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Review article

Current epidemiology and implication for microbiological diagnosis of cryptococcal infections in Korea

Myeong Hee Kim
Department of Laboratory Medicine, Kyung Hee University College of Medicine and Kyung Hee University Hospital at Gangdong, Seoul, Korea

Abstract

Cryptococcosis is a major invasive fungal infection affecting both immunocompromised and immunocompetent hosts worldwide and is mainly caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. *C. neoformans* accounts for 90% of all infections and primarily causes central nervous system infections. Although *C. gattii* is primarily found in tropical and subtropical regions, infections have recently been reported in temperate areas such as Korea. Genetic studies in Korea indicated that most *C. neoformans* strains are of the VNI-ST5 type and show genetic homogeneity. In contrast, genetic studies on *C. gattii* are limited. Cryptococcosis is diagnosed using microscopy, serological tests, culture, and molecular tests. Although it can be detected in the cerebrospinal fluid or body fluids using the India ink method, confirmation through culture is essential for a definitive diagnosis. Cryptococcal antigen testing is economical, highly sensitive, and specific; therefore, it is widely used for the diagnosis of cryptococcosis. Molecular methods have recently been introduced; however, their applications remain limited. The recommended treatment for cryptococcal infections includes amphotericin B alone or in combination with flucytosine or fluconazole. Secondary resistance to flucytosine and fluconazole is common. The Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing recommend the broth microdilution method. Several commercial methods for antifungal susceptibility testing have been developed and are currently in use; however, more data are required to establish breakpoints.

Keywords: Cryptococcosis, *Cryptococcus gattii*, *Cryptococcus neoformans,* Laboratory diagnosis

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Corresponding to
Myeong Hee Kim
E-mail: meikim96@hanmail.net

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Introduction

There are more than 30 identified species of *Cryptococcus*, with *Cryptococcus neoformans* and *Cryptococcus gattii* being the major pathogens causing cryptococcosis [1,2]. These two pathogens exhibit different characteristics in terms of geographical distribution, epidemiology, and clinical symptoms. Infections caused by *C. neoformans* primarily affect immunocompromised hosts by targeting the central nervous system and causing meningoencephalitis. In contrast, *C. gattii* infects immunocompetent hosts and predominantly affects the respiratory system [3-5].

C. neoformans is widely distributed globally, accounting for 90% of cryptococcal infections, and is commonly found in natural environments, particularly in bird droppings and soil [6]. In contrast, *C. gattii* has

been associated with eucalyptus trees in tropical and subtropical climates until the late 1990s. However, it has recently been found in temperate regions, such as North America [7,8], and infections have rarely been reported in Asia, including Korea [9-13].

In Korea, the incidence of cryptococcal infections has been increasing among immunocompromised patients, including those undergoing treatment for malignant diseases, organ transplant recipients, and those with acquired immune deficiency syndrome (AIDS). In this review, we aimed to comprehensively examine recent epidemiological and diagnostic methods for cryptococcal infections.

Pathogens and epidemiology

Cryptococcosis is associated with high morbidity and mortality. Human immunodeficiency virus (HIV) and AIDS are major risk factors in sub-Saharan Africa. However, there are an increasing number of cryptococcosis reports among non-HIV-associated immunocompromised risk groups and immunocompetent individuals [14]. For example, *C. neoformans* infections have been reported in immunocompetent patients in Korea [15-17].

The major causative agents of cryptococcosis are *C. neoformans* and *C. gattii*, which are classified into five serotypes based on the antigenicity of their capsules: serotype A, *C. neoformans*; serotype D, *C. deneoformans*; serotype AD, a hybrid of *C. neoformans/C. deneoformans*; and serotypes B and C, *C. gattii*. Subsequent genetic studies using molecular tests have revealed variations within *C. neoformans* and *C. gattii*, leading to the reclassification of *C. neoformans* into *C. neoformans* and *C. deneoformans*, and *C. gattii* into five different species: *C. gattii, C. bacillisporus, C. decagattii, C. deuterogattii,* and *C. tetragattii* [18]. These two species are divided into the following eight molecular types based on genetic differences: serotype A—VNI and VNII; serotype AD—VNIII; serotype D—VNIV; serotypes B and C— VGI, VGII, VGIII, and VGIV [19,20]. Globally, *C. neoformans* VNI is the most common genotype, followed by *C. neoformans* VNII, *C. deneoformans* VNIV, *C. bacillisporus* VGIII, *C. gattii* VGI, *C. neoformans/deneoformans* hybrid VNIII, and *C. gattii* VGII. Among HIV-infected individuals, *C. neoformans* VNI is the most common genotype, followed by *C. gattii* VGII. In Korea, *C. neoformans* VNI is the most frequently isolated genotype, and other molecular types identified are VNIV, VGI, VGII, and VGIII [12,13]. The International Society of Human and Animal Mycoses working group proposed a multilocus sequence typing (MLST) method to obtain global genotype data, highlighting its high discriminatory power and interlaboratory reproducibility. The proposed international standard genetic loci are *CAP59*, *GPD1, LAC1, PLB1*, *SOD1, URA5*, and *IGS1* [19]. In Asia, including Korea, Japan, and China, studies using MLST have indicated that *C. neoformans* VNI-ST5, which has low genetic diversity, is the most common [10,12,21-25] (Table 1). Among the few genotyping studies in Korea, some have reported that among the 46 clinical specimens collected between 2008 and 2012, 95.7% were *C. neoformans* VNI-ST5, with the remainder being the VNI-ST31 genotype [13]. In another study involving 140 clinical specimens collected from 1993 to 2014, *C. neoformans* VNI-ST5 was predominant, and ST31 and ST127 were also identified; the three isolates of *C. gattii* were identified as ST57, ST7, and ST113 [12]. Research indicates that *C. neoformans* is genetically homogeneous and that genotypes appearing in the environment are closely related to those found in clinical specimens [12]. *C. gattii* infections are rare in Korea; therefore, information on the molecular characteristics of *C. gattii* strains is limited.

Table 1. Distribution of molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii* isolates from Korea, Japan, and China by multilocus sequence typing

Country	Source	Molecular type																				Reference
		VNI										VNII	VNIV	VGI		VGII					VGIII	
		ST4	ST5	ST31	ST32	ST38	ST53	ST63	ST191	ST359	ST360	ST43	ST127	ST57	ST159	ST7	ST21	ST44	ST126	ST173	ST113	
Korea	Clinical		32	4									1	1		1					1	[12]
	Environmental		7	3																		
Korea	Clinical		6													2						[21]
Japan	Clinical		31		1							3										[22]
	Environmental	4	4																			
China	Clinical		34	3				1		2	1				1			1				[10]
China	Clinical		22	1				1														[23]
China	Environmental		5	54		1	10		8					3								[24]
China	Clinical															2.	1	2	1	1		[25]

Abbreviation: ST, sequence type.

Laboratory diagnosis

Cryptococcal infection can be diagnosed by microscopic observation of encapsulated yeast-like fungi in tissues or body fluids, such as cerebrospinal fluid (CSF), and confirmed by culture. Antigen (Ag) testing is also sensitive and specific, aiding in diagnosis.

C. neoformans can be detected in CSF or body fluids using the India ink method. The capsule of *C. neoformans* is composed of polysaccharides, such as glucuronoxylomannan and galactoxylomannan, which are best observed using India ink staining. The ink particles do not penetrate the capsule and form a halo around the cells [26]. However, its sensitivity is less than 90% and in cases of low fungal burden, such as during early infection (≤ 1000 CFU/mL), sensitivity decreases further, necessitating confirmation by culture [27]. Additionally, a positive microscopic finding should only be considered a treatment failure or relapse if it is accompanied by clinical deterioration or a positive culture result in patients undergoing treatment [14].

It is recommended to culture a large volume (5-10 mL) of CSF with a culture positivity rate of 90%-95% [27]. In addition to CSF, *C. neoformans* can also be cultured from blood, sputum, pleural fluid, urine, and prostate fluid. The positivity rate in blood cultures is approximately 10%-30%, and it is beneficial to perform a tissue biopsy alongside CSF and blood cultures, owing to the low sputum positivity rate in pulmonary cryptococcosis (10%-20%) [27]. *C. neoformans* forms colonies within approximately three days of culture and appears as cream-beige-colored mucoid colonies on Sabouraud dextrose agar (SDA) due to the encapsulation of yeast cells. Birdseed agar containing caffeic acid, produces brown and black colonies. Microscopically, they appear as round yeasts on SDA and as budding yeasts with thick dark walls on cornmeal-tween 80 agar.

Detecting cryptococcal Ag is a highly reliable method for diagnosing cryptococcosis [28,29]. Cryptococcal Ag can be detected in the serum, plasma, or CSF using latex agglutination, with sensitivity and specificity both exceeding 90% despite some manufacturer variability, although the sensitivity is somewhat lower for *C. gattii* detection [30-32]. Serum Ag tests revealed 66% and less than 10%-30% positivity in systemic and pulmonary cryptococcosis cases, respectively [27]. Due to their similar antigenicity to *Trichosporon beigelii*, false positives can occur in the serum and CSF of patients with disseminated trichosporonosis [28]. Rare false positives have also been reported in patients with gram-negative bacterial sepsis or cancer [28,33]. Moreover, false negatives may occur when the fungal burden is very low, there is a prozone-like phenomenon, or the capsule is not well-developed [34].

Molecular diagnostic methods have not been widely developed, owing to the availability, high sensitivity, and low cost of cryptococcal Ag testing. The FilmArray meningitis/encephalitis panel (Biomerieux) is a multiplex polymerase chain reaction assay used to detect *C. neoformans* and *C. gattii* in CSF for the diagnosis of cryptococcal meningitis. Meta-analysis suggested that this test has high diagnostic accuracy, with a sensitivity and specificity of 90% and 97%, respectively. The false positive and negative rates were found to be 11.4% and 2.2%, respectively [35]. However, the Filmarray M/E panel is limited because it cannot differentiate between *C. neoformans* and *C. gattii*. Next-generation sequencing can detect low fungal burdens before clinical symptoms appear [36]. However, these molecular methods are not widely used because of their high costs and limited availability.

Antifungal susceptibility test

Resistance to antifungal agents among *Cryptococcus* spp. is relatively common and often associated with prior drug exposure [37]. Secondary resistance frequently arises when flucytosine or fluconazole is used as a monotherapy, owing to the use of amphotericin B either alone or in combination with flucytosine or fluconazole, which is recommended for the treatment of cryptococcal meningitis or other invasive infections [14].

Triazoles and amphotericin B target fungal cells either by directly attacking or altering the synthesis of the enzyme Erg 11 or by depleting ergosterol, respectively [37-40]. Flucytosine resistance can occur through genetic mutations that prevent drug uptake or disrupt the target nucleic acid synthesis pathways. Additionally, cell capsule formation may alter the cell walls and induce resistance. Fluconazole resistance arises from modification of efflux pumps [37,39]. *Cryptococcus* spp. are inherently resistant to echinocandins because of cellular changes that enable rapid or transient adaptation, leading to resistance to these agents [41].

Breakpoints (BPs) and epidemiological cut-off values (ECVs) play important roles in predicting clinical outcomes. The development of BPs requires pharmacokinetic and pharmacodynamic data from animal models, whereas ECVs require clinical and microbiological outcome data obtained from clinical trials. ECVs are a new interpretive endpoint that classify strains as wild-type and non-wild-type without categorizing them as susceptible or resistant, which is not the same as susceptibility or resistance. ECVs can be developed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M57 document [42]. There is no established clinical minimum inhibitory concentration (MIC) BP for fluconazole against *Cryptococcus* spp. and there are insufficient data to suggest that high MICs are correlated with poor outcomes [14]. Accurate species identification is necessary to interpret the ECVs proposed by the CLSI. The cutoff values for *C. neoformans* VNI, *C. gattii* VGI, and *C. deuterogattii* VGII are 8 μg/mL, 16 μg/mL, 32 μg/mL, respectively [42]. An increase in MIC by more than two-fold during treatment suggests the development of resistance, necessitating more thorough clinical monitoring [14]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has not proposed an ECV for fluconazole; however, it provides ECVs for *C. neoformans* with amphotericin B (1 mg/L), posaconazole (0.5 mg/L), and voriconazole (0.5 mg/L). Additionally, EUCAST provided ECVs for *C. gattii* with amphotericin B (0.5 mg/L) and posaconazole (1 mg/L) [43].

The CLSI and EUCAST recommend the broth microdilution (BMD) method [43,44]. Several commercial methods have been developed based on this reference method and are currently in use, including VITEK 2 (bioMérieux), Sensititre YeastOne panel/plate (Thermo Fisher Scientific), and the E-test (bioMérieux). These methods are convenient, effective, and widely used [45]. However, it is necessary to accumulate suitable clinical data to establish the BPs for these commercial methods.

Conclusions

Cryptococcus spp., which are known to cause infections in immunocompromised individuals, have recently been reported to infect immunocompetent hosts. Additionally, infections by *C. gattii* have been reported in temperate regions, indicating a shift in the epidemiology of *Cryptococcus* spp. infections. A recent study in Korea confirmed the epidemiological homogeneity between clinical isolates and environmental strains. However, research on genotypes in Korea remains limited. Antifungal susceptibility testing uses various commercial methods based on the BMD method; however, BPs have not yet been established. Thus, the collection of clinical data is considered necessary for appropriate diagnosis and treatment.

Ethics statement

It is not a human population study; therefore, approval by the Institutional Review Board or the obtainment of informed consent is not required.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

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Data availability

None.

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