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Clinical and microbiological characteristics of cryptococcosis at an university hospital in China from 2013 to 2017



Lan-fang Fang^{a,1}, Piao-piao Zhang^{a,1}, Jie Wang^b, Qing Yang^a, Ting-ting Qu^{a,*}

^a Zhejiang University School of Medicine, The First Affiliated Hospital, State Key Laboratory for Diagnosis and Treatment of Infectious Disease, Hangzhou, Zhejiang, China

^b Zhejiang University School of Medicine, The First Affiliated Hospital, Respiratory Department, Hangzhou, Zhejiang, China

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ABSTRACT

Background: This study aims to explore the epidemiology, clinical profile and strain characteristics of cryptococcosis from 2013 to 2017 in a major teaching hospital in China.

Methods: Trends in antifungal drug susceptibility of 217 consecutive non-repetitive cryptococcal isolates collected from patients of an university hospital in China were analyzed between 2013 and 2017. Of those, 98 isolates were conserved for identification by internal transcribed spacer (ITS) sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) system. Multilocus sequence typing (MLST) was used to designate molecular types. Clinical characteristics of the 98 patients with cryptococcosis during the period of 2013–2017 were retrospectively evaluated.

Results: There was a trend for gradual increase in the MIC range of fluconazole was from 2013 to 2017. The conserved 98 clinical cryptococcal isolates included 97 *C. neoformans* and one *C. gattii*, and 90 (91.8%) isolates belonged to ST5 genotype VNI. Out of the 98 patients with cryptococcosis, 28 (28.6%) were HIV-infected and 32 (32.7%) had no underlying diseases. HIV-infected patients had higher mortality than HIV-uninfected patients (28.6% vs 14.3%, $p = 0.147$).

Conclusions: Most of the patients with cryptococcosis were not HIV-infected in this study, while patients with HIV had a higher mortality. Reduced susceptibility to fluconazole was observed among *C. neoformans* isolates, most of them belonged to ST5 genotype VNI having an impact on the effective dose of fluconazole.

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* Corresponding author at: 79# Qingchun East Road, Hangzhou 310001, China.

E-mail address: qutingting@zju.edu.cn (T. Qu).

¹ Lan-fang Fang and Piao-piao Zhang contributed equally to this work.

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Introduction

Cryptococcosis is a significant invasive fungal infection with noteworthy morbidity and mortality, primarily caused by *Cryptococcus neoformans* (*C. neoformans*) and *Cryptococcus gattii*.^{1,2} Although cryptococcosis may occur in healthy hosts,³ the majority of infections occur in patients with significant underlying predisposing factors, such as organ transplantation, hematologic malignancies, and advanced human immunodeficiency virus (HIV) disease.^{2,4-8} In Europe and the in United States about 80% of the cryptococcosis cases were associated with HIV/AIDS.^{2,9} Nonetheless, in China, the majority of patients with cryptococcosis are not infected with HIV.^{10,11} This infection has been increasing steadily over the past years in China.

In recent years, there is a growing concern among clinicians regarding the potential for the emergence of antifungal resistance among *C. neoformans* because of high rates of fungal persistence and frequent disease relapse.¹²⁻¹⁴ Several reports on the emergence of resistance to amphotericin B, fluconazole, flucytosine, or itraconazole in *C. neoformans* during treatment has been published.^{15,16} Consequently, widespread increase in fluconazole resistance among *C. neoformans* became a public health concern calling for more-widespread surveillance. However, studies on the trends in antifungal drug susceptibility of *Cryptococcus* are limited in China. In 2016, Fan et al. reported a proportional increase of cryptococcal isolates with non-wild-type MICs to fluconazole (non-WT, MIC > 8 µg/ml) in China from 2009 to 2014,¹⁵ but clinical characteristics of these cryptococcal isolates were not collected. Now, the trend in antifungal drug susceptibility of clinical *Cryptococcus* strains in China is still uncertain, and further research on the correlation of the antifungal drug susceptibility trend and clinical outcomes is necessary.

In this study, we analyzed the trends in antifungal drug susceptibility of 217 consecutive cryptococcal isolates from a medical center in China between 2013 and 2017, of which 98 isolates were conserved for molecular evaluation. The present study also assessed the clinical manifestations of the 98 patients during the study period.

Material and methods

Cryptococcal isolates

This was a cross-sectional retrospective study in a 2500-bed teaching hospital in east China. A total of 217 non-repetitive *Cryptococcus* clinical strains were consecutively isolated from *Cryptococcus* culture-proven patients from 2013 to 2017 in this hospital. Antifungal drug susceptibility was detected for all 217 clinical strains.

Out of these 217 strains, 98 isolates were randomly selected and stored in 25% glycerol at -80°C until use and were maintained on yeast peptone dextrose (1% yeast extract, 2% peptone, 2% glucose) agar at 25°C for species identification and MLST during this study.

Antifungal susceptibility studies

The antifungal drug susceptibility of the total 217 *Cryptococcus* strains was detected by ATB FUNGUS 3 kit (BioMérieux France). *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as quality control organisms. The interpretive criteria for susceptibility to fluconazole and flucytosine were according to the manufacturer's instructions of ATB FUNGUS 3 kit. For fluconazole: susceptible, 4 µg/ml; susceptible dose dependent, 8 µg/ml; resistant, 16 µg/ml; and for flucytosine, susceptible, 4 µg/ml; intermediate, 8-16 µg/ml; resistant, 32 µg/ml. As there are currently no standard clinical breakpoints for *Cryptococcus spp.*, we used the epidemiological cut-off values as recommended by previous studies: voriconazole, 0.125 µg/ml; itraconazole, 0.25 µg/ml; and amphotericin B, 1 µg/ml.^{15,17,18}

DNA extraction

Among the 98 conserved clinical cryptococcal strains genomic DNA was extracted using a commercial kit (Sangon Biotech Co., Ltd, Shanghai, China).

Identification of *Cryptococcus* species and variety

Amplification of the rDNA internal transcribed spacer (ITS) region was performed as previously described.^{15,19} The PCR products were sequenced in both directions using the DNA analyzer ABI 3730XL system (Applied Biosystems, Foster City, CA). The obtained ITS sequences of *Cryptococcus* isolates were compared against those contained in the Centraalbureau voor Schimmelcultures (CBS) Fungal Biodiversity Centre database by using BIOLOMICSNET software (<http://www.cbs.knaw.nl/collections/BioLoMICSSequences.aspx>).

Bruker Biotyper MALDI-TOF MS (Bruker, Daltonik, Bremen, Germany) was also used to identify the conserved 98 *Cryptococcus* clinical strains. According to the manufacturer's recommendations and as Buchan et al. described, 16 identification by plate-grown isolates (on-plate protein extraction method) was applied. The procedure was performed as previously reported.¹⁵

Multilocus sequence typing (MLST) and mating type analysis

For MLST analysis, DNA fragments of seven unlinked genes that included IGS1, GPD1, CAP59, URA5, PLB1, LAC1, and SOD1 were amplified by PCR from the 98 preserved *Cryptococcus* clinical strains. Alleles and sequence types (STs) were assigned by using the fungal MLST database (<http://mlst.mycologylab.org/>). Molecular types (i.e., VNI to VNIV for *C. neoformans* and VGI to VGIV for *C. gattii*) were assigned according to isolates' STs and were queried against the online MLST database (<http://mlst.mycologylab.org/>).

Patient selection and data collection

Clinical characteristics of the 98 cryptococcosis patients with positive *Cryptococcus* culture were retrospectively evaluated.

Table 1 – In vitro susceptibility to five antifungal agents of 217 cryptococcal isolates from an university hospital during a 5-year period (2013–2017).

Antifungal agents	MIC range ($\mu\text{g/ml}$)	MIC ₅₀	MIC ₉₀
Amphotericin B	0.5–8	0.5	0.5
Fluconazole	1–16	2	4
Flucytosine	0.5–32	4	4
Itraconazole	0.06–1	0.125	0.25
Voriconazole	0.05–4	0.064	0.25

Demographic and clinical data including predisposing factors, infection types and outcome, were abstracted from the electronic record.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS, Version 22.0, Chicago, IL, USA) was used for the analysis. Two groups (HIV and non-HIV patients) were specified and investigated. Descriptive data are reported as either mean \pm SD, median (interquartile range) or number and percentage. Difference between continuous variables was compared by Student's *t*-test and non-parametrical tests. Categorical variables were compared by chi-square analysis. A $p < 0.05$ was considered statistically significant.

Ethics and consent statements

This was a retrospective study. All isolates present in this study had been stored in the Department of Microbiology,

the First Affiliated Hospital, Zhejiang University School of Medicine, China. The Institutional Review Board of the First Affiliated Hospital, College of Medicine, Zhejiang University waived the need for informed consent and granted permission to use the strains to perform all experiments in this study.

Results

Microbiologic characterization of the cryptococcal isolates

Trends in antifungal drug susceptibility of 217 cryptococcal isolates from 2013 to 2017

Table 1 summarizes the *in vitro* susceptibilities to the five antifungal agents in use (amphotericin B, fluconazole, flucytosine, itraconazole, and voriconazole) of the 217 *Cryptococcus* isolated at an university hospital between 2013 and 2017. All of the 217 *Cryptococcus* isolates were highly susceptible to amphotericin B (99.1% susceptible) over the entire 5-year period. Also, it was shown that susceptibility to fluconazole decreased from 1.0% in 2013 to 75.0% in 2017. The MIC range of fluconazole showed gradual increasing trend shifting from 1 to 4 $\mu\text{g/ml}$ in 2013–2014 to 1–8 $\mu\text{g/ml}$ in 2015, and to 1–16 $\mu\text{g/ml}$ in 2016–2017 (Fig. 1).

Identification, multilocus sequence typing (MLST) and molecular types

According to MALDI-TOF MS results, the conserved 98 clinical cryptococcal strains included 97 *C. neoformans* strains (96 *var. grubii* strains and one *var. neoformans* strain) and one *C. gattii*

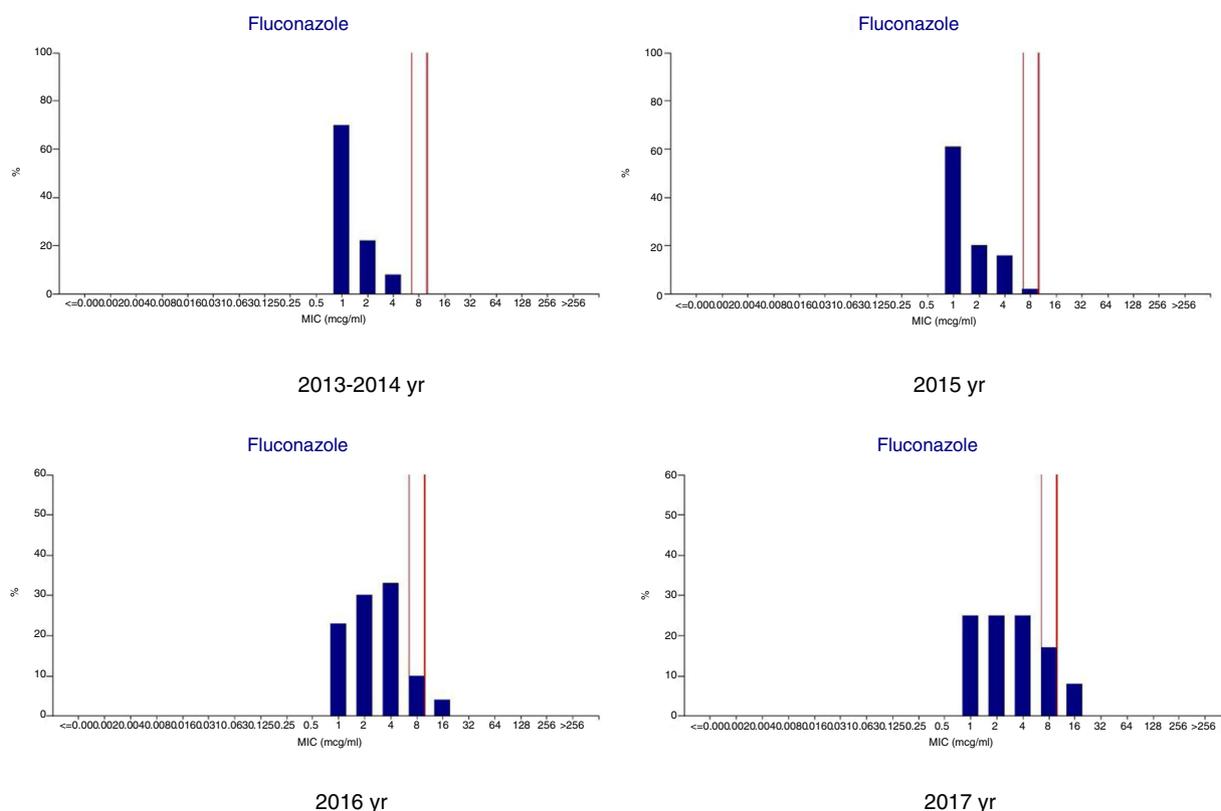


Fig. 1 – Trends in fluconazole susceptibility of cryptococcal isolates from 2013 to 2017.

Table 2 – MLST and molecular type results of 98 clinical cryptococcal isolates.

ST	Genotype	Variety	N (%)
5	VNI	<i>var. grubii</i>	90 (91.8%)
31	VNI	<i>var. grubii</i>	3 (3.1%)
81	VNI	<i>var. grubii</i>	2 (2.0%)
359	VNI	<i>var. grubii</i>	1 (1.0%)
328	VGII	<i>C. gattii</i>	1 (1.0%)
326	VNIV	<i>var. neoformans</i>	1 (1.0%)

strain, which are consistent with the rDNA ITS region amplification results. rDNA ITS region amplification are in agreement with those of MALDI-TOF MS.

MLST and molecular type results of the 98 clinical cryptococcal isolates are shown in Table 2. MLST analysis revealed that all 98 cryptococcal isolates belonged to six sequence types, including ST5 (90 isolates, 91.8%), ST31 (3 isolates, 3.1%), ST81 (2 isolates, 2.0%), ST326 (1 isolates, 1.0%), ST328 (1 isolates, 1.0%), and ST359 (1 isolates, 1.0%). Molecular type VNI (*var. grubii*) was recovered in 96 clinical cryptococcal isolates belonging to ST5, ST31, ST81, and ST359. The type VGII (*C. gattii*) was found in one clinical cryptococcal isolate belonging to ST328. While the type VNIV (*var. neoformans*) was found in one clinical cryptococcal isolate belonging to ST326.

Patients' characteristics

Demographic data and clinical characteristics of the 98 *Cryptococcus* culture-proven patients of the study are shown in

Table 3. Among the 98 patients with cryptococcosis, 28 (28.6%) were male HIV-infected patients and 70 (71.4%) non-HIV-infected patients. There was no apparent sex difference in non-HIV-infected patients with cryptococcosis. HIV-infected patients were younger than non-infected patients (38.4 ± 2.4 vs 50.7 ± 1.7 years). Seventeen (13.7%) patients were on corticosteroids or immunosuppressives, 12 (12.2%) patients had undergone organ transplantation, diabetes mellitus was found in nine (9.2%) patients, but 32 (32.7%) had no underlying diseases.

Among the 70 non-HIV-infected patients with cryptococcosis, 43 (61.4%) patients were diagnosed as cryptococcal meningitis, 17 (17/70, 24.3%) with pulmonary cryptococcosis, and eight (11.4%) patients with disseminated cryptococcosis. However, the incidence rate of disseminated cryptococcosis among the 28 HIV-infected patients was 35.7% (10/28), significantly higher than that in the 70 non-HIV-infected group ($p < 0.05$). Except for the 10 HIV-infected patients infected with disseminated cryptococcosis, the remaining 18 HIV-infected patients were all diagnosed as cryptococcal meningitis. The overall mortality rate was 18.4%, but higher among HIV-infected patients (28.6%) than in non-HIV-infected patients ($p = 0.147$), Table 3.

Discussion

In the past years, the incidence of cryptococcosis in China is on the rise, and the fungal persistence and frequent disease

Table 3 – Demographic features and underlying diseases of HIV-infected and uninfected cryptococcosis patients from hospital-based studies.

Parameter	HIV-uninfected	HIV-infected	Total
No. of Patients	70	28	98
Sex			
Male	57.1% (40/70)	100% (28/28)	69.4% (68/98)
Female	42.9% (30/70)	0	30.6% (30/98)
Age, years (Ref)	50.7 \pm 1.7 (18,81)	38.4 \pm 2.4 (23,70)	47.2 \pm 1.5 (18,81)
Predisposing factors ^a			
HIV/AIDS			28.6% (28/98)
Corticosteroid or immunosuppressive	24.3% (17/70)	0	17.3% (17/98)
Autoimmune disease	5.7% (4/70)	0	4.1% (4/98)
Liver cirrhosis or liver failure	5.7% (4/70)	7.1% (2/28)	6.1% (6/98)
Diabetes mellitus	11.4% (8/70)	3.6% (1/28)	9.2% (9/98)
Tuberculosis	2.9% (2/70)	3.6% (1/28)	3.1% (3/98)
Chronic kidney disease	2.9% (2/70)	0	2.0% (2/98)
Solid malignancy	1.4% (1/70)	0	1.0% (1/98)
Hematologic malignancy	1.4% (1/70)	0	1.0% (1/98)
Organ transplantation	17.1% (12/70)	0	12.2% (12/98)
Pregnancy	1.4% (1/70)	0	1.0% (1/98)
No predisposing factors	45.7% (32/70)	0	32.7% (32/98)
Cryptococcal infection types			
Cryptococcal meningitis	61.4% (43/70)	64.3% (18/28)	62.2% (61/98)
Pulmonary cryptococcosis	24.3% (17/70)	0	17.4% (17/98)
Infections in other sites	2.9% (2/70)	0	2.0% (2/98)
Disseminated cryptococcosis	11.4% (8/70)	35.7% (10/28)	18.4% (18/98)
Outcome			
Survival	85.7% (60/70)	71.4% (20/28)	81.6% (80/98)
Death	14.3% (10/70)	28.6% (8/28)	18.4% (18/98)

^a In some cases, patients had more than one predisposing factor.

relapse of cryptococcosis is the main clinical problem. Several reports of the emergence of resistance to the antifungal agents in HIV-infected patients have been published.^{12,16,20} However, in China, cryptococcosis mostly occurs in patients without HIV infection, and there are limited data on the molecular epidemiology of cryptococcosis in China. In our study, the susceptibility to the five antifungal agents in use (amphotericin B, fluconazole, flucytosine, itraconazole, and voriconazole) of 217 cryptococcal isolates from patients admitted to an university hospital between 2013 and 2017 were evaluated. The trend in the MIC range of fluconazole showed gradual increase from 2013 to 2017, while 16 *C. neoformans* strains were dose-dependent susceptible to fluconazole (MIC 8 µg/ml) and five *C. neoformans* strains with non-wild-type MICs to fluconazole (MIC 16 µg/ml) were detected in 2016–2017. This phenomenon was worth to be conveyed to clinicians, especially in cryptococcosis cases with fungal persistence and relapse. The Infectious Diseases Society of America (IDSA) recommends amphotericin B and 5-flucytosine as the preferred agents for the induction therapy, whereas the azoles (especially fluconazole) are used in the consolidation and maintenance phases of therapy, or as primary prophylaxis.²¹ However, in resource limited settings, azoles are often used as initial therapy.²² In the past years in our hospital, a relatively low fluconazole dose (400 mg per day and even 200 mg per day) was commonly administrated for therapy or prophylaxis of fungal infection, which might lead to the gradual upward MIC drifts of fluconazole. In several reports, high-dose of fluconazole (800 mg per day or even 1200 mg per day) was confirmed more efficacious to treat cryptococcosis.^{22,23} Higher doses of fluconazole and longer durations of treatment are suggested.^{21,23} The gradual upward MIC drifts of fluconazole might also explain the reason that some patients with cryptococcosis were cured or improved with higher dose of fluconazole. Amphotericin B maintained 1.0% susceptibility in the period of 2013–2016, but one isolate was shown to be resistant to amphotericin B (MIC 8 µg/ml) in 2017.

As the results of species and varieties identification by MALDI-TOF MS and ITS sequencing, the conserved 98 clinical cryptococcal strains included 97 *C. neoformans* strains (96 *var. grubii* strains and one *var. neoformans* strain) and one *C. gattii* strain. Analysis of MLST showed that 91.8% of the isolates belonged to ST5 genotype VNI, which was the main ST of clinical *C. neoformans* in China, consistent with the related reports.¹⁵ *C. gattii*, an emerging agent of cryptococcosis, was initially reported only in tropical and subtropical zones and rarely reported in China.²⁴ The patient infected with *C. gattii* had been worked in Cambodia and suffered before going back to China. So, this case of *C. gattii* cryptococcosis might have been imported.

In previous reports, about 80% of the cryptococcosis cases were associated with HIV/AIDS in Europe and the United States.^{9,25} In contrast, in our study most of patients (71.4%) with cryptococcosis were not HIV-infected; moreover, 32.7% of the cryptococcosis patients had no underlying diseases, as shown in other reports from China.^{6,26} All of the 28 HIV-infected cryptococcosis patients with were male, and younger than the patients without HIV. There was no apparent sex difference in the cryptococcosis patients without HIV. The incidence rate of disseminated cryptococcosis, as well as the

mortality rate, among HIV-infected patients were significantly higher than that in patients without HIV infection.

In contrast to the findings of Lee et al., no relationship between MICs of antifungal agents and outcome of patients with cryptococcosis could be demonstrated in this study. The discrepancy might be due to different choices of treatment regimens.¹⁴ In this study, four of the five patients infected with *C. neoformans* with higher MICs for fluconazole were still cured or improved following the regimen recommended by IDSA with amphotericin B and 5-flucytosine as the preferred agents for the induction therapy and fluconazole or voriconazole used in the consolidation and maintenance phases. Moreover, the patient infected with *C. neoformans* resistant to amphotericin B (MIC 8 µg/ml) responded to voriconazole. However, the shifting trend in MIC ranges of antifungal agents, especially fluconazole, warrants attention as it was also reported in other countries and might be related to the dose selection of fluconazole for effective therapy.²⁷ Hence, the dynamic real time surveillance of susceptibility for antifungal agents is necessary in clinic practice.

This study has some limitations. It was a single center retrospective study conducted in a major teaching hospital in China. Hence, the results in this study are exploratory. In future, further investigations with larger samples including more hospitals in China will be necessary to generalize the results for the Chinese population.

Disclosure

The authors report no conflicts of interest in this work.

REFERENCES

- Maziarz EK, Perfect JR. Cryptococcosis. *Infect Dis Clin North Am.* 2016;30:179–206, <http://dx.doi.org/10.1016/j.idc.2015.10.006>. PubMed PMID: 26897067; PubMed Central PMCID: PMC5808417.
- El Fane M, Badaoui L, Ouladlarsen A, Sodqi M, Marih L, Chakib A, et al. [Cryptococcosis during HIV infection]. *J Mycol Med.* 2015;25:257–62, <http://dx.doi.org/10.1016/j.mycmed.2015.09.008>. PubMed PMID: 26515783.
- Chau TT, Mai NH, Phu NH, Nghia HD, Chuong LV, Sinh DX, et al. A prospective descriptive study of cryptococcal meningitis in HIV uninfected patients in Vietnam - high prevalence of *Cryptococcus neoformans var grubii* in the absence of underlying disease. *BMC Infect Dis.* 2010;10:199, <http://dx.doi.org/10.1186/1471-2334-10-199>. PubMed PMID: 20618932; PubMed Central PMCID: PMC2910700.
- Ssekitoleko R, Kanya MR, Reingold AL. Primary prophylaxis for cryptococcal meningitis and impact on mortality in HIV: a systematic review and meta-analysis. *Future Virol.* 2013;8, <http://dx.doi.org/10.2217/fvl.13.71>. PubMed PMID: 24368930; PubMed Central PMCID: PMC3869998.
- Fang W, Chen M, Liu J, Hagen F, Ms A, Al H, et al. Cryptococcal meningitis in systemic lupus erythematosus patients: pooled analysis and systematic review. *Emerg Microbes Infect.* 2016;5:e95, <http://dx.doi.org/10.1038/emi.2016.93>. PubMed PMID: 27599471; PubMed Central PMCID: PMC5113055.
- Li Y, Fang W, Jiang W, Hagen F, Liu J, Zhang L, et al. Cryptococcosis in patients with diabetes mellitus II in

- mainland China: 1993-2015. *Mycoses*. 2017;60:706-13, <http://dx.doi.org/10.1111/myc.12645>. PubMed PMID: 28857298.
7. Sloan DJ, Parris V. Cryptococcal meningitis: epidemiology and therapeutic options. *Clin Epidemiol*. 2014;6:169-82, <http://dx.doi.org/10.2147/CLEP.S38850>. PubMed PMID: 24872723; PubMed Central PMCID: PMCPMC4026566.
 8. Wirth F, Azevedo MI, Goldani LZ. Molecular types of *Cryptococcus* species isolated from patients with cryptococcal meningitis in a Brazilian tertiary care hospital. *Braz J Infect Dis*. 2018;22:495-8, <http://dx.doi.org/10.1016/j.bjid.2018.11.002>. PubMed PMID: 30508502.
 9. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis*. 2017;17:873-81, [http://dx.doi.org/10.1016/S1473-3099\(17\)30243-8](http://dx.doi.org/10.1016/S1473-3099(17)30243-8). PubMed PMID: 28483415; PubMed Central PMCID: PMCPMC5818156.
 10. Chen M, Xu Y, Hong N, Yang Y, Lei W, Du L, et al. Epidemiology of fungal infections in China. *Front Med*. 2018;12:58-75, <http://dx.doi.org/10.1007/s11684-017-0601-0>. PubMed PMID: 29380297.
 11. McClelland EE, Hobbs LM, Rivera J, Casadevall A, Potts WK, Smith JM, et al. The role of host gender in the pathogenesis of *Cryptococcus neoformans* infections. *PLoS One*. 2013;8:e63632, <http://dx.doi.org/10.1371/journal.pone.0063632>. PubMed PMID: 23741297; PubMed Central PMCID: PMCPMC3669355.
 12. Nascimento E, Vitali LH, Kress M, Martinez R. *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*. 2017;59:e49, <http://dx.doi.org/10.1590/S1678-9946201759049>. PubMed PMID: 28793019; PubMed Central PMCID: PMCPMC5626223.
 13. de Carvalho Santana R, Schiave LA, Dos Santos Quaglio AS, de Gaitani CM, Martinez R. Fluconazole Non-susceptible *Cryptococcus neoformans*, Relapsing/Refractory Cryptococcosis and Long-term Use of Liposomal Amphotericin B in an AIDS Patient. *Mycopathologia*. 2017;182:855-61, <http://dx.doi.org/10.1007/s11046-017-0165-1>. PubMed PMID: 28656554.
 14. Lee CH, Chang TY, Liu JW, Chen FJ, Chien CC, Tang YF, et al. Correlation of anti-fungal susceptibility with clinical outcomes in patients with cryptococcal meningitis. *BMC Infect Dis*. 2012;12:361, <http://dx.doi.org/10.1186/1471-2334-12-361>. PubMed PMID: 23253817; PubMed Central PMCID: PMCPMC3546060.
 15. Fan X, Xiao M, Chen S, Kong F, Dou HT, Wang H, et al. Predominance of *Cryptococcus neoformans* var. *grubii* multilocus sequence type 5 and emergence of isolates with non-wild-type minimum inhibitory concentrations to fluconazole: a multi-centre study in China. *Clin Microbiol Infect*. 2016;22:887 e1-e9, <http://dx.doi.org/10.1016/j.cmi.2016.07.008>. PubMed PMID: 27432767.
 16. Mdodo R, Moser SA, Jaoko W, Baddley J, Pappas P, Kempf MC, et al. Antifungal susceptibilities of *Cryptococcus neoformans* cerebrospinal fluid isolates from AIDS patients in Kenya. *Mycoses*. 2011;54:e438-42, <http://dx.doi.org/10.1111/j.1439-0507.2010.01946.x>. PubMed PMID: 21535451; PubMed Central PMCID: PMCPMC3150616.
 17. Espinel-Ingroff A, Aller AI, Canton E, Castanon-Olivares LR, Chowdhary A, Cordoba S, et al. *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*. 2012;56:5898-906, <http://dx.doi.org/10.1128/AAC.01115-12>. PubMed PMID: 22948877; PubMed Central PMCID: PMCPMC3486550.
 18. Espinel-Ingroff A, Chowdhary A, Cuenca-Estrella M, Fothergill A, Fuller J, Hagen F, et al. *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*. 2012;56:3107-13, <http://dx.doi.org/10.1128/AAC.06252-11>. PubMed PMID: 22391546; PubMed Central PMCID: PMCPMC3370763.
 19. McTaggart L, Richardson SE, Seah C, Hoang L, Fothergill A, Zhang SX. Rapid identification of *Cryptococcus neoformans* var. *grubii*, *C. neoformans* var. *neoformans*, and *C. gattii* by use of rapid biochemical tests, differential media, and DNA sequencing. *J Clin Microbiol*. 2011;49:2522-7, <http://dx.doi.org/10.1128/JCM.00502-11>. PubMed PMID: 21593254; PubMed Central PMCID: PMCPMC3147830.
 20. Kammalac Ngouana T, Dongtsa J, Kouanfack C, Tonfack C, Fomena S, Mallie M, et al. Cryptococcal meningitis in Yaounde (Cameroon) HIV infected patients: Diagnosis, frequency and *Cryptococcus neoformans* isolates susceptibility study to fluconazole. *J Mycol Med*. 2015;25:11-6, <http://dx.doi.org/10.1016/j.mycmed.2014.10.016>. PubMed PMID: 25467817.
 21. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. *Clin Infect Dis*. 2010;50:291-322, <http://dx.doi.org/10.1086/649858>. PubMed PMID: 20047480; PubMed Central PMCID: PMCPMC5826644.
 22. Srichatrapimuk S, Sungkanuparph S. Integrated therapy for HIV and cryptococcosis. *AIDS Res Ther*. 2016;13:42, <http://dx.doi.org/10.1186/s12981-016-0126-7>. PubMed PMID: 27906037; PubMed Central PMCID: PMCPMC5127046.
 23. Longley N, Muzoora C, Taseera K, Mwesigye J, Rwebembera J, Chakera A, et al. Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. *Clin Infect Dis*. 2008;47:1556-61, <http://dx.doi.org/10.1086/593194>. PubMed PMID: 18990067.
 24. Xue X, Wu H, Wang K, Cao J, Shen D. Cryptococcosis by *Cryptococcus gattii* in China. *Lancet Infect Dis*. 2015;15:1135-6, [http://dx.doi.org/10.1016/S1473-3099\(15\)00301-1](http://dx.doi.org/10.1016/S1473-3099(15)00301-1). PubMed PMID: 26461947.
 25. Schmiedel Y, Zimmerli S. Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and *Pneumocystis pneumonia*. *Swiss Med Wkly*. 2016;146:w14281, <http://dx.doi.org/10.4414/sm.w.2016.14281>. PubMed PMID: 26901377.
 26. Yuchong C, Fubin C, Jianghan C, Fenglian W, Nan X, Minghui Y, et al. Cryptococcosis in China (1985-2010): review of cases from Chinese database. *Mycopathologia*. 2012;173:329-35, <http://dx.doi.org/10.1007/s11046-011-9471-1>. PubMed PMID: 21979866.
 27. Govender NP, Patel J, van Wyk M, Chiller TM, Lockhart SR, Group for Enteric R, et al. Trends in antifungal drug susceptibility of *Cryptococcus neoformans* isolates obtained through population-based surveillance in South Africa in 2002-2003 and 2007-2008. *Antimicrob Agents Chemother*. 2011;55:2606-11, <http://dx.doi.org/10.1128/AAC.00048-11>. PubMed PMID: 21444707; PubMed Central PMCID: PMCPMC3101403.