ORIGINAL RESEARCH

Cryptococcosis in Southern China: Insights from a Six-Year Retrospective Study in Eastern Guangdong

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Objective: Cryptococcosis is a fatal infection that can affect both immunocompetent and immunocompromised patients, and it is little understood in China's various regions. This research aimed to look at the epidemiology, risk factors, and antifungal susceptibility pattern of *Cryptococcus neoformans* in eastern Guangdong, China.

Methods: A six-year (2016–2022) retrospective study was conducted at Meizhou People's Hospital, China. Demographical, clinical, and laboratory data of cryptococcal patients were collected from hospital records and statistically analyzed using the chi-square and ANOVA tests.

Results: Overall, 170 cryptococcal infections were recorded, of which meningitis accounted for 78 (45.88%), cryptococcemia for 50 (29.41%), and pneumonia for 42 (24.7%). The number of cases increased 8-fold during the study duration. The median age of patients was 58 years (Inter quartile range: 47–66), and the high proportion of cases was from the male population (n = 121, 71.17%). The underlying diseases were identified only in 60 (35.29%) patients, of which 26 (15.29%) were severely immunocompromised, and 26 (15.29%) others were mildly immunocompromised. A statistically significant difference was reported for chronic renal failure, and anemia ($p < 0.05$) persisted in cases of three infection types. A high number of non-wild type (NWT) isolates were found against amphotericin B (n=13/145, 8.96%), followed by itraconazole (n=7/136, 5.15%) and voriconazole (n=4/158, 2.53%). Only six isolates (3.79%) were multidrug-resistant, four of which were from cryptococcemia patients. Compared to meningitis and pneumonia, cryptococcemia revealed a higher percentage of NWT isolates ($p < 0.05$).

Conclusion: In high-risk populations, cryptococcal infections require ongoing monitoring and management.

Keywords: cryptococcosis, *Cryptococcus neoformans*, epidemiology, risk factors, antifungal susceptibility

Introduction

Cryptococcosis is a severe fungal infection caused by various encapsulated yeast species known as *Cryptococcus*.[1] It is an opportunistic infection that frequently affects people with weakened immune systems, such as those with HIV/AIDS, solid organ or bone marrow transplant recipients, or people with other immune-compromising illnesses like malignancies and diabetes. Cryptococcus genus includes ten species, of which seven are pathogenic to animals and humans. Among these, *Cryptococcus neoformans* and *Cryptococcus gattii* are the two main species that cause cryptococcosis in humans, with *C. neoformans* being the most prevalent species globally.[2] Meningitis, pneumonia, fungemia, endocarditis, and skin lesions are the clinical infections induced by *Cryptococcus* species. It is also known to cause severe morbidity and mortality worldwide.[3]

Every year, an estimated 223,100 new cases of cryptococcal meningitis are diagnosed worldwide, resulting in approximately 181,100 deaths.[4] Similarly, the prevalence of cryptococcal infections in China is comparatively significant, and their burden varies in different geographic locations.[5] Due to the lack of a proper surveillance system, the exact burden

for the whole country is still to determine.[6] However, some studies reported up to 43% mortality rate of *C. neoformans* in the Chinese population. The disease is most commonly documented in HIV-infected people, although it can also develop in non-HIV patients with other immunosuppressive conditions like organ transplants and malignancies.[7,8]

Antifungal agents are the cornerstone of cryptococcosis treatment. A combination of amphotericin B and 5-flucytosine is advised as a first-line treatment for patients with severe illness.[9,10] Following that, fluconazole consolidation therapy is administered to finish a 10- to 12-week course of treatment.[11] However, the rise of drug-resistant strains and the toxicities associated with antifungal drugs necessitate antifungal susceptibility testing.[12,13] Based on their antifungal resistance mechanisms and hazardous properties, the WHO classified *C. neoformans* at the top of the critical fungal priority group (https://www.who.int/publications/i/item/9789240006355, accessed on 22 February 2023). The testing can help in selecting the best antifungal treatment and stop the emergence of drug resistance.[12,13]

The clinical traits, risk factors, and antifungal susceptibility patterns of cryptococcal infections have not been extensively studied in China.[14,15] Therefore, a retrospective analysis of patients diagnosed with cryptococcosis in a Chinese hospital during the last six years would provide valuable insights into the disease's epidemiology and management. The research could aid in identifying disease risk factors and determining antifungal susceptibility patterns of isolates. The findings could help in the development of effective strategies for the prevention and treatment of cryptococcosis in China and around the world, where it is still a significant public health issue.

Methods

This retrospective study was conducted at Meizhou People's Hospital located in Meizhou city, eastern Guangdong, South China and included all identified cryptococcal disease cases from January 2016 to December 2022. The hospital is a tertiary (A) level hospital containing 3600 beds and providing healthcare service to around 20 million people in the region.[16] The study received ethical approval from the hospital, which adhered to the standards of the Helsinki Declaration (Letter number: 2021-C-106). Hospital electronic health records were used to collect patient demographics and clinical data, including age, gender, specimen source, department location, baseline, clinical features of cryptococcal disease, HIV serology, and antifungal drug susceptibility test results.

Definition

The diagnosis of cryptococcal disease was based on World Health Organization guidelines (https://www.who.int/publications/i/item/9789240052178, accessed on 22 February 2023). This study classified cryptococcal cases based on specimen source and showed clinical features. Cryptococcal meningitis has been identified in cases where *Cryptococcus neoformans* has been isolated from cerebrospinal fluid (CSF) samples, and patients have experienced symptoms such as headache, fever, stiff neck, nausea, vomiting, photophobia, and confusion. The cases where *C. neoformans* was cultured from respiratory specimens or patients who developed symptoms such as cough, shortness of breath, fever, chest pain, and sputum production were classified as cryptococcal pneumonia. Finally, cryptococcal fungemia was classified for cases where *C. neoformans* was isolated from the blood, and the patients experienced symptoms such as fever, chills, headache, fatigue, and an altered mental state and did not show any symptoms of meningitis and pneumonia.[1]

Laboratory Isolation and Identification of *Cryptococcus* Species

During the study duration, 773,800 patients visited the hospital, of which 170 cases were of cryptococcosis. The species were isolated from different specimen types, like CSF, blood, tissue biopsy, BAL fluid, urine, sputum, ascites fluid, and pus. One species was counted from each patient specimen, and only the first positive culture was selected for the patients having repeated positive cultures. According to routine laboratory protocols, all the samples were first cultured on Sabouraud Dextrose Agar as selective media and then overnight enriched on Brain Heart Agar at 35 °C to identify the species of each patient sample. A single mucoid creamy colony was picked from each plate and viewed under a microscope at 400X magnification. MALDI-TOF MS (Bruker Daltonik, Bremen, Germany) was used to confirm species according to the manufacturer's instructions. The identification process was performed using the Bruker library program Spectra (version 4.0.0.1, which contained 5627 entries) preinstalled on Bruker Biotyper (version 3.1; Bruker.1). The manufacturer's recommended rating standards were used to determine the level

of identification: Rating 2.000 or more indicated species-level identification, a score between 1700 and 1999 indicated genus-level identification, and a score below 1700 indicated that the species could not be identified.

Antifungal Susceptibility Testing

Antifungal susceptibility testing (AST) was performed using the ATB fungal 3 kit (bioMérieux SA, France) according to the manufacturer's instructions. The tested antifungal drugs were fluconazole, itraconazole, voriconazole, 5-flucytosine, and amphotericin B. *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as quality control strains to ensure the accuracy of the results. The results were interpreted as wild type (WT) or non-wild type (NWT) based on the epidemiological cut-off values set by clinical and laboratory standards institutes guidelines for *Cryptococcus* species.[17]

Statistical Analysis

Patient demographics and clinical data were obtained from electronic medical records of the hospital surveillance system and documented in an Excel spreadsheet (2016). Qualitative data were presented as absolute numbers and relative percentages, while quantitative data were expressed as median and interquartile ranges. The characteristics of the three types of cryptococcal infections (meningitis, mycosis, and pneumonia) were analyzed using the chi-square test for categorical variables and ANOVA for continuous variables, with *p*-values less than 0.05 being considered statistically significant. Data analysis and visualization were done with GraphPad Prism v.8.0.2.

Results

Distribution of *Cryptococcus* Species

A total of 170 cryptococcal infections caused by *C. neoformans* were reported in the six-year study duration. The most number of cases were reported in the year 2021 (n = 56, 32.94%), followed by 2018 (n = 40, 23.53%) and 2020 (n = 17.65%), while only 7 (4.17%) cases were reported in 2016. Fluctuations in the number of cases from year to year were reported; however, an 8-fold increase occurred from 2016 to 2021. Regarding the hospital's different departments, a large number of cases were reported from ICU (n = 67, 39.41%), followed by neurology (n = 43, 25.29%), while only 3 (1.76%) cases were reported from pediatrics and only one (0.59%) from surgery department. A total of 78 (45.88%) species were isolated from CSF, 50 (30%) from blood, and only one (0.59%) from the pus sample. The complete presentation of cases from different hospital departments and sample sources is depicted in Figure 1.

## Demographic and Clinical Characteristics

Out of 170 cases, the total number of male cases was comparatively higher than female cases at the ratio of 121:49. Most of the cases occurred in older populations; the median age of the study population was 58 at the range of 5 years to 91 years. Among different age groups, no cases in neonatal and infants were detected, while only 9 cases (5.29%) were detected in children (ages: 2–17). The highest cases (n = 49, 28.82%) were detected in the senior age group of 61 to 70 years (Figure 2).

Based on sample sources and clinical manifestations, three types of cryptococcal infection were identified in the study population. Among these, the highest number of cases was reported for cryptococcal meningitis (n = 78, 45.88%), followed by cryptococcal fungemia (n = 50, 29.41%) and then cryptococcal pneumonia (n = 42, 24.7%). The proportion of male cases was higher in all three cryptococcal infection types than in females. According to the X^2 test comparison, there was no statistically significant difference in the distribution of the three infections by gender (*p* > 0.05). For cryptococcal pneumonia, a high number of cases were reported in the age group 51 to 60 (n = 15/42, 35.71%), followed by the age group 41 to 50 (n = 11/42, 26.19%). While for cryptococcal meningitis and fungemia, a high number of cases were reported in the age group 51 to 60, which are n = 25/78 (32.05%) and 15/50 (30%), respectively. The statistically significant difference in the distribution of infections for the age groups 51 to 60 and 71 to 80 was reported in the current study (*p* < 0.05).

Only 60 (35.29%) cases were reported having underlying diseases among the total cryptococcal cases. Of these, 12 (7.05%) patients had autoimmune disorders, of which 8 (4.7%) had systemic lupus erythematosus, and 4 (2.35%) had rheumatoid arthritis. Similarly, chronic renal failure was reported in 11 (6.47%) cases, followed by 8 (4.7%) cases each for cancer and diabetes and 7 (4.11%) cases for liver cirrhosis. None of the cryptococcosis patients was HIV positive in
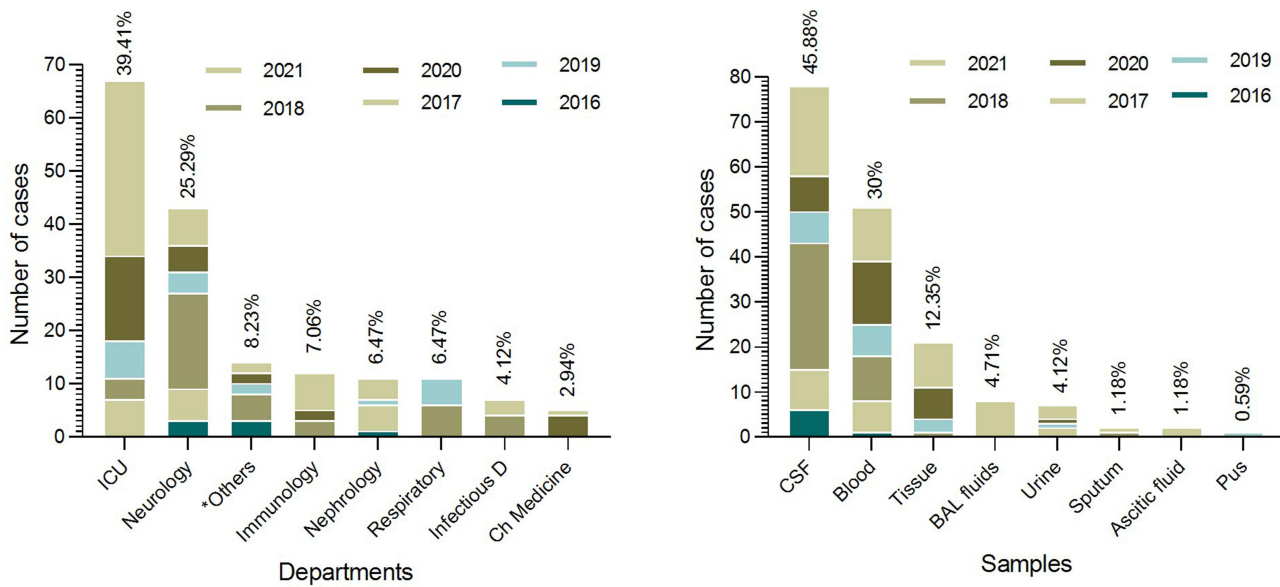
Figure 1 The number of cryptococcal cases detected from different departments and sample sources during the study duration (2016 to 2021). *Other departments represent 4 cases in hepatology, 3 cases (each) from pediatrics and general practice departments, and 1 case (each) from surgery, hematology, stomatology, and cardiology.
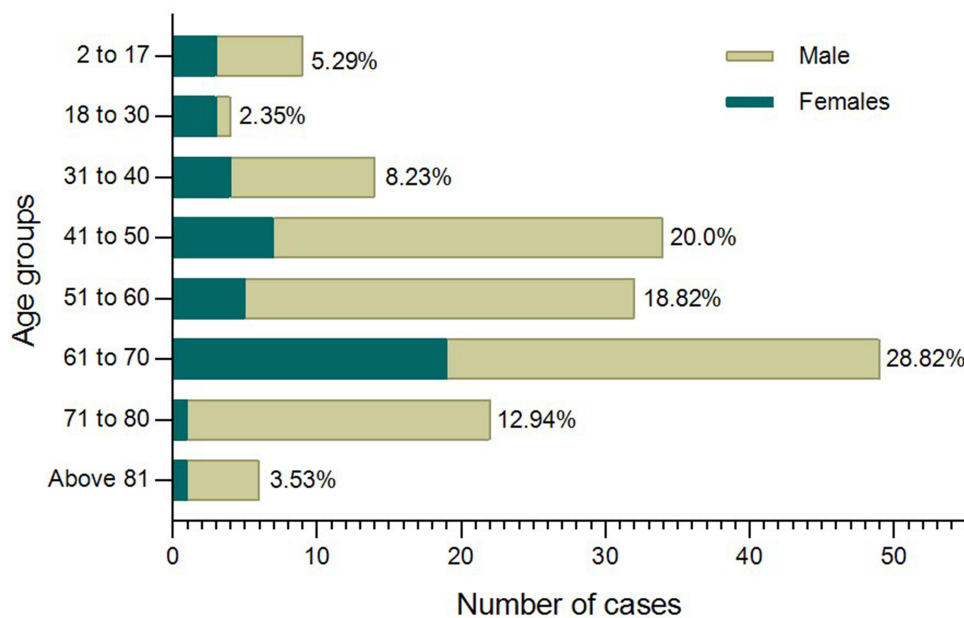
Figure 2 Distribution of cryptococcal cases based on different age groups and gender during the study duration (2016 to 2021).

the current study. According to the X^2 test comparing underlying status distribution in three infection types, a statistically significant difference was reported for chronic renal failure and anemia ($p<0.05$). Chronic renal failure was present in 10% of the patients with cryptococcal fungemia and 9.52% with cryptococcal pneumonia. Similarly, the results showed that anemia was more common in patients with cryptococcal fungemia (6%) than in patients with cryptococcal meningitis (0%) or pneumonia (0%). However, the distribution of some underlying conditions, such as autoimmune disorders, malignancy, diabetes, liver cirrhosis, cardiac diseases, and benign prostatic hyperplasia, was not statistically significant in three cryptococcal infection types ($p > 0.05$).

Among the clinical manifestations, the fever was found in high proportion among three types of cryptococcal infections (64–70%). However, some other manifestations were associated with specific infection types, like headache

(n = 74, 94.87%), neck stiffness (n = 40, 51.28%), and altered mental status (n = 23, 29.48%) were found in high proportion in cryptococcal meningitis. Similarly, fatigue (n = 22, 44%) was associated with cryptococcal fungemia, while cough (n = 36, 85.71%) and shortness of breath (n = 23, 54.76%) were found in high numbers in cryptococcal pneumonia. The X^2 test comparing the distribution of clinical manifestations in three different infection types revealed that, except fever, all others were statistically different ($p < 0.05$). The patient demographic data, underlying conditions, and clinical symptoms for each of the three forms of cryptococcal infections are presented in Table 1.

Table 1 Demographical and Clinical Characteristics Associated with Cryptococcal Meningitis, Cryptococcal Fungemia, and Cryptococcal Pneumonia During the Six-Year Study Duration

Variables	Total (n=170)	Cryptococcal Meningitis (n=78, 45.88%)	Cryptococcal Fungemia (n=50, 29.41%)	Cryptococcal Pneumonia (n=42, 24.7%)	X^2, df	p value
Gender						
Male	121 (71.17%)	52 (66.66%)	37 (74%)	32 (76.19%)	1.482, 2	0.476
Female	49 (28.82%)	26 (33.33%)	13 (26%)	10 (23.8%)	1.482, 2	0.476
M age (IQR)	58 (47–66)	58 (42–67)	63 (48–73)	54 (49–63)	–	0.6713
Age group						
<17	9 (5.29%)	4 (5.12%)	3 (6%)	2 (4.76%)	0.077, 2	0.9619
18 to 30	4 (2.35%)	3 (3.84%)	1 (2%)	0 (0%)	1.796, 2	0.4074
31 to 40	14 (8.23%)	9 (11.53%)	4 (8%)	1 (2.38%)	3.035, 2	0.2193
41 to 50	34 (20%)	13 (16.66%)	10 (20%)	11 (26.19%)	1.548, 2	0.4613
51 to 60	32 (18.82%)	14 (17.94%)	3 (6%)	15 (35.71%)	13.26, 2	0.0013
61 to 70	49 (28.82%)	25 (32.05%)	15 (30%)	9 (21.42%)	1.549, 2	0.4608
71 to 80	22 (12.94%)	6 (7.69%)	14 (28%)	2 (4.76%)	14.47, 2	0.0007
80 above	6 (3.52%)	4 (5.12%)	0 (0%)	2 (4.76%)	2.602, 2	0.2722
Underlying Status						
Chronic renal failure	11 (6.47%)	2 (2.56%)	5 (10%)	4 (9.52%)	53.70, 2	<0.0001
Systemic lupus erythematosus	8 (4.7%)	4 (5.12%)	3 (6%)	1 (2.38%)	0.724, 2	0.6963
Cancer	8 (4.7%)	4 (5.12%)	3 (6%)	1 (2.38%)	0.724, 2	0.6963
Diabetes	8 (4.7%)	5 (6.41%)	3 (6%)	0 (0%)	2.766, 2	0.2508
Liver cirrhosis	7 (4.11%)	4 (5.12%)	2 (4%)	1 (2.38%)	0.524, 2	0.7694
Rheumatoid arthritis	4 (2.35%)	1 (1.28%)	1 (2%)	2 (4.76%)	1.477, 2	0.4778
Heart disease	4 (2.35%)	3 (3.84%)	1 (2%)	0 (0%)	1.796, 2	0.4074
Fainting and collapse	3 (1.76%)	1 (1.28%)	2 (4%)	0 (0%)	2.300, 2	0.3166
Anemia	3 (1.76%)	0 (0%)	3 (6%)	0 (0%)	7.329, 2	0.0256
Hairy cell leukemia	2 (1.17%)	0 (0%)	2 (4%)	0 (0%)	4.857, 2	0.0882
Benign prostatic hyperplasia	1 (0.58%)	0 (0%)	0 (0%)	1 (2.38%)	3.066, 2	0.2159
Leukopenia	1 (0.58%)	0 (0%)	0 (0%)	1 (2.38%)	3.066, 2	0.2159
Clinical manifestation						
Fever	113 (66.47%)	51 (65.38%)	35 (70%)	27 (64.28%)	0.411, 2	0.8144
Headache	98 (57.64%)	74 (94.87%)	20 (40%)	4 (9.52%)	90.48, 2	<0.0001
Neck stiffness	56 (32.94%)	56 (71.79%)	0 (0%)	0 (0%)	98.50, 2	<0.0001
Nausea and vomiting	47 (27.64%)	40 (51.28%)	7 (14%)	0 (0%)	42.49, 2	<0.0001
Fatigue	37 (21.76%)	0 (0%)	22 (44%)	15 (35.71%)	41.02, 2	<0.0001
Cough	36 (21.17%)	0 (0%)	0 (0%)	36 (85.71%)	139.2, 2	<0.0001
Altered mental status	30 (17.64%)	23 (29.48%)	7 (14%)	0 (0%)	16.98, 2	0.0002
Shortness of breath	27 (15.88%)	0 (0%)	4 (8%)	23 (54.76%)	64.57, 2	<0.0001
Photophobia	24 (14.11%)	24 (30.76%)	0 (0%)	0 (0%)	32.96, 2	<0.0001
Seizures	6 (3.52%)	6 (7.69%)	0 (0%)	0 (0%)	7.336, 2	0.0255
Chest pain	6 (3.52%)	0 (0%)	0 (0%)	6 (14.28%)	18.95, 2	<0.0001

Abbreviations: X^2, Chi-square; df, degree of freedom; p value, probability value.
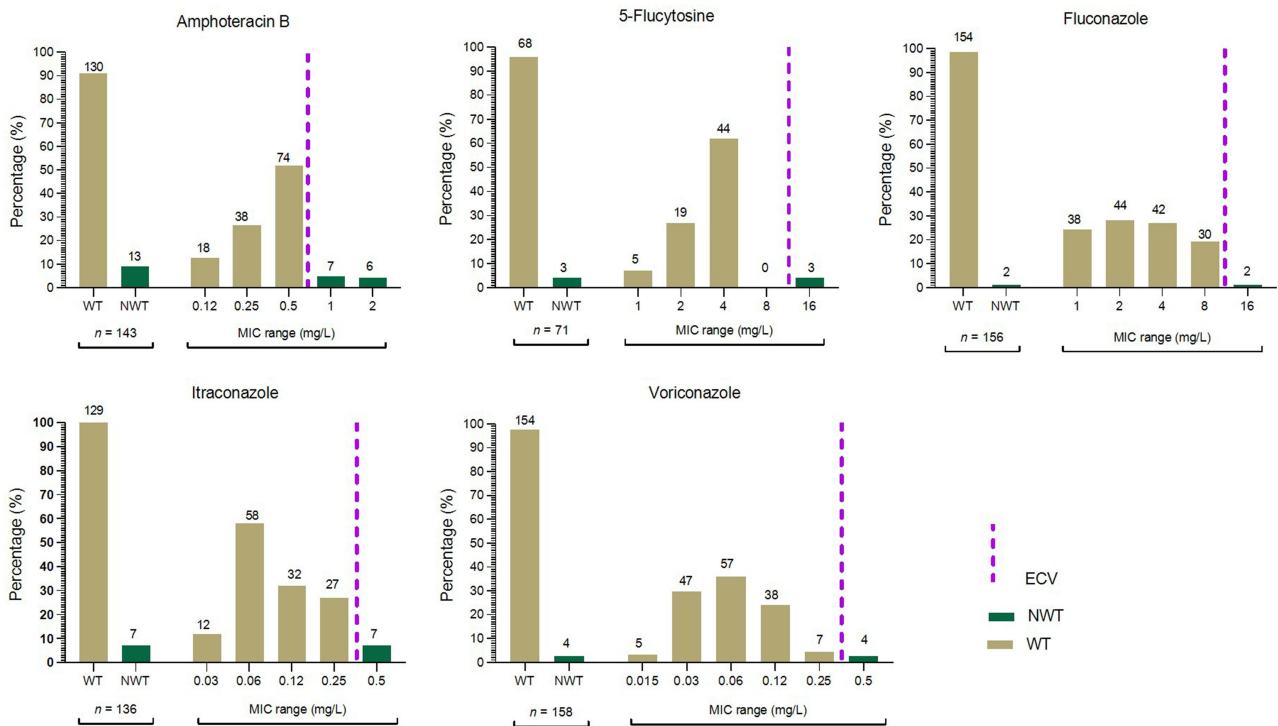
Figure 3 The distribution of WT/NWT and MIC ranges of different antifungal agents against the tested *C. neoformans* isolates tested in the current study. The brown bars indicate WT isolates, while the green bars are for NWT, and the purple line shows the ECVs for each antifungal agent according to CLSI guidelines (M57s). The ECV for amphotericin B is 0.5 mg/L, for 5-flucytosine and fluconazole is 8 mg/L, and for itraconazole, and voriconazole is 0.25 mg/L.

Antifungal Susceptibilities Profiles

Among the tested antifungal agents, a high number of NWT isolates were reported against amphotericin B (n = 13/145, 8.96%), followed by itraconazole (n = 7/136, 5.15%) and voriconazole (n = 4/158, 2.53%), while only 2 (4.11%) isolates showed resistance against fluconazole (Figure 3). In the overall tested isolates, only six (3.79%) isolates were MDR and showed resistance to two or more than two antifungal agents.

Furthermore, we analyze the antifungal susceptibilities in different cryptococcal infection types (Table 2). The results showed that four of the six MDR isolates were from cryptococcal fungemia. The X^2 statistic for MDR was 3.912, indicating that there may be some association between cryptococcal fungemia and the emergence of MDR isolates; however, the p-value of 0.1414 indicates that this association is not statistically significant. Moreover, the findings demonstrated that resistance to amphotericin B was associated with cryptococcal fungemia at a considerably more significant proportion (22.22%) than the other types of infection (p-value = 0.0009). Similarly, resistance to 5-flucytosine occurred at a greater rate in cryptococcal pneumonia infection (12.5%), but the association was only marginally significant (p-value = 0.041).

Table 2 Antifungal Susceptibilities Profiles of *C. neoformans* Causing Cryptococcal Meningitis, Fungemia, and Pneumonia

Variables	Total	Cryptococcal Meningitis	Cryptococcal Fungemia	Cryptococcal Pneumonia	X^2	p value
MDR	6/158 (3.79%)	1/77 (1.29%)	4/49 (8.16%)	1/32 (3.12%)	3.912	0.1414
Amphotericin B (NWT)	13/145 (8.96%)	2/69 (2.89%)	10/45 (22.22%)	1/31 (3.22%)	14.05	0.0009
5-flucytosine (NWT)	3/73 (4.11%)	0/25 (0%)	0/24 (0%)	3/24 (12.5%)	6.388	0.041
Fluconazole (NWT)	2/155 (1.29%)	1/76 (1.32%)	1/47 (2.12%)	0/32 (0%)	0.677	0.7127
Itraconazole (NWT)	7/136 (5.15%)	4/68 (5.88%)	3/41 (7.32%)	0/27 (0%)	1.668	0.4343
Voriconazole (NWT)	4/158 (2.53%)	1/77 (1.29%)	3/49 (6.12%)	0/32 (0%)	3.866	0.1447

Abbreviations: X^2, Chi-square; p value, probability value; MDR, multi-drug resistant; NWT, non-wild types.

Discussion

In the current study, 170 cryptococcosis cases were reported in the past six years, of which 45.88% were meningitis, 29.41% were fungemia, and 24.7% were pneumonia. Typically, *Cryptococcus* is transmitted by inhalation route and causes pneumonia and fungemia, and has a proclivity to spread to the brain, where it manifests as meningitis.[18] Cryptococcal meningitis is the globally predominately reported cryptococcosis followed by pneumonia and then cryptococcemia.[19,20] The current study found meningitis in most in number compared to other infection types, the same as the earlier finding from China.[14,21] However, the high proportion of fungemia than pneumonia is a matter of concern. As cryptococcemia is a severe threat to the immunocompromised population, early detection and treatment are critical for better outcomes.[22] *C. neoformans* caused all cases; no *C. gattii* was detected in the present study. The studies showed that *C. neoformans* is predominantly reported worldwide and mainly infects immunosuppressed individuals, while the *C. gattii* is endemic to only a few regions of the world and infects immune-competent individuals.[23,24]

The cryptococcal infection in this study was found in high proportion in the male population compared to female, 121:49. A total of 71.7% of cases were detected from the male population, which is inconsistent with the previous findings, ie, 60.9% from Hunan, China, 75.6% from Wuhan, China, 73% from Beijing China, 79.6% from Colombia, and 69% from Brazil.[14,18,25–27] The high proportion in the male population might be due to their lifestyle and weak immune system compared to the female population.[19] In the present study, the median age of patients was 58 (IQR; 47–66). Specifically, for cryptococcal meningitis and fungemia, 32.05% and 30% of cases were reported in the age group 61 to 70 years, respectively. The high proportion of cases in the elderly population is similar to earlier findings from China, India, and Taiwan.[14,19,28] The elderly population is more vulnerable to infections because of age-related changes in the immune system, increased risk for underlying conditions, and increased immunosuppressive medication.[19] For cryptococcal pneumonia, 35.71% and 26.19% cases were reported in the age group 51 to 60 and 41 to 50, respectively. Comparatively to the other two types of infections, cryptococcal pneumonia is found in all adult age group patients.[29] A previous 20-year retrospective study from China reported a mean age of 49.3 (SD: 14.3) for cryptococcal pneumonia patients.[24] The occurrence of cryptococcal pneumonia in all age patients is solely related to the subject's weakened immune systems.[30]

Both immunocompetent and immunocompromised persons can develop cryptococcosis.[31] Patients with cryptococcal infections may have predisposing factors that vary geographically and alter over time.[20,32] HIV/AIDS is considered a leading risk factor for cryptococcosis globally. However, in the current study, no cryptococcosis patients were HIV/AIDS positive. Other risk factors, like immunosuppressive therapy, advanced age, severe underlying status, solid organ transplant, hematological malignancies, prolonged corticosteroid treatment, and other immunocompromised statuses, may contribute to cryptococcosis.[33] In the current study, as mentioned in Table 1, most cases are in advanced age, and severe underlying diseases are reported, which might be the reason for possible immunosuppressive statuses and the cause of cryptococcosis. Similar to our study, some previous studies reported extra-pulmonary cryptococcoses in HIV-negative patients from different world regions.[34–37] Moreover, in 64.71% of patients, no apparent underlying diseases were reported. This is nearly similar to previous studies from China, in which 71% and 60% of cases showed no apparent risk factor.[38,39] In the present study, 26 (15.29%) cases were severely immunocompromised (Cancer (n = 8, 4.7%), autoimmune disorder (n = 12, 7.06%), hematological malignancies (n = 6, 3.53%)) and 26 (15.29%) cases were mildly immunocompromised (chronic renal failure (n = 11, 6.47%), diabetes (n = 8, 4.7%), liver cirrhosis (n = 7, 4.11%)). The degree of immunosuppression is undetermined for the other 8 underlying statuses of patients. In previously published studies, both these severe and mildly immunosuppressive states showed an increased risk of cryptococcosis in patients.[40–45]

Cryptococcosis is highly fatal if not diagnosed and treated promptly. Its clinical manifestation is frequently ambiguous.[27] In the current study, fever (n = 113, 66.47%) was found in high proportion, followed by headache (n = 98, 57.64%) and neck stiffness (n = 56, 32.94%), while in six cases (3.52%), chest pain and seizures were reported. Studies revealed that various cryptococcal infections showed different clinical manifestations.[46] According to our data, except for the fever, all other clinical manifestations significantly varied with the type of infection. In cryptococcal meningitis, the headache was found in high proportion, followed by neck stiffness and fever, similar to the previously published report.[47] Fever is a well-known clinical presentation of cryptococcemia, which is reported in our study (n = 35/

50, 70%). While in cryptococcal pneumonia, the cough, shortness of breath, and chest pain were predominant clinical presentations.[24]

The advent of *C. neoformans* with resistance or high MICs exceeding ECVs is also causing concern. Antifungal susceptibility indicated species-specific differential sensitivity, although acquired resistance remained unusual.[48] In the present study, six isolates were MDR, of which four were reported in cryptococcal fungemia, indicating the severity of cryptococcemia.[49] The high numbers of NWT isolates were reported against amphotericin B (8.96%), similar to a previous study reported from China, in which 8.6% of isolates were NWT. However, the proportion of NWT isolates against 5-flucytosine and fluconazole in the present study is lower than the previously published literature from China.[14,50] Worldwide, the estimated prevalence of 5-flucytosine NWT *C. neoformans* isolates is 1–2% but may exceed up to 7% in some studies, and same like in our study, the 4.11% isolates were 5-flucytosine NWT.[51]

Nonetheless, another study from Zhejiang, China, reported only 2% fluconazole NWT isolates, which is closer to our finding (1.29% of isolates).[52] In our finding, the itraconazole and voriconazole NWT *C. neoformans* isolates were 5.15% and 2.53%, respectively. A rise in azole resistance among *C. neoformans* is pervasive worldwide. According to the ARTEMIS DISK Global Antifungal Surveillance Project, azole resistance in *C. neoformans* developed gradually between 1997 and 2007 from 7.3% to 11.7%.[53] Significantly, azole-resistant isolates of *C. neoformans* were uncommon in the United Kingdom and the United States; nevertheless, isolates from Spain, Africa, Cambodia, and were reported to be becoming more resistant.[54] In line with this, reports from Taiwan and Uganda for the past ten years show a rise in azole minimum inhibitory concentrations (MIC) and a decrease in azole susceptibility among *C. neoformans* isolates.[55] The proportion of NWT isolates in the current study and published literature varies depending on several factors, such as the method used for AST determination, the study population and their underlying conditions, and the geographical distribution of *C. neoformans* isolates.[56–58]

The limitation of the current study is its retrospective design, which is based on the available records, and some variables might be missing to finalize a concrete conclusion. Furthermore, the data was collected from a single center which might vary from the other center, indicating that the results might not apply to all locations. However, the current study is the first of its kind, which will provide baseline information for future research work and guidelines for healthcare workers to manage cryptococcosis in the regions.

Conclusion

The study provides important insights into the distribution and clinical characteristics of *Cryptococcus* species infections over six years. The results indicate that *C. neoformans* was the predominant species causing cryptococcal infections, while meningitis was found in high proportion, followed by fungemia and pneumonia. A total of 8-fold increase in cryptococcosis occurred during the study duration. A high number of cases were reported in the male population and senior elderly age group, indicating their immunocompromised status and vulnerability to get infections. Underlying diseases were present in a minority of cases, with autoimmune disorders and chronic renal failure being the most common and all of the cases were HIV negative. A total of 6 MDR isolates were reported, with a high proportion of NWT isolates against amphotericin B. Overall, the isolates recovered from fungemia cases were more resistant than meningitis and pneumonia. The study highlights the need for continued surveillance and management of cryptococcal infections, especially in high-risk populations.

Data Sharing Statement

All the data are presented in the manuscript; any raw data can be available by request to first author (email: bilal. microbiologist@yahoo.com).

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Meizhou People's Hospital (protocol code; 2021-C-106 and date of approval; 25-12-2021).

Patient Consent Form

Patient consent was waived due to the retrospective nature of study and information was collected from the hospital record as a secondary data, and no personal image or figure of patients is included in this study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest.

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