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Virulence factors of *Candida* spp and molecular mechanisms of resistance to azoles expressed by *Candida tropicalis*

Ana Elisa Rojas Rodríguez[1]
Leidy Yurany Cárdenas Parra[2]
Yuliana Zapata Serna[3]
Jorge Enrique Pérez Cárdenas[4]

Abstract

Introduction: Due to the epidemiological panorama of candidiasis caused by *Candida tropicalis* and the marked resistance generated towards azoles, it is necessary to deepen the knowledge of virulence and drug resistance mechanisms. Objective: To synthesize the virulence factors of *Candida* spp. and the molecular mechanisms of azole resistance expressed *by Candida tropicalis*. Materials and methods: The bibliographical search were conducted in the PubMed database and manuscripts were selected according to the critical analysis criteria proposed by the PRISMA instrument. The guiding question for the search was: What are the virulence factors of *Candida* spp, and the azole resistance mechanisms expressed by the species *C. tropicalis?* The results were organized into two categories: Virulence factors of *Candida* spp and molecular mechanisms of resistance to azoles. Results: The virulence factors of *Candida* spp. are represented by toxin and enzyme production, biofilm formation, environmental modification, filamentation, and hyphal growth. The mechanisms of resistance to azoles expressed by *C. tropicalis* are mainly determined by overexpression of the *ERG11* and *MDR1* genes and by mutations in the *ERG11* gene. Conclusion: Virulence factors are similar among *Candida* species and the molecular mechanisms of resistance to azoles expressed by *C. tropicalis* fundamentally result in decreased affinity for the pharmacological target and lower intracellular concentration of the drug.

Key words: *Candida tropicalis*, Virulence Factors, Drug Resistance, Azoles, Antifungals (Source: MeSH).

[1] (MSc). Universidad Católica de Manizales, Manizales, Colombia. arojas@ucm.edu.co
ORCID: 0000-0002-8421-633X.**Google Scholar**
[2] (MSc). Universidad de Caldas, Manizales, Colombia. leidy.cardenas@ucaldas.edu.co
ORCID: 0000-0001-7505-8539.**Google Scholar**
[3] (MSc). Universidad Católica de Manizales, Manizales, Colombia. yzapata@ucm.edu.co
ORCID: 0000-0002-7771-7179.**Google Scholar**
[3] (MSc). Universidad de Caldas, Manizales, Colombia. labmicro@ucaldas.edu.co
ORCID: 0000-0002-7829-6505. **Google Scholar**

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Factores de virulencia de *Candida* spp y mecanismos moleculares de resistencia a los azoles expresados por *Candida tropicalis*

Resumen

Introducción: Debido al panorama epidemiológico de la candidiasis producida por *Candida tropicalis* y la marcada resistencia generada hacia los azoles, se hace necesario profundizar en el conocimiento de la virulencia y mecanismos de resistencia a fármacos. Objetivo: Sintetizar los factores de virulencia de *Candida* spp. y los mecanismos moleculares de resistencia a azoles expresados por *Candida tropicalis*. Materiales y métodos:
Se realizó una búsqueda bibliográfica en la base de datos Pubmed y los manuscritos fueron seleccionados según los criterios de análisis crítico propuestos por el instrumento PRISMA. La pregunta orientadora de la búsqueda fue: ¿Cuáles son los factores de virulencia de Candida spp y los mecanismos de resistencia a los azoles expresados por la especie *C. tropicalis*? y los resultados se organizaron en dos categorías: Factores de virulencia de *Candida* spp y mecanismos moleculares de resistencia a azoles. Resultados: Los factores de virulencia de *Candida* spp. están representados por la producción de toxinas y enzimas, la formación de biopelículas, la modificación del medio ambiente, la filamentación y el crecimiento hifal; por otro lado, los mecanismos de resistencia a los azoles expresados *por C. tropicalis* están determinados principalmente por la sobreexpresión de los genes *ERG11* y *MDR1* y por mutaciones en el gen *ERG11*. Conclusiones: Los factores de virulencia son similares entre las distintas especies de *Candida* y los mecanismos moleculares de resistencia a los azoles expresados por *C. tropicalis* se traducen fundamentalmente en una menor afinidad por la diana farmacológica y una menor concentración intracelular del fármaco.

Palabras clave: Candida tropicalis, Factores de virulencia, Resistencia a medicamentos, Azoles, Antifúngicos (Fuente: DeCS, BIREME)

Introduction

The incidence of life-threatening fungal infections caused by *Candida* species has increased significantly in recent decades, currently ranking fourth among causes of sepsis [1,2]. Notably, *C. tropicalis* has become an important health care-associated fungus, particularly in intensive care units, likely due to the immunodeficiency of patients, use of invasive biomedical equipment, prolonged antibiotic therapy, recent surgeries and cross-contamination, which also poses a significant risk [3-5]. *C. tropicalis* is frequently isolated from the bloodstream and urinary tract, especially in cancer patients, those undergoing bone marrow transplants, and individuals with acute leukemia, neutropenia, or receiving antineoplastic therapy [2,6].

The prevalence of non-albicans *Candida* species varies by geographic location. *C. tropicalis* is the second most frequently isolated species in Asia and Latin America, while in the United States, its prevalence as a sepsis-causing agent, ranges between 2-24% and in India, *C. tropicalis* is the most common cause of candidemia, with isolation in 67-90% of cases [7-11]. The Group for the Control of Antimicrobial Resistance in Bogotá (Grupo para el Control de la Resistecia a los Antimicrobianos, GREBO) conducted a study in eight hospitals between 2001 and 2002 found that of 1,194 fungal isolates, *C. albicans* was the most common (57%) followed by *C. tropicalis* (14%) [12].

C. tropicalis is a diploid yeast of the family Ascomycota, class Hemiascomycetes. It forms spherical to ovoid blastoconidia and

pseudohyphae in branched chains (13). Its genome is 14.6 Mb, with 6258 protein-coding genes and a guanine-cytosine content of 33.1%. Even though the exact number of chromosomes is uncertain, Doi et al. reported 12 chromosomes per cell [13,14]. Although *C. tropicalis* primarily reproduces asexually, it can reproduce parasexually under stress conditions [14,15]. Current pharmacological treatments for *Candida* infections include polyenes, azoles, and echinocandins [16]. Azoles, such as fluconazole and voriconazole, are fungistatic and inhibit the synthesis of ergosterol by binding to the heme group of enzymes like 14-alpha demethylase, which prevents fungal growth [17,18].

The increasing prevalence of candidemia, its impact on mortality, and the increase in drug-resistance strains require a review of resistance mechanisms, especially to azoles due to their frequent use [19,20]. Common resistance mechanisms include mutations in the *ERG11* gene, which encodes 14-alpha demethylase, and overexpression of genes that produce efflux pumps (ABC and MFS transporters) that reduce intracellular concentrations of azoles [21,22].

In this context, the objective of this review is to provide a comprehensive and up-to-date overview of the factors contributing to the pathogenicity of *Candida* spp. and how *C. tropicalis* develops resistance to azoles.

Materials and Methods

A review was conducted with the following guiding question for the search: What are the virulence factors of *Candida* spp. and the azole resistance mechanisms expressed by *C. tropicalis* species? The search was conducted in the PubMed database. The search terms used were *Candida tropicalis*, Virulence Factors, Resistant, Azoles (DeCS) // *Candida tropicalis*, Virulence Factors, Resistant, Azoles (MeSH). These terms were combined with the Boolean operator "AND" in "all fields". A filter was applied to find studies between 2013 and 2022. The search operations were (*Candida tropicalis*) AND virulence factors and (azole resistance) AND *Candida tropicalis*.

The search was conducted in June 2018 and repeated in December 2022 to include other updated studies on the corresponding topic, obtaining a total of 587 studies (168 on virulence mechanisms of *Candida* spp and 419 on resistance mechanisms to azoles expressed by *Candida tropicalis*). Articles that included in the title or abstract mechanisms of virulence by Candida spp and/or resistance to azoles expressed mainly by the *C. tropicalis* species directly became eligible, and those that included frequency or prevalence of virulence of any *Candida* species and/or resistance to any drug from the azole group by any *Candida* species were included for further review and, if related and relevant, were included in the eligible list. Critical reading analysis and quality assessment of studies was performed according to the elements proposed by the PRISMA reports [23].

Two sections of results were established based on the findings. The first section corresponds to virulence mechanisms, and the second addresses the molecular elements conferring resistance to azoles in *C. tropicalis*. The inclusion criteria for the selected manuscripts were, specifically, that they addressed virulence mechanisms of *Candida* spp. and/or azoles resistance, with a particular attention to *C. tropicalis*. Furthermore, only manuscripts available in the PubMed database were included according to the criteria established for each phase of the search. Manuscripts only presenting epidemiological data on *Candida* spp. infections, without addressing virulence mechanisms and/or azoles resistance, particularly in *C. tropicalis*, were excluded.

In addition, manuscripts of experimental studies focused on the evaluation of changes

in virulence factors, resistance to azoles, and experiments with drugs or substances with antifungal potential which did not include specific molecular elements that explain the general mechanisms of resistance of *C. tropicalis* to azoles, were also excluded. Similarly, manuscripts of experimental studies with azoles that were not related to the resistance mechanisms specifically expressed by C. tropicalis to these antifungals were excluded (see Figure 1).

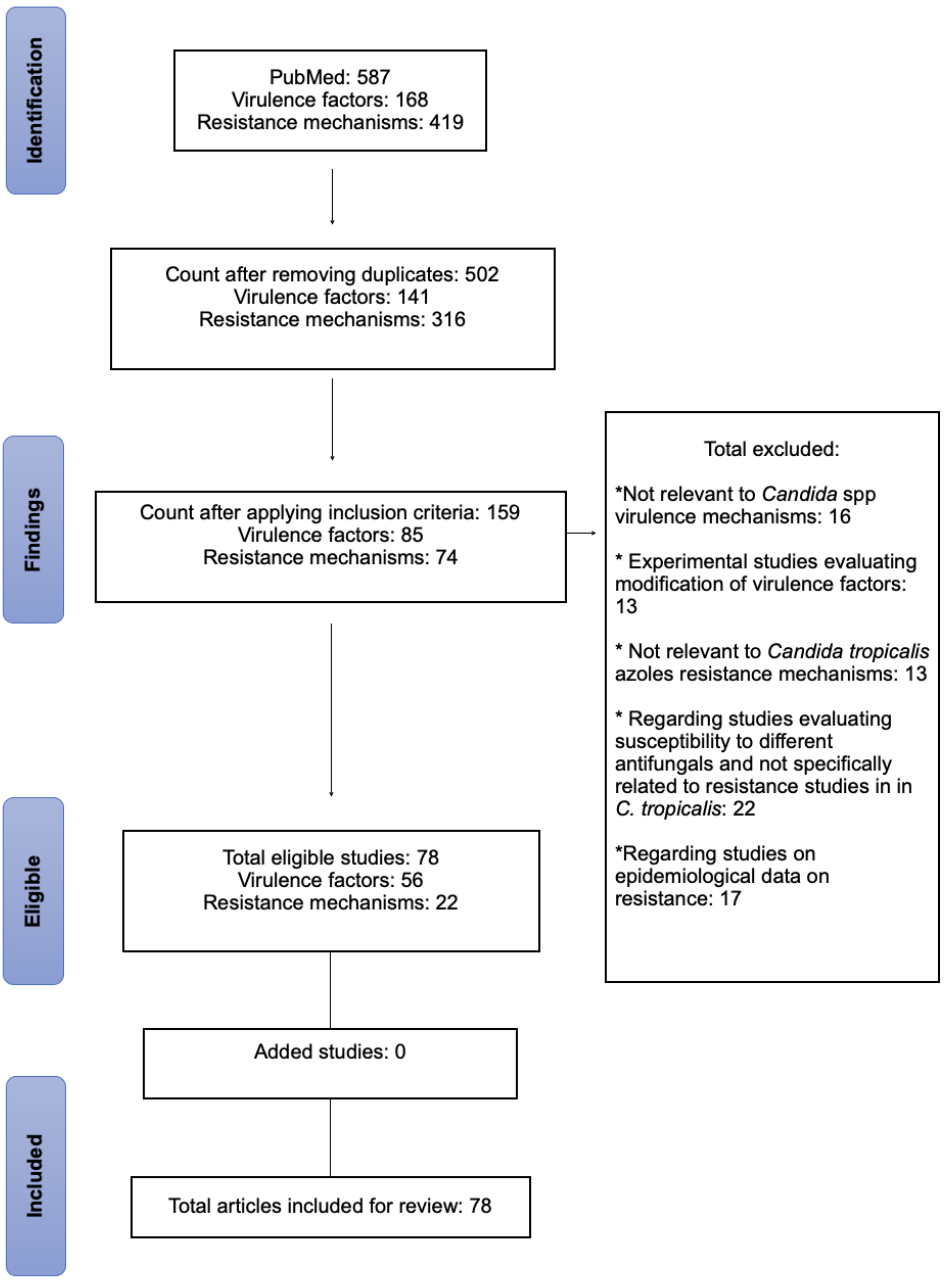

Figure 1. Review process of articles-studies based on the PRISMA method

Results and Discussion

Virulence factors contributing to the pathogenicity of *C. tropicalis* (Table 1)

Table 1. Virulence factors of *Candida* spp.

Virulence factor	Generated effect
Toxin Production [24-27] Production of toxic substances produced by *Candida* spp. that confer pathogenic character to the microorganism and avoid the immune response of the host.	The mechanisms of the humoral immune response are altered and can even induce the lysis of necrotic cells, thus affecting the immune response capacity to control *Candida* spp. infection.
Detection and regulation of pH [28-31] Production and/or use of short-chain carboxylic acids, as a product of the metabolism of sugar by *Candida* spp. Generation and extrusion of ammonia, as a product of amino acid degradation. This is an exclusive virulence mechanism of *C. albicans*. No information on species other than *Candida -albicans* is found in the literature.	Carboxylic acids such as acetate and lactate are used by *Candida* to survive, since in some environmental niches where it colonizes and infects, they are often the only source of carbon and, of course, an adaptive alternative to nutrient restriction for the fungus. The acidic environment generated by the production of carboxylic acids causes: • Irritation and inflammation of the mucosa where *Candida* spp is found. • It prevents the colonization of commensal microorganisms and favors that of aciduric microorganisms. • The enzymes released in the acidic environment by *Candida* spp, cleave IgA secreted by the host and facilitate its adherence to the tissue. *Candida* spp secretes phospholipase, a lytic enzyme that destroys host cell membranes. Specifically, *C. albicans* can detect the pH of the medium and adapt using two beta-glucosidase proteins (Phr1 and Phr2) of the cell wall. Phr1 is expressed in alkaline and neutral media and Phr2 in acidic media. It can also alkalize the extracellular pH and auto-induce the formation of hyphae, through a process in which the fungus absorbs amino acids in the absence of glucose (starvation), degrades them and exports the resulting ammonia, which alkalizes the medium and induces the formation of hyphae.

Metabolic flexibility [29,32] Modification of the energy production pathway, from glycolysis to gluconeogenesis, and activation of the glyoxylate cycle, is described as a particular virulence mechanism of *C. albicans*. No information on species other than *C. albicans* is found in the literature.	When *C. albicans* is phagocytosed by macrophages and neutrophils, the nutritional environment of the fungus is restricted in glucose. Therefore, as a survival factor, glycolysis is inactivated, and the energy-yielding pathway is induced instead of mediated by gluconeogenesis and the glyoxylate cycle. On the other hand, lipids and amino acids begin to serve as an energy source within the phagocytes.
Response to environmental stress [29,33,34] Heat shock, osmotic, oxidative, and nitrosative stress responses detected and transmitted by mitogen-activated protein kinase (MAP) pathways through sequential phosphorylation are characteristic *of C. albicans*. No information related to *non-albicans* species is found in the literature.	The heat shock response is mediated by heat shock proteins, which prevent the unfolding and aggregation of proteins harmful to the fungus and cause its death. They also play other roles in morphological plasticity and biofilm formation. The response to osmotic stress occurs through the formation and intracellular accumulation of solute glycerol through the action of glycerol-3-phosphatase and glycerol-3-phosphate dehydrogenase. Glycerol counteracts water loss of the fungus. Oxidative stress generated by peroxide, superoxide anions, and hydroxyl radicals substantially affects the life of the fungus. Therefore, *C. albicans* activates detoxification and survival mechanisms mediated by catalase and superoxide dismutase. Neutrophils produce reactive nitrogen species that induce nitrosative stress in the phagocytosed fungus, generaring biological processes that promote the activation of the flavohemoglobin-related protein Yhb1, whose function is to detoxify *Candida* spp from the action of reactive nitrogen species.
Filamentation [13,29,30,32,35–40] Morphological plasticity consists in the modification of the yeast form to the form of pseudohyphae and hyphae.	pH > 7, starvation, presence of serum or N-acetylglucosamine, physiological temperature, and CO_2, among others, promote a process of transition and adaptive morphological change, by which the fungus changes from its yeast-like state to one of pseudo-hyphae and hyphae. It has been proposed that a greater capacity for filamentation (morphological plasticity) is associated with more efficient biofilm formation and, on the other hand, the formation of hyphae within phagocytes favor escape by mechanical forces.

Enzyme production [6,13,24,29,37,38,41-71] Hydrolases: Proteases, phospholipases, and lipases. Others: Hemolysin Coagulase Enolase Phytases	Hydrolytic enzymes degrade host cell membranes, allowing tissue invasion and spread of infections. When yeasts are phagocytosed, the pH within the vacuole favors the activation of proteases (aspartyl proteases), which promote adhesion, tissue damage, active penetration into the host cell, and affect the immune response by destroying immunoglobulins, cytokines, complement factors, collagen, keratin, and mucin. Proteases are also described as molecules that provide nutrients for fungal survival. Phospholipases alter the host cell membrane and promote *Candida* spp invasion. Lipases promote fungal cell survival in macrophages and mitigate the inflammatory response of the host. Hemolysin uses the iron contained in host hemoglobin and activates complement to opsonize the surface of red blood cells, destroying them and facilitating the invasion of hyphae in systemic candidiasis. Coagulase binds to plasma fibrinogen and initiates a series of reactions that cause coagulation. It is a recognized virulence factor for *C. albicans* and *C. tropicalis*. Enolase is a cytosolic enzyme involved in several functions. However, the most important function conferring pathogenicity to *Candida* spp is unclear. It has been proposed that *C. albicans* enolase increases the ability to cross human brain endothelial cells and, through interaction with extracellular matrix proteins, laminin and fibronectin, mediates adhesion. In addition, enolase has been found in biofilms, suggesting its participation in the formation of their structure. Phytase hydrolyzes phytate, a component rich in inorganic phosphorus and myoinositol, essential nutrients for *C. albicans*. In addition, phytase is known to be essential for carrying out the process of cell filamentation, adhesion, and invasion: especially penetration of human epithelium.

Adhesion and biofilm formation [13,29,36,38,50,55,58-61,64-78] Biofilms are structures formed by a community of microorganisms adhering to solid surfaces of either biotic or abiotic nature. Their consolidation requires several processes: adhesion, colonization, growth, cell proliferation and, finally, the formation of pseudohyphae and/or hyphae, which will result in the formation of exopolymeric extracellular matrix, that protects the fungus and facilitates its pathogenesis.	Adhesion is one of the main virulence mechanisms of *Candida* spp. and occurs in two stages: primary adhesion between yeast and surfaces, generally through non-specific hydrophobic interactions (the cell wall of *Candida* spp contains hydrophobic proteins that tend to adhere to plastic materials and host proteins such as laminin, fibrinogen, and fibronectin; and the second stage refers to the specific adhesion that occurs by the interaction between the biotic surface, host cells or other microorganisms and specific adhesins. The most representative adhesins are those of agglutinin-like sequence, which are part of a family of glycoproteins located on the surface of the yeast cell wall. Biofilm forms in two phases: in the first phase the fungal blastospores adhere to an inert or living surface and recruit new planktonic cells. In the second phase, hyphae and pseudohyphae are formed and the extracellular polymeric matrix, rich in carbohydrates and proteins, is synthesized. The biofilm limits contact with antifungal drugs, cells, and molecules of the host immune response.

Molecular Mechanisms of azole resistance

Azoles are drugs frequently used to treat infections caused by *Candida* spp., due to their low cost, oral availability and good pharmacological safety profile in terms of low toxicity. However, the intrinsic and developed resistance of fungi to this therapeutic alternative has been widely described [79]. The main resistance mechanisms expressed by *Candida* spp. include point mutations in the *ERG11* gene, which encodes proteins that catalyze the 14-alpha-demethylation of lanosterol leading to the biosynthesis of ergosterol, a key component of the fungal membrane. The *ERG11* mutation results in decreased affinity of the azole for the enzyme 14-alpha-demethylase (pharmacological target) and alters the direct ligand-receptor relationship, which implies a reduction in the effectiveness of fluconazole for infection control [80,81]. Another associated resistance mechanism is related to the overexpression of genes encoding the ATP-binding cassette (ABC) and major facilitator (MF) pumps, which are recognized as antifungal pumping systems or permeability barriers, since they remove part of the active ingredient from the interior of the fungal cell, limiting its pharmacological effectiveness. ABC transporters are encoded by *CDR* (*Candida* Drug Resistance) genes and MF transporters are encoded by *MDR* (Multidrug Resistance) genes [82-84]. Finally, as a resistance mechanism, *Candida* spp. develops alternative pathways to keep the celtoxic intermediate productsional, which are not interrupted by the action of azoles, and which are not susceptible to the accumulation of intermediate products toxic to the fungus [85].

Table 2 summarizes the azole resistance mechanisms expressed by *C. tropicalis*, as documented in the literature.

Table 2. Molecular mechanisms of azole resistance expressed by *C. tropicalis*

Resistance mechanism	**The functional basis of resistance**
Modifications in the expression and/or mutations in the *ERG* gene	
Overexpression of the *ERG11* gene in resistant strains and *ERG11* gene mutations [86,87-103]: * *A339T* mutation * *C461T* mutation * *A395T* Mutation (*Y132F* substitution in *ERG11p*) * *V125A* mutation * *Y257H* mutation * *G464S* mutation * *T224C* mutation * *G263A* mutation * *S154F* Mutation * *G464D* substitutions Overexpression of *ERG1, ERG2, ERG3* [97]	The genes *ERG1* and *ERG7, ERG24, ERG25, ERG26, ERG27, ERG6, ERG2, ERG3, ERG5, ERG4* and especially *ERG11*, regulate the synthesis of ergosterol. Overexpression and mutations of the *ERG11* gene, as described in the literature, constitute one of the most studied resistance mechanisms. The functional basis of resistance is attributed to a decrease in the affinity of the azoles for the pharmacological target lanosterol 14 alpha demethylase enzyme and, consequently, the drug-target relationship and inhibitory activity of the enzyme are altered, with consequent drug resistance. Overexpression of *ERG1, ERG2*, and *ERG3* genes has been found in resistant strains in other studies. However, a significantly higher expression in resistant strains of *C. tropicalis* was first identified in the study by Paul S, et al (2022) [95]. The functional basis of resistance generated by overexpression of *ERG1* and *ERG2*, is not exactly known.
ERG3 gene mutations [96,104] * *S258F* mutation * *C773T* mutation * *A334G* mutation	The *ERG3* gene encodes for the enzyme C-5 sterol desaturase, and its mutation causes the inactivation of this enzyme, which favors the detoxification of the fungal cell of toxic intermediates generated in the ergosterol biosynthesis pathway in the presence of azole-inhibitory drugs on the lanosterol 14α-demethylase enzyme and the function of the C-5 sterol desaturase enzyme on 14α-demethylated products. This confers pharmacological resistance. These toxic products are called 14alpha-methyl sterols, especially 14α -methyl fecosterol.

Modifications in expression and/or mutations in the *CDR* gene	
Overexpression of the *CDR1* gene in strains resistant, non-sensitive, or less sensitive to fluconazole [88-90,94,97,105].	The *CDR* gene encodes drug extrusion pumps of the ABC (ATP-Binding cassette) transporters superfamily. The functional basis of the resistance generated by the overexpression of *CDR* genes is an increased efflux of azoles from the interior of the fungal cell to the outside, with the consequent decrease in the concentrations of drug available to carry out its function.
Overexpression of *CDR2* and/or *CDR3* genes in resistant strains [97,106].	
Modifications in the expression and/or mutations in the *MDR* gene	
Overexpression of the *MDR1* gene in resistant, non-sensitive, or less sensitive strains to fluconazole [88-90,92,94,107,108].	The *MDR* gene encodes drug extrusion pumps of the MF (Major Facilitators) transporter superfamily. The functional basis of the resistance generated by the overexpression of *MDR* genes consists of an increased efflux of azoles from the interior of the fungal cell to the exterior, with a consequent decrease in the drug concentrations available to carry out its function.
***ERG11* transcription factor**	
Overexpression of the *UPC2* gene in resistant strains [86,89,97,106,107].	The *UPC2* gene regulates the expression of the *ERG11* gene and overexpression of *UPC2* increases the expression of *ERG11*.
Transcriptional activator of *CDR1*	
Overexpression of the *TAC1* gene in resistant strains [89,97,106].	The *TAC1* gene regulates the expression of the *CDR1* gene and overexpression of *TAC1* increases the expression *of CDR1*.
Modifications in the expression of the *MKC1* gene	
Overexpression of the *MKC1* gene in resistant strains [97].	The *MKC1* gene encodes for mitogen-activated protein kinase involved in cell wall synthesis and morphological transition: filamentation. A possible mechanism of *C. tropicalis* resistance to azoles, based on overexpression of the *MKC1* gene in resistant strains, has been described in the study by Paul S, et al. (2022) [95]. However, the functional basis of the resistance conferred to *C. tropicalis* is not described.

Discussion

Research on virulence factors in *Candida* spp. has focused primarily on *C. albicans* compared to other species, possibly due to its epidemiological predominance as a causative agent of infections within this genus [12]. *C. albicans* has been extensively used as a model to study the mechanisms that facilitate colonization, persistence, and pathogenicity in human hosts. Studies have reported virulence mechanisms such as the ability to produce toxins, regulate intracellular pH, metabolically adapt to different environments, respond to environmental stress, and develop structures like biofilms and filamentous forms. Overall, virulence in *Candida* spp. is a multifaceted characteristic involving a series of complex biological processes that enable these fungi to survive in the host, evade immune responses, and resist antifungal treatments [13].

Despite these advances, there is an urgent need to expand research towards other *Candida* species to better understand the particularities of each virulence mechanism and their variations between species. This is crucial for advancing the development of more effective therapeutic strategies against fungal infections. Similarities among *Candida* species regarding toxin production, pH regulation, filamentation, enzyme production, adhesion, and biofilm formation have been identified. Among the virulence mechanisms, biofilm formation and morphological plasticity are probably the most representative and studied, being biologically robust. However, it would be highly beneficial to advance in applicative research that explores alternatives for pharmacological intervention, new therapeutic targets, and the development of antifungals capable of overcoming the pathogenicity and resistance mechanisms of *Candida* spp., through more precisely targeted therapeutic interventions [24-32, 35-71].

Regarding some virulence mechanisms, there is insufficient information for species other than albicans or no mechanisms have been reported in the literature related to specific adaptations such as ammonia generation in pH regulation, energy acquisition through gluconeogenesis and glyoxylate cycle activation instead of glycolysis as a metabolic flexibility process. The same lack of information occurs regarding responses to environmental stress mediated by heat shock proteins, intracellular glycerol formation, detoxification mechanisms activated by catalase and superoxide dismutase activity, and activation of proteins that detoxify reactive nitrogen species, depending on the type of environmental stress (thermal, osmotic, oxidative, and/or nitrosative) [31-34]. This lack of information in literature constitutes a significant opportunity for research, both at the basic and applied levels, which would allow for a detailed comparative analysis of the virulence factors differentiated by species of *Candida*. A deeper understanding of these cellular and molecular aspects could reveal new therapeutic alternatives and innovative strategies to combat these infections and counteract the growing resistance to antifungal drugs.

Moreover, resistance to azoles in *C. tropicalis* represents a significant challenge in treating fungal infections, given the widespread use of these antifungals due to their relatively low cost and acceptable safety profile. Documentation of intrinsic and acquired resistance in this species highlights the complexity of the mechanisms that favor this phenomenon, including point mutations in the *ERG11* gene, overexpression of genes related to ergosterol synthesis, and increased activity of efflux pumps, specifically those encoded by *CDR* and *MDR*, belonging to the ABC and MFS families, respectively [87-108].

The current problematic scenario invites the academic and scientific community to urgently develop new therapeutic strategies and diagnostic methods to address resistance to azoles in *C. tropicalis*. Based on the literature

reviewed in this manuscript, the authors believe that future resistance research should focus on several key areas: first, to understand in detail the molecular mechanisms leading to resistance, including the identification of new mutations in key genes such as *ERG11* and others related to ergosterol synthesis; second, to explore how alternative pathways for maintaining cell membrane integrity could be intercepted or inhibited by new antifungal agents; and third, to improve the early detection of resistance through the development of rapid and accurate diagnostic methods that allow timely adjustment of treatment.

Conclusions

Virulence factors have been identified especially in *C. albicans*, whose role in other species requires further study. To know the virulence mechanisms of *Candida* spp. is essential not only for understanding their pathogenicity, but also because they can be considered promising pharmacological targets for infection control and future effective alternatives for addressing the resistance phenomenon.

Resistance to azoles generated by *C. tropicalis* is primarily attributed to overexpression or mutations of the *ERG11* gene, which modifies the binding of the drugs to the molecular target of action, and to positive regulation of the *MDR1* gene, a mechanism that causes a decrease in effective intracellular concentrations of azoles. However, studies are required to help establish more precisely the molecular mechanisms responsible for resistance, as well as the functional-biological basis that determines it, in order to provide new evidence that should be considered in terms of pharmacological efficacy in the control of resistant candidiasis.

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