



# *In vitro* Antifungal Susceptibility Profiles of *Cryptococcus neoformans* var. *grubii* and *Cryptococcus gattii* Clinical Isolates in Guangxi, Southern China

## OPEN ACCESS

### Edited by:

Keke Huo,  
Fudan University, China

### Reviewed by:

Piriyaporn Chongtrakool,  
Mahidol University, Thailand  
Lujuan Gao,  
Fudan University, China

### \*Correspondence:

Wan-qing Liao  
liaowanqing@sohu.com  
Cun-wei Cao  
caocunwei@yeah.net

† These authors have contributed  
equally to this work and share first  
authorship

### Specialty section:

This article was submitted to  
Antimicrobials, Resistance  
and Chemotherapy,  
a section of the journal  
Frontiers in Microbiology

Received: 11 May 2021

Accepted: 05 July 2021

Published: 10 August 2021

### Citation:

Al-Odaini N, Li X-y, Li B-k,  
Chen X-c, Huang C-y, Lv C-y,  
Pan K-s, Zheng D-y, Zheng Y-q,  
Liao W-q and Cao C-w (2021) *In vitro*  
Antifungal Susceptibility Profiles of  
*Cryptococcus neoformans* var. *grubii*  
and *Cryptococcus gattii* Clinical  
Isolates in Guangxi, Southern China.  
*Front. Microbiol.* 12:708280.  
doi: 10.3389/fmicb.2021.708280

Najwa Al-Odaini<sup>1,2†</sup>, Xiu-ying Li<sup>1,2†</sup>, Bing-kun Li<sup>1,2</sup>, Xing-chun Chen<sup>3</sup>, Chun-yang Huang<sup>1,2</sup>,  
Chun-ying Lv<sup>1,2</sup>, Kai-su Pan<sup>1,2</sup>, Dong-yan Zheng<sup>1,2</sup>, Yan-qing Zheng<sup>2,4</sup>, Wan-qing Liao<sup>5\*</sup>  
and Cun-wei Cao<sup>1,2\*</sup>

<sup>1</sup> Department of Dermatology and Venerology, First Affiliated Hospital, Guangxi Medical University, Nanning, China, <sup>2</sup> Guangxi Health Commission Key Lab of Fungi and Mycosis Research and Prevention, Nanning, China, <sup>3</sup> The People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China, <sup>4</sup> Fourth People's Hospital of Nanning, Nanning, China, <sup>5</sup> Shanghai Key Laboratory of Medical Fungal Molecular Biology, Second Military Medical University, Shanghai, China

This study analyzed the *in vitro* drug sensitivity of *Cryptococcus* spp. from Guangxi, Southern China. One hundred three strains of *Cryptococcus* were recovered from 86 patients; 14 were HIV positive and 72 were HIV negative. Ninety-two strains were identified as *Cryptococcus neoformans* var. *grubii*, while 11 strains were identified as *Cryptococcus gattii* (5 *C. gattii sensu stricto* and 6 *Cryptococcus deuterogattii*). The recovered strains were tested against commonly used antifungal drugs (fluconazole, amphotericin B, 5-fluorocytosine, itraconazole, and voriconazole) and to novel antifungal drugs (posaconazole and isavuconazole) using CLSI M27-A4 method. The results showed that all isolates were susceptible to most antifungal drugs, of which the minimum inhibitory concentration (MIC) ranges were as follows: 0.05–4 μg/ml for fluconazole, 0.25–1 μg/ml for amphotericin B; 0.0625–2 μg/ml for 5-fluorocytosine, 0.0625–0.25 μg/ml for itraconazole, 0.0078–0.25 μg/ml for voriconazole, 0.0313–0.5 μg/ml for posaconazole, 0.0020–0.125 μg/ml for isavuconazole for *C. neoformans* var. *grubii* isolates, and 1–16 μg/ml for fluconazole, 0.125–1 μg/ml for 5-fluorocytosine, 0.25–1 μg/ml for amphotericin B, 0.0625–0.25 μg/ml for itraconazole, 0.0156–0.125 μg/ml for voriconazole, 0.0156–0.25 μg/ml for posaconazole, and 0.0078–0.125 μg/ml for isavuconazole for *C. gattii* isolates. Furthermore, some *C. neoformans* var. *grubii* isolates were found to be susceptible-dose dependent to 5-fluorocytosine and itraconazole. In addition, a reduction in the potency of fluconazole against *C. gattii* is possible. We observed no statistical differences in susceptibility of *C. neoformans*

var. *grubii* and *C. gattii* in the tested strains. Continuous observation of antifungal susceptibility of *Cryptococcus* isolates is recommended to monitor the emergence of resistant strains.

**Keywords:** *Cryptococcus gattii*, variant identification, *in vitro* drug sensitivity, *C. neoformans* var. *grubii*, Guangxi

## INTRODUCTION

Cryptococcosis is a common opportunistic invasive fungal infection caused mainly by *Cryptococcus neoformans* (*C. neoformans*, serotypes A, AD, and D) and *Cryptococcus gattii* (*C. gattii* serotypes B and C) (Guinea et al., 2010). The latter mainly affects otherwise healthy individuals, whereas *C. neoformans* is more common in immunocompromised patients (Park et al., 2009; Guinea et al., 2010; Maziarz and Perfect, 2016). Although the incidence of cryptococcosis has declined with the introduction of highly active antiretroviral therapy (HAART), immunocompromised individuals remain at risk, and mortality rate remain unacceptably high despite the continued research on cryptococcosis (Park et al., 2009; Selb et al., 2019). *C. neoformans* and *C. gattii* are genetically related to each other; however, they vary in the ecological niche geographic distribution, natural habitat, host infectivity, and pathogenicity (Gutch et al., 2015). For instance, *C. neoformans* has a global distribution, while *C. gattii* strains are more common in North America and Australia (Ellis and Pfeiffer, 1990). Moreover, *C. gattii* seems to be less sensitive to therapy and requires more aggressive management than *C. neoformans* (Speed and Dunt, 1995). China has the world's largest population with large humid tropical and subtropical regions rich with vegetation, a climate favorable for the growth and spread of fungi. In addition, the number of immunocompromised (HIV and non-HIV) populations in China has been increasing in the past several decades, leading to an increase in the incidence and prevalence of aggressive fungal infections such as cryptococcosis, which is a heavy burden to public health (Fang et al., 2015). Furthermore, cryptococcosis can be easily misdiagnosed due to the vague and diversity of the clinical manifestations, leading to the delay of proper treatment. Therefore, early diagnosis and timely treatment play an important role in the prognosis of the disease. Several studies have been performed to investigate the microbiological, epidemiological, and clinical characteristics of *C. neoformans* and *C. gattii* strains in China (Chen et al., 2008, 2018; Feng et al., 2008; Wu et al., 2015, 2021; Fang et al., 2020; Jin et al., 2020; Xu et al., 2021). Unfortunately, relatively little is known about the pathogenic *Cryptococcus* species and their sensitivity to antifungal chemotherapy in Guangxi, Southern China. Guangxi province is located in the subtropical zone with a warm, humid climate where cryptococcosis is significantly common but might be severely underreported. Recently, new antifungal agents have been introduced, suggesting that the

antifungal susceptibility profiles need to be researched and updated. Therefore, the current study aims to analyze the clinical characteristics of *Cryptococcus* infection and *in vitro* drug susceptibility of *Cryptococcus* spp. in Guangxi, Southern China.

## MATERIALS AND METHODS

### Ethics Statement

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. The clinical data in this study were obtained with written consent from the patients or their families, and data collected concerning them was anonymized.

### Isolates and Clinical Data

Between May 2014 and May 2018, clinical isolates of *Cryptococcus* spp. from patients admitted to the First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, the Fourth People's Hospital of Nanning, and People's Hospital of Guangxi Zhuang Autonomous Region were collected and recovered for this study. We assessed the patients' medical records to collect clinical information. Strains with insufficient clinical data of patients were excluded.

### Strains Identification

#### Activation and Identification

A total of 120 *Cryptococcus* strains were taken out of the refrigerator at  $-80^{\circ}\text{C}$ , resuscitated at  $20^{\circ}\text{C}$  for 24 h, transferred to Sabouraud dextrose agar (SDA) medium, and incubated at  $27^{\circ}\text{C}$  for 48–72 h, then transferred onto L-canavanine-glycine-bromothymol blue (CGB) medium for 3–7 days to differentiate *C. neoformans* from *C. gattii* species as previously mentioned (Klein et al., 2009). Three consecutive purifications were made using the streak plate technique to ensure the growth and purity of the strains. Multiple colonies were transferred to SDA medium for cultivation at  $27^{\circ}\text{C}$  and enrichment for later use.

#### Extraction of *Cryptococcus Neoformans* Protein

A sterile loop full of the purified isolated strains (about 5 mg) was added to an Eppendorf tube containing 300  $\mu\text{l}$  of distilled  $\text{H}_2\text{O}$  and was mixed thoroughly. Nine hundred microliters of absolute ethanol was added to the tube, mixed, and centrifuged at 12,000 r/min for 2 min. After discarding the supernatant, the tube was centrifuged again at 12,000 r/min for 2 min and later placed at room temperature to dry. Following the addition of 50  $\mu\text{l}$  of 70% formic acid and 50  $\mu\text{l}$  of acetonitrile, the tube was centrifuged for 2 min at 12,000 r/min. The supernatant was then transferred to a new tube for use.

**Abbreviations:** FLC, fluconazole; AmB, amphotericin B; ITC, itraconazole; 5-FC, 5-flucytosine; VOC, voriconazole; POS, posaconazole; ISA, isavuconazole; SDA, Sabouraud dextrose agar medium; CGB, L-canavanine-glycine-bromothymol blue; MALDI-TOF MS, matrix-assisted laser desorption ionization-time of flight mass spectrometry; ECVs, epidemiological cutoff values.

## Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Identification

The samples were overlaid with 1  $\mu$ l of matrix solution consisting of a saturated solution of  $\alpha$ -cyano-4-hydroxycinnamic acid in 50% acetonitrile–2.5% trifluoroacetic acid and again allowed to air dry prior to analysis. For each isolate, a spectrum with a mass-to-charge range of 2,200–22,000 Da was generated as an average of 240 laser shots in an automatic acquisition mode. When poor spectra (fewer than 10 well-defined peaks above 1,000 arbitrary units) were obtained, analysis was repeated with an extra wash step during the protein extraction procedure, which improved the quality of the spectra. The MALDI Biotyper 3.0 database was used to compare the data and record the mass spectrometry identification results. According to the instruction manual, a log score  $\geq 1.70$  indicates correct identification; a log score  $< 1.70$  indicates that the identification is incorrect or unable to be identified (McTaggart et al., 2011).

## DNA Extraction and ITS Sequencing

As a reference “gold standard,” 20 strains of *Cryptococcus* were randomly selected to extract DNA for ITS sequencing. Strains were cultured on SDA medium at 27°C for 72 h for DNA extraction according to the operation steps of the fungal genomic DNA extraction kit produced by Jiangsu Kangwei Century Biotechnology Co., Ltd (Changping, Beijing, China). According to the operating manual, the concentration of genomic DNA is determined using a nucleic acid protein analyzer and then placed in a refrigerator at  $-20^{\circ}\text{C}$  for later use. Ribosomal RNA (rRNA) internal transcribed spacer Region (rITS) sequencing is performed on the extracted DNA using primer sets (upstream primer sequence ITS1: 5'-TCCGTAGGTGAACCTGCGG-3') and (downstream primer sequence ITS4: 5'-TCCTCCGCTTATTGATATGC-3'). A total volume of 50  $\mu$ l (Taq-Mix, 25  $\mu$ l; upper material, 2  $\mu$ l; downstream primer, 2  $\mu$ l; at least 100 ng of DNA extract and sterile deionized water) was used to perform polymerase chain reaction (PCR) for 35 cycles at 95°C with 3 min initial denaturation, 30 s denaturation at 95°C, 30 s annealing at 55°C, 2 min extension at 72°C, and final extension cycle for 10 min at 72°C, and then stored at 4°C. A total of 6  $\mu$ l of PCR products was separated slowly into 1% agar gel spotting hole electrophoresis at 120 V for 30 min. The agar gel was placed under the UV gel imager to observe whether the PCR product and marker are clear and record the band length. PCR products are then sent with clear bands under electrophoresis to Guangzhou Kinco Biotechnology Co., Ltd. for two-way sequencing. Results were uploaded to the National Center for Biotechnology Information (NCBI) Genebank database<sup>1</sup> for comparison and identification.

## Antifungal Susceptibility Test

### Antifungal and Suspensions Preparation

The antifungal susceptibility testing was assessed by the checkerboard broth microdilution method performed according to the Clinical and Laboratory Standards Institute (CLSI) protocol M27-A4 (Clinical and Laboratory Standards Institute (CLSI), 2017). The minimum inhibitory concentration (MIC)

value was determined for fluconazole (FLC), amphotericin B (AmB), 5-fluorocytosine (5-FC), itraconazole (ITC), voriconazole (VOC), posaconazole (POS), and isavuconazole (ISA). Antifungal drugs were provided as powders with known potency from Sigma Chemical Co. (St. Louis, MO, United States). Stock solutions were prepared as follows: FLC and 5-FC were dissolved in sterile distilled water to a drug storage solution of 1,280  $\mu\text{g/ml}$ , while AmB, ITC, VOC, POS, and ISA were dissolved in dimethyl sulfoxide (DMSO) (Sigma Chemical Co., United States) into a stock solution of 1,600  $\mu\text{g/ml}$  and stored at  $-20^{\circ}\text{C}$  until needed. The final concentration ranges were 0.125–64  $\mu\text{g/ml}$  for FLC, 0.00156–8  $\mu\text{g/ml}$  for AmB and 5-FC, 0.0020–1 g/ml for ITC, VOC, POS, and ISA, respectively. The yeast inocula were adjusted to a concentration of  $1 \times 10^3$ – $5 \times 10^3$  cfu/ml in Roswell Park Memorial Institute (RPMI) 1640 medium as measured by a hemocytometer, and an aliquot of 0.1 ml was added to each well containing various concentrations of antifungal drugs. The 96-well plates were incubated at 37°C. The assays were read 72 h after inoculation. *Candida parapsilosis* ATCC 22019 was used as a quality control strain for susceptibility tests.

### MIC and MIC Interpretations

According to the CLSI M27-A4 protocol, the MIC of AmB was defined as the lowest concentration that produced complete growth inhibition, while the MIC for other antifungal agents were defined as the lowest concentrations at which there was 50% inhibition of growth ( $\geq 50\%$ ) compared with that of drug-free control (optical clear). The MIC<sub>50</sub> and MIC<sub>90</sub>, on the other hand, are the concentrations capable of inhibiting the growth of isolates by 50 and 90%, respectively (Favalessa et al., 2014). The interpretive MIC criteria for FLC were as follows: susceptible (S),  $\leq 8$   $\mu\text{g/ml}$ ; susceptible-dose dependent (SDD), 16–32  $\mu\text{g/ml}$ ; and resistance (R),  $\geq 64$   $\mu\text{g/ml}$ ; for 5-FC,  $\leq 4$   $\mu\text{g/ml}$  (S), 8–16  $\mu\text{g/ml}$  (SDD), and  $\geq 32$   $\mu\text{g/ml}$ ; for ITC,  $\leq 0.125$   $\mu\text{g/ml}$  (S), 0.25–0.5  $\mu\text{g/ml}$  (SDD), and  $\geq 1$   $\mu\text{g/ml}$ ; for VOR,  $\leq 1$   $\mu\text{g/ml}$  (S) based on previous studies (Pfaller et al., 2005; Dias et al., 2006; Bertout et al., 2013; Gutch et al., 2015). For *Cryptococcus*, interpretative criteria have not been defined for POS, ISA, and AmB; hence, data available for *Candida* spp. were used, as previously reported (Revankar et al., 1988; Rodríguez-Tudela et al., 1995; Pfaller et al., 1999). Based on the recommendation of previous studies, the ECVs for *C. neoformans* var. *grubii* were 8.0  $\mu\text{g/ml}$  for FLC, 1.0  $\mu\text{g/ml}$  for AmB, 4  $\mu\text{g/ml}$  for 5FC, 0.125  $\mu\text{g/ml}$  for ITC, 1.0  $\mu\text{g/ml}$  for VOR, 0.5  $\mu\text{g/ml}$  for POS, and 0.25  $\mu\text{g/ml}$  for ISA; and that for *C. gattii* were 8  $\mu\text{g/ml}$  for FLC, 4  $\mu\text{g/ml}$  for 5FC, 0.5  $\mu\text{g/ml}$  for AmB, ITC, VOR, and POS, and 0.25  $\mu\text{g/ml}$  for ISA (Espinel-Ingroff, 2012; Espinel-Ingroff et al., 2012, 2015; Lockhart et al., 2012). The susceptibility of each *Cryptococcus* spp. isolate was determined in triplicate at different times for optimal results.

### Statistical Analysis

Statistical analysis was performed using SPSS 17.0 (SPSS Inc., Chicago, IL, United States) where  $p < 0.05$  was considered statistically significant.

<sup>1</sup><http://www.ncbi.nlm.nih.gov/>

## RESULTS

### Isolates and Clinical Data

A total of 103 strains from 86 patients were included in this study (Table 1). Fifty-nine of them were male, and twenty-seven were female. The age distribution ranged from 21 to 84 years. Of the 86 patients, 14 were HIV positive and 72 were HIV negative. A total of 55 had no underlying diseases, 21 had liver diseases, 7 were diabetic, 4 had systemic lupus erythematosus (SLE), 1 had a history of immunosuppressing therapy, 3 had liver cancer, and 1 had rheumatoid arthritis. All 86 patients came from 13 different cities in Guangxi province, Southern China (Figure 1). Of the 103 strains, 17 were episode strains (i.e., strains recovered from different parts of the same patient simultaneously or from the same part at different times). In total, 91 were isolated from cerebrospinal fluid, 7 were from lung tissue, 3 were from alveolar lavage, and 2 from the skin.

### Strain Identification

Eleven strains successfully turned canavanine-glycine-bromothymol blue (CGB) medium to blue and were consequently identified as *C. gattii*. The remaining 92 strains were identified as *C. neoformans* var. *grubii* according to the MALDI-TOF MS technique, with mass spectrum scores  $\geq 1.7$ . The DNA results on the database comparison result were 100% consistent with the MALDI-TOF MS identification result, making the results of MALDI-TOF MS in our study credible. The identification of *C. gattii* strains and their genotypes were analyzed in detail in our previous study using multilocus sequence typing (MLST) technique, where five isolates were identified as *C. gattii sensu stricto* (*C. gattii* s.s.), and six were identified as *C. deuterogattii* serotype (Huang et al., 2020).

### Drug Susceptibility Results

The seven antifungal agents tested retained activity against all isolates. The MIC, MIC<sub>50</sub>, and MIC<sub>90</sub> values are presented in Table 2.

#### *C. neoformans* var. *grubii*

The MIC ranges of the 92 *C. neoformans* var. *grubii* strains for the 7 drugs were as follows: FLC, 0.05–4  $\mu\text{g/ml}$ ; AmB, 0.25–1  $\mu\text{g/ml}$ ; 5-FC, 0.0625–2  $\mu\text{g/ml}$ ; ITC, 0.0625–0.25  $\mu\text{g/ml}$ ; VOC, 0.0078–0.25  $\mu\text{g/ml}$ ; POS, 0.0313–0.5  $\mu\text{g/ml}$ ; and ISA, 0.0020–0.125  $\mu\text{g/ml}$ . All 92 clinical strains of *C. neoformans* var. *grubii* were susceptible to FLC, AmB, VOC, POS, and ISA; 91 strains (98.91%) were susceptible to 5-FC, while 1 strain (1.09%) was susceptible-dose dependent; 85.87% (79/92) of the strains were susceptible to ITC; and the remaining 14.13% (13/92) strains were susceptible-dose dependent to ITC.

#### *C. gattii*

Similar to *C. neoformans* var. *grubii*, none of the strains demonstrated resistance to the antifungal drugs (Table 2). However, we observed an MIC value above the ECVs to FLC against a single *C. deuterogattii* isolate.

**TABLE 1** | Demographic and clinical characteristics of the 86 patients with cryptococcosis.

Variables	<i>C. neoformans</i> var. <i>grubii</i> (n = 75)	<i>Cryptococcus gattii</i> (n = 11)
Age, year, average	46.58	38.09
Gender, male	51	8
HIV (+), n	14	–
<b>Underlying diseases</b>		
None, n	51	4
Diabetes mellitus, n	7	–
HBV, n	13	5
HCV, n	1	2
Liver diseases <sup>a</sup> , n	4	1
Liver cancer, n	3	–
Corticosteroids administration, n	1	–
Autoimmune diseases <sup>b</sup> , n	4	1
<b>Clinical diagnosis</b>		
Cryptococcal meningitis	47	10
Pulmonary cryptococcosis	8	1
Disseminated cryptococcosis	20	–
<b>Clinical symptoms</b>		
Neurological abnormalities <sup>c</sup> , n	31	1
Headache, n	61	9
Fever, n	51	9
Cough, n	13	2
Vomiting, n	39	6
Abnormal vision, n	2	2
Skin lesions, n	1	–
<b>Treatment outcome</b>		
Survived, n	47	10
Died, n	27	1
Unknown, n	1	–
<b>Specimen source</b>	<b>(n = 92)</b>	<b>(n = 11)</b>
CSF, n	81	10
Lung tissue, n	6	1
BALF, n	3	–
Skin tissue, n	2	–

n, number; HBV, hepatitis B virus; HCV, hepatitis C virus; CSF, cerebrospinal fluid; BALF, bronchoalveolar lavage fluid.

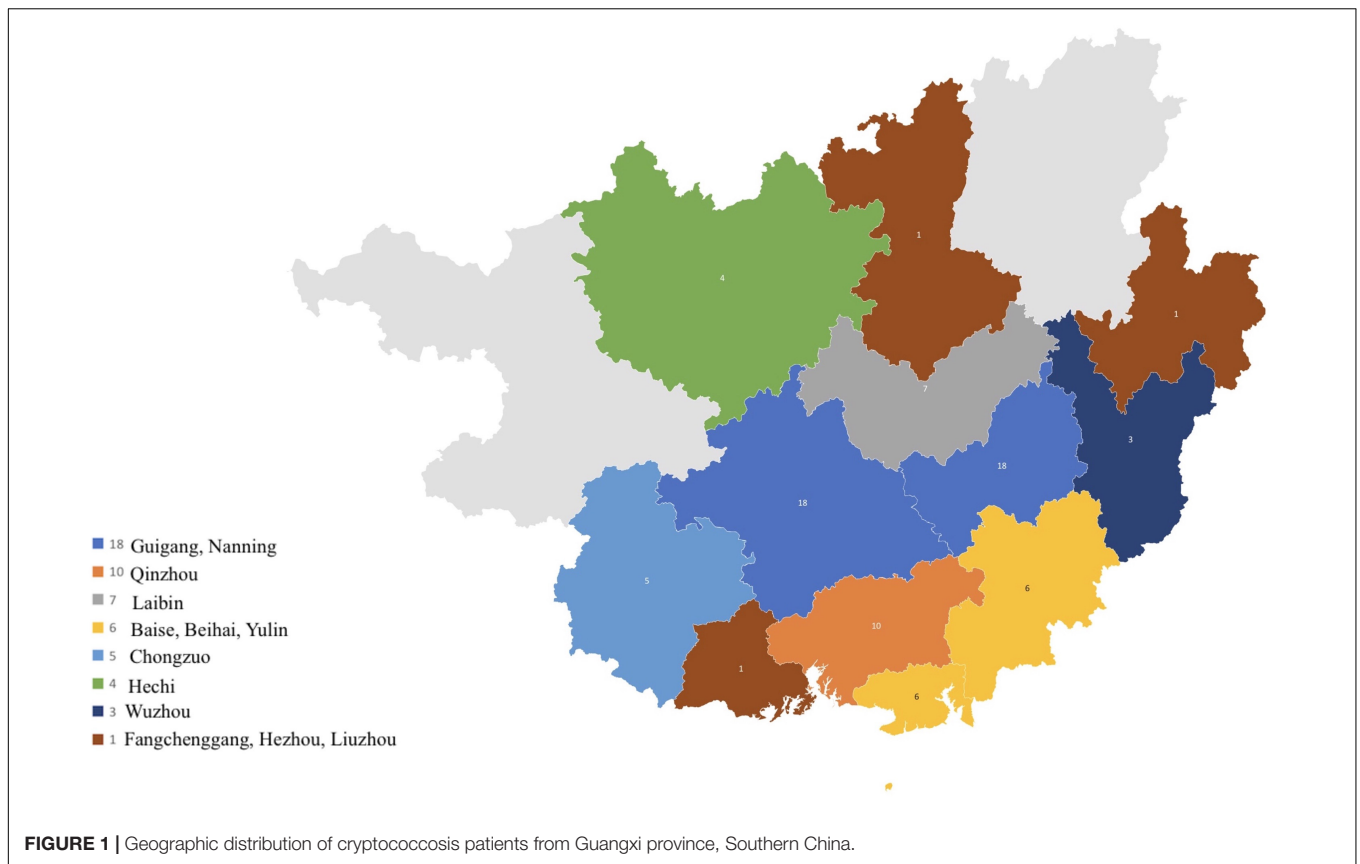
<sup>a</sup>Liver cirrhosis, fatty liver.

<sup>b</sup>Systemic lupus erythematosus, rheumatoid arthritis.

<sup>c</sup>Convulsion, altered consciousness, epilepsy.

## DISCUSSION

This study analyzed the epidemiology and *in vitro* antifungal susceptibility profiles of *Cryptococcus* spp. in a large-scale population from Guangxi, Southern China. Consistent with previous reports from China, our study showed that the prevalence of cryptococcosis caused by *C. neoformans* is higher than those caused by *C. gattii* (Chen et al., 2008; Fang et al., 2015; Wu et al., 2015). In addition, the disease was more observed among otherwise healthy individuals.



**FIGURE 1** | Geographic distribution of cryptococcosis patients from Guangxi province, Southern China.

**TABLE 2** | Antifungal susceptibilities for 130 *Cryptococcus* isolates.

Isolates and antifungal	MIC range	GM	MIC <sub>50</sub>	MIC <sub>90</sub>
	( $\mu\text{g/ml}$ )	( $\mu\text{g/ml}$ )	( $\mu\text{g/ml}$ )	( $\mu\text{g/ml}$ )
<b><i>C. neoformans</i> var. <i>grubii</i> (n = 92)</b>				
FLC	0.05–4	1.57	2	4
AmB	0.25–1	0.75	1	1
5-FC	0.0625–2	0.57	0.5	1
ITC	0.0625–0.25	0.08	0.0625	0.125
VOC	0.0078–0.25	0.03	0.0313	0.0625
POS	0.0313–0.5	0.13	0.125	0.25
ISA	0.0020–0.125	0.02	0.0156	0.0625
<b><i>Cryptococcus gattii</i> (n = 11)</b>				
FLC	1–16	2.57	2	4
AmB	0.25–1	0.47	0.5	0.5
5-FC	0.125–1	0.28	0.25	0.5
ITC	0.0625–0.25	0.18	0.25	0.25
VOC	0.0156–0.125	0.05	0.0625	0.125
POS	0.0156–0.25	0.07	0.0625	0.25
ISA	0.0078–0.125	0.04	0.0625	0.125

MIC<sub>50</sub> and MIC<sub>90</sub>, the concentration capable of inhibiting the growth of isolates by 50 and 90%, respectively. GM, geometric means.

Furthermore, our study demonstrated that *C. neoformans* was responsible for the infection in immunocompetent, which is contrary to the HIV-associated cryptococcosis cases reported

in the United States, Africa, and Europe (Dromer et al., 1996; Moosa and Coovadia, 1997; Hajjeh et al., 1999). Similar to reports from other parts of China, *C. neoformans* var. *grubii* was the dominant pathogenic strain in Guangxi (Chen et al., 2008).

In recent years, MALDI-TOF MS has emerged as a rapid, accurate, and cost-effective method for microbial identification and diagnosis (Singhal et al., 2015). Additionally, MALDI-TOF MS can replace some traditional identification methods, which improves clinical diagnosis and treatment. Furthermore, MALDI-TOF MS can help predict drug-resistant fungal isolates by identifying inflicting fungal species (McTaggart et al., 2011). Moreover, MALDI-TOF MS correctly identified 100% of *Cryptococcus* spp. in previous studies (Firacative et al., 2012; van Belkum et al., 2015; Cheng et al., 2016). Similarly, our study demonstrated that MALDI-TOF MS method is reliable, as we obtained a 100% consistency with results obtained using DNA sequencing.

The antifungal agents tested retained activity against all *Cryptococcus* isolates. Both *C. neoformans* var. *grubii* and *C. gattii* showed susceptibility to amphotericin B (MIC, 0.25–1  $\mu\text{g/ml}$ ). These results are consistent with previous reports (Thompson et al., 2009; Favalessa et al., 2014; Nascimento et al., 2017); however, greater MIC values are reported in the literature and were associated with treatment failure (Lozano-Chiu et al., 1998; Perkins et al., 2005).

Studies conducted previously have shown low MIC<sub>50</sub> and MIC<sub>90</sub> values for fluconazole against *C. neoformans* (Datta et al., 2003; Tay et al., 2006; Lia et al., 2012). In this study, the MIC<sub>50</sub> and MIC<sub>90</sub> values for fluconazole were 2–4 µg/ml; however, higher MIC<sub>50</sub> and MIC<sub>90</sub> values (4–8 and 2–128 µg/ml) have been reported for *C. neoformans* isolates (Sar et al., 2004; Favalessa et al., 2014).

High MIC values ( $\geq 64$  µg/ml) have been reported for fluconazole against *C. gattii* (Sar et al., 2004; Tay et al., 2006; Soares et al., 2008; Lia et al., 2012), in contrast to this study, where we determined MIC values of  $\leq 16$  µg/ml. It is worth mentioning that MIC  $\geq 16$  µg/ml is believed to be the resistance cutoff for fluconazole; this value was observed from a single *C. gattii* (*C. deuterogattii*) isolate in this study. This observation brings the resistance concerns to attention and suggests that FLC might lose its potency, especially if used as a single treatment.

The new azoles (voriconazole, posaconazole, and isavuconazole) showed high antifungal activity against all *C. neoformans* and *C. gattii* strains, with low MIC values of  $\leq 0.5$  µg/ml, which is consistent with previous reports (Pfaller et al., 2003, 2009; Sabatelli et al., 2006; Illnait-Zaragozi et al., 2008; Torres-Rodríguez et al., 2008). Itraconazole, on the other hand, showed high activity against all isolates with low MIC values of  $\leq 0.25$ , which is similar or lower than those of previous reports (Alves et al., 2001; Souza et al., 2010; Chowdhary et al., 2011; Herkert et al., 2018); however, 13 *C. neoformans* strains (14.13%) showed to be susceptible-dose dependent to itraconazole. Similarly, *C. neoformans* strains from Serbia showed to be susceptible-dose dependent to itraconazole (Arsic Arsenijevic et al., 2014). Nevertheless, itraconazole is rarely used as a single drug, especially in the treatment of cryptococcal meningitis, due to low concentration in the cerebrospinal fluid.

Studies conducted in several parts of the world have shown MIC values of  $\leq 64$  µg/ml for 5-FC against *C. neoformans* and *C. gattii* (Thompson et al., 2009; Chowdhary et al., 2011; Arsic Arsenijevic et al., 2014). In this study, all isolates were susceptible to 5-FC with MIC values  $\leq 2$  µg/ml; however, one *C. neoformans* strain (1.09%) was susceptible-dose dependent to 5-FC.

We observed no statistically significant difference between *C. neoformans* var. *grubii* and *C. gattii*, nor between *C. gattii* s.s. and *C. deuterogattii* isolates against all seven antifungal drugs ( $p > 0.05$ ). Overall, according to CLSI M27-A4, most strains in this study were of wild type, except for one that might acquire resistance to FLC. A follow-up study is required to confirm if it was of non-wild type.

## REFERENCES

- Alves, S. H., Oliveir, L., Costa, J. M., Lubeck, I., Casali, A. K., and Vainstein, M. H. (2001). In vitro susceptibility to antifungal agents of clinical and environmental *Cryptococcus neoformans* isolated in Southern of Brazil. *Rev. Inst. Med. Trop. Sao Paulo* 43, 267–270. doi: 10.1590/s0036-46652001000500006
- Arsic Arsenijevic, V., Pekmezovic, M. G., Meis, J. F., and Hagen, F. (2014). Molecular epidemiology and antifungal susceptibility of Serbian *Cryptococcus neoformans* isolates. *Mycoses* 57, 380–387.

In summary, cryptococcosis is a complicated disease that can be easily misdiagnosed due to the lack of specificity of the clinical manifestations. In China, healthy individuals are more prone to the infection. *C. neoformans* var. *grubii* is the most predominant strain in Guangxi. MALDI-TOF MS is a rapid and reliable method to identify *Cryptococcus* spp. All isolates showed no resistance to commonly used antifungal drugs and were highly susceptible to the new triazoles. Antifungal susceptibility tests are desirable to early detect any resistant strains in order to ensure proper and successful therapy of cryptococcosis.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

X-YL, NA-O, C-YL, and C-YH contributed to the data collection. X-YL, X-CC, B-KL, K-SP, Y-QZ, and D-YZ contributed to the laboratory work. NA-O and X-YL wrote the manuscript. W-QL and C-WC supervised and evaluated the process of the study. All authors contributed to manuscript revision and read and approved the submitted version.

## ACKNOWLEDGMENTS

This study was supported by the National Natural Science Foundation of China (81960567), grants from the Natural Science Foundation of Guangxi Province of China (2020GXNSFGA238001), and The First Affiliated Hospital of Guangxi Medical University Provincial and Ministerial Key Laboratory Cultivation Project: Guangxi Key Laboratory of Tropical Fungi and Mycosis Research (No. YYZS2020006). The funders had no role in study design, data, collection and analysis, decision to publish, or preparation of the manuscript.

- Bertout, S., Drakulovski, P., Kouanfack, C., Krasteva, D., Ngouana, T., and Dunyach-Rémy, C. (2013). Genotyping and antifungal susceptibility testing of *Cryptococcus neoformans* isolates from Cameroonian HIV-positive adult patients. *Clin. Microbiol. Infect.* 19, 763–769. doi: 10.1111/1469-0691.12019
- Chen, J., Varma, A., Diaz, M. R., Litvintseva, A. P., Wollenberg, K. K., and Kwon-Chung, K. J. (2008). *Cryptococcus neoformans* strains and infection in apparently immunocompetent patients, China. *Emerg. Infect. Dis.* 14, 755–762. doi: 10.3201/eid1405.071312

- Chen, Y., Yu, F., Bian, Z. Y., Hong, J. M., Zhang, N., Zhong, Q. S., et al. (2018). Multilocus sequence typing reveals both shared and unique genotypes of *Cryptococcus neoformans* in Jiangxi Province, China. *Sci. Rep.* 24:1495.
- Cheng, K., Chui, H., Domish, L., Hernandez, D., and Wang, G. (2016). Recent development of mass spectrometry and proteomics applications in identification and typing of bacteria. *Proteomics Clin. Appl.* 10, 346–357. doi: 10.1002/prca.201500086
- Chowdhary, A., Singh Randhawa, H., Sundar, G., Kathuria, S., Prakash, A., Khan, Z., et al. (2011). In vitro antifungal susceptibility profiles and genotypes of 308 clinical and environmental isolates of *Cryptococcus neoformans* var. grubii and *Cryptococcus gattii* serotype B from north-western India. *J. Med. Microbiol.* 60(Pt 7), 961–967. doi: 10.1099/jmm.0.029025-0
- Clinical and Laboratory Standards Institute (CLSI) (2017). *Reference Method for Broth. (Dilution)Antifungal Susceptibility Testing of Yeasts. Approved Standard*, 4th Edn. Wayne, PA: CLSI.
- Datta, K., Jain, N., Sethi, S., Rattan, A., Casadevall, A., and Banerjee, U. (2003). Fluconazole and itraconazole susceptibility of clinical isolates of *Cryptococcus neoformans* at a tertiary care centre in India: a need for care. *J. Antimicrob. Chemother.* 52, 683–686. doi: 10.1093/jac/dkg399
- Dias, A. L. T., Mastumoto, F., Melhem, M. S. C., da Silva, E. G., Auler, M. E., de Siqueira, A. M., et al. (2006). Comparative analysis of Etest and broth microdilution method (AFST-EUCAST) for trends in antifungal drug susceptibility testing of Brazilian *Cryptococcus neoformans* isolates. *J. Med. Microbiol.* 55, 1693–1699. doi: 10.1099/jmm.0.46789-0
- Dromer, F., Mathoulin, S., Dupont, B., and Laporte, A. (1996). Epidemiology of cryptococcosis in France: a 9-year survey (1985–1993). French Cryptococcosis study group. *Clin. Infect. Dis.* 23, 82–90. doi: 10.1093/clinids/23.1.82
- Ellis, D. H., and Pfeiffer, T. (1990). Natural habitat of *Cryptococcus neoformans* var. gattii. *J. Clin. Microbiol.* 28, 1642–1644. doi: 10.1128/jcm.28.7.1642-1644.1990
- Espinel-Ingroff, A. E. A. (2012). *Cryptococcus neoformans*-*Cryptococcus gattii* species complex: an international study of wild-type susceptibility endpoint distributions and epidemiological cutoff values for amphotericin B and flucytosine. *Antimicrob. Agents Chemother.* 56, 3107–3113. doi: 10.1128/aac.06252-11
- Espinel-Ingroff, A., Aller, A. I., Canton, E., Castañón-Olivares, L. R., Chowdhary, A., and Cordoba, S. (2012). *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. *Antimicrob. Agents Chemother.* 56, 5898–5906. doi: 10.1128/aac.01115-12
- Espinel-Ingroff, A., Chowdhary, A., Gonzalez, G. M., Guinea, J., Hagen, F., Meis, J. F., et al. (2015). Multicenter study of isavuconazole MIC distributions and epidemiological cutoff values for the *Cryptococcus neoformans*-*Cryptococcus gattii* species complex using the CLSI M27-A3 broth microdilution method. *Antimicrob. Agents Chemother.* 59, 666–668. doi: 10.1128/aac.04055-14
- Fang, L. F., Zhang, P., Wang, J., Yang, Q., and Qu, T. T. (2020). Clinical and microbiological characteristics of cryptococcosis at a university hospital in China from 2013 to 2017. *Braz. J. Infect. Dis.* 24, 7–12. doi: 10.1016/j.bjid.2019.11.004
- Fang, W., Fa, Z., and Liao, W. (2015). Epidemiology of *Cryptococcus* and cryptococcosis in China. *Fungal Genet. Biol.* 78, 7–15. doi: 10.1016/j.fgb.2014.10.017
- Favalessa, O. C., de Paula, D. A., Dutra, V., Nakazato, L., Tadano, T., and Lazera Mdos, S. (2014). Molecular typing and in vitro antifungal susceptibility of *Cryptococcus* spp from patients in Midwest Brazil. *J. Infect. Dev. Ctries* 8, 1037–1043. doi: 10.3855/jidc.4446
- Feng, X., Bo, L., and Daming, R. (2008). Analysis of variants, genotypes and mating types of 110 clinical strains of *Cryptococcus neoformans* in China. *Chin. J. Microbiol. Immunol.* 12, 193–197.
- Firacative, C., Trilles, L., and Meyer, W. (2012). MALDI-TOF MS enables the rapid identification of the major molecular types within the *Cryptococcus neoformans*/*C. gattii* species complex. *PLoS One* 7:e37566. doi: 10.1371/journal.pone.0037566
- Guinea, J., Hagen, F., Peláez, T., Boekhout, T., Tahoune, H., Torres-Narbona, M., et al. (2010). Antifungal susceptibility, serotyping, and genotyping of clinical *Cryptococcus neoformans* isolates collected during 18 years in a single institution in Madrid, Spain. *Med. Mycol.* 48, 942–948. doi: 10.3109/13693781003690067
- Gutch, R. S., Nawange, S. R., Singh, S. M., Yadu, R., Tiwari, A., Gumasta, R., et al. (2015). Antifungal susceptibility of clinical and environmental *Cryptococcus neoformans* and *Cryptococcus gattii* isolates in Jabalpur, a city of Madhya Pradesh in Central India. *Braz. J. Microbiol.* 46, 1125–1133. doi: 10.1590/s1517-838246420140564
- Hajjeh, R. A., Conn, L., Stephens, D. S., Baughman, W., Hamill, R., and Graviss, E. (1999). Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus-infected persons. cryptococcal active surveillance group. *J. Infect Dis.* 179, 449–454. doi: 10.1086/314606
- Herkert, P. F., Meis, J., Lucca, de Oliveira Salvador, G., Rodrigues Gomes, R., Aparecida Vicente, V., et al. (2018). Molecular characterization and antifungal susceptibility testing of *Cryptococcus neoformans* sensu stricto from southern Brazil. *J. Med. Microbiol.* 67, 560–569. doi: 10.1099/jmm.0.000698
- Huang, C., Tusi, C., Chen, M., Pan, K., Li, X., and Wang, L. (2020). Emerging *Cryptococcus gattii* species complex infections in Guangxi, southern China. *PLoS Negl. Trop. Dis.* 14:e0008493. doi: 10.1371/journal.pntd.0008493
- Illnait-Zaragozi, M. T., Martínez, G. F., Curfs-Breuker, I., Fernández, C. M., Boekhout, T., and Meis, J. F. (2008). In vitro activity of the new azole isavuconazole (BAL4815) compared with six other antifungal agents against 162 *Cryptococcus neoformans* isolates from Cuba. *Antimicrob. Agents Chemother.* 52, 1580–1582. doi: 10.1128/aac.01384-07
- Jin, L., Cao, J. R., Xue, X. Y., Wu, H., Wang, L. F., and Guo, L. (2020). Clinical and microbiological characteristics of *Cryptococcus gattii* isolated from 7 hospitals in China. *BMC Microbiol.* 20:73.
- Klein, K. R., Hall, L., Deml, S. M., Rysavy, J. M., Wohlfiel, S. L., and Wengenack, N. L. (2009). Identification of *Cryptococcus gattii* by use of L-canavanine glycine bromothymol blue medium and DNA sequencing. *J. Clin. Microbiol.* 47, 3669–3672. doi: 10.1128/jcm.01072-09
- Lia, M., Liao, Y., Chena, M., Pana, W., and Weng, L. (2012). Antifungal susceptibilities of *Cryptococcus* species complex isolates from AIDS and non-AIDS patients in Southeast China. *Braz. J. Infect. Dis.* 16, 175–179. doi: 10.1590/s1413-86702012000200012
- Lockhart, S. R., Bolden, C. B., DeBess, E. E., Marsden-Haug, N., Worhle, R., Thakur, R., et al. (2012). *Cryptococcus gattii* PNW Public health working group. epidemiologic cutoff values for triazole drugs in *Cryptococcus gattii*: correlation of molecular type and in vitro susceptibility. *Diagn Microbiol. Infect Dis.* 73, 144–148. doi: 10.1016/j.diagmicrobio.2012.02.018
- Lozano-Chiu, M., Paetznick, V., Ghannoum, M. A., and Rex, J. H. (1998). Detection of resistance to amphotericin B among *Cryptococcus neoformans* clinical isolates: performances of three different media assessed by using E-test and national committee for clinical laboratory standards M27-A methodologies. *J. Clin. Microbiol.* 36, 2817–2822. doi: 10.1128/jcm.36.10.2817-2822.1998
- Maziarz, E. K., and Perfect, J. (2016). Cryptococcosis. *Infect. Dis. Clin. North Am.* 30, 179–206.
- McTaggart, L. R., Lei, E., Richardson, S. E., Hoang, L., Fothergill, A., and Zhang, S. X. (2011). Rapid identification of *Cryptococcus neoformans* and *Cryptococcus gattii* by matrix-assisted laser desorption/ionization-time of flight mass spectrometry. *J. Clin. Microbiol.* 49, 3050–3053. doi: 10.1128/jcm.00651-11
- Moosa, M. Y. S., and Coovadia, Y. (1997). Cryptococcal meningitis in Durban, South Africa: a comparison of clinical features, laboratory findings, and outcome for human immunodeficiency virus (HIV)-positive and HIV-negative patients. *Clin. Infect. Dis.* 24, 131–134. doi: 10.1093/clinids/24.2.131
- Nascimento, E., Vitali, L., Kress, M. R. V. Z., and Martinez, R. (2017). *Cryptococcus neoformans* and *C. gattii* isolates from both HIV-infected and uninfected patients: antifungal susceptibility and outcome of cryptococcal disease. *Rev. Inst. Med. Trop. Sao Paulo* 59:e49.
- Park, B. J., Wannemuehler, K., Marston, B. J., Govender, N., Pappas, P. G., and Chiller, T. M. (2009). Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 23, 525–530. doi: 10.1097/qad.0b013e3283222fac
- Perkins, A., Gomez-Lopez, A., Mellado, E., Rodriguez-Tudela, J. L., and Cuenca-Estrella, M. J. (2005). Rates of antifungal resistance among Spanish clinical isolates of *Cryptococcus neoformans* var. *neoformans*. *J. Antimicrob. Chemother.* 56, 1144–1147. doi: 10.1093/jac/dki393
- Pfaller, M. A., Diekema, D., Gibbs, D. L., Newell, V. A., Bijie, H., and Dzierzanowska, D. (2009). Results from the ARTEMIS DISK Global antifungal

- surveillance study, 1997 to 2007: 10.5-year analysis of susceptibilities of noncandidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. *J. Clin. Microbiol.* 47, 117–123. doi: 10.1128/jcm.01747-08
- Pfaller, M. A., Messer, S., Boyken, L., Hollis, R. J., Rice, C., Tendolkar, S., et al. (2003). In vitro activities of voriconazole, posaconazole, and fluconazole against 4,169 clinical isolates of *Candida* spp. and *Cryptococcus neoformans* collected during 2001 and 2002 in the ARTEMIS global antifungal surveillance program. *Diagn. Microbiol. Infect. Dis.* 48, 201–205. doi: 10.1016/j.diagmicrobio.2003.09.008
- Pfaller, M. A., Messer, S., Boyken, L., Rice, C., Tendolkar, S., Hollis, R. J., et al. (2005). Global trends in the antifungal susceptibility of *Cryptococcus neoformans* (1990 to 2004). *J. Clin. Microbiol.* 43, 2163–2167. doi: 10.1128/jcm.43.5.2163-2167.2005
- Pfaller, M. A., Zhang, J., Messer, S. A., Brandt, M. E., Hajjeh, R. A., Jessup, C. J., et al. (1999). In vitro activities of voriconazole, fluconazole, and itraconazole against 566 clinical isolates of *Cryptococcus neoformans* from the United States and Africa. *Antimicrob. Agents Chemother.* 43, 169–171. doi: 10.1128/aac.43.1.169
- Revankar, S. G., Kirkpatrick, W. R., McAtee, R. K., Fothergill, A. W., Redding, S. W., and Rinaldi, M. G. (1988). Interpretation of trailing endpoints in antifungal susceptibility testing by the national committee for clinical laboratory standards method. *J. Clin. Microbiol.* 36, 153–156. doi: 10.1128/jcm.36.1.153-156.1998
- Rodríguez-Tudela, J. L., Martínez-Suárez, J. V., Dronza, F., Laguna, F., Chaves, F., and Valencia, E. (1995). Correlation of in-vitro susceptibility test results with clinical response: a study of azole therapy in AIDS patients. *J. Antimicrob. Chemother.* 35, 793–804. doi: 10.1093/jac/35.6.793
- Sabatelli, F., Patel, R., Mann, P. A., Mendrick, C. A., Norris, C. C., Hare, R., et al. (2006). In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. *Antimicrob. Agents Chemother.* 50, 2009–2015. doi: 10.1128/aac.00163-06
- Sar, B., Monchy, D., Vann, M., Keo, C., Sarthou, J. L., and Buisson, Y. (2004). Increasing in vitro resistance to fluconazole in *Cryptococcus neoformans* Cambodian isolates: April 2000 to March 2002. *J. Antimicrob. Chemother.* 54, 563–565. doi: 10.1093/jac/dkh361
- Selb, R., Fuchs, V., Graf, B., Hamprecht, A., Hogardt, M., Sedlacek, L., et al. (2019). Molecular typing and in vitro resistance of *Cryptococcus neoformans* clinical isolates obtained in Germany between 2011 and 2017. *Int. J. Med. Microbiol.* 309:151336. doi: 10.1016/j.ijmm.2019.151336
- Singhal, N., Kumar, M., Kanaujia, P. K., and Virdi, J. S. (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front. Microbiol.* 6:791.
- Soares, B. M., Santos, D. A., Kohler, L. M., da Costa César, G., de Carvalho, I. R., dos Anjos Martins, M., et al. (2008). Cerebral infection caused by *Cryptococcus gattii*: a case report and antifungal susceptibility testing. *Rev. Iberoam. Micol.* 25, 242–245.
- Souza, L. K., Santos, J. A., Costa, C. R., Faganello, J., Vainstein, M. H., Chagas, A. L., et al. (2010). Molecular typing and antifungal susceptibility of clinical and environmental *Cryptococcus neoformans* species complex isolates in Goiania. Brazil. *Mycoses* 53, 62–67. doi: 10.1111/j.1439-0507.2008.01662.x
- Speed, B., and Dunt, D. (1995). Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clin. Infect. Dis.* 21, 28–34. doi: 10.1093/clinids/21.1.28
- Tay, S. T., Tanty Haryanty, T., Ng, K. P., Rohani, M. Y., and Hamimah, H. (2006). In vitro susceptibilities of Malaysian clinical isolates of *Cryptococcus neoformans* var. *grubii* and *Cryptococcus gattii* to five antifungal drugs. *Mycoses* 49, 324–330. doi: 10.1111/j.1439-0507.2006.01242.x
- Thompson, G. R., Wiederhold, N. P., Fothergill, A. W., Vallor, A. C., Wickes, B. L., and Patterson, T. F. (2009). Antifungal susceptibilities among different serotypes of *Cryptococcus gattii* and *Cryptococcus neoformans*. *Antimicrob. Agents Chemother.* 53, 309–311. doi: 10.1128/aac.01216-08
- Torres-Rodríguez, J. M., Alvarado-Ramírez, E., Murciano, F., and Sellart, M. (2008). MICs and minimum fungicidal concentrations of posaconazole, voriconazole and fluconazole for *Cryptococcus neoformans* and *Cryptococcus gattii*. *J. Antimicrob. Chemother.* 62, 205–206. doi: 10.1093/jac/dkn132
- van Belkum, A., Chatellier, S., Girard, V., Pincus, D., Deol, P., and Dunne, W. M. (2015). Progress in proteomics for clinical microbiology: MALDI-TOF MS for microbial species identification and more. *Expert Rev. Proteomics* 12, 595–605. doi: 10.1586/14789450.2015.1091731
- Wu, S. Y., Lei, Y., Kang, M., Xiao, Y. L., and Chen, Z. X. (2015). Molecular characterisation of clinical *Cryptococcus neoformans* and *Cryptococcus gattii* isolates from Sichuan province. China. *Mycoses* 58, 280–287.
- Wu, S., Kang, M., Liu, Y., Chen, Z. X., Xiao, Y. L., and He, C. (2021). Molecular epidemiology and antifungal susceptibilities of *Cryptococcus* species isolates from HIV and non-HIV patients in Southwest China. *Eur. J. Clin. Microbiol. Infect. Dis.* 40, 287–295. doi: 10.1007/s10096-020-04013-4
- Xu, X., Du, P., Wang, H., Yang, X., Liu, T., and Zhang, Y. (2021). Clinical characteristics, *Cryptococcus neoformans* genotypes, antifungal susceptibility, and outcomes in human immunodeficiency virus-positive patients in Beijing, China. *J. Int. Med. Res.* 49:3000605211016197.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Al-Odaini, Li, Li, Chen, Huang, Lv, Pan, Zheng, Zheng, Liao and Cao. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.