Impact on antifungal susceptibility patterns of previous vs. revised Clinical and Laboratory Standards Institute breakpoints for *Candida* species isolated from candidemia: Experience of two tertiary care institutions in Japan.

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SUMMARY

Background: Candidemia has a high mortality rate. Identifying prognostic factors of candidemia, based on each regional data, is essential for the better management. The Clinical and Laboratory Standards Institute (CLSI) recently revised *Candida* species-specific breakpoints (R-BP) for antifungal agents. Few studies have investigated the detection performance of resistance in *Candida* species by comparing the R-BP and previous species no-specific CLSI breakpoint (P-BP) among patients with candidemia. The primary objective was to investigate the impact of the R-BP on the antifungal susceptibility patterns of *Candida* species, while the secondary objective was to identify the prognostic factors of candidemia.

Methods: A total of 193 *Candida* species isolated from 187 patients with candidemia between January 2007 and December 2016 were examined. Susceptibility based on CLSI M27-A3 was defined as the P-BP, and that based on species-specific CLSI M59 or M60 breakpoint was defined as the R-BP. Multivariate *Cox's* hazard analysis was performed to identify prognostic factors within 30 days of the diagnosis of candidemia.

Results: A significant difference was observed in the susceptibility rate to fluconazole (FLCZ) (P-BP; 93.0% vs. R-BP; 79.4%), to voriconazole (VRCZ) (P-BP; 97.2% vs. R-BP; 91.0%). The susceptibilities of *C. parapsilosis*, *C. glabrata*, and *C. tropicalis* to azole antifungal agents were markedly lower with the R-BP. Based on the R-BP, antifungal therapy was regarded as inappropriate for approximately 10% of patients. The 30-day mortality rate was 29.4%. In a multivariate *Cox's* hazard analysis, age, lung disease, *C. albicans*, and the absence of antifungal therapy were associated with a high mortality rate, whereas serum albumin, *C. parapsilosis*, surgical wards, the removal of central venous catheter (CVC), and follow-up blood culture tests to confirm the clearance of *Candida* species were associated with a lower mortality rate.

Conclusions: Early initiation of antifungal therapy, removal of CVC and follow-up blood culture

tests are essential for improving the outcome. The R-BP efficiently detected non-susceptible strains to FLCZ and VRCZ, particularly in non-*albicans Candida* species. The present results support the importance of antifungal susceptibility tests and interpretations based on the R-BP among patients with candidemia.

KEY WORDS: Antifungal susceptibility, Candidemia, Clinical and Laboratory Standards Institute M59 M60, Prognostic factors of candidemia

INTRODUCTION

Candidemia, which is defined as the isolation of *Candida* species from blood cultures, has a high mortality rate [1]. This infectious disease has a potentially significant impact on patients because its associated complications, such as endophthalmitis and infective endocarditis [2]. Geographical variations has been observed in species distribution among patients with candidemia [3]. Therefore, investigations on the prognostic factors of candidemia based on each regional practice data and microbiological features are essential for the better management.

Treatment failures associated with *Candida* species showing resistance to widely used antifungal agents have recently been reported [4]. Therefore, the treatment guidelines for invasive candidiasis published by the Infectious Disease Society of America (IDSA) strongly recommended susceptibility tests to antifungal agents for isolated *Candida* species [2]. The previously published Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoints (CLSI M27-A3) were the established criteria, regardless of *Candida* species [5, 6]. In 2012, CLSI revised the susceptibility breakpoint (CLSI M27-S4) to improve the detection accuracy of resistant *Candida* species [6]. The most prominent change in the CLSI M27-S4 breakpoint from the CLSI M27-A3 breakpoint is that each *Candida* species has different susceptibility breakpoints for widely used antifungal agents. On the other hand, one of the disadvantages of the CLSI M27-S4 breakpoint is that the susceptibility breakpoint is not defined for *Candida* species with infrequently isolated and not widely used antifungal agents due to a lack of data. Therefore, some experts recommend using the CLSI M27-S4 breakpoint and epidemiological cut-off values (ECVs) in combination to improve the detection performance of resistance in infrequently isolated *Candida* species [7].

Recently, CLSI published M60 and M59 as the revision of clinical breakpoint and ECVs for *Candida* species, respectively [8, 9]. Although this revision was a minor change compared to that from CLSI M27-A3 to M27-S4, the susceptibility evaluation based on the latest breakpoint is extremely important to monitor the emergence of *Candida* species that exhibited resistant to

antifungal agents. Few studies have investigated the detection performance of resistance in *Candida* species by comparing the revised species-specific and previous species no-specific CLSI breakpoints among patients with candidemia in Japan. In order to promote the appropriate use of antifungal agents, it is important to investigate the detection performance of resistance in *Candida* species according to the revised CLSI breakpoint. Therefore, the primary objective of the present study was to assess the impact of the species-specific revised CLSI breakpoint on the antifungal drug susceptibility patterns of *Candida* species isolated from candidemia, while the secondary objective was to identify the prognostic factors of candidemia.

MATERIALS AND METHODS

Study design and population

One hundred and eighty-seven patients diagnosed with candidemia in Aomori Prefectural Central Hospital and Hirosaki University Hospital between January 2007 and December 2016 were enrolled in the present study. We defined a case of candidemia as a patient with at least one positive blood culture for *Candida* species.

Ethical approval

The study protocol was approved by the Ethics Committee of Hirosaki University Graduate School of Medicine, and the Ethics Committee of Aomori Prefectural Central Hospital. Patient consent was not required because of the retrospective nature of this study. We examined patient medical records while assuring anonymity.

Mycological data collection

Mycological data were collected from the blood culture records of each patient. Methods for identifying *Candida* species in each institution were as follows. At Aomori Prefectural Central Hospital, blood cultures were performed using the Bac T/ALERT 3D blood culture system (SYSMEX; bioMerieux, Lyon, France). Blood culture bottles with suspected yeast-like fungi by microscopy were subcultured on CHROM agar *Candida* culture medium (Kanto Chemical, Tokyo, Japan) and incubated at 30°C for 48 h. The identification of each *Candida* species was performed using the ID32C system (SYSMEX; bioMerieux, Lyon, France) between January 2007 and August 2014. The identification kit was changed to the VITEK 2 YST ID Card (SYSMEX; bioMerieux, Lyon, France) from September 2014. At Hirosaki University Hospital, blood cultures were performed using the Bac T/ALERT blood culture system (bioMerieux, Lyon, France) between January 2007 and September 2009. The blood culture system was changed to the Bac T/ALERT 3D

blood culture system (SYSMEX; bioMerieux, Lyon, France) from October 2009. Blood culture bottles with suspected yeast-like fungi by microscopy were subcultured on CHROM agar *Candida* culture medium (Kanto Chemical, Tokyo, Japan) and incubated at 35°C for 48 hours. The identification of each *Candida* species was performed using the VITEK 120 YBC Card (bioMerieux, Lyon, France) between January 2007 and September 2009. The identification kit was changed to the VITEK 2 YST ID Card (SYSMEX; bioMerieux, Lyon, France) between October 2009 and January 2015, and the MALDI Biotyper (Bruker Daltonics, Bremen, Germany) has been used since February 2015. The same method was used for drug susceptibility tests at the two institutions. Antifungal susceptibility tests were performed using the yeast-like fungal drug susceptibility kit ASTY (Kyokuto Pharmaceutical Industrial, Tokyo, Japan). The antifungal susceptibility of each agent was assessed based on the minimum inhibitory concentration (MIC) after an incubation at 35°C for 48 hours. We defined seventeen unidentifiable *Candida* species as other *Candida* species. CLSI M60, species-specific antifungal agent drug susceptibility breakpoints, were used to assess susceptibility [8]. CLSI M59, revised species-specific ECVs, were applied to assess susceptibility in *Candida* species that have no breakpoint in CLSI M60 [9]. We defined the results of susceptibility based on CLSI M60 or CLSI M59 as the revised CLSI breakpoint (R-BP). Previously published ECVs were applied to the *Candida* species have no breakpoint in CLSI M59 or CLSI M60 (e.g., *Candida guiiliermondii* (*C. guiiliermondii*) to voriconazole (VRCZ) and amphotericin B (AMB), *Candida pelliculosa* (*C. pelliculosa*) to fluconazole (FLCZ) and VRCZ) [7]. According to the previous study, susceptibility based on conventional species non-specific CLSI M27-A3 was defined as the previous CLSI breakpoint (P-BP) [10]. A comparison of susceptibility criteria based on the revised and previous CLSI breakpoints is shown in Table 1. CLSI M27-A3 was applied to other *Candida* species due to the lack of a species-specific breakpoint [6]. According to previous studies, we defined a strain that showed resistance to two or more antifungal agents in the same class as cross-resistant *Candida* species [11].

In the susceptibility analysis, five *Candida albicans* (*C. albicans*) to VRCZ and three to FLCZ, micafungin (MCFG), and AMB were excluded due to a lack of data. Two *Candida parapsilosis* (*C. parapsilosis*) to VRCZ, two *Candida glabrata* (*C. glabrata*) to VRCZ and one to FLCZ and MCFG, two *C. guilliermondii* to VRCZ and one to FLCZ, and two other *Candida* species to VRCZ and one to FLCZ and MCFG were also excluded due to a lack of susceptibility data.

Primary outcomes and evaluation of appropriateness of candidemia treatments

The primary outcome of the present study was the 30-day mortality rate after the diagnosis of candidemia, according to previous studies [12]. The dosages of initial antifungal agents, two sets of blood cultures, examination of serum β-D glucan, follow-up blood cultures to confirm the clearance of *Candida* species, appropriate therapy based on susceptibility tests, and ophthalmological consultations to rule out endophthalmitis were evaluated for the management of candidemia. Appropriate dosages of antifungal agents were evaluated according to the IDSA candidiasis guidelines [2]. When isolated *Candida* species were treated with antifungal agents to which they were susceptible based on the R-BP, the therapy administered to patients was regarded as appropriate [13]. Inappropriate therapy was defined as a patient who received antifungal agents for a non-susceptible or non-wild-type species.

Definitions and clinical data

We evaluated age, sex, complications, and the main disease. The administration of cyclosporine, tacrolimus, or systemic steroids was defined as immunosuppressive therapy [14]. Patients who received total parenteral nutrition (TPN) were defined as TPN. Patients administered anticancer agents were defined as anticancer therapy. Patients with a neutrophil count < 500 cells/μL were regarded as having neutropenia. Patients with mechanical ventilation at the submission of the blood culture test were defined as mechanical ventilation. Patients who required hemodialysis or those with

serum creatinine ≥ 3.0 mg/dL were regarded as renal failure. Patients who had more than five-fold the upper limit for aspartate transaminase, alanine transaminase, or gamma glutamyl transpeptidase were regarded as liver failure. Patients with underlying diseases, such as asthma, pneumonia, interstitial pneumonia, and lung cancer, were regarded as lung disease.

Patients who underwent surgery within 90 days prior to the submission of blood cultures were defined as patients with a history of surgery. *Candida* colonization was defined as patients with the isolation of *Candida* species from non-sterile tissues, such as stools.

Statistical analysis

Results are expressed as means±standard deviation or n(%). Continuous data were analyzed using the Student's *t*-test, and categorical data were analyzed using the χ^2 test. Significant items in the univariate analysis were selected for inclusion in a multivariate *Cox's* hazard analysis to identify prognostic factors within 30 days of the diagnosis of candidemia. $P<0.05$ was considered to be significant. All statistical analysis were performed using Excel-Toukei 2012 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

RESULTS

Epidemiology and species distribution of candidemia

A total of 187 patients were enrolled in the present study. The overall incidence of candidemia was 0.045 cases/1,000 inpatients. The number of total *Candida* isolates was 193 because six patients were diagnosed with a mixed infection of two *Candida* species. The combinations of *Candida* species among patients with mixed infections were as follows: two patients with *C. albicans* and *C. glabrata*, and one each with *C. albicans* and other *Candida* species, *C. guilliermondii* and other *Candida* species, *C. guilliermondii* and *C. parapsilosis*, *C. glabrata* and *C. parapsilosis*. The distribution of isolated *Candida* species was as follows: *C. albicans* (n=76, 39.3%), *C. parapsilosis* (n=53, 27.4%), *C. glabrata* (n=20, 10.3%), *C. guilliermondii* (n=18, 9.3%), *Candida tropicalis* (*C. tropicalis*) (n=7, 3.6%), *Candida krusei* (*C. krusei*) (n=1, 0.5%), *C. pelliculosa* (n=1, 0.5%), and other *Candida* species (n=17, 8.8%).

Susceptibility patterns of isolated *Candida* species based on previous and revised CLSI breakpoints

The susceptibility patterns of the *Candida* species isolated in the present study were as follows: a significant difference was observed in the susceptibility rate to FLCZ (P-BP; 93% (173/186) vs. R-BP; 79.4% (148/186), χ^2 test, $p < 0.01$), as well as to VRCZ (P-BP; 97.2% (174/179) vs. R-BP; 91.0% (163/179), χ^2 test, $p = 0.024$), respectively. No significant difference was observed in that to MCFG (P-BP; 95.7% (179/187) vs. R-BP; 94.6% (177/187), χ^2 test, $p = 0.8$). All isolates were susceptible to AMB. Table 2 shows a comparison of the susceptibility rates of each *Candida* species between the revised and previous CLSI breakpoints. A significant difference was observed in the susceptibility rate in *C. glabrata* to FLCZ (P-BP; 78.9% vs. R-BP; 0%, χ^2 test, $p < 0.01$). The susceptibility rate in *C. parapsilosis* to FLCZ (P-BP; 96.2% vs. R-BP; 86.7%) was slightly reduced with the revised CLSI breakpoint. Similar results were observed for the susceptibility rate in *C.*

tropicalis to FLCZ (P-BP; 85.7% vs. R-BP; 28.5%), *C. tropicalis* to VRCZ (P-BP; 85.7% vs. R-BP; 42.8%), as well as *C.glabrata* to VRCZ (P-BP; 100% vs. R-BP; 77.7%). Susceptibility rates in *C. albicans* and *C. guilliermondii* were affected less by the R-BP. Three *Candida* species (*C. guilliermondii*, *C. parapsilosis,* and *C. tropicalis*) were identified as cross-resistant according to the R-BP. Only one *C. guilliermondii* was regarded as cross-resistant according to the P-BP. Two of the other *Candida* species were regarded as cross-resistant.

Treatment patterns of candidemia, and impact of the revised CLSI breakpoint on appropriate antifungal therapy

Treatment patterns among 187 patients diagnosed with candidemia are shown in Table 3. In order to diagnose candidemia, two sets of blood cultures were obtained from 67.3% of patients. Serum β-D glucan was examined in 78.0% of cases. Ophthalmological consultations to rule out endophthalmitis were performed on 34.2% of patients. A total of 90.3% patients received systemic antifungal therapy, with 45.4% patients receiving MCFG as the initial antifungal treatment. Approximately 50% of patients received an appropriate dosage of antifungal therapy. Sixteen patients (8.5%) received inappropriate antifungal therapy according to the R-BP. A total of 80% of patients received appropriate therapy based on the antifungal susceptibility test.

Prognostic factors among patients with candidemia

We divided 187 patients with candidemia into survivors (n=132) and non-survivors (n=55) 30 days after the diagnosis of candidemia, and compared their clinical backgrounds and treatments (Table 4). The 30-day mortality rate was 29.4% (55/187). In the univariate analysis, significant differences were observed in age, serum albumin, *C. albicans*, *C. parapsilosis*, mechanical ventilation, renal failure, lung disease, intensive care wards, surgical wards, central venous catheter (CVC) removal, the absence of antifungal therapy, ophthalmological consultations, and follow-up blood culture tests

to confirm the clearance of *Candida* species between surviving and non-surviving patients. A significant difference was not observed for appropriate antifungal therapy.

The multivariate *Cox's* hazard analysis identified factors that had significant relationships with death: an advanced age (hazard ratio (HR); 1.02, 95% confidence interval (CI); 1.0-1.04, $p<0.01$), *C. albicans* (HR; 2.11, 95% CI; 1.03-4.35, $p=0.041$), lung disease (HR; 2.83, 95% CI; 1.57-5.12, $p<0.01$), and the absence of antifungal therapy (HR; 5.47, 95% CI; 2.31-12.9, $p<0.01$) were significant prognostic factors for a high mortality rate, whereas serum albumin (HR; 0.35, 95% CI; 0.20-0.61, $p<0.01$), *C. parapsilosis* (HR; 0.21, 95% CI; 0.059-0.79, $p=0.021$), surgical wards (HR; 0.41, 95% CI; 0.18-0.91, $p=0.03$), the removal of CVC (HR; 0.33, 95% CI; 0.17-0.64, $p<0.01$), and follow-up blood culture tests to confirm the clearance of *Candida* species (HR; 0.49, 95% CI; 0.25-0.93, $p=0.029$) were associated with a lower mortality rate (Table 5).

DISCUSSION

Candidemia is one of the most serious nosocomial infections because of its high mortality rate. Its incidence has been increasing due to the development of immunosuppressive therapy and the aging of populations in developed countries [15]. A number of *Candida* species may cause candidemia, which shows different drug susceptibility patterns. Therefore, examining the detection performance of *Candida* species that show resistance to antifungal agents by the species-specific R-BP is extremely important for a better understanding of appropriate antifungal therapy among patients with candidemia.

Species distribution and susceptibility patterns of each *Candida* species to antifungal agents

Among the *Candida* species isolated from blood cultures in the present study, *C. albicans* accounted for 39.3%, *C. parapsilosis* 27.4%, *C. glabrata* 10.3%, *C. guilliermondii* 9.3%, *C. tropicalis* 3.6%, *C. krusei* 0.5%, *C. pelliculosa* 0.5%, and other *Candida* species 8.8%. This distribution pattern was similar to the findings of a large-scale surveillance recently performed in Japan, and *C. albicans*, *C. parapsilosis*, and *C. glabrata* accounted for approximately 80% of all species isolated [16].

Although antifungal susceptibility patterns to most *Candida* species based on the R-BP were similar to those of previous studies, the susceptibility rate in *C. tropicalis* to FLCZ was only 28.5%, which was lower than that reported previously [17]. *C. tropicalis* has been identified as a causative species among patients with neutropenia as well as hematological malignancies [18]. Appropriate antifungal therapy is required for these patients because candidemia among patients with neutropenia is associated with a high mortality rate [19]. Therefore, special attention needs to be paid to the susceptibility test results for azole class antifungal agents among patients from whom *C. tropicalis* was isolated from blood culture tests.

Impact of the revised CLSI breakpoint on susceptibility patterns of isolated *Candida* species

We compared the susceptibility rate according to the CLSI M59 or M60 with that of CLSI M27-A3 because the revision from CLSI M27-S4 to M60 was a minor change. In comparisons of susceptibility patterns based on the revised and previous CLSI breakpoints, significant differences were observed to FLCZ from 93% (P-BP) to 79.5% (R-BP), as well as to VRCZ from 97.2% (P-BP) to 91.0% (R-BP), respectively. This was the most interesting result of the present study. From the CLSI M27-S4 breakpoints published in 2012, *C. glabrata* has been defined as being dose-dependent susceptible to FLCZ having a MIC value less than 32 μg/mL, while the MIC value more than 64 μg/mL was defined as resistant, which means the susceptible of *C. glabrata* to FLCZ was substantially reduced [6]. CLSI M59 also revised ECVs of *C.glabrata* to VRCZ [9]. From the CLSI M59, *C.glabrata* has been defined as being wild-type to VRCZ having a MIC value less than 0.25 μg/mL, which was lower than that of the P-BP[6, 9]. Therefore, the reduction observed in the susceptibility rate to FLCZ and VRCZ based on the R-BP may be associated with a change in the susceptibility cut-off value for *C. glabrata*.

In addition to the FLCZ and VRCZ susceptibility rate in *C. glabrata*, the R-BP reduced susceptibility rates to azole antifungal agents in *C. parapsilosis* and *C. tropicalis*. On the other hand, it did not exert marked effects on MCFG. This result was consistent with previous findings, which indicated that the R-BP efficiently detected azole resistant strains in non-*albicans Candida* species [10]. Azole antifungal agents are clinically important drugs among patients who perform oral switches of therapy due to their high bioavailabilities. The IDSA candidiasis guidelines published in 2016 strongly recommended performing drug susceptibility tests on isolated *Candida* species to azole antifungal agents among all patients with candidemia [2]. The results of the present study indicated the importance of drug susceptibility tests and interpretations based on the R-BP among patients with candidemia.

Treatment patterns and prognostic factors among patients with candidemia

Approximately 30% of patients were subjected to ophthalmological consultations to detect fungal endophthalmitis after a diagnosis of candidemia. Although this study was retrospective in nature and conducted by two tertiary care hospitals, the rate of ophthalmological consultations was markedly lower than that reported previously in Japan [20]. Endophthalmitis is one of the most important complications in candidemia because it causes blindness among patients who delay appropriate therapy [2]. Therefore, special education on the importance of ophthalmological consultations may be required for physicians involved in the treatment of candidemia. Approximately 10% of the 169 patients treated with systemic antifungal therapy were considered to have received inappropriate therapy based on the R-BP. However, a statistical analysis associated with death showed that appropriate antifungal therapy based on susceptibility tests was not a significant factor for a poor prognosis.

González-Lara MF et al. reported that approximately 10% of patients treated with systemic antifungal therapy were considered to have received inappropriate therapy based on the CLSI M27-S4 breakpoint, which was not a significant factor for death [13]. The present results were consistent with previous findings. On the other hand, a meaningful statistical analysis may not have been performed because only 16 patients received inappropriate antifungal therapy. Further studies are needed to examine the relationship between mortality rates and inappropriate antifungal therapy based on the R-BP.

In a multivariate *Cox's* hazard analysis, age, lung disease, *C. albicans*, and the absence of antifungal therapy were associated with a high mortality rate, whereas serum albumin, *C. parapsilosis*, surgical wards, the removal of CVC, and follow-up blood culture tests to confirm the clearance of *Candida* species were associated with a lower mortality rate. Among these factors, the absence of antifungal therapy, the removal of CVC, and follow-up blood culture tests were

associated with medical procedures. Regarding the duration of antifungal therapy among candidemia without endophthalmitis, at least 14 days of therapy after confirmation of the clearance of blood culture tests is recommended in the IDSA published candidiasis guidelines [2]. Takesue et al. reported that compliance with an appropriate duration of antifungal therapy was a prognostic factor for the treatment of candidemia [21]. Based on these results, follow-up blood culture tests are important for assessing an appropriate duration of antifungal therapy, and this medical procedure may reflect the quality of antifungal therapy. The compliance of follow-up blood culture tests in the present study was approximately 55%, which indicates that an educational program is required to inform physicians of its importance. Besides follow-up blood culture tests, antifungal therapy and CVC removal were significant factors for a favorable outcome in the present study. These results were similar to those of previous studies [22]. Therefore, the early initiation of antifungal therapy and removal of CVC are essential clinical procedures for improving the outcome of candidemia.

Strengths and limitations of this study

The major strengths and limitations of this study were as follows.

The majority of recent studies that focused on the antifungal susceptibility patterns and prognostic factors of candidemia in Japan were based on a monocentric retrospective analysis [14, 20]. The findings of a monocentric retrospective analysis are generally not sufficiently reliable because the number of cases was limited and it was not possible to exclude some local factors. The present study was conducted by two tertiary care institutions with similar clinical standards. In addition to the study design, the same antifungal susceptibility test methods were performed at the two institutions. This may be a strength of the present study. Therefore, the results obtained on the prognosis of candidemia as well as the susceptibility patterns of isolated *Candida* species in the present study were more reliable than those from previously published monocentric retrospective studies in Japan.

Some indicators reflecting general conditions (e.g., the acute physiology and chronic health

evaluation II score (APACHE II score)) have been reported as prognostic factors in the treatment of candidemia [12]. In the present study, difficulties were associated with calculating the APACHE II score in some patients due to a lack of clinical data. Evaluations of the impact of inappropriate antifungal therapy in consideration of general conditions were difficult, which was a limitation in the present study. This may have been due to the retrospective study design. A prospective multicentric study considering general conditions is needed in order to overcome this issue.

CONCLUSION

The early initiation of antifungal therapy, removal of CVC and follow-up blood culture tests are essential clinical procedures for improving the outcome of candidemia. The R-BP significantly reduced the susceptibility rate of *Candida* species to FLCZ and to VRCZ. The R-BP was more efficient than the P-BP at detecting azole non-susceptible species in non-*albicans Candida* species, such as *C. parapsilosis*, *C. glabrata*, and *C. tropicalis*. The present results support the importance of antifungal susceptibility tests and interpretations based on the R-BP among patients with candidemia.

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Table 1. Comparison of previous and revised CLSI breakpoints and epidemiological cut-off values.

C. albicans	Previous CLSI breakpoint (CLSI M27-A3)			Previous epidemiological cut off values		Revised CLSI breakpoint (CLSI M60)			Revised Epidemiological cut off values (CLSI M59)	
	S	S-DD or I	R or NS	WT	non-WT	S	S-DD or I	R	WT	non-WT
FLCZ	≤ 8	16-32	≥ 64	≤ 0.5	> 0.5	≤ 2	4	≥ 8	≤ 0.5	> 0.5
VRCZ	≤ 1	-	≥ 4	≤ 0.03	> 0.03	≤ 0.12	0.25-0.5	≥ 1	≤ 0.03	> 0.03
MCFG	≤ 2	-	> 2	≤ 0.03	> 0.03	≤ 0.25	0.5	≥ 1	≤ 0.03	> 0.03
AMB	-	-	-	≤ 2	> 2	-	-	-	≤ 2	> 2

C. parapsilosis	Previous CLSI breakpoint (CLSI M27-A3)			Previous epidemiological cut off values		Revised CLSI breakpoint (CLSI M60)			Revised Epidemiological cut off values (CLSI M59)	
	S	S-DD or I	R or NS	WT	non-WT	S	S-DD or I	R	WT	non-WT
FLCZ	≤ 8	16-32	≥ 64	≤ 2	> 2	≤ 2	4	≥ 8	≤1	> 1
VRCZ	≤ 1	-	≥ 4	≤ 0.12	> 0.12	≤ 0.12	0.25-0.5	≥ 1	≤ 0.03	> 0.03
MCFG	≤ 2	-	> 2	≤ 4	> 4	≤ 2	4	≥ 8	≤ 4	> 4
AMB	-	-	-	≤ 2	> 2	-	-	-	≤ 2	> 2

C. glabrata	Previous CLSI breakpoint (CLSI M27-A3)			Previous epidemiological cut off values		Revised CLSI breakpoint (CLSI M60)			Revised Epidemiological cut off values (CLSI M59)	
	S	S-DD or I	R or NS	WT	non-WT	S	S-DD or I	R	WT	non-WT
FLCZ	≤ 8	16-32	≥ 64	≤ 32	> 32	-	≤ 32	≥ 64	≤ 8	> 8
VRCZ	≤ 1	-	≥ 4	≤ 0.5	> 0.5	-	-	-	≤ 0.25	> 0.25
MCFG	≤ 2	-	> 2	≤ 0.03	> 0.03	≤ 0.06	0.12	≥ 0.25	≤ 0.03	> 0.03
AMB	-	-	-	≤ 2	> 2	-	-	-	≤ 2	> 2

C. tropicalis	Previous CLSI breakpoint (CLSI M27-A3)			Previous epidemiological cut off values		Revised CLSI breakpoint (CLSI M60)			Revised Epidemiological cut off values (CLSI M59)	
	S	S-DD or I	R or NS	WT	non-WT	S	S-DD or I	R	WT	non-WT
FLCZ	≤ 8	16-32	≥ 64	≤ 2	> 2	≤ 2	4	≥ 8	≤1	> 1
VRCZ	≤ 1	-	≥ 4	≤ 0.06	> 0.06	≤ 0.12	0.25-0.5	≥ 1	≤ 0.12	> 0.12
MCFG	≤ 2	-	> 2	≤ 0.12	> 0.12	≤ 0.25	0.5	≥ 1	≤ 0.06	> 0.06
AMB	-	-	-	≤ 2	> 2	-	-	-	≤ 2	> 2

C. guilliermondii	Previous CLSI breakpoint (CLSI M27-A3)			Previous epidemiological cut off values		Revised CLSI breakpoint (CLSI M60)			Revised Epidemiological cut off values (CLSI M59)	
	S	S-DD or I	R or NS	WT	non-WT	S	S-DD	R	WT	non-WT
FLCZ	≤ 8	16-32	≥ 64	≤ 8	> 8	-	-	-	≤ 8	> 8
VRCZ	≤ 1	-	≥ 4	≤ 0.25	> 0.25	-	-	-	-	-
MCFG	≤ 2	-	> 2	≤ 2	> 2	≤ 2	4	≥ 8	≤ 2	> 2
AMB	-	-	-	≤ 2	> 2	-	-	-	-	-

C. krusei	Previous CLSI breakpoint (CLSI M27-A3)			Previous epidemiological cut off values		Revised CLSI breakpoint (CLSI M60)			Revised Epidemiological cut off values (CLSI M59)	
	S	S-DD or I	R or NS	WT	non-WT	S	S-DD	R	WT	non-WT
FLCZ	≤ 8	16-32	≥ 64	≤ 64	> 64	-	-	-	-	-
VRCZ	≤ 1	-	≥ 4	≤ 0.5	> 0.5	≤ 0.5	1	≥ 2	≤ 0.5	> 0.5
MCFG	≤ 2	-	> 2	≤ 0.12	> 0.12	≤ 0.25	0.5	≥ 1	≤ 0.25	> 0.25
AMB	-	-	-	≤ 2	> 2	-	-	-	≤ 2	> 2

C. pelliculosa	Previous CLSI breakpoint (CLSI M27-A3)			Previous epidemiological cut off values		Revised CLSI breakpoint (CLSI M60)			Revised Epidemiological cut off values (CLSI M59)	
	S	S-DD or I	R or NS	WT	non-WT	S	S-DD	R	WT	non-WT
FLCZ	≤ 8	16-32	≥ 64	≤ 4	> 4	-	-	-	-	-
VRCZ	≤ 1	-	≥ 4	≤ 0.25	> 0.25	-	-	-	-	-
MCFG	≤ 2	-	> 2	-	-	-	-	-	-	-
AMB	-	-	-	-	-	-	-	-	-	-

Note; FLCZ, fluconazole; VRCZ, voriconazole; MCFG, micafungin; AMB, amphotericin B; S, susceptible; NS, non-susceptible; S-DD, susceptible dose-dependent; I, intermediate; R, resistant; WT, wild-type; CLSI, Clinical and Laboratory Standards Institute. Each value means minimum inhibitory concentration (μg/mL).

Table 2. Susceptibility rates for *Candida* species isolated from candidemia using previous and revised CLSI breakpoints.

	Previously published CLSI clinical breakpoints			Revised CLSI clinical breakpoints (M60) or epidemiological cutoff values (M59)			*P* value
	S(%)	S-DD(%)	R or NS(%)	S or WT(%)	I or S-DD(%)	R or non-WT(%)	
C.albicans							
FLCZ (n=73)	73(100)	0	0	72(98.6)	1(1.3)	0	1
MCFG (n=73)	73(100)	ND	0	72(98.6)	0	1(1.3)	1
VRCZ (n=71)	70(98.5)	ND	1(1.5)	70(98.5)	0	1(1.5)	1
AMB (n=73)	ND	ND	ND	73(100)	0	0	NA
C.parapsilosis							
FLCZ (n=53)	51(96.2)	2(3.7)	0	46(86.7)	3(5.6)	4(7.5)	0.16
MCFG (n=53)	51(96.2)	ND	2(3.7)	50(94.3)	2(3.7)	1(1.8)	1
VRCZ (n=51)	51(100)	ND	0	48(94.1)	2(3.9)	1(2.0)	0.24
AMB (n=53)	ND	ND	ND	53(100)	0	0	NA
C.glabrata							
FLCZ (n=19)	15(78.9)	4(21)	0	ND	19(100)	0	< 0.01
MCFG (n=19)	18(94.7)	ND	1(5.2)	18(94.7)	0	1(5.2)	1
VRCZ (n=18)	18(100)	ND	0	14(77.7)	0	4(22.2)	0.1
AMB (n=20)	ND	ND	ND	20(100)	0	0	NA
C.tropicalis							
FLCZ (n=7)	6(85.7)	0	1(14.2)	2(28.5)	2(28.5)	3(42.8)	0.1
MCFG (n=7)	7(100)	ND	0	7(100)	0	0	1
VRCZ (n=7)	6(85.7)	ND	1(14.2)	3(42.8)	3(42.8)	1(14.2)	0.26
AMB (n=7)	ND	ND	ND	7(100)	0	0	NA

C.guilliermondii							
FLCZ (n=17)	14(82.3)	2(11.7)	1(5.8)	14(82.3)	0	3(17.6)	1
MCFG (n=18)	16(88.8)	ND	2(11.1)	16(88.8)	2(11.1)	0	1
VRCZ (n=16)	15(93.7)	ND	1(6.2)	14(87.5)	0 [b]	2(12.5)	1
AMB (n=18)	ND	ND	ND	18(100)	0 [b]	0	NA
C.krusei							
MCFG (n=1)	1(100)	ND	ND	1(100)	ND	ND	NA
AMB (n=1)	ND	ND	ND	1(100)	ND	ND	NA
C.pelliculosa							
FLCZ (n=1)	1(100)	ND	ND	1(100) [b]	ND	ND	NA
VRCZ (n=1)	1(100)	ND	ND	1(100) [b]	ND	ND	NA
other *C.* species [a]							
FLCZ (n=16)	13(81.2)	1(6.2)	2(12.5)	ND	ND	ND	NA
MCFG (n=16)	13(81.2)	0	3(18.7)	ND	ND	ND	NA
VRCZ (n=15)	13(86.6)	0	2(13.3)	ND	ND	ND	NA

Note: [a] CLSI M27-A3 breakpoints were applied to other *C.* species due to the lack of species-specific susceptibility breakpoints.

[b] Previously published epidemiological cutoff values were applied to these species due to the lack of breakpoint in CLSI M59 or M60.

ND, not determined; NA, not applicable; FLCZ, fluconazole; VRCZ, voriconazole; MCFG, micafungin; AMB, amphotericin B; S, susceptible; NS, non-susceptible; S-DD, susceptible dose-dependent; I, intermediate; R, resistant; WT, wild-type; CLSI, Clinical and Laboratory Standards Institute.

Table 3. Treatment patterns for candidemia.

Total number of cases	187
Diagnosis for candidemia	
Two sets of blood cultures	126(67.3)
Examination of serum β-D glucan	146(78.0)
Follow-up blood cultures to confirm the clearance of *Candida* species	104(55.6)
Ophthalmological consultations to rule out endophthalmitis	64(34.2)
Antifungal therapy	
Patients treated with antifungal therapy	169(90.3)
Absence of antifungal therapy	18(9.6)
FLCZ	59(31.5)
MCFG	85(45.4)
L-AMB	12(6.4)
VRCZ	7(3.7)
CPFG	3(1.6)
ITCZ	3(1.6)
Patients with appropriate dosages of antifungal agents based on IDSA guidelines	90(48.1)
Appropriateness of therapy based on susceptibility	
Appropriate therapy based on susceptibility	149(79.6)
Inappropriate therapy based on the susceptibility test	16(8.5)
Excluded due to a lack of data	4(2.1)

Note: Data are expressed as the number (%) of patients.

FLCZ, fluconazole; VRCZ, voriconazole; MCFG, micafungin; L-AMB, liposomal amphotericin B; CPFG, caspofungin; ITCZ, itraconazole; IDSA, Infectious Diseases Society of America.

Table 4. Comparison of various parameters between survivors and non-survivors.

	Non-survivors	Survivors	*P* value
Number of cases	55	132	
Clinical demographics			
Age	67.8±15.9	55.8±21.7	< 0.01
Serum albumin (mg/dL)	2.16±0.6	2.69±0.63	< 0.01
Sex (male)	37(67.3)	82(62.1)	0.5
Presence of CVC	46(83.6)	118(89.4)	0.39
Immunosuppressive therapy	17(30.9)	35(26.5)	0.54
Anticancer therapy	16(29.1)	43(32.6)	0.64
Total parenteral nutrition	43(78.2)	107(81.1)	0.65
Mechanical ventilation	19(34.5)	15(11.4)	< 0.01
Renal failure	11(20)	6(4.5)	< 0.01
Liver failure	17(30.9)	29(22)	0.195
Solid tumor	16(29.1)	55(41.7)	0.1
Hematological tumor	11(20)	13(9.8)	0.098
Lung disease	25(45.5)	24(18.2)	< 0.01
Diabetes mellitus	9(16.4)	20(15.2)	0.98
Organ transplantation	6(10.9)	11(8.3)	0.78
Neutropenia	7(12.7)	10(7.6)	0.4
Intensive care wards	13(23.6)	6(4.5)	< 0.01
Internal medicine wards	22(40)	50(37.9)	0.78
Surgical wards	8(14.5)	53(40.2)	< 0.01
Hematological wards	11(20)	15(11.4)	0.18

Emergency department	1(1.8)	3(2.3)	0.71
Pediatric wards	0	6(4.5)	0.24
History of surgery	12(21.8)	39(29.5)	0.27
Clinical practices			
Removal of CVC	19(34.5)	96(72.7)	< 0.01
Absence of antifungal therapy	12(21.8)	5(3.8)	< 0.01
Appropriate dosages of initial antifungal agents	21(38.2)	69(52.3)	0.078
Appropriate therapy based on susceptibility tests	39(70.9)	110(83.3)	0.054
Ophthalmological consultations	9(16.4)	55(41.7)	< 0.01
Follow-up blood culture tests to confirm the clearance of *Candida* species	24(43.6)	80(60.6)	0.033
FLCZ	13(23.6)	46(34.8)	0.132
MCFG	23(41.8)	62(47)	0.51
L-AMB	3(5.5)	9(6.8)	0.98
VRCZ	0	7(5.3)	0.18
ITCZ	2(3.6)	1(0.8)	0.43
CPFG	2(3.6)	1(0.8)	0.43
Microbiological characteristics of *Candida* species			
C. albicans	33(60)	41(31.1)	< 0.01
C. parapsilosis	3(5.5)	48(36.4)	< 0.01
C. glabrata	5(9.1)	13(9.8)	0.91
C. tropicalis	3(5.5)	4(3)	0.7
C. guilliermondii	2(3.6)	14(10.6)	0.2
C. krusei	0	1(0.8)	0.65

other *C.*species	6(10.9)	9(6.8)	0.52
Mixed infection by *Candida* species	2(3.6)	4(3)	0.81
Resistance to FLCZ	4(7.3)	8(6.1)	0.98
Resistance to MCFG	2(3.6)	4(3)	0.8
Resistance to VRCZ	2(3.6)	7(5.3)	0.91

Note: Comparison of parameters between survivors and non-survivors 30 days after the diagnosis of candidemia. Data are expressed as the number (%) of patients.

CVC, central venous catheter; FLCZ, fluconazole; VRCZ, voriconazole; MCFG, micafungin; L-AMB, liposomal amphotericin B; CPFG, caspofungin; ITCZ, itraconazole.

Table 5. Risk factors for death among patients with candidemia according to the *Cox's* regression analysis.

	Hazard ratio	95% CI	*P* value
Age	1.02	1.0-1.04	< 0.01
Serum albumin	0.35	0.21-0.61	< 0.01
C. albicans	2.11	1.03-4.35	0.041
C. parapsilosis	0.22	0.059-0.79	0.021
Mechanical ventilation	1.97	0.94-4.11	0.07
Renal failure	0.73	0.31-1.66	0.45
Lung disease	2.84	1.57-5.12	< 0.01
Intensive care wards	1.68	0.72-3.91	0.23
Surgical wards	0.41	0.18-0.91	0.03
Removal of CVC	0.33	0.17-0.64	< 0.01
Absence of antifungal therapy	5.47	2.31-12.9	< 0.01
Follow-up blood culture tests to confirm the clearance of *Candida* species	0.49	0.25-0.93	0.029
Ophthalmological consultations	0.55	0.22-1.35	0.19

Note: Each factor was selected from a univariate analysis between survivors and non-survivors.

CVC, central venous catheter; CI, confidence interval.