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ANTIFUNGAL RESISTANCE: A GROWING CONCERN

Resistencia antifúngica: una creciente preocupación

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ABSTRACT

Globally, the increasing number of drug-resistant human pathogens represents a major threat to public health. Among these pathogens, fungi that have acquired resistance to the already scarce arsenal of antifungals are of particular significance, as they present therapeutic challenges that increase morbidity and mortality rates. Particularly, most mycoses are opportunistic since they mainly affect hosts with a weakened immune system, including patients with cancer, hematological malignancies, prolonged neutropenia, solid organ transplants, HIV/AIDS, patients in intensive care units, using central venous catheters or on dialysis, using corticosteroids, among others. In most cases, fungal infections have a significant medical and economic burden that outweighs the burden of the underlying disease alone and changes the outcome. In addition, the treatment for mycoses, which consists of four classes of antifungals described several decades ago, polyenes, flucytosine, azoles, and echinocandins, continues to be a major challenge. With the increase in patients at risk, the incidence of mycoses is therefore a growing concern. Considering as well, the scarcity of drugs, together with toxicity, the high price of some formulations, the low availability in low-resource countries, and the development of resistance, there is an urgent need to discover new antifungals or therapeutic strategies or to modify the existing molecules with antifungal activity. This reflection article reveals that various of the most common human fungal pathogens have had the ability to acquire antifungal resistance as antifungal drugs are developed.

Keywords: Antifungal Agents, Drug Resistance, Fungal, Invasive Fungal Infections, Mycoses, Public health.

RESUMEN

Mundialmente, el creciente número de patógenos humanos resistentes a fármacos representa una importante amenaza para la salud pública. Entre estos patógenos, los hongos que han adquirido resistencia al escaso arsenal de antifúngicos son de particular significancia, porque presentan desafíos terapéuticos que aumentan la morbilidad y mortalidad. Particularmente, la mayoría de las micosis son oportunistas porque afectan especialmente a huéspedes con un sistema inmunológico debilitado, incluyendo pacientes con cáncer, neoplasias hematológicas y neutropenia prolongada, trasplante de órganos sólidos, VIH/SIDA, pacientes en unidades de cuidado intensivo, usando catéteres venosos centrales o en diálisis, usando corticosteroides, entre otros. Generalmente, las infecciones fúngicas tienen una carga médica y económica significativa que supera la carga de la enfermedad subyacente por sí sola y modifica el panorama de su desenlace. Por otro lado, el tratamiento para las micosis, que consiste en cuatro clases de antifúngicos descritos ya hace varias décadas, polienos, flucitosina, azoles y equinocandinas, sigue siendo un desafío importante. Con el incremento de pacientes en riesgo, la incidencia de las micosis es entonces una preocupación creciente. Considerando además la escasez de fármacos, su toxicidad, el alto precio de algunas formulaciones, la poca disponibilidad en países de escasos recursos y el desarrollo de resistencia, existe una necesidad urgente de descubrir nuevos antifúngicos o estrategias terapéuticas o de modificar las moléculas existentes con actividad antimicótica. En este artículo de reflexión se revela que varios de los hongos patógenos humanos más comunes han tenido la capacidad de adquirir resistencia antifúngica a medida que se desarrollan drogas antifúngicas.

Palabras clave: Antifúngicos, Farmacorresistencia Fúngica, Infecciones Fúngicas Invasoras, Micosis, Salud Pública.

INTRODUCTION

Around the world, invasive fungal infections continue to be responsible for a substantial number of deaths, significant morbidity and increased medical costs among critically ill and immunocompromised patients, whose population is constantly increasing (Bongomin et al., 2017). Added to this are the difficulties in suspecting, diagnosing, and successfully treating these infections (Firacative, 2020). Of the four major classes of antifungal drugs that are currently in use, as monotherapies or in combination, three classes have poor fungicidal activity, which at times leads to treatment failure (Perfect, 2017). Toxicity, mainly hepatotoxicity, and nephrotoxicity, together with a range of adverse side effects associated with most antifungals, represent further limitations for their use (LiverTox, 2012a). In addition, despite the efforts to search for new classes of antifungal agents, which is very challenging since there are common features between fungi and humans on the cellular level, resistance to these drugs has appeared over time, as these therapeutic options are developed (Perfect and Ghannoum, 2020). Even though fungi do not possess plasmids or transposons that are transferred from strain to strain, as it occurs in bacteria, these eukaryotic pathogens have developed diverse tools that allow them to survive in the hosts under antifungal treatment (Perfect and Ghannoum, 2020). This reflection article aims to recall the definition of invasive fungal infections, to refer to the major mechanisms associated with antifungal drug resistance, and, mainly, to reveal the decreased antifungal susceptibility, that various of the most common human fungal pathogens have to the scarce arsenal of existing antimycotic drugs, which is mainly related to the emergence of antimicrobial resistance. This resistance phenomenon is therefore a global threat to public health, as it leads to the failure of the treatment of many invasive fungal infections, an everyday problem faced by physicians, and could even hamper several clinical developments and lead to death.

INVASIVE FUNGAL INFECTIONS

Among the six million fungal species estimated to be widely distributed in the environment, several hundred of them, including yeasts and molds, can affect humans in various ways. From superficial infections of the hair, skin, nails, or mucosal surfaces, which are usually benign and represent more a cosmetic problem, fungi can also cause invasive infections affecting various organs of the body and which can cause death or substantial morbidity if not diagnosed and treated on time (Brown et al., 2012; Kohler et al., 2017). Since humans are generally naturally resistant to most invasive fungal infections due to their sophisticated immune systems, most cases of these mycoses occur in people with a serious underlying disease or condition, disregarding age, sex and place of residence (von Lilienfeld-Toal et al., 2019). Additionally, advances in medical care and treatments for various illnesses that can lead to impaired immune function have also increased the number of susceptible patients or people at-risk for developing fungal infections (Firacative, 2020; Kohler et al., 2017). This is how medical interventions, prolonged survival of premature infants, long therapies for bacterial infections, chemotherapy regimens to treat cancer, the increased number of stem cell and solid organ transplants, large use of immunosuppressive therapies and biological agents in people with autoimmune diseases, HIV/AIDS, tuberculosis, COVID, among other factors, contribute significantly to the rise of infections caused by fungal pathogens (Firacative, 2020). Lastly, some socio-economic and geo-ecological characteristics, including exposure to a high number of infectious fungal particles in their natural habitat, and even natural disasters and war, are also important scenarios that influence the incidence and prevalence of fungal diseases worldwide (Benedict and Park, 2014). It is estimated that more than 300 million people are affected by serious fungal infections every year in the world, resulting in more than 1.5 million deaths (Bongomin et al., 2017).

Simultaneously with the increase, not only in the number but also in the diversity of people critically ill and immunocompromised, the rates and spectrum of invasive fungal infections are also increasing and are becoming a major problem for human health. These infections continue to have mortality rates that remain unacceptably high, with significant economic burdens, despite advances in medical technology, improved diagnosis, earlier administration of antifungal treatments, and more effective preventive therapies (Badiee and Hashemizadeh, 2014). Added to this, the growing appearance of antimicrobial resistance, which includes species of human pathogenic fungi that are resistant to the already small number of available treatments, is a factor that negatively impacts public health, being one of the greatest challenges of modern medicine (CDC, 2019; Perfect, 2017). Finally, as fungi are metabolically similar to mammalian cells, the development and discovery of novel products to treat fungal pathogens is defiant, considering that some of these compounds can have limited efficacy owing to toxicity and that generally, fungi have fewer pathogen-specific targets for antimicrobials, compared with bacteria and virus (Ostrosky-Zeichner et al., 2010).

Among at-risk and immunocompromised patients, the yeast *Candida albicans* together with other species of this genus such as *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, and *Candida auris*, the filamentous fungus *Aspergillus fumigatus*, and the encapsulated yeast *Cryptococcus neoformans*, along with its sibling species *Cryptococcus gattii*, are the main fungal pathogens responsible for most cases of serious mycoses in the world: candidiasis, invasive aspergillosis and cryptococcosis, respectively (Bongomin et al., 2017; Brown et al., 2012; Cortes et al., 2013; Kohler et

al., 2017; Kwon-Chung et al., 2014; Schmiedel and Zimmerli, 2016). Globally, particularly after medical interventions, *Candida* infections appear among the most common causes of health care-associated bloodstream infections, due to the disruption of the normal skin barriers that allows direct access of the yeast to the body's interior (McCarty and Pappas, 2016). Among lung-transplant recipients and patients with hematological malignancies, who present with severe neutropenia (low neutrophil count), invasive infection by *Aspergillus* will be responsible for the death of more than half of the patients (Baddley, 2011). Moreover, after tuberculosis, meningitis caused by *Cryptococcus* is the second leading cause of death in people living with HIV, who present defects in cell-mediated immunity, accounting for about 19 % of AIDS-related mortality (Rajasingham et al., 2022). Altogether, it is estimated that yearly there are 750.000 cases of candidiasis, 300.000 cases of invasive aspergillosis and 152.000 cases of cryptococcal meningitis, with mortality rates that, in some cases, can exceed 70 % (Bongomin et al., 2017; Firacative, 2020).

Although the prognosis and outcome of these infections depend largely on the underlying medical condition of the patients and the time at which the diagnosis is made, the choice of early empirical therapy or prophylaxis is also important to reduce mortality and morbidity associated with these invasive fungal infections, especially when dealing with strains or species that are resistant to antifungals.

ANTIFUNGAL RESISTANCE

Apart from the importance that fungi represent to human health, as formerly stated, there is a negative impact on the efficacy of treatment of fungal infections through the emergence of resistance to essential drugs (WHO, 2022b). Antifungal resistance is defined as the ability of a fungus of a given species, through various mechanisms, to grow at a concentration of the antifungal drug that is equal to or greater than the concentration that stops growth or kills most fungal isolates of the same species (Fisher et al., 2022; Perfect and Ghannoum, 2020). In a resistant species or isolate, the drug–target interaction is directly or indirectly affected by different mechanisms, including mutations of the genes encoding target binding sites, overexpression of the amount of target available and/or drug efflux activity, among others (Fisher et al., 2022). On the other hand, tolerance to antifungals, also referred to as heteroresistance, is the ability of a subpopulation of cells of a susceptible isolate to grow, albeit slowly, in the presence of increasing concentrations of the antifungal, until it becomes resistant (Sionov et al., 2009). Some species, in addition, have intrinsic resistance to some antifungals, because they do not present the drug-binding target, or they present other mechanisms of resistance which are observed in all members of the same species (Fisher et al., 2022).

Currently, there are four families or major classes of antifungals widely used for the treatment of fungal infections: flucytosine, polyenes, azoles, and echinocandins. However, although these drugs continue to have great clinical utility, their effectiveness, either as monotherapy or in combination for prophylaxis, or as empirical or preventive therapy, has decreased significantly due to the appearance of isolates or species that are resistant to antifungals, which have emerged over time and simultaneously as new therapeutic options are developed (Fig. 1).

Flucytosine, or 5-fluorocytosine, which interferes with DNA synthesis, is known since 1957 (Duschinsky et al., 1957) but it was approved by the FDA (Food and Drug Administration) until 1971, for the treatment of serious infections by species of *Candida* and *Cryptococcus* (LiverTox, 2012b). However, shortly after its approval, in 1973, the first isolates of *C. neoformans* resistant to this drug were reported (Block et al., 1973) and later on resistant isolates of *C. albicans* (Abdul-Samad et al., 1989), *C. parapsilosis* (Pawlik and Filip, 1993) and more recently, *C. auris* (Chowdhary et al., 2014). Currently, the use of flucytosine as monotherapy is not recommended due to the high risk of development of resistance, so its use is almost exclusive to patients with cryptococcal meningitis, in whom flucytosine is always administered in combination with amphotericin B (WHO, 2022a).

Regarding polyenes, amphotericin B, used since 1959 (Dutcher et al., 1959), remains, until today, the most potent fungicide with the broadest spectrum among antifungal agents in clinical use (Perfect, 2017). This drug, which binds strongly to ergosterol, the principal sterol in fungal membranes, form complexes that create membrane pores, which lead to leakage of intracellular constituents (Almeida et al., 2017). However, the toxicity of amphotericin B deoxycholate limits its use, and although some less toxic liposomal formulations exist, the high price of these formulations, like that of 5-fluorocytosine, limits its availability in low-resource countries (Mourad and Perfect, 2018). Additionally, resistance to amphotericin B has been reported since the early 1970s in isolates of *C. tropicalis* (Woods et al., 1974), *C. krusei* (Safe et al., 1977), *C. albicans* (Jakab et al., 1990), *C. glabrata* (Law et al., 1994), *C. parapsilosis* (Pawlik and Filip, 1993; Seidenfeld et al., 1983), *C. auris* (Sarma et al., 2013), and in general in various species of *Candida* (Ghannoum and Rice, 1999), as well as in isolates of *C. neoformans* (Kelly et al., 1994) and *A. fumigatus* (Sachs et al., 1990).

Among the azoles, which disrupt the fungal cell membrane, fluconazole was patented in 1981, approved for commercial use in 1988, approved by the FDA in 1990 (LiverTox 2012a; CDC, 2019; Sachs et al., 1990) and it is currently used for the treatment of candidiasis and cryptococcosis. In this last mycosis, fluconazole is used alone or in combination with amphotericin B as a substitute for 5-fluorocytosine when it is

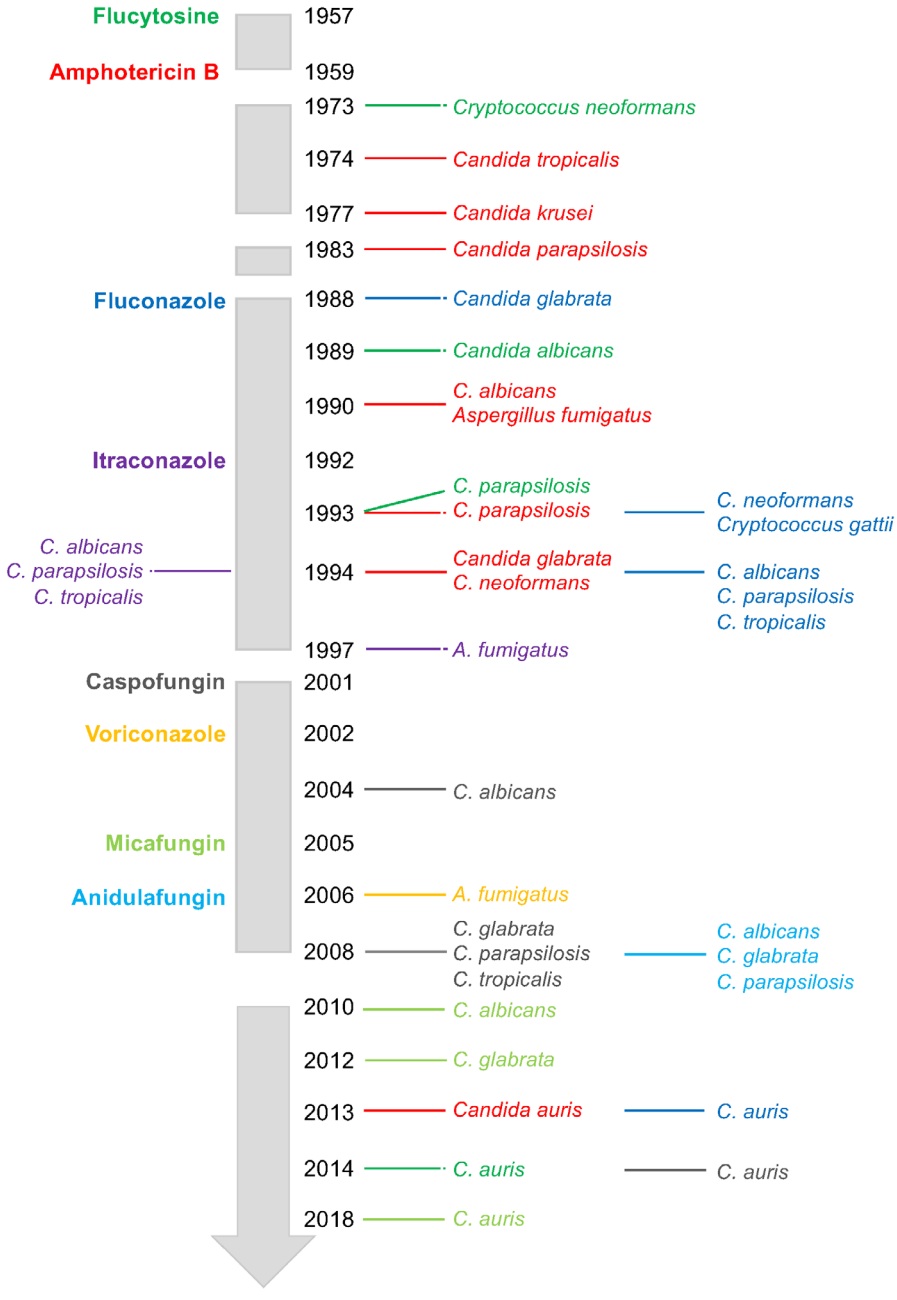

Figure 1. Representation of the emergence of antifungal resistance over time. The year in which each antifungal was launched and the year and fungal species in which resistance to each drug was reported, are indicated.

The color of the font of each species represents the resistance to each antifungal drug written with the same color.

not available in the induction phase, as well as monotherapy in the consolidation and maintenance phases (Mourad and Perfect, 2018; Woods et al., 1974). Like other azoles, however, fluconazole has poor penetration in various sites of the body, due to its oral formulation, and some species of fungi show resistance and heteroresistance, including isolates of *C. glabrata* (Warnock et al., 1988), *C. albicans* (White and Goetz, 1994), *C. parapsilosis*, *C. tropicalis* (Arias et al., 1994), *C. auris* (Sarma et al., 2013), *C. neoformans* (Tanaka et al., 1993), and *C. gattii* (Peetermans et al., 1993). Importantly too, *C. krusei* and *A. fumigatus* have intrinsic resistance to fluconazole, which prevents its use in mycoses caused by these two species (Cortes et al., 2013).

Other azoles such as itraconazole, known since 1992 and administered orally, is a triazole that is used to treat a wide spectrum of fungal infections, including candidiasis and aspergillosis (LiverTox, 2012a). However, isolates resistant to this azole have been reported since shortly after its approval as an antifungal drug, in *C. albicans*, *C. glabrata*, *C. parapsilosis* (Arias et al., 1994) and *A. fumigatus* (Denning et al., 1997). Another triazole that has been used since 2002 mainly for the treatment of candidiasis and invasive aspergillosis is voriconazole, which is also used as empiric antifungal therapy in patients with neutropenia and persistent fever, as well as preventive antifungal therapy in individuals at high risk of develop invasive fungal infections (PubChem, 2004a). However, isolates of *A. fumigatus* resistant to this azole have also been reported since 2006 (Howard et al., 2006).

Finally, the echinocandins, which include caspofungin, micafungin, and anidulafungin that inhibit the β-(1,3)-d-glucan synthesis that builds the fungal cell wall (Szymański et al., 2022), are a relatively new class of parenterally administered antifungal agents that are the therapy of choice or are used for prevention of invasive aspergillosis and systemic *Candida* infections (Pappas et al., 2016). Because they have very little toxicity to the host, echinocandins are used very frequently in the hospital setting, especially to treat infections caused by fungal species resistant to fluconazole, such as some species of *Candida* (Pfaller, et al., 2003a; 2003b). However, like other antifungal drugs, echinocandins are also ineffective in treating isolates that develop resistance. After the introduction of caspofungin on the market in 2001 (Morris and Villmann, 2006; PubChem, 2004b), isolates resistant to this antifungal have been reported in *C. albicans* (Hernandez et al., 2004), *C. parapsilosis*, *C. glabrata*, *C. tropicalis* (Pfaller et al., 2008) and *C. auris* (Chowdhary et al., 2014). Similarly, isolates of *C. albicans* (Niimi et al., 2010), *C. glabrata* (Niimi et al., 2012) and *C. auris* (Khan et al., 2018) have also shown resistance to micafungin, after its introduction in 2005 (Morris and Villmann, 2006). More recently, with the approval of anidulafungin in 2006 (Morris and Villmann, 2006), *Candida* species resistant to this echinocandin have been reported, particularly isolates of *C. albicans*, *C. parapsilosis*, and *C. glabrata* (Pfaller et al., 2008). Echinocandins are also ineffective in the treatment of species that present natural or intrinsic resistance to these drugs, such as *C. neoformans* and *C. gattii* (Denning, 2003).

REFLECTION

Not only complexities in diagnosing fungal infections abound in the health care system, but also the challenges regarding the treatment, including affordability and drug safety, are a substantial medical concern. To further complicate this issue, the constant increase of fungal pathogens that are resistance to antifungal drugs in the clinical setting is of high impact, as this reduces the drug options available to treat the infections they cause. When resistance to certain drug appears, other therapeutic options must be sought, as higher doses do not help to fight resistant microorganisms but rather may end up strengthening them. Limited medications and treatment failure imply prolonged illness or even death of the patients, especially when the infection is caused by strains or species that are resistant to all currently available antifungal agents, as it has been reported with *C. auris* (Perfect and Ghannoum, 2020). As resistance emerges, new therapies must therefore become available to meet these difficulties. However, antifungal research and development is challenging, costly and slow. Considering that both fungi and humans are eukaryotic organisms that share many cellular enzymes and metabolic pathways, any new potential antifungal drug must comply with certain levels of safety, this is high tolerability and low or no toxicity, to ensure avoiding damaging the human cells. To the afore mentioned is added the use of fungicides as pesticides to control diseases of vegetable crops, which directly or indirectly affects human health. For instance, human and animal waste, along with waste from pharmaceutical manufacturing and agricultural cultivation, can introduce antifungals and antifungal-resistant species into the environment, which then become the causative agent of diverse mycoses (CDC, 2019). Given that mortality and morbidity remain high in invasive fungal infections, despite various management strategies, and keeping in mind that many of these infections can be challenging, it is of great importance to use all available tools to fight against fungal pathogens. Moreover, highlighting again the increasing emergence of antimicrobial resistance, the discovery and development of new, more potent, less toxic and with a broad-spectrum antifungal agent, or the implementation of new strategies for the treatment of mycoses, should be our goal and highest priority, to reduce the negative impact of these infections on human health. Clearly, improved surveillance, better diagnostic tools and laboratory capacity, antifungal resistance monitoring, investments on research, development and innovation, as well as public-health interventions are urgently needed in the field of mycology, as it is emphasized in the fungal priority pathogens list recently published by the World

Health Organization (WHO, 2022b). Antifungal resistance is among us and to overcome this growing concern in the clinics, we must begin being aware of what this emerging phenomenon actually represents.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

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