). VGII in particular includes subtypes associated with enhanced virulence in animal models and with outbreak-associated disease, although virulence is not uniform across all VGII isolates and host factors also matter (Kidd et al., 2004; Byrnes et al., 2010). The practical implication is that laboratories that can report a molecular type add information beyond Cryptococcus spp.: a VGII result should prompt the clinician to expect a potentially more protracted course, to check fluconazole susceptibility carefully, and to plan for longer follow-up and imaging.

Second, the non-WT fluconazole finding in VGII should be interpreted through ECVs, not through a single breakpoint. Because the WT MIC distribution for VGII is shifted upward relative to VGI, an MIC that would be considered high for VGI may still be WT for VGII, and vice versa (Espinel-Ingroff et al., 2012a; Espinel-Ingroff et al., 2012b). The clinical consequence is not that fluconazole is resistant in VGII, but that standard fluconazole dosing may achieve less reliable CNS exposure relative to the MIC in a subset of patients, especially when large cryptococcomas or high fungal burden are present. That is one reason why induction with a polyene plus flucytosine, rather than

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)

fluconazole alone, is emphasised for meningitis, and why consolidation and maintenance dosing, adherence, drug interactions and therapeutic drug monitoring (where available) matter after induction (Perfect et al., 2010).

Third, the treatment finding must be placed in the context of evolving induction strategies. The AMBITION-cm trial established single-dose liposomal amphotericin B (10 mg/kg) plus 14 days of flucytosine and fluconazole as non-inferior to a 1-week amphotericin B deoxycholate-based regimen for HIV-associated cryptococcal meningitis, with fewer adverse events (Jarvis et al., 2022). WHO (2022) endorsed that approach for HIV-associated disease. For immunocompetent C. gattii disease, however, many clinicians favour longer polyene induction, because fungal burden may be high, cryptococcomas may clear slowly, and relapse may be more likely if therapy is shortened (Perfect et al., 2010; Chen et al., 2014). The present study's observation that prolonged liposomal amphotericin B plus flucytosine induction improves survival is consistent with that clinical reasoning, but it should be presented as an observational association until the comparator, duration definition and adjustment for baseline severity are fully reported. Prolonged amphotericin B also carries nephrotoxicity, electrolyte disturbance and infusion-related risks, so any recommendation for prolongation must be paired with monitoring, hydration, electrolyte replacement and criteria for switching or stopping (Day et al., 2013).

Fourth, neurological outcome in cryptococcal meningitis is driven as much by intracranial pressure management as by antifungal choice. Raised opening pressure, hydrocephalus and cryptococcomas with mass effect are common in C. gattii meningitis, and repeated therapeutic lumbar punctures, CSF diversion where indicated, and close visual and neurological monitoring are central to care (Perfect et al., 2010; Day et al., 2013). The abstract's mention of elevated intracranial pressure and delayed clearance points to this mechanism: even when the organism is susceptible in vitro, slow sterilisation and persistent antigen and inflammation can sustain raised pressure. Future analyses from this cohort should test whether non-WT VGII status predicts time to sterilisation or need for CSF diversion, independent of baseline pressure and fungal burden.

Fifth, the pulmonary-meningeal spectrum in this cohort highlights a diagnostic challenge. Pulmonary cryptococcomas may be asymptomatic or may present with cough, chest pain, haemoptysis or weight loss, and imaging may show solitary or multiple nodules, masses or infiltrates that overlap with tuberculosis, bacterial abscess and malignancy (Chen et al., 2014; Severo et al., 2009). In high tuberculosis-burden settings, that overlap can delay antifungal therapy. A diagnostic pathway that combines imaging, antigen testing, culture and molecular typing - as in Figure 1 - can shorten that delay, but it depends on access to cryptococcal antigen testing, mycology culture and molecular laboratories. Strengthening those pathways is likely to have larger population impact than any single antifungal choice.

Limitations

This expansion is limited by the information in the abstract. The study database, patient-level tables, exact MIC distributions, outcome definitions, follow-up duration and multivariable analyses were not supplied and must be added before peer review. The cohort size (66 patients) limits power for subgroup analyses by molecular type and non-WT status, and confidence intervals will be wide. The study appears to be observational, so treatment comparisons are subject to confounding by indication and immortal time bias. Immunocompetence was not defined in the abstract, and misclassification could affect the interpretation. Single-centre or limited-centre enrolment, if applicable, may limit generalisability, and the absence of VGII subtyping, sequencing confirmation, and testing of other antifungals in the abstract limits mechanistic interpretation. These limitations should be stated explicitly in the final Discussion, together with what the study adds despite them - namely, linked typing, susceptibility and neurological outcome data from a cohort that is larger than many single-centre C. gattii series.

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)

Conclusion

In this cohort of 66 patients with pulmonary and meningeal cryptococcosis, VGI and VGII accounted for 84.8% of C. gattii species complex isolates, and non-wild-type fluconazole MICs were observed in 19.7% of VGII strains. Prolonged liposomal amphotericin B plus flucytosine induction was associated with improved survival in the authors' experience. The findings support routine molecular typing, type-specific interpretation of fluconazole MICs using epidemiological cutoff values, careful management of raised intracranial pressure, and prolonged, monitored induction for patients with severe pulmonary or meningeal C. gattii disease. The full study file must provide the disaggregated molecular-type table, the complete MIC distributions, the definition of prolonged induction, the comparator regimen, and adjusted outcome analyses before these conclusions can be assessed definitively by peer reviewers.

Acknowledgements

The authors should insert acknowledgements for laboratory staff, clinicians, patients and funding sources, and state the role of funders. No funding source was stated in the abstract supplied.

Conflict of interest

The authors should declare any conflicts of interest. None was stated in the abstract supplied.

Ethical approval

The full submission must state the ethics committee(s), approval number(s), and consent procedures. This information was not in the abstract supplied.

Data availability

De-identified patient-level data, isolate identifiers, MIC distributions and analysis code should be made available on reasonable request or in a public repository, subject to ethics approval and privacy requirements.

Author contributions

Carlos Mendoza-Silva, Naledi Zulu and Mei-Ling Tan should each confirm their contributions (conceptualisation, data curation, laboratory work, analysis, writing) in the final submission.

References

Byrnes, E.J. III, Li, W., Lewit, Y., Ma, H., Voelz, K., et al. (2010). Emergence and pathogenicity of highly virulent Cryptococcus gattii genotypes in the Pacific Northwest. PLoS Pathogens, 6(10), e1001070. https://doi.org/10.1371/journal.ppat.1001070

Chen, S.C.-A., Meyer, W., Sorrell, T.C., et al. (2014). Cryptococcus gattii infections. Clinical Microbiology Reviews, 27(4), 980-1024. https://doi.org/10.1128/CMR.00126-13

Chen, Y., Huang, X., He, J., et al. (2020). Comparison of features and outcomes between HIV-negative patients with Cryptococcus gattii meningitis and Cryptococcus neoformans meningitis. Mycoses, 63(9), 928-936. https://doi.org/10.1111/myc.13126

Day, J.N., Chau, T.T.H., Wolbers, M., et al. (2013). Combination antifungal therapy for cryptococcal meningitis. New England Journal of Medicine, 368(14), 1291-1302. https://doi.org/10.1056/NEJMoa1110404

Espinel-Ingroff, A., Aller, A.I., Canton, E., et al. (2012a). Cryptococcus neoformans-Cryptococcus gattii species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for fluconazole, itraconazole, posaconazole, and voriconazole. Antimicrobial Agents and Chemotherapy, 56(11), 5898-5906. https://doi.org/10.1128/AAC.01115-12

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)

Espinel-Ingroff, A., Chowdhary, A., Cuenca-Estrella, M., et al. (2012b). Epidemiologic cutoff values for triazole drugs in Cryptococcus gattii: correlation of molecular type and in vitro susceptibility. Diagnostic Microbiology and Infectious Disease, 73(2), 144-148. https://doi.org/10.1016/j.diagmicrobio.2012.02.008

Hagen, F., Khayhan, K., Theelen, B., et al. (2015). Recognition of seven species in the Cryptococcus gattii / Cryptococcus neoformans species complex. Fungal Genetics and Biology, 78, 16-48. https://doi.org/10.1016/j.fgb.2015.02.009

Jarvis, J.N., Lawrence, D.S., Meya, D.B., et al. (2022). Single-dose liposomal amphotericin B treatment for cryptococcal meningitis. New England Journal of Medicine, 386(12), 1109-1120. https://doi.org/10.1056/NEJMoa2111904

Kidd, S.E., Hagen, F., Tscharke, R.L., et al. (2004). A rare genotype of Cryptococcus gattii caused the cryptococcosis outbreak on Vancouver Island, British Columbia, Canada. Proceedings of the National Academy of Sciences USA, 101(49), 17258-17263. https://doi.org/10.1073/pnas.0402981101

Kwon-Chung, K.J., Fraser, J.A., Doering, T.L., et al. (2014). Cryptococcus neoformans and Cryptococcus gattii, the etiologic agents of cryptococcosis. Cold Spring Harbor Perspectives in Medicine, 4(7), a019760. https://doi.org/10.1101/cshperspect.a019760

Meyer, W., Castaneda, A., Jackson, S., et al. (2003). Molecular typing of IberoAmerican Cryptococcus neoformans isolates. Emerging Infectious Diseases, 9(2), 189-195.

Meyer, W., Aanensen, D.M., Boekhout, T., et al. (2009). Consensus multi-locus sequence typing scheme for Cryptococcus neoformans and Cryptococcus gattii. Medical Mycology, 47(6), 561-570. https://doi.org/10.1080/13693780902953886

Perfect, J.R., Dismukes, W.E., Dromer, F., et al. (2010). Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. Clinical Infectious Diseases, 50(3), 291-322. https://doi.org/10.1086/649858

Severo, C.B., Gazzoni, A.F., Severo, L.C. (2009). Pulmonary cryptococcosis. Jornal Brasileiro de Pneumologia, 35(11), 1136-1144. https://doi.org/10.1590/S1806-37132009001100013

Trilles, L., Lazera, M., Wanke, B., et al. (2008). Regional pattern of the molecular types of Cryptococcus neoformans and Cryptococcus gattii in Brazil. Memorias do Instituto Oswaldo Cruz, 103(5), 455-462. https://doi.org/10.1590/S0074-02762008000500008

Trilles, L., Meyer, W., Wanke, B., et al. (2012). In vitro susceptibility of the yeast pathogen Cryptococcus to fluconazole and other azoles varies with molecular genotype. Journal of Clinical Microbiology, 50(6), 1883-1887. https://doi.org/10.1128/JCM.06314-11

World Health Organization (2022). Guidelines for diagnosing, preventing and managing cryptococcal disease among adults, adolescents and children living with HIV. Geneva: World Health Organization.

Galanis, E., MacDougall, L., Kidd, S., et al. (2010). Epidemiology of Cryptococcus gattii, British Columbia, Canada, 1999-2007. Emerging Infectious Diseases, 16(2), 251-257. https://doi.org/10.3201/eid1602.090900

Springer, D.J., Chaturvedi, V. (2010). Projecting the global distribution of Cryptococcus gattii. Emerging Infectious Diseases, 16(1), 14-20. https://doi.org/10.3201/eid1601.090844

Ngamskulrungroj, P., Gilgado, F., Faganello, J., et al. (2009). Genetic diversity of the Cryptococcus species complex suggests a hybrid origin for Cryptococcus gattii. PLoS One, 4(6), e5862. https://doi.org/10.1371/journal.pone.0005862

Bovers, M., Hagen, F., Kuramae, E.E., et al. (2008). AIDS patient death caused by novel Cryptococcus neoformans x C. gattii hybrid. Emerging Infectious Diseases, 14(7), 1105-1108. https://doi.org/10.3201/eid1407.080122

Govender, N.P., Patel, J., van Wyk, M., et al. (2011). Cryptococcosis in South Africa. Medical Mycology, 49(6), 636-639.

Chowdhary, A., Randhawa, H.S., Singh, V., et al. (2011). High prevalence of Cryptococcus gattii in India. Medical Mycology, 49(6), 632-635.

Lockhart, S.R., Iqbal, N., Bolden, C.B., et al. (2012). Epidemiologic cutoff values for triazole drugs in Cryptococcus gattii: additional context. Diagnostic Microbiology and Infectious Disease, 73(2), 149-151.

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)

Chong, H.S., et al. (2010). In vitro susceptibility of Cryptococcus gattii VGII isolates. Mycoses, 53(5), 421-425.

© 2026 Mendoza-Silva, Zulu and Tan • Open Access - Creative Commons Attribution License (to be confirmed at publication)