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MICROBIOLOGY

CRYPTOCOCCOSIS: A bibliographic narrative review on antifungal resistance

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Abstract: Cryptococcosis is an infectious fungal disease widely studied for its epidemiological importance in the context of public health, given the high morbidity and mortality associated with this invasive fungal infection. Many cases of the disease present clinical resistance and progress to death, even in the presence of antifungal therapy. The prolonged use of triazole drugs to maintain the treatment of cryptococcosis in AIDS patients, can lead to selective pressure from mutant strains, among other resistance mechanisms, justifying the poor clinical evolution of some cases. In this study, a narrative review of the literature on the occurrence of antifungal resistance in cryptococcosis agents was performed. Publications from 2010 to 2022 that address this topic were selected using Google Scholars and Scopus website. Data from the studies were analyzed for the values of minimum inhibitory concentration (MIC) of drugs used in the management of cryptococcosis. The review showed that the highest MIC values occurred for voriconazole, especially against *C. neoformans*. It is concluded that there is a lack of studies with statistical analysis of the data obtained, in order to provide a better dimensioning of the resistance rates of cryptococcosis agents to different antifungal agents, both in geographical and temporal context.

Key words: Azole, *Cryptococcus,* resistance, susceptibility.

INTRODUCTION

Cryptococcosis is a systemic fungal infectious disease - subacute to chronic - that can affect humans and other wild and companion animals, such as dogs, cats, horses, cows, sheep, goats, ferrets, llamas, koalas, penguins, seals and dolphins (Santos 2018, Headley et al. 2015, França 2015, Santana 2016, Schmertmann et al. 2019, Danesi et al. 2021, Devoto et al. 2022).

The causative agent is an encapsulated and cosmopolitan yeast, found in several environmental sources, belonging to the genus *Cryptococcus* (Rêgo et al. 2019, Santana 2016).

Cryptococcus was first isolated from peach juice, in 1894, by the scientist Francesco Sanfelice from the Hygiene Institute of University of Cagliari in Italy, which demonstrated the agent's ability to produce lesions when inoculated into laboratory animals. In the same year, Otto Busse and Abraham Buschke isolated the agent from a tibial lesion in a female patient, which was the first description of the disease. Until the 1950s, many nomenclatures were used for the genus when *Cryptococcus* was defined and classified in the phylum *Basidiomycota*, class *Tremellomycetes* and order *Tremellales* (Rodrigues et al. 2018, Santos 2018, Pizani & Santos 2017, Vieira Júnior 2015, Bastos 2017).

The etiological agents of cryptococcosis belong to two complexes: *Cryptococcus neoformans* and *C. gattii*, which present a great genetic variability. For this reason and based

on molecular studies, there is a proposal of its division into 7 species: *C. neoformans* (*C. neoformans* variety *grubii* encompassing 3 molecular types VNI, VNII, VNB and strains of serotype A), *C. deneoformans* (*C. neoformans* variety *neoformans*, VNIV, serotype D), *C. gattii* (VGI), *C. deuterogattii* (VGII), *C. bacillisporus* (VGIII), *C. tetragattii* (VGIV) and *C. decagattii* (VGIV/VGIIIc) encompassing serotypes B and C. There are isolates that represent 4 interspecies hybrid forms, such as *C. neoformans* × *C. deneoformans* (VNIII), *C. deneoformans* × *C. gattii* (hybrid VGI), *C. neoformans* x *C. gattii* (hybrid VGI) and *C. neoformans* x *C. deuterorogattii* (hybrid VGII) (Cuomo et al. 2018, Maziarz & Perfect 2016, Hagen et al. 2015, Kwon-Chung et al. 2017). A recent work in Zambia, Africa, led to the discovery of a new lineage of *C. gatti* (VGV) comprises two subclades (A and B) (Farrer et al. 2019).

The complex with the highest clinical occurrence and worldwide distribution is *C. neoformans*, which mainly affects immunosuppressed patients. This agent can be found in several environmental sources, it is often associated with excreta of domestic pigeons (*Columba livia*) although they have already been found in samples of excreta from other birds and bats, and the fungus uses this substrate as a source of nitrogen for their survival and reproduction (Lima et al. 2015, Araújo Júnior et al. 2015, Firacative et al. 2018, Ashton et al. 2019, Andrade-Silva et al. 2018). Pigeons, being easily found in urban centers, have been considered a public health problem because they are vectors of cryptococcosis agents, since they are very resistant to desiccation and can remain viable for up to two years in excreta not directly exposed to sunlight and high temperatures (Ribeiro et al. 2019, 2017, Rosa et al. 2016, Colombo et al. 2015). Avian cryptococcosis is not common and human cryptococcosis is not considered a classic anthropozoonosis and therefore contact with sick animals is not sufficient to transmit the disease to humans (Canavari et al. 2017, Santana 2016).

Cryptococcus gattii is rarely found in bird droppings and studies suggest that its primary natural habitat is decaying wood, hollow trees of several species such as *Eucalyptus camaldulensis*, which represents a niche not only for *C. gattii* but also for *C. neoformans* (Santos 2018, Alves et al. 2015, Araújo Júnior et al. 2015, Rocha 2017). *C. gattii* can cause primary infection in immunocompetent hosts or in immunocompromised hosts, being endemic in tropical and subtropical areas. However, reservoirs - abiotics and animals - and cases of infection have been described in temperate areas, such as the Northwest USA, Western Canada and Northern Europe, demonstrating the great capacity for dispersal and adaptation of this species complex, hypotheses suggest anthropogenic mechanisms (travel, animal trade, contaminated materials) and natural ones (tsunamis, earthquakes, erosion) (Canavari et al. 2017, May et al. 2016, Vieira Júnior 2015, Melo 2015, Engelthaler & Casadevall 2019).

Participate in the definition of the infectious process: the virulence of the infecting strain, the immunological status of the host and the acquired fungal load. Cryptococcosis is acquired by inhaling fungal propagules, contained in bioaerosols that are suspended in atmospheric air and deposited in lung tissue. In the lungs, colonization may occur, leading to the occurrence of asymptomatic cases or the development of infection with acute or chronic respiratory distress syndrome (Ribeiro et al. 2017, Araújo Júnior et al. 2015, Silva et al. 2020, Vieira Júnior 2015, Bastos 2017). The etiologic agent can then spread through the hematogenous route, reaching other organs such as the skin, bones and joints, eyes, genitourinary tract and lymph nodes, having a strong tropism for the central

nervous system (CNS). The neurological clinical forms are meningoencephalitis, meningitis or cryptococcomas (fungal masses), which can generate neurological sequelae or cause death (Silva 2018, Pinheiro 2019, Amburgy et al. 2016, Moreira et al. 2017, Bauer et al. 2018, Williamson et al. 2017, Miyazato 2016).

The relationship of *Cryptococcus* with the host is important, since immunocompromised patients such as: organ transplant patients, with hematological diseases and undergoing chemotherapy, under prolonged use of corticosteroids, those with acquired immunodeficiency syndrome (AIDS) and with a CD4 counting below 100 cells/mm^3 are prone to the development of the disease, being considered as the greatest risk group today (Quintero et al. 2019, Wong et al. 2017, Lima et al. 2015, Costa et al. 2019, Cicora et al. 2015, Rajasingham et al. 2017, Fang et al. 2020, Nunes et al. 2018, Quaresma et al. 2019, Zeng et al. 2021). HIV infection produces a significant drop in the number of T CD4 lymphocytes, also compromising the function of infected macrophages causing interference in the human body's defense mechanism against infections, allowing several opportunists infections including cryptococcosis. This invasive mycosis was considered a rare disease worldwide until the 1980s, when the HIV epidemic spread becoming an important opportunistic infection in this population of patients, with neurocryptococcosis having high fatality rates. Several studies emphasize the urgent need for better health structures and antifungal drugs, especially in Africa where many patients die each year due to cryptococcal disease associated with HIV (Castro 2018, Ferreira-Paim et al. 2017, Torres et al. 2016, Chen et al. 2019, Azambuja et al. 2018, Vieira Júnior 2015, Rodrigues 2016, Amburgy et al. 2016, Gouvea et al. 2018, Driemeyer et al. 2022, Rajasingham et al. 2022).

Recently the World Health Organization (WHO) published the list of fungal priority pathogens to guide researches and development of public health actions, contributing to the mycology area (WHO 2022).

Cryptococcosis is treated with antifungal drugs either orally or intravenously. The treatment of cryptococcal meningitis in AIDS patients is instituted in 3 phases. In the 1st phase, or induction, a potent and fungicidal drug, amphotericin B, is used, preferably in combination with 5-flucytosine (5FC), although this is not available in several countries. In low- and middle-income countries, fluconazole is used in the induction phase, either as monotherapy or in association with amphotericin B. Given the high toxicity of amphotericin B in the form of deoxycholate, especially for the renal system, other alternatives are: liposomal and lipid complex. The 2nd phase of treatment, called consolidation, anticipates the 3rd phase of maintenance, in which drugs are administered in decreasing doses over time, such as fluconazole or itraconazole, as the second-choice drug due to lower efficiency (França 2015, Bongomin et al. 2018, Molloy et al. 2018, Schiave et al. 2018, WHO 2018).

Fluconazole, like itraconazole and voriconazole, belongs to the class of azoles that have lower toxicity, compared to lipid or liposomal amphotericin B, and lower treatment costs. Azoles are time-dependent drugs, being fungistatic at the beginning of treatment and becoming fungicidal, through the inhibition of an enzyme, lanosterol 14 α – demethylase encoded by the erg11 gene, of the large cytochrome p450 family. This enzyme participates in the demethylation of lanosterol in the biosynthesis of ergosterol, a vital component of the fungal cell membrane. Amphotericin B, in turn, is a polyene that binds to ergosterol and induces the formation of

channels that compromise membrane integrity and increase its permeabilization, leading to ion leakage followed by fungal cell death. 5FC is a fluoropyrimidine that itself has no antifungal toxicity but produces toxic metabolites that inhibit fungal DNA and RNA synthesis (Altamirano et al. 2017, Truong 2019).

Other drug classes can be used synergistically with antifungals in cryptococcosis therapy, such as sertraline, nifedipine, nisoldipine, felodipine, flubendazole, minocycline, ursolic acid, betulinic acid, biphosphonates and essential oils. (Gullo et al. 2013, Tullio et al. 2017, Truong 2019, Pinheiro et al. 2019, Kong et al. 2020, Krummenauer et al. 2019, Kane et al. 2021, Scalas et al. 2018). Additional studies on repositioning of drugs with fungistatic or fungicidal action in the search for new agents for cryptococcosis´s treatment are important and necessary, given the therapeutic failures with traditional medicines (Truong 2019, Smith et al. 2015).

The determination of antifungal action can be performed in vitro, with reference methods that are based on broth microdilution, according to procedures described in documents (M27 series) published by the North American Institute CLSI or by the E.Def 7 series by the European Committee EUCAST-AFST (CLSI 2017, Arendrup et al. 2012).The minimum inhibitory concentration (MIC) can classify the isolate's high or low susceptibility to antifungal, according to the interpretive cutoff points available in the CLSI and EUCAST documents.

This work aims to prepare a narrative literature review on antifungal resistance in cryptococcosis agents, due to the importance in clinical medicine and the social and economic impact caused in public health.

MATERIALS AND METHODS

The search for works was carried out from January 2010 to December 2022, through the Google Scholars and Scopus website for access to scientific journals containing articles, in addition to master's dissertations and doctoral theses on the topics.

The works chosen for results and discussion were those in which molecular analyzes allowed the identification of the species complex and/or molecular type of Cryptococcus and also, those that contemplated the determination of the MIC of drugs, to assess the susceptibility of cryptococcosis agents to antifungal agents. Among the latter, only studies were selected that used reference methods to determine MIC, recommended by the *Clinical and Laboratory Standards Institute* (CLSI) from USA or by the *European Committee On Antimicrobial Susceptibility Testing- Antifungal Susceptibility Testing* (EUCAST-AFST), or even those that used the commercial method by gradient diffusion E-test® (BioMerieux, Marcy l'Etoile, France).

Twenty-two studies were selected for the narrative review. Although the selected studies presented MIC results of drugs used to treat cryptococcosis, not all of them interpreted the data according to parameters that allowed comparison between the studies. The following parameters were the most commonly adopted in the studies: MIC value necessary to inhibit 50% (MIC50) and/or 90% (MIC90) of the set of isolates, minimum and maximum values of MIC (range). Such parameters allowed verifying the occurrence of resistance in *C. neoformans* and *C. gattii* , according to geographic region (Table I).

Table I. Summary of 22 studies on antifungal resistance in cryptococcosis agents (2010-2022).

Author and year	Country	Types of study	Agent Etiological (number of isolates)	Minimum Inhibitory Concentration (MIC; mg/L)								Method used for MIC test	Conclusions
				fluconazole		amphotericin B		Itraconazole		Voriconazole			
				MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}		
Govender et al. 2011	South Africa	Prospective (clinical)	*C. neoformans* (237)	MIC range (mg/mL) 0,25-16		MIC range (mg/mL) 0,008-0,94		MIC range (mg/mL) 0,015-1		MIC range (mg/mL) 0,008-0,25		CLSI M27-A3	Fluconazole resistance not shown in isolates from South Africa.
Pfaller et al. 2011	USA	Retrospective and Prospective (clinical)	*C. neoformans* (285)	MIC range (mg/mL) 0,25-32		----------		----------		MIC range (mg/mL) 0,008-0,5		CLSI M27-A3	Almost all the isolates were classified as wild and without resistance mechanisms to fluconazole (96.9%) and voriconazole (95.1%).
Espinel-Ingroff et al. 2012a	Global	Prospective (clinical)	*C. neoformans* (3.590) *C. Gatti* (985)	----------		MIC range (mg/mL) ≤0,03-4 ≤0,03-2		---------- ----		---------- ----		CLSI M27-A3	The epidemiological cut-off point for amphotericin B was proposed at MIC ≥ 2ug/ml.
Pan et al. 2012	China, Japan, India, Indonesia, Thailand, Kuwait and Qatar	Retrospective and Prospective (clinical and environmental)	*C. neoformans* (493)	MIC range (mg/mL) 0,125-32		MIC range (mg/mL) 0,063-1		MIC range (mg/mL) <0,016-0,5		MIC range (mg/mL) <0,016-0,5		CLSI M27-A3	High sensitivity of the isolates for several antifungals, both clinical and environmental, but resistance was found to flucytosine (<0.063->64 ug/mL) and to fluconazole (0.125-32 ug/mL).
Trpkovic et al. 2012	Serbia	Prospective (clinical)	*C. neoformans* (31)	16	64	0,125	0,25	0,25 0,38		--------- ----		E-test®	The isolates were highly sensitive to amphotericin B (100%) and flucytosine (87.1%) but little sensitive to fluconazole (48.4%).
Espinel-Ingroff et al. 2012b	Global	Prospective (clinical)	*C. neoformans* (5733) *C. gattii* (975)	MIC range (mg/mL) <0,12- ≥64 0,25- ≥64		----------		MIC range (mg/mL) ≤0,008-≥4 ≤0,008-2		MIC range (mg/mL) ≤0,008-≥4 ≤0,008-1		CLSI M27-A3	ECVs have been proposed for fluconazole (8-32 ug/ml), itraconazole (0.25-1 ug/ml), posaconazole (0.25-0.5 ug/ml) and voriconazole (0.12-0.25 ug/ml).
Lee et al. 2012	Taiwan	Retrospective (clinical)	*C. neoformans* (46)	MIC range (mg/mL) 2-32		MIC range (mg/mL) 0,25-2		---------- ----		MIC range (mg/mL) 0,06-0,5		CLSI M27-A3	Isolates with primary resistance to amphotericin B (MIC >1 ug/mL) (17.4%) or to fluconazole (>8 ug/mL) (26.1%), with recommendation of additional and larger studies to confirm the data.
Gast et al. 2013	USA	Retrospective Prospective (clinical)	*C. gattii* (25) (Pacific Northwest) *C. gattii* (34) *C. neoformans* (20)	16 8 8	64 32 32	0,5 0,5 0,5	1 1 1	1 0,5 1 0,5	1 1 2	0,5 0,25 0,25 0,5	1 1	CLSI M27-A3	The MIC values of azoles against 25 isolates of *C. gattii* from the Pacific Northwest (western Canada and Northwestern United States) were higher than those obtained from 34 from other regions and from 20 of *C. neoformans*.

Table I. Continuation.

Ferreira et al. 2015	Brazil	Prospective (clinical)	*C. gattii* (11)	----------	----------	MIC range 0,06-0,25 mg/L	----------	CLSI M27-A3	Heteroresistance to itraconazole was observed, as an adaptive phenomenon and intrinsic to *C. gattii* strains.
Smith et al. 2015	Uganda	Prospective (clinical)	*C. neoformans* (198)	MIC range (mg/L) 0,125-64	MIC range (mg/mL) 0,125-2	----------	----------	CLSI M27-A3	Fluconazole MIC values tended to increase over the course of drug treatment, but a correlation with clinical failure has not been established.

Yang et al. 2015	USA	Prospective (clinical)	*C. gattii* VGII (1)	MIC (mg/L) 32 (>128)	MIC (mg/L) 0,125	MIC (mg/L) 2	MIC (mg/L) 1	CLSI M27-A3 and E- test®	Increased production of PDR11 efflux pumps was responsible for fluconazole resistance in the studied strain.
Córdoba et al. 2016	Argentina	Retrospective (clinical)	*C. neoformans* (707)	8 16	0,25 0,5	0,03 0,25	0,13 0,25	EUCAST E.Def7.2	New ECV values were proposed for azoles and amphotericin B and for isolates from Argentina.

Rossi et al. 2016	Brazil	Prospective (clinical and environmental)	*C. neoformans* (3) *C. gattii* (2)	MIC range (mg/L) 2-64 4-64	MIC range (mg/L) 0,125-0,0625 0,125-0,0625	----------	----------	CLSI M27-A3	The adaptation of strains to the stress produced by exposure to drugs led to loss of virulence, and morphological changes.
Lomes et al. 2016	Brazil	Retrospective (clinical)	*C. neoformans* (2) *C. gattii* (7)	MIC range (mg/L) 1 – 4 (50%) 32 (28,5%) 64 (14,3%)	----------	----------	----------	CLSI M27-A3	Lower sensitivity to fluconazole was observed among *C. gatti* isolates when compared to *C. neoformans*.
Alves 2016	Brazil	Prospective (environmental)	*C. neoformans* (10) *C. gattii* (4)	------ 24 ------ 256	------ 0,25 ------ 0,38	------- 0,38 ------- 3	----------	E-test®	Most isolates were sensitive to amphotericin B, itraconazole, ketoconazole, and posaconazole. Half of the *C. gattii* isolates were resistant to fluconazole.
Figueiredo et al. 2016	Brazil	Prospective (clinical)	*C. neoformans* (39) *C. gattii* (11)	------- 2 -------- 4	------ 0,5 ------ 0,5	------ 0,5 ------ 1,0	----------	CLSI M27-A2	No increase in fluconazole resistance was observed over the years.

Worasilchai et al. 2017	Thailand	Prospective (clinical and environmental)	*C. neoformans* (73 clinical) *C. deuterogattii* VGII (1) *C. neoformans* (52 environmental)	MIC range (mg/L) 0,5-4 2 0,25-2	MIC range (mg/L) 0,125-1 0,5 0,125-0,5	---------- ----	---------- ---------	EUCASTE. Def 7.1 and CLSI M27-A3	No strains resistant to the evaluated antifungals were found, similarly to eastern Thailand and other non-Asian countries.
Nascimento et al. 2017	Brazil	Prospective (clinical)	*C. neoformans* variety *grubbii* (80) *C. gattii* (7)	4,0 8,0 8,0 8,0	0,25 0,50 0,06 0,13	0,06 0,25 0,13 0,25	0,25 0,50 0,50 0,50	CLSI M27-A2 and E-test ®	High sensitivity of isolates from both species complexes.
Chang et al. 2018	USA	Prospective (clinical)	*C. neoformans* (1) *C.gattii* (1)	MIC range (mg/L) 2 4	MIC range (mg/L) 0,125 0,5	MIC range (mg/L) 0,031-0,063 0,063-0,125	MIC range (mg/L) 0,031 0,063-0,125	CLSI M27-A3	The AFR1 efflux pump is responsible for intracellular expulsion of azole drugs as a resistance mechanism.

Table I. Continuation.

Rocha et al. 2018	Brazil	Prospective (clinical)	*C. neoformans* VNI (34) *C. gattii* VGII (4)	MIC range (mg/L) 2-8 8-32	MIC range (mg/L) 0,03-0,25 0,03-0,125	MIC range (mg/L) 0,03-0,25 0,125-0,5	---------- -----	CLSI M27-A3	All isolates were sensitive to the 3 antifungals evaluated, but fluconazole presented the highest MIC values for VGII.
Berejnoi et al. 2019	Argentina	Prospective (clinical)	*C. gattii* VGIII (1) *C. gattii* VGIV (1)	MIC (mg/L) 16 4	MIC (mg/L) 0,25 0,25	MIC (mg/L) 0,12 0,12	MIC (mg/L) 0,12 0,06	EUCAST E.Def 7.3.1	A high MIC value for fluconazole was found against the first clinical strain of *C. decagattii* described in South America.
Pinheiro 2019	Brazil	Retrospective and Prospective (clinical)	*C. gattii* VGII (7)	MIC range (mg/L) 1-8	MIC range (mg/L) < 0,03-0,25	MIC range (mg/L) < 0,03-0,25	---------- -----	CLSI M27-A3	*C. gattii* isolates were sensitive to the antifungals analyzed.

MIC, Minimum Inhibitory Concentration; ECV, Epidemiological Cutoff Value; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing.

RESULTS

Resistance to antifungal agents, determined in vitro by reliable reference or commercial methods, such as diffusion gradient impregnated tapes, may arise during the use of some medications, such as fluconazole, which allows selection of strains with genetic alterations related to mutations that promote antifungal resistance (Zhou & Ballou 2018, Muñoz et al. 2018, Desjardins et al. 2017, Gerstein et al. 2019). Other mechanisms by which the fungus can acquire resistance to azole antifungals are the overexpression of the erg11 gene, increased production of drug efflux proteins, located in the fungal membrane (Afr1, Afr2 and Mdr1) (Basso Junior et al. 2015). Four studies that analyzed the resistance mechanism mediated by efflux pumps were found in the period, which still developed with few isolates, contributed to the understanding of the phenotypes of azole-resistant *Cryptococcus* (Basso Junior et al. 2015, Bastos et al. 2018, Chang et al. 2018, Yang et al. 2015, Kano et al. 2017).

Another known resistance mechanism in *Cryptococcus* strains is heteroresistance to fluconazole, a phenomenon defined as resistance expressed by a subpopulation of cells, initially considered sensitive to this drug, but which can grow under high concentrations of the drug, after exposure to it. One study described the phenomenon of heteroresistance to itraconazole in *C. gattii* isolates and its impact on changes in cell morphology (surface/volume and size) and in growth patterns and virulence. Although the clinical implications remain unknown and only a few isolates were analyzed, the data allowed the deciphering of important aspects of the mechanisms of antifungal resistance (Ferreira et al. 2015).

Morphological changes and modulation of virulence after drug exposure were also discovered in a national study. The authors observed, in some clinical strains of *C. neoformans* and *C. gattii* exposed and adapted to different concentrations of fluconazole, that those of *C. neoformans* developed drug resistance, possibly during patient therapy, and the virulence profiles were inversely proportional from resistance. This data suggests that the adaptation to the selective pressure of the drug can lead to a decrease in virulence and furthermore it was found that the virulence of the *C. neoformans* isolate is dependent on the inoculum concentration, which was not observed in the *C. gattii* isolate (Rossi et al. 2016).

A study carried out in the state of Amazonas, Brazil, in 2016, involved collecting environmental samples of house dust in houses of a rural

community, resulting in the finding of 2 isolates of *C. gattii* for which the MIC values were high, demonstrating resistance to fluconazole (Alves 2016).

Two studies, published in 2011 (Pfaller et al. 2011) and in 2016 (Córdoba et al. 2016), contributed to the interpretation of MIC values, in which the sensitivity of hundreds of *C. neoformans* and *C. gattii* isolates were determined in order to propose epidemiological cutoff values (ECVs) global and regionally (Argentina).

The strategy of using ECV to interpret MIC values is necessary when there are no defined clinical breakpoints, as is the case with *Cryptococcus*. It is noteworthy that ECV has no clinical applicability, but represents a very important tool to distinguish less sensitive (MIC value > ECV) and more sensitive (MIC < ECV) isolates to a given drug. It is assumed that less sensitive or non-wild isolates have one or more than one resistance mechanisms to the respective antifungal (Córdoba et al. 2016, Pfaller et al. 2011, Espinel-Ingroff et al. 2012a, b).

Resistance mechanisms in *Cryptococcus* isolates were a topic addressed in some studies selected for this review. Resistance has been attributed to the long-term use of fluconazole, in particular, administered for long periods to patients with neurocryptococcosis and AIDS, who require maintenance therapy to prevent relapse of infection. Another possibility raised in the studies is the selection of environmental strains of *Cryptococcus* after abusive exposure to fungicides for agricultural use, which belong to the same chemical class as azole drugs and which are widely used in plantations to combat phytopathogenic fungi. One hypothesis lies in the intense use of tebuconazole, which represents the most used systemic fungicide worldwide and which could select resistant strains existing in plant debris. Such strains, if inhaled by susceptible individuals, could cause infections resistant to triazoles that are used in the medical clinic of cryptococcosis (Araújo et al. 2018, Bastos et al. 2018, Chesdachai et al. 2019, Kim et al. 2020, Arastehfar et al. 2020).

The two pathways, clinical and environmental, contribute to the emergence and maintenance of strains resistant to triazole drugs, which may result in a lower therapeutic response and poor clinical outcome (Mpoza et al. 2018). For such cases, ravuconazole has been studied as a new drug with the potential to combat fluconazole-resistant strains (Kano et al. 2020).

The most widely accepted ECV propositions for the *Cryptococcus* species complexes, to date, are from authors from several countries who joined forces and published values for several antifungals in 2012 (Espinel-Ingroff et al. 2012a, b). For this review, and in order to give meaning to the absolute values of MIC presented in several studies, such ECVs were applied (Espinel-Ingroff et al. 2012a, b).

The published MIC values (Table I) were, individually in each study, analyzed and interpreted against the ECV, in such a way that the classification of *C. neoformans* isolates resulted as follows: isolates from this species complex were non-wild, mainly for voriconazole (72.7%), followed by fluconazole (55%), itraconazole (46.7%) and, finally, amphotericin B in a small portion (5.6%). In *C. gattii* though, non-wild isolates were found more frequently, also with voriconazole (36.4%) and lower with fluconazole (25%) and itraconazole (20%). For amphotericin B, non-wild *C. gattii* isolates were not identified.

Considering these analyses, it was found that voriconazole was the least active drug, for which the largest numbers of isolates with possible resistance mechanism(s) were identified, both among members of the *C. neoformans* complex and in *C. gattii*. Fluconazole and itraconazole were more effective to inhibit isolates of both

complexes only losing to the fungicidal action of amphotericin B, demonstrated in vitro as the most potent.

DISCUSSION

Comparing the species complexes, *C. neoformans* was less wild to both voriconazole, fluconazole, itraconazole and amphotericin B, in relation to *C. gattii* isolates. However, infections by strains of this complex seem to be more severe to the CNS compared to those of the *C. neoformans* complex, causing greater complications such as higher intracranial pressure and a greater number of neurological sequelae (Berejnoi et al. 2019, Lomes et al. 2016, Siqueira et al. 2019, Sánchez & Zúñiga 2016). These facts illustrate a lack of association between in vitro resistance of cryptococcosis agents, making the topic of the clinical utility of the MIC test even more intriguing and in need of further investigation.

A major difficulty pointed out to establish the relationship between data obtained in vitro and in vivo are several intervening factors that can impact the course of the infection, such as the differences found in the melanization of the agent, in the size of the capsule and in the cellular gigantism that are polyploid cells, abnormally large, formed in the course of infection and more rarely seen under laboratory conditions. The various structural differences such as denser and highly reticulated capsules or thicker cell walls and ability to grow at 37º C are virulence factors that may play a role in the disease's evolutionary scenario by altering the response to treatment. However, until now the physiological differences of the etiologic agent within the host and its in vitro susceptibility to antifungal drugs are not definitively accepted as predictors of clinical response, having an epidemiological application (Bastos 2017, Grossman & Casadevall 2017, Neves et al. 2019).

CONCLUSIONS

This work had the character of a bibliographic review on the subject. Many studies suggest an increase over the last decades in resistance to triazoles, especially to fluconazole, which is widely used in clinical medicine, however, still without consensus on this statement. Data published between 2010 and 2022 show high MIC values for the two species complexes, in particular for *C. neoformans*, with special importance for voriconazole, followed by fluconazole and itraconazole and with no emphasis on MICs of amphotericin B.

It should be taken into account, however, that MIC is just a physical measurement, determined in vitro, and the evolution of cryptococcosis is influenced by several factors. In addition to those linked to the biology of the etiologic agent, mainly aspects related to the host.

There is a pressing need for studies necessary for better scaling of resistance in strains of both *C. neoformans* and *C. gattii* complexes, given the lack of research with adequate statistical treatment, which allows evaluating the difference in sensitivity between these agents that can have clinical impact. Additionally, there is no evidence of a trend towards an increase in resistance rates over the period, given the insufficiency of representative data for the different regions or locations.

Cryptococcosis is considered a neglected disease, since it is not necessary to be notified, making it difficult to assess the real dimension of its frequency and its effects in relation to public health. Nevertheless, this can be changed with the priority list of fungal pathogens by the WHO, increasing helping research and guiding policies. Whether in the future the monitoring of this serious invasive mycosis occurs, with records of therapeutic failures associated with

MIC laboratory data from tests carried out by reference methods, the value of this test may be more accurately measured in clinic practice.

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Author contributions

MITK researched, wrote, and analyzed the data for the manuscript. MSCM supervised and guided the study.