Resistance beyond antibiotics – antifungal resistance

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Fungal infections are important complications in adults and children receiving cancer treatments such as myelosuppressive chemotherapy. This article reviews the epidemiology of invasive fungal disease, the measurement of antifungal resistance and its epidemiology, and the impact of antifungal resistance on patients with cancer. It also offers recommendations on how to move forward to better understand and contain antifungal resistance.

Infections are important complications in adults and children receiving myelosuppressive chemotherapy, with the highest risk among those with profound and prolonged neutropenia *(1)*. The most important pathogens associated with morbidity and mortality during chemotherapy-induced myelosuppression are bacterial and fungal organisms. Clinical practice guidelines have been developed for this patient population, focusing on the prevention of infection *(2,3)*, and empiric management of fever *(4)*. Antimicrobial resistance (AMR) is an increasingly important issue, resulting in morbidity, mortality and increased health-care utilization. While growing attention has been focused on antibacterial resistance in patients with cancer, less attention has been focused on antifungal resistance, which can result in substantial morbidity and mortality *(5)*. Consequently, the objectives of this article are to review the epidemiology of invasive fungal disease (IFD), the measurement of antifungal resistance and the epidemiology and the impact of antifungal resistance among patients with cancer.

One of the major challenges in addressing antifungal resistance is the limited number of antifungal pharmacological agents available. Developing drug therapy for fungi is challenging, in part, because fungi are eukaryotes and, thus, are more similar to human cells than bacteria or viruses. Fungal cellular structure includes an outer cell wall and an inner plasma membrane. There are three general classes of pharmacological agents that can be used to treat IFD, namely polyenes, azoles and echinocandins:

- Polyenes include conventional and lipid formulations of amphotericin B and work by binding to ergosterol, which is located in the fungal cells' inner plasma membrane.
- Azoles include triazoles such as fluconazole, itraconazole, voriconazole, posaconazole and isavuconazole. Azoles inhibit lanosterol-14α-demethylase, thereby blocking ergosterol production. This enzyme is encoded by ERG11 in yeasts and CYP51 in moulds.
- Echinocandins include caspofungin, micafungin and anidulafungin. Their antifungal activity arises from preventing synthesis of *(1,3)* β-d-glucan, which is an important component of fungal cells' outer cell walls.

Epidemiology of invasive fungal disease

Fungi are an important cause of infection worldwide and affect more than 2 million people per year, with documented rates of IFD increasing over time *(6)*. Most patients with IFD are immunocompromised, such as patients with cancer or haematopoietic stem cell transplant (HSCT) recipients. Once infection occurs, outcomes are poor, with mortality rates often exceeding 50% *(7)*. IFD accounts for more than 1 million deaths per year. Moreover, IFD incidence and mortality are almost certainly underestimated because of diagnostic challenges and the limited availability of diagnostic tools, particularly in low- and middle-income countries.

Fungi can be broadly categorized as yeasts or moulds. The most common yeast causing IFD is invasive *Candida spp*., which is linked to mortality rates as high as 40% *(8)*. In high-income countries, invasive candidiasis is the most common IFD among hospitalized patients *(8)*. Infection with *Candida spp*. may cause fungemia and metastatic organ involvement of the kidney, liver, spleen and eye, for example.

Moulds are organisms frequently found in soil, water and vegetation. The most common mould to cause IFD is *Aspergillus* spp. Frequent sites of infection include the lungs, sinuses and skin. Angioinvasion is common in immunocompromised patients *(9)* and pulmonary invasive aspergillosis can result in life-threatening haemoptysis. In contrast to *Candida* infection, fungemia is uncommon with *Aspergillus* infection.

Measurement of antifungal resistance

The standard approach to assessing antibacterial resistance is to determine *in vitro* minimum inhibitory concentration (MIC) for various drug and organism combinations. However, the ability to determine MIC is not ubiquitous for fungal isolates.

There is far greater uncertainty in defining breakpoints for fungal isolates in comparison to bacterial isolates for several reasons *(10)*. Fungi grow at a much slower rate than bacteria, prolonging the timeframe needed to determine susceptibility. More importantly, many fungal isolates do not grow in culture. Instead, they are detected through histopathology or through use of biomarkers such as galactomannan or fungal polymerase chain reaction (PCR). This challenge impedes our understanding of the epidemiology of antifungal resistance. Even among fungal isolates that can be grown in culture, breakpoints do not exist for many drug and organism combinations. In addition, correlation with clinical outcomes is challenging because isolate sensitivity is only one of many factors that impact on infection control and survival. Other factors impacting these outcomes including resolution of neutropenia, extent of IFD and patient comorbidities. Finally, fungal infection often occurs within specific microenvironments such as lung cavities, and thus, *in vitro* conditions may be markedly different compared to clinical conditions.

Nonetheless, there are breakpoints that exist for some *Candida* and *Aspergillus* spp. Fungal breakpoints were first established for *Candida* spp. Breakpoints were later established for *Aspergillus* spp. by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) *(11)*.

Antifungal resistance

Antifungal resistance is increasing over time and presents a major problem. Antifungal resistance may result in IFDs that cannot be treated with our current armamentarium of antifungal agents and may be associated with worse clinical outcomes. One study evaluated 67 episodes of candidemia in adult patients with acute leukemia *(12)*. Almost all (94%) were receiving antifungal prophylaxis at the time of candidemia. Non-susceptibility to caspofungin and multidrug resistance were both statistically significantly associated with an increase in 14-day all-cause mortality.

The two general types of antifungal resistance are intrinsic and acquired resistance *(13)*. Intrinsic resistance is inherent; it is genetically coded and, thus, is not dependent on environmental exposure to antifungal agents. An example is the resistance of *Aspergillus fumigatus* to fluconazole. In contrast, acquired resistance is developed as a consequence of exposure to antifungal agents.

There are several factors that have had an undesirable impact on rates of resistance over time and consequently have increased resistance to antifungal agents *(13)*. Exposure to antifungal agents is increasing as a result of both medical and agricultural use. Insufficient antifungal drug exposure may also increase antifungal resistance. This can result from inappropriate prescribing practices or the presence of biofilms, foreign bodies such as catheters and abscesses.

Two important examples of antifungal resistance include fluconazole-resistant *Candida* spp. and azole-resistant *Aspergillus* spp. *Candida* spp. is a common cause of infection in patients with cancer. Increasing prevalence of intrinsically resistant non-albicans spp. (such as *C. glabrata*, *C. parapsilosis* and *C. tropicalis*) and acquired fluconazole resistance are problematic *(14)*. Examples include infection with *C. krusei*, intrinsically resistant to fluconazole, and *C. glabrata*, with acquired resistance to fluconazole. The increase in non-albicans *Candida* infection has been associated with a decline in *C. albicans* infection.

Candida auris is a recently identified species that is often multidrug resistant and may be resistant to all classes of antifungal agents *(15)*. It was the first globally-emergent fungal pathogen with both multidrug resistance and the potential for nosocomial transmission. It emerged almost concurrently on four continents, suggesting that resistant *C. auris* arose from widespread exposure to antifungal agents rather than arising from a common source. It has been responsible for hospital outbreaks. The Centers for Disease Control and Prevention (CDC) issued an alert in June 2016 *(16)* requesting the reporting of identified cases to the local health units and to the CDC *(17)*.

Azole-resistant *Aspergillus* spp. is also an escalating problem, with an increase in pan-azole-resistant *Aspergillus fumigatus* *(18)*. A common mechanism of azole resistance in *Aspergillus* spp. is a mutation of the Cyp51A gene. This mutation alters the lanosterol 14-α-demethylase enzyme, which is targeted by azoles *(19)*. The mutation often confers cross-resistance to azoles in general. Azole-resistant *Aspergillus* may arise where there is high regional prevalence of resistance or in single cases of long-term exposure to azole therapy *(20)*. It has also been linked to agricultural azole utilization. The epidemiology of azole-resistant *Aspergillus* spp. has been evaluated by the Surveillance Collaboration on Aspergillus Resistance in Europe (SCARE) Network *(21)*. In one study, 22 centres from 19 countries participated from January 2009 to January 2011; each centre screened for azole resistance for 12 months. Among 3,788 *Aspergillus* isolates screened, *A. fumigatus* was identified in 77.6% of cases. Prevalence of azole resistance among patients with *A. fumigatus* was 3.2%. Among 195 cases with invasive aspergillosis, azole resistance was found in 10 (5.1%) cases.

Because of the importance of IFD and the consequence of increasing resistance to antifungal agents, it is important that antifungal resistance be more strongly emphasized in policy related to AMR. In the absence of such efforts, it is expected that resistance to antifungal agents will continue to rise and

result in greater morbidity and mortality among patients with cancer.

Future needs

With the increasing prevalence of both IFD and antifungal resistance, there are several areas that require attention in the near future. First, a basic understanding of the epidemiology of antifungal resistance is lacking. Without this knowledge, empiric and therapeutic antifungal strategies are challenging, and patients may receive initial antifungal therapy that is suboptimal. To address this problem, non-culture approaches to measuring antifungal resistance need to be developed and systematically implemented. As IFD is rare, well-designed, multicentre, observational studies describing the extent of antifungal resistance and factors associated with increased resistance are required. As there is geographical variation in IFD prevalence, international collaboration will be necessary to fully understand antifungal resistance epidemiology.

Secondly, new effective approaches to treat resistant fungi must be identified. This will require the development, evaluation and licensing of new antifungal agents. As the infection is rare, treatment trials will require a multicentre design. Examples of agents currently in clinical trials include ibrexafungerp, rezafungin and oteseconazole *(18)*.

Thirdly, strategies to reduce antifungal resistance are required, including the development of standards and clinical practice guidelines focused on antifungal use. There are a number of antifungal stewardship programmes that have been established and these will likely be important in limiting the spread of antifungal resistance *(22)*.

Conclusions

Antifungal resistance is a growing problem in patients with cancer. Increasing intrinsic and acquired resistance have been observed and the latter has been linked to rising exposure related to medical and agricultural use. Pan-resistant isolates such as *C. auris* have begun to emerge and are of major concern. Future efforts should focus on establishing multicentre collaborations, advancing non-culture approaches to detect antifungal resistance and establishing antifungal stewardship programmes. ■

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