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The Effect of Cumulative Length of Hospital Stay on the Antifungal Resistance of Candida Strains Isolated from Critically Ill Surgical Patients

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Abstract

Fluconazole is the first line of therapy for the management of candidiasis. However, fluconazole-resistant strains pose an emerging challenge in everyday clinical practice. In this study, we sought to determine whether cumulative length of hospital stay (CLOS) is a predictive factor for the acquisition of non-susceptible *Candida* strains to fluconazole. Thirty-three critically ill emergency surgery patients with 56 *Candida* isolates were enrolled in this prospective study. We divided our isolates according to their minimum inhibitory concentration (MIC) to fluconazole using 8 mcg/ml as a cutoff. We then compared the two groups with respect to basic demographics, antifungal agents prescribed, number of wide-spectrum antibiotics, duration of central venous catheter placement, elapsed time to positive culture, duration of prior hospital stay, and length of hospital stay. Non-susceptible fluconazole samples belonged to patients with a significantly longer prior hospital stay and a longer CLOS ($P = 0.02$ and 0.01 , respectively). The difference between the 2 groups regarding non-albicans strains was statistically significant ($P < 0.001$). By fitting a non-parametric receiver-operating characteristics (ROC) curve into our analysis, CLOS ≥ 29 days predicted the occurrence of non-susceptible strains with 90% sensitivity and 79.6% specificity (correct classification 81.5%). A CLOS ≥ 29 days is a strong predictor for the isolation of non-susceptible *Candida* isolates to fluconazole among critically ill emergency surgery patients. Clinicians should consider the duration of previous hospital stay when deciding on empiric antifungal therapy.

Keywords

Candida; Risk factor; Fluconazole; Emergency surgery; Resistance; Candidiasis

Introduction

Candida spp. are the most common cause of human fungal infections, representing nearly 96% of all opportunistic mycoses [1, 2]. Up to 2% of intensive care unit (ICU) patients suffer from invasive candidiasis, the incidence of which shows an alarming increase during recent years [3, 4]. Overall, *Candida* spp. are the 4th most common cause of hospital acquired bloodstream infections in the United States and 7th in Europe, and approximately 10% of all ICU-acquired bloodstream infections are caused by *Candida* spp. [5–7].

Numerous protocols detailing the use of azoles for curative, pre-emptive, or prophylactic treatment of invasive candidiasis have been developed during the previous decades. Since its introduction in the 1990s, fluconazole has proven to be a reliable, cost-effective, and clinically safe antifungal agent against *Candida* infections [8]. Currently, it is being used in nearly 67% of all cases of candidemia in North American hospitals [9]. In the past 15 years, several studies from various settings and diverse populations have investigated clinical risk factors leading to the emergence of fluconazole resistance among *Candida* spp. or the acquisition of intrinsically resistant strains such as *C. glabrata* or *C. krusei* [10–21]. The vast majority of these studies are in high-risk populations such as patients with human immunodeficiency virus (HIV) infection, neutropenia, or bone marrow transplant recipients [19, 20, 22]. Unfortunately, the investigation of specific risk factors in the ICU setting is limited [12, 13].

Interestingly, no previous studies have examined the impact of cumulative length of hospital stay (CLOS) in the acquisition of *Candida* strains with increased antifungal resistance. This correlation is particularly important because it may provide realtime information on the likelihood of antimicrobial resistance in these patients. Especially, tertiary referral centers often accept patients who have spent long periods in other hospitals, and attention should be given to the influence of prior hospitalizations to infectious processes and antimicrobial resistance. This is especially important in *Candida* because colonization is probably the single most important predisposing factor for *Candida* infection. In our study, we investigated cumulative length of hospital stay in this population, as an independent risk factor for the development of antifungal resistance and/or the selection of *Candida* spp. that are resistant to fluconazole. This study focused on patients with nontraumatic surgical illnesses, a population that has an established high risk for systemic candidiasis [23].

Patients and Methods

Design of Study

We conducted a prospective, single-center, observational cohort study to analyze the antifungal susceptibilities of 56 *Candida* isolates to fluconazole with respect to CLOS. All isolates originated from critically ill emergency surgery patients admitted between September 1, 2007 and March 1, 2008. Patients were admitted directly to Massachusetts General Hospital or transferred from another facility. Investigators who made the MIC determinations were blinded to the study objective. We selected this particular population of critically ill surgical patients in order to increase the yield of *Candida* isolation (critical illness predisposes to the development of *Candida*). For the purposes of this study, we

defined as emergency surgical disease all processes causing obstruction, perforation, bleeding, or inflammation that required an emergent intervention [23, 24]. Data on the demographic and clinical characteristics of the patients were prospectively collected during their hospital stay. One patient was excluded from the study due to insufficient data. The Massachusetts General Hospital Investigational Review Board (IRB) approved the study protocol. Those *Candida* strains with an MIC > 8 mcg/ml to fluconazole were considered as 'non-susceptible', and those with an MIC less than 8 mcg/ml were considered as 'susceptible'. Of note is that all isolates belonged to patients that were either infected or colonized by *Candida*. CLOS was defined as the sum, in days, of all the lengths of previous hospitalizations of each patient 3 months prior to hospitalization including the length of index hospitalization. Characteristics such as CLOS, antifungal consumption, days of central venous catheter (CVC) placement, and number of wide-spectrum antibiotics reflected the period before each positive culture during the current hospital stay. As noted earlier, we decided to limit our review of patient data up to 3 months because (a) *Candida* strains can retain antifungal resistance and remain preserved in the environment [25, 26] and (b) in pilot case reviews, we found that extracting information for length of hospitalizations older than 3 months was not reliable.

Fungal Isolation and Identification

All *Candida* samples originated from 31 patients colonized and 2 patients infected by *Candida*. By definition, all positive blood cultures for *Candida* were considered infections. All others were difficult to evaluate whether they represented colonization or infection since no standard and widely accepted definitions exist for other forms of *Candida* infections. Thus, we defined all other strains as colonizing. Fluconazole susceptibility testing was performed on every *Candida* sample. Specimens were taken for diagnostic purposes upon the clinician's request. The samples included intra-abdominal fluid (from drains or abdominal collections), blood, tissue, wound, and urine. *Candida* isolates were identified preliminary to the species level using differential established assays. A combination of CHROMagar *Candida* (CHROMagar, Paris, France) and modified molybdate agar plates, containing $P_2O_5 \cdot 20MoO_3 \cdot xH_2O$, were used to identify *C. albicans*, *C. tropicalis*, and *C. parapsilosis* [27]. *C. glabrata* isolates were further confirmed using a rapid assimilation of trehalose assay [28]. The differential media identification assays employed in this research are limited to preliminary species identification and do not provide definitive identification of the *Candida* strain.

Drug Susceptibility Testing and Minimum Inhibitory Concentration (MIC) Determinations

Antifungal susceptibilities to fluconazole were determined according to the CLSI (Clinical and Laboratory Standards Institute, formerly known as the National Committee on Clinical Laboratory Standards) method M-27A with broth microdilution techniques and reading of the endpoints at 48 h [29, 30]. According to the CLSI protocol, isolates with MIC values ≤ 8 mcg/ml are considered susceptible to fluconazole. MICs between 16 and 32 mcg/ml indicate susceptible but "dose-dependent" isolates. Isolates for which MICs were ≥ 64 mcg/ml were considered resistant [31, 32].

Statistical Analysis

Categorical data were compared using the chi-square test. Continuous variables were compared using the Mann–Whitney test. ‘Stata V8’_ was used for statistical analysis, and a P value less than 0.05 was considered significant. CLOS, the parameter of interest, was considered as a test variable for the development of non-susceptible *Candida* strains and a receiver-operating characteristics (ROC) curve was fitted to define its predictive value.

Results

During the 6-month study period, 56 *Candida* isolates were obtained from 33 eligible patients. The mean (SD) age was 57.7 years (± 18.6) with a male predominance (19 male vs. 14 female). The median length of stay during the current hospitalization before a positive culture for *Candida* spp. was 11 days (range 0 to 89 days), and the median CLOS was 20 days (range: 0 to 105). Upon comparison of the ‘susceptible’ and the ‘non-susceptible’ groups (Table 1), there were no significant differences regarding demographics, antifungal agents prescribed, number of wide-spectrum antibiotics, duration of CVC placement, and elapsed time to positive culture.

Candida cultures were obtained from urine (n = 33, 59.8%), intra-abdominal fluid (n = 13, 23.2%), superficial wounds (n = 5, 8.9%), blood (n = 3, 5.4%), intraabdominal abscess (n = 1, 1.8%), and bile (n = 1, 1.8%).

As shown in Table 1, the ‘non-susceptible’ strains belonged to patients who had a longer length of prior hospital stays in an outside hospital (P = 0.02) and longer CLOS (P = 0.01). *Candida* cultures were obtained from urine (n = 33, 59.8%), intra-abdominal fluid (n = 13, 23.2%), superficial wounds (n = 5, 8.9%), blood (n = 3, 5.4%), intra-abdominal abscess (n = 1, 1.8%), and bile (n = 1, 1.8%). Regarding the type of surgical interventions, 16 of the patients underwent laparotomy alone (48.5%), 2 of them underwent combined laparotomy and orthopedic surgery (6.1%), 1 combined laparotomy and craniotomy (3%), 1 combined laparotomy and thoracotomy (3%), 1 combined thoracotomy and craniotomy (3%), 2 combined orthopedic surgery (6.1%), 8 other types of invasive procedures (24%), and 2 of them underwent no surgical intervention (6.1%).

In terms of previous hospitalizations, 50% of *Candida* isolates (n = 28) were obtained from patients who had a prior hospitalization. The median length of current nosocomial stay for patients with previous hospitalization was 11 days (range 1–90 days), and the other 46.6% of the isolates (n = 26) belonged to patients without prior admission (46.4%). Data on previous admissions were not available for 2 records (3.6%) and were included as belonging to patients with no previous hospitalizations. In Fig. 1, we show each one of the *Candida* MICs with respect to CLOS.

Candida isolates were identified to the species level where *C. albicans* accounted for the majority of the samples (71%, n = 40), and *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* accounted for 14% (n = 8), 9% (n = 5), and 4% (n = 2), respectively. One non-*albicans* isolate could not be identified by the phenotypic assays employed in this study (n = 1, 2%). In addition, among all 56 *Candida* isolates, 44 (78.6%) were susceptible to fluconazole and

12 (21.4%) were non-susceptible. Of note, all the *Candida* strains exhibited susceptibility to amphotericin B (results not shown). The susceptible group comprised 36 *C. albicans* vs. 8 non-*albicans* strains, while the non-susceptible group included 2 *C. albicans* and 10 non-*albicans* isolates. The difference between the 2 groups regarding non-*albicans* strains was statistically significant (P value 0.001). Of the patients enrolled, 27.2% had at least one positive culture with a non-susceptible strain. Interestingly, of the 5 out of 33 patients who died (15.1%), 3 had a culture positive for a non-susceptible *Candida* strain (2 *C. glabrata* and 1 *C. albicans* strains) and 2 of them harbored susceptible strains (both *C. albicans*), suggesting a higher mortality trend for the non-susceptible group (3 out of 9 patients with a non-susceptible strain vs. 2 out of 24 with a non-susceptible strain, $P = 0.07$). More specifically, empiric antifungals (fluconazole and micafungin) were administered to 1 patient with a fluconazole-susceptible non-infecting *C. albicans* strain (urine sample, CLOS = 83) and to 2 patients who harbored non-susceptible to fluconazole *C. glabrata* strains with an MIC = 8 mcg/ml (1 from the blood and 1 from the urine with CLOS of 29 and 90, respectively). The other 2 deceased patients did not receive empiric antifungals, and one harbored a *C. albicans* urine strain (CLOS = 11) and the other a *C. glabrata* non-susceptible urine strain (CLOS undefined). The patient with the undefined CLOS was the one originally excluded from the analysis; however, his history suggested a prolonged surgical history in Iran, which warranted his continuance of care in the US. Unfortunately, we could not analyze the attributable mortality of the deceased patients.

We analyzed the effect of CLOS in the acquisition of non-susceptible *Candida* strains by fitting a nonparametric receiver-operating characteristics (ROC) curve for CLOS (Fig. 2). This analysis allows the identification of a specific value from which we can predict the acquisition of non-susceptible fluconazole strains with a clinically acceptable sensitivity and specificity. In our study, the AUC was 0.87, therefore CLOS may be considered a good discriminating variable. A cutoff value of C 29 days predicted the acquisition of *Candida* strains non-susceptible to fluconazole with 90% sensitivity and 79.6% specificity (i.e., a positive *Candida* culture from a critically ill surgical patient with a CLOS C 29 days has a 90% probability to be non-susceptible to fluconazole).

Discussion

The results of this study demonstrate that a CLOS C 29 days can predict the isolation of a nonsusceptible *Candida* strain among critically ill emergency surgery patients with a 90% sensitivity and 79.6% specificity (Fig. 2). This is a new correlation, which may prove to have important therapeutic repercussions. Early initiation of empiric antifungal therapy is probably the single most important intervention in the management of candidiasis and can decrease mortality from above 30 to 10% [33]. However, the identification of *Candida* to the species level and the determination of *Candida* MICs to common antifungal agents requires time; thus, the clinician may be required to initiate treatment before the identification and the antifungal resistance profile is available. In the ICU, inadequate initial fluconazole dosing among patients with bloodstream candidiasis has been linked to substantially increased mortality ($P = 0.004$) [34]. This study suggests that a CLOS C 29 days predicts with a high probability an increased likelihood of non-susceptible to fluconazole *Candida* that would necessitate appropriate adjustments in antifungal regimens and dosing [35].

The predictive value of CLOS as a risk factor for non-susceptible *Candida* strains is supported by experimental studies. *Candida* species have the ability to survive outside a living host and remain in the environment for long periods of time, thus being able to contaminate skin surfaces and the hands of healthcare professionals [26, 36, 37]. Also, in vitro studies have shown that various *Candida* spp. such as *C. albicans*, *C. tropicalis*, and *C. glabrata* can develop fluconazole resistance when cultured in fluconazole-rich media and retain this resistance for a period of time [38–41]. Even though it is generally accepted that most patients develop *Candida* infection from their own flora, the above-mentioned studies also suggest that nosocomial or ICU fungal strains are spread to the environment by colonized or infected patients and may remain there for a long time while possibly retaining their antifungal resistance. Additionally, certain *Candida* spp. lose their antifungal resistance to fluconazole over time [38, 42], suggesting that recent previous hospital stays may be important predisposing factors for a re-hospitalized patient to be infected or colonized with strains with increased fluconazole resistance.

In conclusion, the exact calculation of the length of recent hospitalizations is often ignored in clinical practice. Combining the length of previous and current hospital stays, a CLOS 29 days may be a strong predictor for the development of a fluconazole-resistant *Candida* infection, and it should be taken into account when initiating empirical antifungal therapy. This is particularly important since systemic candidiasis is associated with 30–40% mortality, and effective empiric antifungal therapy is the single most important measure to decrease this dramatic mortality rate. A larger multicenter study is needed in order to evaluate the ramifications of this finding.

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Fig. 1.

Each point represents the occurrence of one positive *Candida* culture. (Asterisk) We excluded 2 cultures (isolates) from a single patient, from the graph due to unknown previous hospitalizations. If more than one culture occurred during a single day, we arbitrarily set these points apart by 0.2 days so that all could be visualized.

Fig. 2.

Non-parametric ROC curve fitted for cumulative stay (CLOS). The area under the curve is 0.87, and a cutoff value 29 days can predict resistant strains with 90% sensitivity and 79.6% specificity (correct classification 81.5%).

Table 1 Comparisons between susceptible and non-susceptible *Candida* strains

	Susceptible group (n = 44)	Non-susceptible group (n = 12)	P value
Age (years)	59 (21 to 82)	77 (38 to 82)	0.10
Male gender	26 (59%)	9 (75%)	0.31
Elapsed time to positive culture (days)	11 (0 to 89)	17.5 (0 to 35)	0.63
CVC duration (days)	8 (0 to 93)	9.5 (0 to 32)	0.85
Prior Hospitalization (OSH) (days)*	0 (0 to 24)	13 (0 to 90)	0.02
<i>C. albicans</i> /non- <i>albicans</i> spp.	36/8 (82%/18%)	2/10 (17%/83%)	<0.001
CLOS ⁻ (days)	17 (0 to 105)	34.5 (27 to 90)	0.01
Number of prior antibiotics during current hospitalization	5 (0 to 9)	6 (3 to 12)	0.10
Prior antifungal agents during current hospitalization	14/44	2/12	0.30

Continuous data are reported as median (range). CLOS equal to zero was described when the patient had no prior hospitalization and exhibited a *Candida* strain during his first day of admission. Prior hospital stay equal to zero referred to a patient with no prior hospitalizations

* Data on 56 available records

⁻ Sum of days to positive culture plus prior hospital stay, CVC central venous catheter, OSH, outside hospital