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RESEARCH

Distribution and antifungal susceptibility of clinical *Trichosporon* spp. isolates: 10 years of single-center experience

Klinik *Trichosporon* spp. izolatlarının dağılımı ve antifungal duyarlılığı: 10 yıllık tek merkez deneyimi

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Abstract

Purpose: The genus *Trichosporon*, which is a yeast-like basidiomycete, is ubiquitous in nature and a part of human microbiota. It's an oppurtunistic fungal pathogen, which was once rare, but increasing dramatically recently, leading to fatal infections. The aim of this study was to determine the prevalence, and antifungal susceptibility of clinical *Trichosporon* spp. isolates, and to determine whether there was a change in incidence during the COVID-19 Pandemic.

Materials and Methods: This was a retrospective cross-sectional descriptive study, conducted between January 1, 2013 and February 5, 2023. Cultures which *Trichosporon* spp. isolated (n=125) were screened, and those met the eligibility criteria were included (n=77). Identification, antifungal susceptibility test results, age, gender records were identified from Microbiology Laboratory Information Management System.

Results: 97.4% of the isolates were *Trichosporon asahii*, and 2.6% were *Trichosporon mucoides*. The most frequent isolation period was the 2016 and 2019 year group (71.4%), not during the COVID-19 Pandemics (19.5%). The most common sample type was urine (58.4%), of whom predominantly (58.4%) hospitalized in the Intensive Care Unit. Amphotericin B MICs were ≤1µg/ml in 68.9% of the isolates. Fluconazole and voriconazole MICs were ≤1µg/ml in 20.0% and 94.8%, respectively. 59.7% of the isolates had a MIC of ≥4µg/ml for flucytosine. Micafungin and caspofungin MICs were ≥4µg/ml in 88.3% and 92.2% of the isolates, respectively. Voriconazole had the strongest in vitro activity, and amphotericin B had lower MICs than expected. The combination therapy of voriconazole and amphotericin B could be a therapeutic option in this setting, as well as monotherapy of voriconazole.

Öz

Amaç: Maya benzeri bir basidiomycete olan *Trichosporon* cinsi, doğada her yerde bulunur ve insan mikrobiyotasının bir parçasıdır. *Trichosporon* spp. bir zamanlar nadir görülen, ancak son zamanlarda dramatik bir şekilde artan ve ölümcül enfeksiyonlara yol açan opurtunistik bir mantar patojenidir. Bu çalışmanın amacı klinik *Trichosporon* spp. izolatlarının prevalansını ve antifungal duyarlılıklarını belirlemek ve COVID-19 Pandemisi'nde insidansında bir değişiklik olup olmadığını tespit etmektir.

Gereç ve Yöntem: Bu, 1 Ocak 2013 ile 5 Şubat 2023 tarihleri arasında yürütülen, retrospektif kesitsel tanımlayıcı bir çalışmadır. *Trichosporon* spp. izole edilmiş olan kültür sonuçları (n=125) tarandı ve uygunluk kriterlerini karşılayanlar (n=77) çalışmaya dahil edildi. Demografik veriler, kültür sonuçları ve antifungal duyarlılık test sonuçları Mikrobiyoloji Laboratuvarı Bilgi Yönetim Sistemi'ndeki kayıtlardan elde edildi.

Bulgular: İzolatların %97.4'ü *Trichosporon asahii* ve %2.6'sı *Trichosporon mucoides* idi. En sık izolasyon dönemi COVID-19 Pandemisinde (%19,5) değil, 2016 ve 2019 yılları arasında (%71.4) olduğu tespit edildi. En yaygın örnek türü idrardı (%58.4) ve bunların büyük çoğunluğu (%58.4) Yoğun Bakım Ünitesi'ndeki hastalara aitti. Amfoterisin B MİK değerleri izolatların %68.9'unda ≤1µg/ml idi. Flukonazol ve vorikonazol MİK'leri sırasıyla %20.0 ve %94.8'de ≤1µg/ml idi. İzolatların %59.7'sinde flusitozin için MİK değeri ≥4µg/ml idi. Mikafungin ve kaspofungin MİK'leri sırasıyla izolatların %88,3'ünde ve %92,2'sinde ≥4µg/ml idi. Vorikonazol en güçlü in vitro aktiviteye sahipti ve amfoterisin B beklenenden daha düşük MİK'lere sahipti. Bu durumda vorikonazol ve amfoterisin B'nin kombinasyon tedavisinin yanı sıra, vorikonazol monoterapisi de terapötik bir seçenek olabilir.

Sonuç: Klinik örneklerden izole edilen *Trichosporon* türleri ve bunların antifungal duyarlılıkları coğrafi bölgeye ve

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Conclusion: *Trichosporon* spp. isolated in clinical specimens and their antifungal susceptibility depend on the geographic region and the anatomic site. Identifying local data will contribute to both the management of these patients, and surveillance studies.
Keywords:. *Trichosporon asahii*, fungal infection, rare yeast, antifungal susceptibility, antifungal resistance, voriconazole, fungemia, COVID-19.

anatomik bölgeye bağlı olarak değişmektedir. Lokal verilerin belirlenmesi hem bu hastaların yönetimine hem de sürveyans çalışmalarına katkı sağlayacaktır.

Anahtar kelimeler: *Trichosporon asahii*, mantar enfeksiyonu, maya, antifungal duyarlılık, antifungal direnç, vorikonazol, fungemi, COVID-19.

INTRODUCTION

In recent years, there's an increase in severe fungal infections with a number of over than 150 million cases per year, and resulting in 1.7 million deaths annually. Especially in the last decade, the fungal pathogenic microorganisms are increasingly resistant to antifungals. The fungal pathogens responsible for severe systemic mycoses are *Candida* spp., *Aspergillus* spp., *Trichosporon* spp., Zygomycetes, Fusarium and Scedosporium, etc. Among them, *Trichosporon* spp., which causes opportunistic infections, has a high mortality rate[1-2].

The genus *Trichosporon,* the arthroconidia-forming basidiomycetous yeast-like fungi, which belongs to the phylum *Basidiomycota*, subphylum *Agaricomycotina*, class *Tremellomycetes, o*rder *Trichosporonales* and family *Trichosporonaceae,* was first described by Beigel in the mid-19th century as a benign causative agent of hair shaft infection. All members were initially classified as *Trichosporon beigelii,* until Gueho et al. revised the taxonomy of 20 species in 1992, including six pathogens; *Trichosporon asahii* (*T. asahii*), *T. mucoides, T. ovoides, T. asteroides, T. cutaneum* and *T. inkin*[3-4]. We now know that there are no less than 50 different species within the genus, 17 of which being medically significant[3].

Trichosporon spp. is broadly spread in nature such as soil, water, plants, birds, etc., mostly in regions with warm and tropical climates. Moreover, it is also present in human body microbiota of the gastrointestinal and oral cavity, and it can temporarily colonize the skin, respiratory tract and vagina. It can cause superficial infection (white piedra), summer-type hypersensitivity pneumonitis and rarely, opportunistic invasive infections especially in immunocompromised patients (i.e., hematological malignancies, neutropenia, mucositis) with incidence rates ranging from 0.01 to 2 cases per million inhabitants[4]. Biofilm production, ability to adhere to abiotic surfaces, thermotolerance, melanin production and secretion of some enzymes play a critical role in the development of infection[5-6]. It was reported that there was a three-fold increase in invasive cases from 2005 to 2015[7].

Trichosporon species are intrinsically resistant to echinocandins, and have higher minimum inhibitory concentrations (MICs) to amphotericin B (AMB) than to azoles. And voriconazole (VRC) has the strongest in vitro activity against *Trichosporon* isolates[8]. Whereas for empirical therapy recommended by current international guidelines propose echinocandins (caspofungin, micafungin) and/or AMB, which is mainly directed to *Candida* spp. for empirical therapy. Thus, inappropriate treatment comes with the higher mortality rates[8-10]. *Trichosporon* spp. is on the rise as an opportunistic pathogen, representing the first or second most common non-*Candida* yeast involved in life-threatening fungal infection with mortality rates of 42% to 90% despite antifungal therapy[9-11]. Accurate and timely identification at the species level and antifungal susceptibility testing are of great importance to overcome re-emergence of this difficult-to-manage opportunistic *Trichosporon* species in recent decades[12-14]. This narrows the option of effective antifungal agents to combat life-threatening infection.

Identifying species distribution and antifungal susceptibility are important for the hospital or regional specificity of the isolates in an epidemiological perspective and for appropriate antifungal treatment. But the data on clinical *Trichosporon* strains have not been thoroughly explored in Türkiye. There are also limited data associated with *Trichosporon* spp. in the international literature. In this study, our primary objective was to determine the prevalence and antifungal susceptibility change of clinical *Trichosporon* spp. isolates in a tertiary care university hospital within 10 years to provide local data for surveillance studies. The subsidiary purpose was to determine whether there was a change in incidence during the COVID-19 Pandemic.

MATERIALS AND METHODS

Study design

This was a retrospective cross-sectional descriptive study conducted in the Microbiology Laboratory of Cukurova University Faculty of Medicine Balcalı Hospital, which is a tertiary care regional hospital with a capacity of 1150 beds. The study protocol was approved by the Cukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (2023/52), and conducted in accordance with the Declaration of Helsinki.

The Microbiology Laboratory Data, which has been carried out by microbiology specialist physicians and certified, experienced laboratory personnel for at least 20 years, was retrospectively screened by comparing it with the Hospital Data Processing Unit records. Cultures which *Trichosporon* spp. isolated (n=125) were screened, and those met the eligibility criteria were included (n=77) to the study. Identification and antifungal susceptibility tests, age, gender records were identified from Microbiology Laboratory Information Management System between January 1, 2013 and February 5, 2023.

Inclusion criteria for the study were; All *Trichosporon* spp. recovered from blood (n=16), other sterile body fluids, including cerebrospinal fluid (n=1), peritoneal fluid (n=1), central venous catheter (n=1), cornea (n=2), pus (n=6), wound (n=5) and urine (n=45) were included in this study. Exclusion criteria were; isolates, which were of the same species, of the same susceptibility profile and the same site of body at a different time were considered as duplicates (n=48) and were excluded.

Identification and antifungal susceptibility

Blood and sterile body fluids were inoculated in fully automated blood culture system BACTEC (Becton-Dickinson, BD, USA), and incubated for five days. The other samples were cultured on 5% sheep blood agar, Sabouraud dextrose agar (SDA), chocolate agar, Mc Conkey agar, and then incubated at 37°C for 24-48 hours.

The suspicious isolates that grew in the SDA were directly examined with both Gram staining and lactophenol cotton blue staining. The identification of the isolates was performed using a fully automated identification system, the Vitek 2 (bioMérieux, Marcy l'Étoile, France) which gave high levels (96.5%) of correct identification as the species were present in the database. The isolates were tested for susceptibilities to six antifungal agents, namely, AMB, fluconazole (FLC), caspofungin (CAS), micafungin (MCA), VRC and 5-flucytosine (5FC), using an automated system, the Vitek 2 Yeast Susceptibility Panel (bioMérieux, Marcy l'Étoile, France) according to the Clinical and Laboratory Standards Institute (CLSI) criteria[15]. Quality control was performed for each run using the *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 strains.

Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics Version 20.0 (IBM Corp. Armonk, USA) statistical software package[16]. Categorical variables such as demographic characteristics, date range, number of isolates, clinical departments and isolation sites were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation and as median and min-max where appropriate. Antifungal (AMB, FLC, CAS, MCA, VRC and 5FC) susceptibility profiles of *Trichosporon* spp. were compared according to age groups, application date, clinical departments and sample types, Pearson Chi Square Test or Fisher's Exact Test was used depending on whether the expected value problem arises or not. The statistical level of significance for all tests was considered to be 0.05.

RESULTS

Characteristics and prevalence rates of *Trichosporon* spp. cultured from different anatomical sites

Seventy seven culture isolates of patients, 61% (n=47) of whom were male, were analyzed. The mean age of the patients were 9.3±28.1 years (range 0-90 years), and the median age was 60 years (range 0-90 years). According to the species distribution of *Trichosporon* spp. in clinical samples, 97.4% (n=75) *Trichosporon asahii* and 2.6% (n=2) *Trichosporon mucoides*. The most isolated date range of *Trichosporon* spp. was between 2016 and 2019, with a ratio of 71.4% (n=55), not the date range of the COVID-19 Pandemics (19.5%). Forty nine of the clinical isolates (63.6%) belonged to the patients aged 50 and over. The most of the clinical isolates (58.4%) were in specimens belonged to the intensive care unit (ICU), and the most common sample types were urine

(n=45, 58.4%) and blood (n=16, 20.8%). Demographic features of the patients and clinical characteristics of 77 *Trichosporon* isolates are summarized in Table 1.

Table 1. Species distribution and characteristics of 77 clinical Trichosporon isolates.

Characteristics	Number	%
Number of isolates		
Trichosporon asahii	75	97.4
Trichosporon mucoides	2	2.6
Total	77	100
Gender		
Female	30	39.0
Male	47	61.0
Age groups (years)		
0-1	7	9.0
2-15	9	11.7
16-49	12	15.6
50-65	23	29.9
65<	26	33.8
Date ranges		
2012-2015	7	9.1
2016-2019	55	71.4
2020-2023	15	19.5
Clinical departments		
Intensive care unit	45	58.4
Internal medicine[a]	22	28.6
Surgical medicine[b]	10	13.0
Isolation sites		
Urine	45	58.4
Blood	16	20.8
Respiratory	6	7.8
Others[c]	10	13.0

[a]Including hematology (n=4), infectious diseases medicine (n=2), cardiology (n=3), gastroenterology (n=1), endocrinology (n=1), rheumatology (n=1), oncology (n=2), pediatrics (n=7) and dermatology (n=1).
[b]Including urinary surgery (n=5), basic surgery (n=1), neurosurgery (n=1), orthopedics (n=1), ophthalmology (n=1) and chest surgery (n=1).
[c]Including wound (n=5), cerebrospinal fluid (n=1), cornea (n=2), catheter (n=1) and peritoneal fluid (n=1).

Antifungal susceptibility tests

Since there are no validated clinical breakpoints to accurately interpret the MICs of the antifungal agents for *Trichosporon* spp., the distribution of MICs for each tested antifungal, and comparison with characteristics of isolates are summarized in Table 2.

The proportional distribution of MIC value ranges for antifungal agents of *Trichosporon* spp. isolates was as follows:

Amphotericin B MICs; ≤1μg/ml, 68.9% (n=53); 1-2μg/ml, 22.1% (n=17); ≥4μg/ml, 9.0% (n=7).

Flucytosine MICs; 0.25–1μg/ml, 16.9% (n=13); 2μg/ml, 23.4% (n=18); 4-8μg/ml, 40.2% (n=31); ≥16μg/ml, 19.5% (n=15).

Fluconazole MICs; 0.25–1μg/ml, 14.3% (n=11); 2μg/ml, 28.6% (n=22); 4-8μg/ml, 24.7% (n=19); ≥16μg/ml, 32.4% (n=25).

Voriconazole MICs; ≤1μg/ml, 94.8% (n=73); 1-2μg/ml, 3.9% (n=3), ≥4μg/ml, 1.3% (n=1).

Micafungin MICs; ≤1μg/ml, 11.7% (n=9); ≥4μg/ml, 88.3% (n=68).

Caspofungin MICs; ≤1μg/ml, 7.8% (n=6); ≥4μg/ml, 92.2% (n=71).

In other words, 68.9% of *Trichosporon* spp. isolates had a MIC value of ≤1μg/ml for AMB, %59.7 had a MIC of ≥4μg/ml for 5FC, 57.1% had ≥4μg/ml for FLC, 94.8% had a MIC of ≤1μg/ml for VRC, 88.3% had a MIC of ≥4μg/ml for MCA and 92.2% had a MIC of ≥4μg/ml for CAS.

Data comparison between antifungal susceptibility profiles and characteristics of 77 Trichosporon isolates are presented in Table 2 (Table 2 is added at the end of the article for better display).

In summary, data comparison between antifungal susceptibility profiles and characteristics of 77 *Trichosporon* isolates showed that there were no statistically significant effects of variables (gender, age groups, year, clinical department and specimen) on MIC levels of antifungal agents ($p>0.05$). There was an exception which was that the clinical sample type affects the MIC level. The MIC levels of antifungal compounds were found to be higher in urine samples ($p=0.000$).

DISCUSSION

Trichosporon spp. have been increasingly encountered in clinical settings, and associated with high fatal outcomes. Furthermore, in contrast with *Candida* spp., it's not a good target for echinocandins which is recommended to be used empirically in case of fungal infection[17-18].

Trichosporon spp. isolated from clinical samples in laboratories was reported to be mostly *T. asahii* (81.2-91.7%), similar to the ratio of 97.4% in this study[18-21].

There is a limited data on the incidence of this pathogen in clinical samples. The most common sample types from which *Trichosporon* spp. were isolated in this study were urine (58.4%) and blood (20.8%). In concordance with this, in a multi-centre study conducted in Brazil, it was reported that the clinical isolates were mostly cultured from urine (55%) and blood samples (25%)[22]. Whereas, in China, the most common sample was blood (36.8%), and ascetic fluid (21.8%) was following it[11]. These differences may be due to regional, clinical and patient characteristics.

There are several opportunistic fungal pathogens reported to be common in COVID-19 pandemic, including *Aspergillus* spp., *Candida* spp., *Cryptococcus neoformans* and mucormycosis[23,24]. When we evaluated whether *Trichosporon* spp. could be one of these opportunistic fungal pathogens for the COVID-19 Pandemic, it was determined that the most frequent period of isolation from clinical samples was not the COVID-19 Pandemic, but in the 2016-2019 period.

The most of the isolates (63.6%) belonged to the patients over 50 years of age. And more than half of the patients (58.4%) were in the ICU, which frequently has patients who are neutropenic, immunocompromised, receiving invasive medical procedures and/or antimicrobial therapy. ICU patients have many risk factors for colonization and development of invasive *Trichosporon* infection. This can occur by endogenous infection via translocation from gut to bloodstream and by horizontal transmission through fomites and inhalation of arthrospores or contamination of bed rails, drawers, air, urinary catheters and endoscopes, as previously reported[12,25]. The risk increases as the length of hospitalization increases. The duration of fungemia development was previously reported between 16 and 47 days[20,26]. Moreover, there were four isolates with a high MIC for VRC in the present study, and all were from the ICU. The MICs of 5FU, FLC, CAS, and MCA were also predominantly higher in ICU isolates.

There are no current validated clinical breakpoints to precisely interpret the MICs of antifungal agents for *Trichosporon* spp., wheter to report susceptible or resistant. *Trichosporon* isolates in present study had high MICs for CAS, MCA and 5FC. The compounds which showed better in vitro activity against *Trichosporon* were VRC (94.8%, ≤1μg/ml), AMB (68.9%, ≤1μg/ml) and FLC (42.9%, ≤2μg/ml), respectively. Up to present, it's known that *Trichosporon* species are intrinsically resistant to echinocandins (CAS and MCA), and have higher MICs to AMB than to azoles. And VRC has the strongest in vitro activity[8]. The results in this study were consistent with this data, except AMB. AMB showed good in vitro activity against *Trichosporon* isolates with predominant MICs of <4μg/ml (91%) in this cohort. The combination therapy of VRC and AMB could be a therapeutic option in this setting, as well as monotherapy of VRC, which is the front-line option[12]. Further researches with genotyping and comparative analysis are required to understand the reasons for this difference in AMB MIC values. There are 15 different genotypes for *T. asahii* have been described around the world, and the majority of them were reported to be genotype 1, so far[18-19,27]. Although high MIC values have not been commonly reported for VRC, which is the first-line antifungal for *T. asahii* isolates, it was reported that genotype 7 had low susceptibility to azoles, and this may be due to selective pressure due to their widespread use[22,28]. Determination of genotypes is important for tracing the origins of health-care associated infections, for local features of the isolates and also for management of patients. MCA and CAS, which had higher MICs

in present study, are associated with unfavorable outcome as a consequence of intrinsic resistance[8,29].

The comparison between antifungal susceptibility profiles and characteristics of *Trichosporon* isolates showed that urine samples affected the MIC value (48.9%, ≥16μg/ml) of FLC. And 44.4% of the isolates, that had a MIC value above 16μg/ml for FLC, belonged to the ICU. The *Trichosporon* species, which are biofilm-producing opportunistic fungi, may colonize disposable instruments, such as urinary catheters[30,31]. Complicated urinary infections caused by *Trichosporon* spp. were previously documented in the ICU patients with urinary catheters[32-34]. The patients in the ICU are under risk for colonization and infections as both they often have compromised immune systems due to comorbidities and they are often treated with invasive medical devices such as urinary catheters[8,25]. It may be appropriate for clinicians to use higher doses of FLC or prefer the other drugs in the treatment of *Trichosporon* spp. associated urinary tract infections in the ICU.

In a recent case report in Türkiye, it was drawn attention to the invasive *Trichosporon* agent, which was not isolated in the hematology service before. It was reported to be detected in the blood cultures of two patients within the same week during construction and renovation work carried out in the hospital[34]. Considering the demolition and planned repair works observed in 11 provinces including Cukurova region affected by the Kahramanmaras Earthquake of February 6, 2023, in Türkiye, encountering rare factors such as *Trichosporon* spp. may be more emerge.

Although we used the Vitek (bioMérieux, Marcy l'Étoile, France) automated system for the identification method instead of the analysis of internal transcribed spacer 1-2 or sequence intergenic spacer 1, it was aimed to reveal and document local epidemiological data, which has not been thoroughly explored in our setting. Additionally, the retrospective nature of the study, unavailability of the sequencing and genotyping, lack of data on clinical features and outcomes may represent limitations of the study.

IThe frequencies and sample distribution of *Trichosporon* spp. and antifungal susceptibility vary according to the regional, clinical and patient characteristics. The vast majority of the isolates (97.4%) were *T. asahii*. The most frequent isolation was between the years, 2016 and 2019 (71.4%), not the period of the COVID-19 pandemics. The most common sample type was urine (58.4%) and blood (20.8%) of whom predominantly (58.4%) hospitalized in the ICU. It was determined that being older than 50 years of age, being an intensive care unit patient and isolation from urine samples may be associated with increased incidence of the infection and elevated MIC levels of antifungal agents. VRC had the strongest in vitro activity against *Trichosporon* spp. and AMB MICs were lower than expected. Thus, the combination therapy of VRC and AMB could be a therapeutic option in this setting, as well as monotherapy of VRC. And it may be appropriate for clinicians to use higher doses of FLC or prefer the other drugs in the treatment of *Trichosporon* spp. associated urinary tract infections in the ICU.

Identifying and reporting local data on this subject will contribute to both the management of these patients and surveillance studies. The investigation of faster diagnostic methods, determination of genetic variations by sequencing, monitoring genotype distribution, antifungal susceptibility profiles and therapeutic drug monitoring should be included in future studies for better clinical management of *Trichosporon* infections. Additionally, it should be kept in mind that natural disasters such as earthquakes and floods seen recently may affect the diversity and frequency of pathogenic microorganisms.

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Table 2. Data comparison between antifungal susceptibility profiles and characteristics of 77 *Trichosporon* isolates.

	MIC* (µg/ml)	Gender			Years of age groups						Date range of isolation				Clinical departments				Sample types			
		Female n* (%)	Male n (%)	Total n (%)	0-1 n (%)	2-14 n (%)	15-49 n (%)	50-65 n (%)	>65 n (%)	Total n (%)	2012-2015 n (%)	2016-2019 n (%)	2020-2023 n (%)	Total n (%)	ICU* n (%)	IM* n (%)	Surgery n (%)	Total n (%)	Urine n (%)	Blood n (%)	Others[a] n (%)	Total n (%)
Amphotericin B	≤1	20 (66.7)	33 (70.2)	53 (68.8)	5 (71.4)	5 (55.6)	9 (75.0)	16 (69.6)	18 (69.2)	53 (68.8)	3 (42.9)	39 (70.9)	11 (73.3)	53 (68.8)	27 (60.0)	16 (72.7)	10 (100)	53 (68.8)	31 (68.9)	12 (75.0)	10 (62.5)	53 (68.8)
	2	7 (23.3)	10 (21.3)	17 (22.1)	1 (14.3)	2 (22.2)	3 (25.0)	4 (17.4)	7 (26.9)	17 (22.1)	2 (28.6)	12 (21.8)	3 (20.0)	17 (22.1)	14 (31.1)	3 (13.6)	0 (0.0)	17 (22.1)	10 (22.2)	3 (18.8)	4 (25.0)	17 (22.1)
	≥4	3 (10.0)	4 (8.5)	7 (9.1)	1 (14.3)	2 (22.2)	0 (0.0)	3 (13.0)	1 (3.8)	7 (9.1)	2 (28.6)	4 (7.3)	1 (6.7)	7 (9.1)	4 (8.9)	3 (13.6)	0 (0.0)	7 (9.1)	4 (8.9)	1 (6.3)	2 (12.5)	7 (9.1)
	Total	30 (100)	47 (100)	77 (100)	7 (100)	9 (100)	12 (100)	23 (100)	26 (100)	77 (100)	7 (100)	55 (100)	15 (100)	77 (100)	45 (100)	22 (100)	10 (100)	77 (100)	45 (100)	16 (100)	16 (100)	77 (100)
p value		0.999			0.725						0.354				0.098				0.934			
Flucytosine	0.25 – 1	5 (16.7)	8 (17.0)	13 (16.9)	1 (13.4)	3 (33.3)	2 (16.7)	4 (17.4)	3 (11.5)	13 (16.9)	1 (14.3)	12 (21.8)	0 (0.0)	13 (16.9)	6 (13.3)	7 (31.8)	0 (0.0)	13 (16.9)	7 (15.6)	2 (12.5)	4 (25.0)	13 (16.9)
	2	6 (20.0)	12 (25.5)	18 (23.4)	2 (28.6)	1 (11.1)	4 (33.3)	3 (13.0)	8 (30.8)	18 (23.4)	2 (28.6)	11 (20.0)	5 (33.3)	18 (23.4)	15 (33.3)	2 (9.1)	1 (10.0)	18 (23.4)	14 (31.1)	1 (6.3)	3 (18.8)	18 (23.4)
	4 – 8	13 (43.3)	18 (38.3)	31 (40.3)	3 (42.9)	3 (33.3)	3 (25.0)	12 (52.2)	10 (38.5)	31 (40.3)	2 (28.6)	20 (36.4)	9 (60.0)	31 (40.3)	18 (40.0)	8 (36.4)	5 (50.0)	31 (40.3)	16 (35.6)	8 (50.0)	7 (43.8)	31 (40.3)
	≥16	6 (20.0)	9 (19.1)	15 (19.5)	1 (14.3)	2 (22.2)	3 (25.0)	4 (17.4)	5 (19.2)	15 (19.5)	2 (28.6)	12 (21.8)	1 (6.7)	15 (19.5)	6 (13.3)	5 (22.7)	4 (40.0)	15 (19.5)	8 (17.8)	5 (31.3)	2 (12.5)	15 (19.5)
	Total	30 (100)	47 (100)	77 (100)	7 (100)	9 (100)	12 (100)	23 (100)	26 (100)	77 (100)	7 (100)	55 (100)	15 (100)	77 (100)	45 (100)	22 (100)	10 (100)	77 (100)	45 (100)	16 (100)	16 (100)	77 (100)
p value		0.948			0.873						0.174				0.048				0.383			
Fluconazole	0.25 – 1	6 (20.0)	5 (10.6)	11 (14.3)	2 (28.6)	1 (11.1)	3 (25.0)	1 (4.3)	4 (15.4)	11 (14.3)	2 (28.6)	8 (14.5)	1 (6.7)	11 (14.3)	3 (6.7)	7 (31.8)	1 (10.0)	11 (14.3)	4 (8.9)	3 (18.8)	4 (25.0)	11 (14.3)
	2	7 (23.3)	15 (31.9)	22 (28.6)	3 (42.9)	4 (44.4)	3 (25.0)	5 (21.7)	7 (26.9)	22 (28.6)	3 (42.9)	12 (21.8)	7 (46.7)	22 (28.6)	10 (22.2)	8 (36.4)	4 (40.0)	22 (28.6)	7 (15.6)	11 (68.8)	4 (25.0)	22 (28.6)
	4 – 8	9 (30.0)	10 (21.3)	19 (24.7)	2 (28.6)	2 (22.2)	2 (16.7)	6 (26.1)	7 (26.9)	19 (24.7)	0 (0.0)	16 (29.1)	3 (20.0)	19 (24.7)	12 (26.7)	4 (18.2)	3 (30.0)	19 (24.7)	12 (26.7)	1 (6.3)	6 (37.5)	19 (24.7)
	≥16	8 (26.7)	17 (36.2)	25 (32.5)	0 (0.0)	2 (22.2)	4 (33.3)	11 (47.8)	8 (30.8)	25 (32.5)	2 (28.6)	19 (34.5)	4 (26.7)	25 (32.5)	20 (44.4)	3 (13.6)	2 (20.0)	25 (32.5)	22 (48.9)	1 (6.3)	2 (12.5)	25 (32.5)
	Total	30 (100)	47 (100)	77 (100)	7 (100)	9 (100)	12 (100)	23 (100)	26 (100)	77 (100)	7 (100)	55 (100)	15 (100)	77 (100)	45 (100)	22 (100)	10 (100)	77 (100)	45 (100)	16 (100)	16 (100)	77 (100)
p value		0.445			0.538						0.285				0.031				0.000			
Voriconazole	≤1	29 (96.7)	44 (93.6)	73 (94.8)	7 (100)	8 (88.9)	11 (91.7)	23 (100)	24 (92.3)	73 (94.8)	6 (85.7)	52 (94.5)	15 (100)	73 (94.8)	41 (91.1)	22 (100)	10 (100)	73 (94.8)	42 (93.3)	16 (100)	15 (93.8)	73 (94.8)
	2	1 (3.3)	2 (4.3)	3 (3.9)	0 (0.0)	0 (0.0)	1 (8.3)	0 (0.0)	2 (7.7)	3 (3.9)	0 (0.0)	3 (5.5)	0 (0.0)	3 (3.9)	3 (6.7)	0 (0.0)	0 (0.0)	3 (3.9)	3 (6.7)	0 (0.0)	0 (0.0)	3 (3.9)
	≥4	0 (0.0)	1 (2.1)	1 (1.3)	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)	1 (14.2)	0 (0.0)	0 (0.0)	1 (1.3)	1 (2.2)	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	1 (6.3)	1 (1.3)
	Total	30 (100)	47 (100)	77 (100)	7 (100)	9 (100)	12 (100)	23 (100)	26 (100)	77 (100)	7 (100)	55 (100)	15 (100)	77 (100)	45 (100)	22 (100)	10 (100)	77 (100)	45 (100)	16 (100)	16 (100)	77 (100)
p value		0.999			0.271						0.183				0.827				0.275			
Micafungin	≤1	5 (16.7)	4 (8.5)	9 (11.7)	1 (14.3)	0 (0.0)	2 (16.7)	3 (13.0)	3 (11.5)	9 (11.7)	1 (14.3) 14.5/ 0.0/ 11.7)	8 (14.5)	0 (0.0)	9 (11.7)	5 (11.1)	4 (18.2)	0 (0.0)	9 (11.7)	7 (15.6) 6.3/ 6.3/ 11.7)	1 (6.3)	1 (6.3)	9 (11.7)
	≥4	25 (83.3)	43 (91.5)	68 (88.3)	6 (85.7)	9 (100)	10 (83.3)	20 (87.0)	23 (88.5)	68 (88.3)	6 (85.7)	47 (85.5)	15 (100)	68 (88.3)	40 (88.9)	18 (81.8)	10 (100)	68 (83.3)	38 (84.4)	15 (93.8)	15 (93.8)	68 (88.3)
	Total	30 (100)	47 (100)	77 (100)	7 (100)	9 (100)	12 (100)	23 (100)	26 (100)	77 (100)	7 (100)	55 (100)	15 (100)	77 (100)	45 (100)	22 (100)	10 (100)	77 (100)	45 (100)	16 (100)	16 (100)	77 (100)

p value		0.300			0.838						0.283				0.352				0.697			
Caspofungin	≤1	3 (10.0)	3 (6.4)	6 (7.8)	1 (14.3)	0 (0.0)	1 (8.3)	2 (8.7)	2 (7.7)	6 (7.8)	1 (14.3)	5 (9.1)	0 (0.0)	6 (7.8)	3 (6.7)	3 (13.6)	0 (0.0)	6 (7.8)	4 (8.9)	1 (6.3)	1 (6.3)	6 (7.8)
	≥4	27 (90.0)	44 (93.6)	71 (92.2)	6 (85.7)	9 (100)	11 (91.7)	21 (91.3)	24 (92.3)	71 (92.2)	6 (85.7)	50 (90.9)	15 (100)	71 (92.2)	42 (93.3)	19 (86.4)	10 (100)	71 (92.2)	41 (91.1)	15 (93.8)	15 (93.8)	71 (92.2)
	Total	30 (100)	47 (100)	77 (100)	7 (100)	9 (100)	12 (100)	23 (100)	26 (100)	77 (100)	7 (100)	55 (100)	15 (100)	77 (100)	45 (100)	22 (100)	10 (100)	77 (100)	45 (100)	16 (100)	16 (100)	77 (100)
p value		0.673			0.929						0.356				0.465				0.999			

* MIC=Minimum inhibitory concentration; n=number; ICU=Intensive care unit; IM=Internal medicine.

aIncluding wound (n=5), sputum (n=4), tracheal aspirate (n=2), cerebrospinal fluid (n=1), cornea (n=2), catheter (n=1) and peritoneal fluid (n=1).