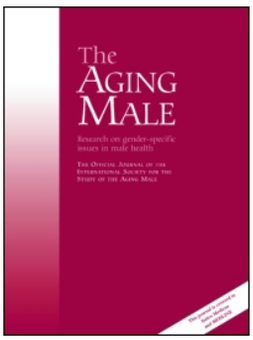

The Aging Male

ISSN: 1368-5538 (Print) 1473-0790 (Online) Journal homepage: https://www.tandfonline.com/loi/itam20

Incidence and clinical outcome of Cryptococcosis in a nation with advanced HIV surveillance program

Fatma Ben Abid, Hussam Abdel Rahman S. Al Soub, Muna Al Maslamani, Wanis Hamad Ibrahim, Hafedh Ghazouani, Abdullatif Al-Khal & Saad Taj-Aldeen

To cite this article: Fatma Ben Abid, Hussam Abdel Rahman S. Al Soub, Muna Al Maslamani, Wanis Hamad Ibrahim, Hafedh Ghazouani, Abdullatif Al-Khal & Saad Taj-Aldeen (2019): Incidence and clinical outcome of Cryptococcosis in a nation with advanced HIV surveillance program, The Aging Male, DOI: 10.1080/13685538.2019.1692198

To link to this article: https://doi.org/10.1080/13685538.2019.1692198

Published online: 19 Nov 2019.

THE AGING MALE
https://doi.org/10.1080/13685538.2019.1692198

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

OPEN ACCESS Check for updates

Incidence and clinical outcome of Cryptococcosis in a nation with advanced HIV surveillance program

Fatma Ben Abid[a], Hussam Abdel Rahman S. Al Soub[b], Muna Al Maslamani[c], Wanis Hamad Ibrahim[d], Hafedh Ghazouani[e], Abdullatif Al-Khal[b] and Saad Taj-Aldeen[f]

[a]Infectious Disease Department, Hamad Medical Corp, Medicine, Hamad General Hospital-HMC, Doha, Qatar; [b]Infectious Diseases Section, Hamad Medical Corp, Medicine, Doha, Qatar; [c]Infectious Diseases, Hamad Medical Corporation, Communicable Diseases Centre, Doha, Qatar; [d]Medicine, Hamad Medical Corporation, Doha, Qatar; [e]Division of Oncology, Hamad Medical Corporation, Doha, Qatar; [f]Hamad Medical Corporation, Doha, Qatar

ABSTRACT

Background: Cryptococcosis is a major opportunistic invasive mycosis that mostly affects immunocompromised patients.

Methods: This was an observational study of all culture-confirmed cases of cryptococcosis conducted in the State of Qatar from January 2005 to December 2016. Cryptococcus fungi were identified using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS).

Results: Fourteen culture-confirmed cases of cryptococcosis were identified during the study period. Four patients had a Human Immunodeficiency Virus (HIV) infection with low CD4 count and five were receiving immunosuppressant medications. The rest of the patients were apparently immuno-competent. The central nervous system was the most common site of infection (57%) followed by bloodstream infection (36%) and pneumonia (14%). One patient had a cryptococcal scrotal infection. Twelve isolates were Cryptococcus neoformans and 2 were Cryptococcus laurentii. All isolates were within the wild type ECV values to amphotericin B and fluconazole. Only 2 patients with bloodstream infection (HIV negative) died. The rest were cured of the infection.

Conclusion: Cryptococcosis is a rare fungal disease in the State of Qatar, mostly diagnosed in Asian immigrants. The central nervous system is the most common site of infection. The presence of the fungus in the blood carries a high mortality.

ARTICLE HISTORY
Received 25 September 2019
Revised 4 November 2019
Accepted 9 November 2019
Published online 15 November 2019

KEYWORDS
Fungus; Cryptococcus; HIV; antifungal

Introduction

Cryptococcosis is a systemic opportunistic fungal infection mainly caused by yeasts belonging to two species complexes: *Cryptococcus neoformans* and *Cryptococcus gattii*. Furthermore, few cases of *Cryptococcus Laurentii* causing human infection were reported in literature. Cryptococcal infections are mostly seen in immunocompromised patients, particularly those with cellular immune defects including advanced HIV infection with predilection of central nervous system [1–4]. The clinical presentation is usually subtle, vague, and indolent over a period of one to two weeks. The prognosis depends on early diagnosis, prompt use of antifungal drugs and reduction of the intracranial pressure. We carried out a retrospective observational study to determine the epidemiological and clinical characteristics of cryptococcosis in the State of Qatar, a country with low HIV prevalence and good surveillance program.

Materials and methods

Study design and population

This was a descriptive study of all culture-confirmed cases (both adult and pediatric cases) of cryptococcosis in the State of Qatar during the period from 1 January 2005 to 31 December 2016. Cases were identified from the data base of the Central Microbiology Laboratory of Hamad General Hospital (HGH) that receives clinical specimens and performs all fungal studies from different hospitals in the country.

Microbiologic methods

The Cryptococcus spp. isolates were stored in cryovials at −80 °C. Each isolate was labeled with specific

CONTACT Fatma Ben Amid fabid@hamad.qa Infectious Disease Department, Hamad Medical Corp, Medicine, Hamad General Hospital-HMC, Doha, Qatar

patient's code and the date of culture. The samples of interest were retrieved and inoculated onto Sabouraud Dextrose Agar (SDA) media and Sabouraud Dextrose Agar plates with chloramphenicol (SDAC) (Difco, USA). Plates were incubated at 35 °C and a single colony was isolated and sub-cultured on SDA plates for 24 h at 30 °C. Cryptococcus spp. isolates were processed for Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS)-based identification of all isolates was performed according to Bruker Daltonics (Bremen, Germany) with the ethanol (EtOH)/formic acid (FA) extraction protocol as reported previously (13). To ensure reproducibility of the spectra, isolates were tested in duplicates and identified by MALDI Biotyper RTC software 3.0 (Bruker Daltonics, Germany). This research was carried out using two databases: the original, commercially available Bruker Daltonics database and the Fungal Biodiversity Center (CBS), Utrecht, The Netherlands in-house build data.

Antifungal susceptibility

The susceptibility of *Cryptococcus* spp. isolates to the antifungal drugs was determined by Sensititre Yeast One method (MCS Diagnostics, Netherlands). The antifungal panel contains amphotericin B (AMB), flucytosine (5-FC), fluconazole (FLU), voriconazole (VOR), posaconazole (POS) and itraconazole (IT). The minimum inhibitory concentrations (MIC) were determined after 72 h of incubation at 35 °C. *Candida krusei* strain ATCC 6258 and *Candida parapsilosis* strain ATCC22019 were used as quality control (QC) strains. Based on the recommendation of other publications, the epidemiological cut-off values (ECVs) of *C. neoformans* for AMB used in the present study was 1 μg/ml; 8 μg/ml for 5-FC and FLU; and 0.25 μg/ml for IT, VOR and POS [5,6]. Isolates with MIC values above the ECVs were considered as non-wild type (non-WT) isolates. Isolates with MIC values equal to the ECVs were considered having reduced susceptibility to antifungal agents.

Statistical analysis

We have used descriptive statistics to summarize patient's demographic, epidemiological, clinical and laboratory characteristics. Mean and standard deviation (SD) were described for the continuous variables with normal distribution. Frequencies and proportions were used for categorical variables. A *p*-value of .05 or less was considered significant. We used STATA version 12.0 (Statacorp, College Station, TX, USA) for exploratory data analysis and descriptive statistics.

Results

Patients and demographic data

A total of 14 cases with culture-confirmed cryptococcal infection were identified over the study period. Baseline demographic and clinical characteristics are summarized in Table 1. The mean annual incidence was 0.65 per 10^6 populations. Males constituted 93% of the diagnosed cases. The median age was 39 years (range: 6 to72). Only one pediatric case was diagnosed with the disease during the study period. The vast majority of patients (93%) were expatriates (mostly from Asian subcontinent). A total of 11 patients (78%) had underlying immunosuppression state (4 patients (28%) had HIV with CD4 count less than 100 cells per μL, 5 patients (35%) were receiving immuno-suppressive medications and 2 (14%) had renal transplant). No apparent risk factor was identified in 3 patients (21%). Subacute manifestation was characteristic, with a median duration of symptoms of 14 days. The most common presenting symptoms were fever (57%), vomiting (50%), headache (43%), photophobia and altered mental status (21%). Central nervous system was the most commonly involved site by the infection in 8 patients (57%) followed by blood stream infection in 5 patients (36%), and pneumonia in 2 (14%). One patient (7%) had a cryptococcal scrotal abscess. Among the isolated Cryptococcus species, 12 were *Cryptococcus neoformans* and 2 were *Cryptococcus laurentii*. The later were isolated from scrotal abscess pus and from blood of two different patients. The patient with scrotal abscess was a 39 year old with no underlying comorbidities underwent incision and drainage with complete resolution of the abscess without antifungal treatment. The other patient with *C. Laurentii* fungemia; was a six year old child with underlying history of congenital muscle dystrophy. He received amphotericin for 2 weeks and recovered completely.

Antifungal susceptibility

The MIC values of the present isolates were determined for six antifungal drugs (Table 1). For the 12 isolates of *C. neoformans* the susceptibility ranges for AMB were (0.12–0.5 μg/ml; 1–8 μg/ml) for FLC; (0.015–0.5 μg/ml for IT); (0.008–0.03 μg/ml) for VOR; (0.03–0.5 μg/ml for POS); and (1–8 μg/ml) for 5-FC.

All isolates of *C. neoformans* were sensitive (WT) to AMB, VOR and FLC. Two isolates (patient # 3) is non-

Table 1. Demographics and clinical features of patients with cryptococcosis and the MIC of the most used antifungal agents for *Cryptococcus* species determined by Sensititre Yeastone method compared to the published ECV values.

Case no.	Gender/ Age (years)	Year of isolation	Patient Origin	Underlying comorbidities	Immuno-suppressive agent treatment	Site of infection	Signs and symptoms	Cryptococcus spp. isolated	MIC μg/ml						Antifungal treatment& duration (days)	Outcome
									5-FC	POS	VOR	IT	FLU	AMB		
1	M/28	2005	India	Renal transplant	Prednisolone cyclosporine	CNS, blood	Fever, headache, N/V, altered mental status, ataxia	*C. Neoformans*	2	0.03	0.015	0.015	2	0.5	AMB (28)	Alive
2	M/39	2007	Indonesia	HIV positive	Non	CNS, Lung	Fever, headache, cough, sputum, SOB (pneumonia)	*C. Neoformans*	4	0.06	0.015	0.03	2	0.25	AMB (16) FLU (30)	Alive
3	M/39	2011	Bangladesh	Non	Non	Scrotum	Scrotal swelling with pus discharge	*C. Laurentii*	2	0.5	0.12	0.025	4	0.25	Non (incision & drainage only)	Alive
4	M/47	2012	India	DM, HTN, CLD	Non	Blood	Fever, Chills & rigors	*C. Neoformans*	Failed to grow						AMP (21)	Died
5	M/66	2013	Jordan	DM, HTN	Non	Lung	Fever, cough, sputum, SOB (pneumonia)	*C. Neoformans*	1	0.06	0.008	0.015	1	0.12	Non	Alive
6	M/27	2014	Philippine	Non	Non	CNS	Fever, headache, photophobia, N/V, neck stiffness.	*C. Neoformans*	4	0.03	0.015	0.015	1	0.5	AMP + Flucytosine (28 days) FLU (28)	Alive
7	F/43	2008	Sudan	DM, HTN, UC	Prednisolone	CNS	Fever, headache, photophobia, Altered mental status, N/V, neck stiffness.	*C. Neoformans*	1	0.06	0.03	0.15	2	0.25	AMP (41)	Alive
8	M/29	2008	Indonesia	HIV positive	Non	CNS, blood	Fever, headache, photophobia, N/V.	*C. Neoformans*	2	0.06	0.015	0.015	2	0.5	AMP (15)	Alive
9	M/19	2009	Philippine	Non	Non	CNS	Headache, N/V, 6th nerve palsy	*C. Neoformans*	4	0.12	0.03	0.03	4	0.25	AMP (39)	Alive
10	M/6	2010	Qatari	CMD	Non	Blood	Not available	*C. Laurentii*	0.25	1	0.5	0.5	2	2	AMP (10)	Alive
11	M/49	2011	Bangladesh	HIV positive	Non	CNS		*C. Neoformans*	4	0.06	0.015	0.015	8	0.5	AMP (14)	Alive
12	M/72	2014	Palestine	HTN, HT, P-ANCA vasculitis	Blood stream infection	Blood	N/V	*C. Neoformans*	8	0.06	0.015	0.06	2	0.25	FLU (5)	Died
13	M/65	2015	Somalia	DM, HTN, CKD, renal transplant	Prednisolone cyclosporine	CNS	Altered mental status, fits.	*C. Neoformans*	1	0.03	0.008	0.015	1	0.25	AMP + Flucytosine (21) FKUC (56)	Alive
14	M/37	2016	India	HIV positive	Non	Lung	Fever, altered mental status, cough, SOB. (pneumonia)	*C. Neoformans*	Not done						FLU (180)	Alive
ECV value *(C. Neoformans)*									**8**	**0.25**	**0.25**	**0.25**	**0.8**	**1**		

5-FC: Flucytosine; POS: Posaconazole; VOR: Voriconazole; IT: Itraconazole; FLU: Fluconazole; AMB: Amphotericin B; M: male; F: Female; DM: diabetes mellitus; HTN: hypertension; CLD: chronic liver diseases; CKD: Chronic kidney disease; UC ulcerative colitis; CMD: congenital muscle dystrophy; HT: hypothyroidism; N: nausea; V: vomiting; SOB: Shortness of breath; BSI: Blood stream Infection; ND: not done
ECV: Epidemiological cutoff values for the most common genotype *Cryptococcus neoformans* (VN1) based on CLSI guidelines M59.

WT for POS and (patient # 12) is with reduced susceptibility for 5-FC (Table 1). Thirteen patients received liposomal amphotericin B during the induction phase for a median duration of 23 ± 10 days. Fluconazole was given during the continuation phase for a median duration of 54 ± 52 days. Liposomal amphotericin B was given to two patients with underlying renal transplant. One patient had only CNS infection and the other one had disseminated infection involving the CNS and the blood. Liposomal amphotericin B was given to these two patients; despite having renal transplant; for life saving with close monitoring of the electrolytes and renal function. Both patients had good recovery with no renal impairment. Only 2 patients (14%) with disseminated disease and blood stream infection (HIV negative) died. The rest of patients were cured from the infection and discharged home.

Discussion

Cryptococcosis is a human opportunistic fungal infection affecting predominantly immuno-compromised patients in particular HIV infection [1,7,8]. Historically, cryptococcosis is rarely seen in immuno-competent patients with no obvious predisposing factors [1,8]. However, with the introduction of anti-retroviral therapy (ART) for HIV infected patients, the infection is becoming more recognized among immuno-competent individuals [8,9]. In our series, cryptococcosis was seen almost equally in patients with HIV infection, patients on immuno-suppressive drugs and in patients apparently immuno-competent. Cryptococcosis is estimated to cause 1 million annual cases globally and nearly 625,000 deaths/year [7,10]. Despite the use of anti-retroviral treatment, the prevalence of cryptococcal infection remains unchanged in low-income and middle-income countries [11]. Rajasingham et al. reported Sub-Saharan Africa to account for 73% of the estimated cryptococcal meningitis cases resulting in 75% mortality rate [11]. Globally, deaths caused by cryptococcal meningitis were much less, accounting for 15% of AIDS-related deaths [11].

The current study reported a low incidence of cryptococcosis in the State of Qatar with non HIV patients being more commonly affected by the disease. A plausible reason for this finding is the very low prevalence of HIV infection in the country. The estimated prevalence was less than 0.1% with only 10 to 15 new cases diagnosed yearly [12]. Taj-Aldeen S *et al* reported that the annual incidence for cryptococcal meningitis in Qatar was 0.43 cases per 100,000 [13]. Qatar has an advanced HIV surveillance program that is responsible for raising awareness about HIV infection among public, free voluntary testing, providing free, high quality care and treatment for HIV-infected subjects. Such efforts could have contributed to early HIV detection and hence, reduce the incidence of opportunistic infections.

The most commonly encountered causative organism of cryptococcal infection is *C. neoformans* with its traditional subtypes; *C. neoformans* – serotypes A, D, and AD and *C. gatti* - serotype B and C. The former is found world-wide and causes cryptococcal meningitis mostly in HIV patients, whereas the latter is geographically restricted; mainly in tropical and subtropical regions; and frequently diagnosed in healthy subjects [14,15]. *C. gatti* is virtually confined to previously healthy hosts and causes more severe disease in such patients [16,17]. Whereas, *C. laurentii* rarely causes infection in human. For instance, *C. laurentii* has been reported in literature in only 19 cases, predominantly of the skin, bloodstream, and central nervous system [10,18]. It has been reported that the most clinically significant risk factors for *C. laurentii* infection are impaired cell-mediated immunity, recent corticosteroid use, and invasive catheter placement.

Central nervous system infection is the most common site of Cryptococcosis. In addition, Cryptococcal meningitis is the most serious manifestation and is the leading invasive opportunistic mycosis of the central nervous system among HIV infected patients with low CD4 count world-wide [1]. The clinical presentation is usually vague and subtle. The most common presenting symptoms are fever and headaches 79% followed by photophobia, transient visual loss, neck pain, vomiting, altered sensorium, seizure and focal neurologic deficit [19,20]. Cranial nerve palsy can occur as well and is most likely due to basal arachnoiditis or due to hydrocephalus. Overt meningeal signs are uncommon. Papilloedema, hydrocephalus, focal deficits and seizures are more commonly seen in immunocompetent hosts. Most of our patients had central nervous system involvement with chronic or subacute presentation prior to diagnosis.

Routine laboratory investigations are usually unhelpful to establish the diagnosis. Lumbar puncture preceded by neuro-imaging is the key for diagnosis for cryptococcal meningitis. Cerebrospinal (CSF) pressure is usually elevated. CSF analysis typically shows lymphocytic pleocytosis with high protein and low glucose in half of the cases. CSF should be tested for India ink, cryptococcal latex agglutination test and fungal culture. The yield of India ink testing is much less in HIV-negative cases compared to HIV-infected

cases (60% and 90% respectively) [19]. This is usually explained by the higher concentration of the yeast in the CSF of AIDS patients as compared to those who are immuno-competent. Latex agglutination test or enzyme-linked immunosorbent assay (ELISA) rarely misses positive cases with the exception of cases with very early disease or in those with very high titers due to the prozone effect [21]. Fungal culture is the gold-standard and is positive in nearly all cases. Nevertheless, cryptococcal culture can take a long time.

The current clinical guideline for cryptococcal meningitis is a combination of intravenous liposomal AMB and oral 5-FC for two to six weeks, followed by maintenance therapy with oral fluconazole for eight weeks [22]. As cryptococcal infection can be life threatening infection, liposomal AMB was given even for two patients with precious kidney transplant. In addition, the intracranial pressure should be maintained below 20 cm H2O. When we apply the recently published ECVs to evaluate the current results, one the isolates in this study showed reduced susceptibility to 5-FC, and all of the isolates were nonresistant to FLU and AMB. Reduced susceptibility to 5-FC or other compounds might results in therapeutic failure.

At present, the resistance of *Cryptococcus* spp. to amphotericin B, fluconazole and flucytosine is less than 1%, but non-susceptible strains have arisen all over the world, suggesting progression to a future broader resistance [23,24]. The lack of susceptibility of *Cryptococcus* spp. to these antifungal agents, in most clinical laboratories may be one of the critical factors determining an adverse outcome in patients with cryptococcosis [25]. It is recommended that antifungal susceptibility testing of *C neoformans* be performed as a routine test in clinical laboratories to improve outcome of patients with invasive cryptococcosis.

A repeat lumbar puncture may be indicated for assessment of the therapeutic response, reducing CSF pressure or confirming fungal elimination [22]. Most patients with cryptococcal meningitis improve with the above treatment. Ventriculo-peritoneal shunting may be considered indicated when previous measures fail or patient shows signs of hydrocyphalus. Various neurological sequelae previously reported include; permanent visual and hearing loss, persisting cranial nerve palsy, and hydrocephalus [1,20]. The current study reported a lower mortality rate of 14% compared to previous studies. [11,21]. A plausible explanation for such a low mortality rate is to the improvement in the diagnosis and care of HIV subjects in this country besides the availability of cryptococcal treatment. Furthermore, the fact that most of the patients in the current study are non-HIV could contribute to the reported lower mortality rate. Previous studies have identified a number of factors that could contribute to poor prognosis in cryptococcal meningitis. Among these factors, is the late diagnosis resulting in a delay in proper treatment (physicians do not think "fungus"). Furthermore, altered sensorium at presentation, high opening CSF pressure >25 cm H2O and high cryptococcal antigen load of more than 1024 can lead to poor outcome [21]. In disseminated HIV cryptococcal infection, molluscum-like cutaneous lesions may be seen [26]. Immuno-compromised individuals, including solid organ transplant recipients, are at higher risk for cryptococcal infections [27].

This current study was the first to describe the epidemiology and clinical outcomes of cryptococcosis in the State of Qatar. Although the incidence of cryptococcal infection was found to be low, the current study emphasized the importance of considering the disease in apparently immuno-competent patients who present with meningitis, particularly those with subacute or chronic symptoms of meningitis.

Conclusion

Cryptococcosis is a rare disease in the State of Qatar that predominantly affects young Asian immigrants. Central nervous system is the most common site of infection. The presence of the fungus in the blood carries a high mortality.

Study limitations

Besides the limitations inherent in retrospective studies, the small sample size identified during the study period limited the detailed description of the clinical characteristics of the study subjects.

Ethical considerations

The study was approved by the Institutional Review Board at Hamad Medical Corporation. A waiver for the requirement to get an informed consent was granted due to the retrospective nature of data collection and analysis.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Perfect JR. The triple threat of cryptococcosis: it's the body site, the strain, and/or the host. MBio. 2012;3.4: e00165-12.

[2] Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS – 100 years after the discovery of *Cryptococcus neoformans*. Clin Microbiol Rev. 1995;8(4):515–548.

[3] Sriwidyani NP, Dewi N, Golden IN, et al. Cerebellar cryptococcal abscess in HIV-negative patient: a case report and literature review. Open Access Maced J Med Sci. 2019;7(8):1353–1355. 29

[4] Poley M, Koubek R, Walsh L, et al. Cryptococcal meningitis in an apparent immunocompetent patient. J Investig Med High Impact Case Rep. 2019;7: 2324709619834578.

[5] Espinel-Ingroff A, Aller AI, Canton E, 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(11):5898–5906.

[6] Espinel-Ingroff A, Chowdhary A, Cuenca-Estrella M, 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(6):3107–3113.

[7] Park BJ, Wannemuehler KA, Marston BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS. 2009;23(4):525–530.

[8] Chan M, Lye D, Win MK, et al. Clinical and microbiological characteristics of cryptococcosis in Singapore: predominance of *Cryptococcus neoformans* compared with *Cryptococcus gattii*. Int J Infect Dis. 2014;26: 110–115.

[9] Chen S, Sorrell T, Nimmo G, et al. Epidemiology and host-and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Clin Infect Dis. 2000;31(2):499–508.

[10] Cheng MF, Chiou CC, Liu YC, et al. *Cryptococcus laurentii* fungemia in a premature neonate. J Clin Microbiol. 2001;39(4):1608–1611.

[11] Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017; 17(8):873–881.

[12] Al Soub H, Al-Khal AL, Al Maslamani M, et al. Epidemiology and the changing face of HIV infection in Qatar. Infect Dis Clin Pract. 2018;26(4):220–223.

[13] Taj-Aldeen SJ, Chandra P, Denning DW. Burden of fungal infections in Qatar. Mycoses. 2015; 58:51–57.

[14] Kwon-Chung KJ, Ashok V. Do major species concepts support one, two or more species within *Cryptococcus neoformans*? FEMS Yeast Res. 2006;6.4:574–587.

[15] Litvintseva AP, Thakur R, Reller LB, et al. Prevalence of clinical isolates of *Cryptococcus gattii* serotype C among patients with AIDS in Sub-Saharan Africa. J Infect Dis. 2005;192(5):888–892.

[16] Mitchell DH, Sorrell TC, Allworth AM, et al. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. Clin Infect Dis. 1995; 20(3):611–616.

[17] Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. Clin Infect Dis. 1995;21(1):28–34.

[18] Smith N, Sehring M, Chambers J, et al. Perspectives on non-neoformans cryptococcal opportunistic infections. J Commun Hosp Intern Med Perspect. 2017; 7(4):214–217.

[19] Satishchandra P, Mathew T, Gadre G, et al. Cryptococcal meningitis: clinical, diagnostic and therapeutic overviews. Neurol India. 2007;55(3):226.

[20] Cox GM, Perfect JR. *Cryptococcus neoformans* var. *neoformans* and *gattii* and *Trichosporon* species. Topley and Wilson's microbiology and microbial infections-medical mycology. 9th ed. New York, NY: Oxford University Press; 1998. p. 461–484.

[21] Bongomin F, Gago S, Oladele RO, et al. Global and multi-national prevalence of fungal diseases—estimate precision. J Fungi. 2017;3(4):57. Dec

[22] Perfect JR, Dismukes WE, Dromer F, 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(3):291–322.

[23] Mahabeer Y, Chang CC, Naidu D, et al. Comparison of Etests and Vitek 2® to broth microdilution for the susceptibility testing of *Cryptococcus neoformans*. Diagn Microbiol Infect Dis. 2014;80(4):294–298.

[24] Gullo FP, Rossi SA, Sardi JC, et al. Cryptococcosis: epidemiology, fungal resistance, and new alternatives for treatment. Eur J Clin Microbiol Infect Dis. 2013;32(11): 1377–1391.

[25] Castanheira M, Messer SA, Rhomberg PR, et al. Antifungal susceptibility patterns of a global collection of fungal isolates: results of the SENTRY Antifungal Surveillance Program (2013). Diagn Microbiol Infect Dis. 2016;85(2):200–204.

[26] Srivastava GN, Tilak R, Yadav J, et al. Cutaneous Cryptococcus: marker for disseminated infection. BMJ Case Reports. 2015;2015:bcr2015210898.

[27] Kothiwala SK, Prajapat M, Kuldeep CM, et al. Cryptococcal panniculitis in a renal transplant recipient: case report and review of literature. J Dermatol Case Rep. 2015;9(3):76.