Emergence of Resistant *Candida* in Neutropenic Patients

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Problems with resistance to antifungal drugs have emerged due to an increase in the incidence of systemic fungal infections and widespread use of antifungal agents. Accordingly, efforts have been made to develop adequate fungal susceptibility tests. The ideal test should have high intra and inter-laboratory reproducibility, good correlation with the clinical outcome, and should be easy to perform. While no such test has yet been developed, advances have been made. Over the past decade, many reports of fungal resistance have been published, most of them in AIDS patients. Though the frequency of resistant strains is still low in neutropenic cancer patients, and is mostly limited to Candida glabrata and Candida krusei, resistance to Candida albicans has also been reported.

Key Words: Candida, resistance, neutropenic.

The incidence of systemic fungal infections has increased dramatically over the past 20 years [1]. Therapy for such infections has been difficult, because of the limited number of available antifungal agents. After the introduction of the azoles, which have a high oral bioavailability and a low incidence of side effects, a new era in the treatment of fungal infections begun. Indeed, fluconazole has proven highly effective in many clinical situations, such as oral and esophageal candidiasis in AIDS patients [2], systemic fungal infections in bone marrow [3] and liver transplant recipients [4], cryptococcosis [5,6] and candidemia [7,8]. However, in the case of oral candidiasis in AIDS patients, although the efficacy rate is very high, relapse frequently occurs. Therefore, many AIDS patients receive fluconazole for long periods of time. This scenario has favored the development of resistance, and many reports of treatment failure have been published over the past decade [9-16]. Efforts have been made to standardize antifungal susceptibility tests for fungi. In this review, we will focus on the limitations of the tools for assessing antifungal susceptibility, and the magnitude of this problem in neutropenic cancer patients.

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Antifungal susceptibility tests

Resistance is a concept derived from an *in vitro* phenomenon that, theoretically, is associated with clinical failure, whereas susceptibility would indicate efficacy. However, this is not necessarily true, and in fact, besides *in vitro* antifungal resistance, there are many reasons for treatment failure. For instance, in the treatment of fungal infections in neutropenic patients, bone marrow recovery is a strong predictor of efficacy, regardless of whether the strain is susceptible or resistant [17-19]. Likewise, breakthrough candidemia, an increasingly reported phenomenon, seems not to be associated with resistant strains, but with the compromised immune status of the host [20]. Other

possible reasons for efficacy failure include infection in non-vascular sites (abscesses, catheters, and prosthesis), poor absorption, and fast elimination or metabolization of the antifungal drug.

Since host factors are often much more important than drug susceptibility, susceptibility does not necessarily predict success. However, susceptibility tests would still be clinically useful if a lack of *in vitro* susceptibility was correlated with treatment failure. In addition, the test should be reproducible. Over the past decade, many methods have been developed, including disk diffusion methods [21], E-test [22], colorimetric methods [23], and broth macro and microdilution methods [24]. The broth macro and microdilution test is the NCCLS (National Committee for Clinical Laboratory Standards) reference method, and provides over 90% intralaboratory and interlaboratory reproducibility [25,26]. Interpretative break points have been established for azoles, based mainly on the analysis of the outcome of oropharyngeal candidiasis in AIDS patients [27]. Therefore, in the case of the azoles, a major limitation for the clinical usefulness of an antifungal susceptibility test is the very limited amount of data on the correlation between MICs and the outcome in invasive candidiasis, especially in neutropenic patients. Likewise, this method remains to be validated for other antifungal agents, including amphotericin B. The correlation between *in vitro* susceptibility tests for amphotericin B and the outcome in candidemia was assessed using the NCCLS method [28]. Among 105 candidemic episodes, 33 had microbiological failure, defined as persistence of *Candida* in the bloodstream after 3 days of amphotericin B treatment. The correlation between MIC and failure was poor. This was mainly because the MIC range was very narrow (0.06 – 2 μg/mL). A resistance breakpoint of =1 μg/mL was suggested.

Although reproducible, the NCCLS standardized method is cumbersome. Thus, alternatives to this method have been sought. Overall, disk diffusion and E-test are effective in identifying susceptible strains. However, these methods do not adequately discriminate between resistant and intermediate or dose-dependent strains. Therefore, they may be useful as screening tests, but confirmation of resistant strains should be made with the NCCLS method.

Antifungal resistance in clinical practice

The first reports of antifungal resistance occurred in patients with mucocutaneous candidiasis treated with ketoconazole [29], but since the AIDS epidemic began, this problem has gained great clinical relevance. The typical background for the development of azole resistance is the prolonged and repeated use of fluconazole for the management of oral and esophageal candidiasis in AIDS patients with low CD4+ cell counts [30]. Patients initially infected by susceptible strains of *Candida albicans* subsequently developed infection by the same genotype of *C. albicans*, but with high MICs. Another pattern of resistance is the acquisition of infection due to azole-resistant non-*albicans* species, such as *Candida krusei* and *Candida glabrata* [31]. However, during the late 90s, the introduction of highly effective antiretroviral therapy exerted a tremendous impact on the natural history of HIV infection and its complications. Indeed, the incidence of opportunistic infections, including oropharyngeal candidiasis due to resistant strains has decreased significantly.

Outside the setting of oropharyngeal candidiasis in AIDS patients, reports of infection by resistant strains have become more frequent. With the widespread use of azoles, especially fluconazole, for prophylaxis in neutropenic cancer patients, this problem has gained increased significance. A phenomenon that has been increasingly reported among patients receiving fluconazole is the shift from highly susceptible to less-susceptible species of *Candida*. Epidemiological studies performed in patients with cancer and fungemia have shown that while the number of cases caused by *Candida albicans* has decreased, the frequency of infection due to *Candida krusei* and *Candida glabrata* has increased substantially [32-35]. While *C. krusei* is considered resistant to fluconazole, the MIC values of fluconazole for the *C. glabrata* isolates

are variable, but are much higher than those reported for C. *albicans*, *Candida tropicalis* and *Candida parapsilosis*.

This increase in the incidence of infection due to less-susceptible *Candida* species has been attributed to the widespread use of fluconazole. For instance, in a large prospective survey conducted in European institutions, antifungal prophylaxis was a strong predictor for non-*albicans* candidemia [35]. In another prospective study, 349 *Candida* isolates obtained from colonization and systemic infections were analyzed [36]. Resistance to fluconazole was observed in 3.4% of *C. albicans* isolates and in 30.7% of *C. glabrata* isolates. Only 2 strains (*C. glabrata* and *C.* krusei) were resistant to amphotericin B (MIC =1 μg/mL). In this study, previous azole exposure was associated with the isolation of azole-resistant *C. albicans* strains, but this association was not observed with *C. glabrata*.

The influence of fluconazole use on the development of azole resistance was further evaluated in 585 bone marrow transplant recipients [37]. Weekly mouthwash samples were obtained, and yielded *Candida* in 256 patients. While *C. albicans* was the most frequent species obtained before fluconazole exposure, the majority of patients who were colonized by *C. glabrata* and *C. krusei* had received fluconazole for a median of 36 days. Ninety-nine percent of *C glabrata* isolates were resistant to fluconazole. Among 30 candidemias occurring during fluconazole prophylaxis, 14 were caused by *C. glabrata*, 6 were due to *C. krusei*, and 2 were due to *C. albicans*, and all strains were highly resistant to fluconazole. *C. albicans* strains that acquire resistance after exposure to fluconazole have been reported by other authors [38], but as shown in other studies [36,39,40], this seems to be rare.

The development of candidemia during antifungal treatment (breakthrough candidemia) is another phenomenon that can be associated with infection due to resistant strains. During the past decade, reports of the occurrence of breakthrough candidemia have been published [20,41-45]. Unfortunately, in the majority of such reports no information on the MICs was provided. In a recent study of 29 breakthrough candidemias occurring among 270 candidemias, MICs were obtained from all patients [46]. The MIC 50s for fluconazole and amphotericin B were similar in breakthrough and non-breakthrough candidemias. Furthermore, the frequency of strains with high MICs in the entire cohort was very low (4 patients with amphotericin B MIC =2, and 4 patients with fluconazole MIC ≥64). Therefore, it seems that in most cases, breakthrough candidemia is not related to the development of infection due to resistant strains.

In Brazil, antifungal susceptibility tests of 200 bloodstream isolates of *Candida* were performed using the NCCLS method [47]. Cancer was the most frequent underlying disease, accounting for 33% of cases, and about 50% of these were neutropenic. The incidence of fluconazole resistance was 2.5%, and resistance was limited to *C. krusei* and *C. glabrata* isolates. Data from an epidemiological study conducted by the same authors showed that the incidence of these two species was low [48]. The low incidence of resistant strains was attributed to the infrequent use of fluconazole in these Brazilian hospitals.

In summary, during the past decade, fluconazole has been extensively used in neutropenic patients. This has resulted in a marked decrease in the incidence of invasive candidiasis, especially in allogeneic bone marrow transplants. However, this was accompanied by a shift from highly susceptible to less susceptible *Candida* species, by a process of selection. Indeed, *C. glabrata* has emerged as an important pathogen, causing fungemia in many countries. In addition, resistance has also emerged as a result of the acquisition of resistance in otherwise susceptible *Candida* species, but at a much smaller magnitude. Finally, the limitations of the present methods for assessing antifungal susceptibility hamper any conclusion about resistance to amphotericin B and preclude their use for therapy guidance in the clinical setting.

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