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RESEARCH ARTICLE

Identification and antifungal susceptibility of *Saprochaete clavata* from invasive infections in Turkey

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ABSTRACT

Saprochaete clavata is an emerging opportunistic pathogen, that causes life-threatening infections, but there are limited evidence and information about the evaluation of *in vitro* antifungal susceptibility test results. The aim of this study was to determine *S. clavata* isolates from clinical specimens and to investigate their *in vitro* antifungal susceptibility. *S. clavata* was identified by API ID20C AUX (BioMérieux, Brussels, Belgium), MALDI TOF (Bruker Daltonik, Germany), and ITS gene region sequencing. *In vitro* susceptibility tests were performed using Sensititre YeastOne (TREK Diagnostic System, East Grinstead, UK). During the study period, 4,736 fungi were isolated from various clinical samples and, *S. clavata* was identified in eight patients with underlying diseases namely, pancreatic neoplasma, acute myeloid leukaemie, follicular lymphoma, cholelithiasis. Anidulafungin and micafungin minimum inhibitory concentration values were 1–2 and 1–4 mg/L, respectively, while those of the azole group antifungals were much lower. This is the first study in Turkey reporting isolation, identification and antifungal susceptibilities of *S. clavata* from clinical specimens. Higher MIC values seen in some isolates suggest that continuous monitoring of sensitivity rates and observation of regional differences will thus be useful guides in determining infection control and antifungal use policies.

KEYWORDS

Saprochaete clavata, antifungal susceptibility, invasive fungal infections

INTRODUCTION

The threat posed by invasive fungal infections in immunosuppressed patients is growing [1, 2]. In addition to *Candida* and *Aspergillus* species, other emerging opportunistic yeasts must also be considered in these patients [1]. *Saprochaete clavata*, formerly known as *Geotrichum clavatum*, is classified in the family Dipodascaceae in the class Saccharomycetales and is phylogenetically related to ascomycetous yeasts [3]. These non-encapsulated fungi produce arthroconidia, are non-fermentative and urease-negative [1, 4, 5]. Although it is known to grow best at temperatures above 30 °C, its ecology, reservoir, and importance in agriculture and food are unknown [3, 6]. *S. clavata* is an important opportunistic pathogen causing pulmonary infections, endocarditis, encephalitis and sepsis especially in immunocompromised patients [1, 3, 7]. Although there may be life-threatening infections due to *S. clavata*, no definitive evidence has been established for treatment options and no limit values have been defined for the evaluation of *in vitro* antifungal susceptibility test results [1].

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The aim of this study was (a) to determine *S. clavata* isolates from clinical specimens and (b) to investigate their *in vitro* antifungal susceptibilities in the Karadeniz Technical University clinical microbiology laboratory between February 2015 and April 2017.

MATERIALS AND METHODS

Study design, setting

Retrospective single-center study on data collected from electronic medical records covering the period from February 2015 to April 2017.

Isolates

S. clavata isolates from various clinical specimens sent from different clinics between February 2015 and April 2017, were included in the study. The isolates were investigated in the clinical microbiology laboratory of the Karadeniz Technical University, Farabi Hospital, and a tertiary referral center in Turkey.

Identification of isolates

Clinical specimens were cultured on 5% sheep blood agar (Salubris, Turkey), Eosin-Methylene-Blue (EMB) agar (Oxoid, UK), chocolate agar (Salubris, Turkey) and Sabouraud dextrose agar (SDA) media and incubated for 24–48 h at 35 °C and at 25 °C. Growing colonies were then evaluated.

Cream-colored colonies forming in the SDA medium were identified as *S. clavata* using API ID 20C AUX (BioMérieux, Brussels, Belgium) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI TOF-MS) (Bruker Daltonik GmbH, Leipzig, Germany) in addition to conventional methods.

DNA isolation

DNA extraction form isolates identified as *S. clavata* with mass spectrometry and biochemical tests was carried out using Bio-Speedy™ DNA-Regular Purification kits (Bioeksen, Turkey) in line with the manufacturer's recommendations.

Internal transcribed spacer (ITS) gene region amplification

ITS gene region amplification was performed using ITS1F′TCCGTAGGTGAACCTGCGG′ and ITS1R ′TCCTC CGCTTATTGATATGC′ primer sets (IDT, USA and Biomers, Germany) [1].

ITS gene region sequencing

BigDye ® terminator v3.1 Cycle Sequencing kits (Applied Biosystems, USA) were used in the sequencing of the ITS gene region, and sequencing procedures were performed with an automated 3130 Genetic Analyzer (Applied Biosystem, USA) device. The sequences were compared using

Table 1. Demographic data for patients with *Saprochaete clavata*

Case no	1	2	3	4	5	6	7	8
Age (years)	65	54	62	18	77	61	89	72
Gender	M	F	F	M	M	M	F	M
Clinical department responsible for treatment	Oncology	Hematology	Hematology	Hematology	Gastroenterology	Internal diseases-ICU	Gastroenterology	General surgery
Underlying disease	Pancreatic neoplasm	AML	Follicular lymphoma	AML	Cholelithiasis	AML	Pancreatic neoplasm	AML
Positive microbiologic specimen	Abscess	Blood	Blood	Blood	Bile	Blood	Bile	peritoneal fluid
Broad spectrum antibiotic therapy	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Antifungal therapy	FZ	Amp-B, VZ	CAS	Amp-B	FZ, CAS	CAS	-	Amp-B
Outcome (as a cause of death)	Died	Unknown	Died	Died	Survived	Survived	Survived	Survived

M: Male; F: Female; ICU: Intensive Care Unit; AML: Acute Myeloid Leukemia; FZ: Fluconazole; Amp-B: Amphotericin-B; VZ: Voriconazole; CAS: Caspofungin.

gene bank (www.ncbi.nlm.nih.gov) and the nucleotide BLAST program (URL-1, 2005), and their homologies were determined.

Antifungal susceptibility testing

In vitro susceptibility tests against fluconazole, voriconazole, amphotericin B, anidulafungin and caspofungin were performed using Sensititre ® YeastOne (TREK Diagnostic System, East Grinstead, UK) based on Clinical and Laboratory Standards Institute (CLSI) standards [8].

RESULTS

To our laboratory 182,842 specimens were sent from various clinics during the study period, and 4,736 fungus species were identified from these isolates. *S. clavata* was identified in eight patients during this process. Except for one patient, all isolates were identified in patients with malignancy. Four fungi were isolated from blood, two from bile, one from abscess, and one from peritoneal fluid. Demographic and clinical data for these patients are shown in Table 1.

Macroscopic image of *S. clavata* on SDA Petri dish after 48 h of incubation at 30 °C and microscopic appearance of *S. clavata* after Gram staining are shown in Fig. 1.

Eight isolates in our study were identified at the species level using API ID 20C AUX (BioMérieux, Brussels, Belgium), MALDI–TOF MS biotyper v3.1 database, and nucleotide sequencing analysis in addition to conventional methods, all of which were in agreement.

Susceptibility testing was performed for all eight isolates. The range of MIC values for all isolates were 0.12–0.25 mg/L for voriconazole, 0.25–0.50 mg/L for posaconazole, 0.50–2.00 mg/L for amphotericin B, 1.00–2.00 mg/L for anidulafungin, and 1.00–4.00 mg for micafungin. MIC values for itraconazole were ranged as 0.12–0.25 mg/L in seven isolates, and it was 32.00 mg/L in one isolate. Flucanazole MIC range were significantly high (16.00–32.00 mg/L) except for two isolates (4.00 and 8.00 mg/L). Similarly, range of MIC values for flucytosine were found to be higher (16.00–32.00 mg/L) except for one isolate (0.25 mg/L). The MIC values of the azole group antifungals were very much lower. Table 2 shows the *in vitro* MIC values for *S. clavata* isolates based on the microdilution method.

DISCUSSION

Since little is known about the etiology, outbreak sources and therapeutic options of *Saprochaete* species, these can lead to life-threatening infections [6, 7, 9]. Several cases of invasive and fatal infections due to *S. clavata* were reported in France during last two decades [10]. During 2016–2017 years, a cancer center in France was faced with an outbreak

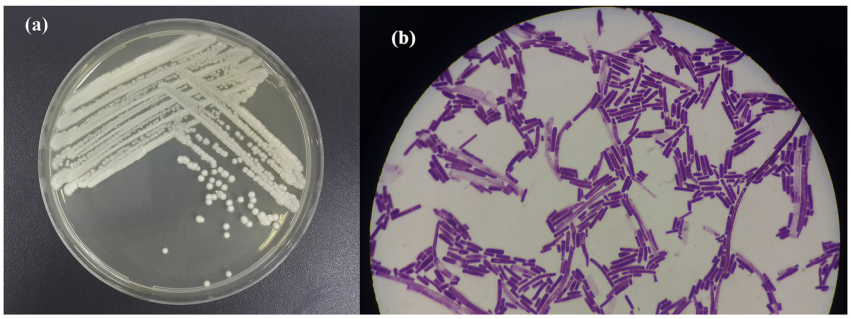

Fig. 1 Macroscopic image of *S. clavata* on SDA petri dish after 48 h of incubation at 30 °C (a). Microscopic appearance of *S. clavata* after Gram staining (Magnification Factor × 1,000) (b). 173 × 63 mm (1,200 × 1,200 DPI)

Table 2. MIC values (mg/L) of amphotericin-B, fluconazole, itraconazole, posaconazole, flucytosine, anidulafungin, micafungin and voricanazole for all *Saprochaete clavata* isolates tested

	MIC values (mg/L)							
Organism No	Amp-B	FZ	IZ	PZ	VZ	FC	AND	MIC
TSC1	0.5	16	0.25	0.5	0.25	16	1	2
TSC2	1	16	32	0.5	0.25	0.25	2	4
TSC3	0.5	4	0.12	0.25	0.12	32	2	1
TSC4	0.5	8	0.25	0.5	0.12	32	2	2
TSC5	0.5	32	0.12	0.5	0.25	16	2	2
TSC6	0.5	16	0.25	0.5	0.25	32	2	1
TSC7	2	32	0.25	0.5	0.25	32	2	1
TSC8	0.5	16	0.25	0.5	0.25	32	1	2

Amp-B: Amphotericin-B; FZ: Fluconazole; IT: Itraconazole; PZ: Posaconazole; FC: Flucytosine; AND: Anidulafungin; MIC: Micafungin; VZ:Voriconazole.

of *S. clavata* infections in patients with malignancies [11]. Limited reports concerning *Saprochaete capitata* have been published in countries such as the USA, Italy, Spain, and Turkey [9–19].

One review of data from various parts of the world reported the most common underlying causes of *S. capitata* infections as being hematological diseases at 91.7%, followed by solid organ tumors at 3.1% [20]. *S. clavata*, which bears a very close similarity to *S. capitata*, caused an outbreak in a hematology department in France [4]. Thirty cases were identified between September 2011 and October 2012 in that outbreak, and the mortality rate of 60% within 60 days at prognosis was particularly striking [4]. Similarly to the previous reports in the literature, in our study hematological diseases were observed in six of eight *S. clavata* cases and solid organ tumors in two, with three of these patients dying during monitoring. And also in our study, it was noteworthy that *S. clavata* was isolated from bile samples of a patient who was not diagnosed with cancer and followed up with cholelithiasis.

S. clavata generally affects patients with hematological malignancies [9, 17, 19]. Risk factors for these patients include chemotherapy-induced neutropenia, presence of central venous catheter, or hematopoietic stem cell transplantation [1, 7]. Risk factors for fungal infection, such as immunosuppressive therapy or long-term stay in the ICU were present in all our patients.

The transmission of *Saprochaete* species have not been fully understood yet. Examination of reported outbreaks shows that the source of infection has not been clearly identified. It has also been suspected that milk products and medical equipment may represent potential sources in invasive fungal diseases, although there is little evidence for this [1]. Moreover, dishwashers and pitchers made available to patients with stem cell transplant or malignancies can be the vector of contamination for *S. clavata*. Environmental samples, including dishwashers and pitchers, grew *S. clavata* at a cancer center in Marseille, France [11]. The portal system is exposed to large numbers of organisms when *Saprochaete* enters the blood circulation through the intestines. Therefore, in agreement with our study, *S. clavata* has frequently been detected in blood and invasive tissue specimens [7].

Biochemical tests frequently misidentify *S. clavata* as *S. capitata*. It is therefore recommended that the molecular sequencing technique be used as a reference method [6]. However, recent advances in pathogen analysis with MALDI-TOF MS have resulted in new possibilities of identification and a high level of discriminatory power [1, 5, 7]. Eight isolates in our study were identified by MALDI–TOF MS biotyper 3.1 database, which were in agreement with nucleotide sequencing analysis.

The Infectious Diseases Society of America (IDSA) recommends the use of echinocandins, voriconazole and amphotericin B in neutropenic patients with prolonged fever [21, 22]. *Saprochaete* species are a pathogen group that can lead to such a manifestation, but no defined antifungal clinical breakpoints have yet been reported for these fungal

Table 3. Summary of some reports on MIC values (mg/L) of antifungal agents against *Saprochaete clavata*

Reference	Method	MIC values (mg/L)									
		FC	VZ	PZ	FZ	IZ	ISVZ	Amp-B	AND	CAS	MIC
Durán Graeff et al. [1] (n:4)	Sensititre Yeast One	0.06–>32	0.016–0.25	0.03–0.25	1–32	0.002–0.25	0.004–0.25	1–2	2–4	>32	0.25–1
Kaplan et al. [3] (n: 8)	EUCAST broth microdilution		0.063–0.5	0.25	16–32	0.25–0.5	0.125–1	0.125–0.5			
Vaux et al. [4] (n:23)	EUCAST broth microdilution	0.125–1	0.06–2	0.125–1	–	–	–	0.125–1	–	1–8	–
Favre et al. [5] (n:1)	EUCAST broth microdilution	0.25	0.5	0.5	32	–	–	–	0.25	≥4	≥4
Del Principe et al. [7] (n:3)	Sensititre Yeast One	–	0.06–0.03	0.125–0.25	2–4	0.12–0.03	–	0.25–0.5	1	8	0.5
Camus et al. [16] (n:1)	Gradient strip test	–	0.064	0.75	12		–	1	–	–	–
Stanzani et al. [9] (n:4)	Micronaut broth microdilution	–	0.063	0.063	8	0.25	–	0.5	1	8	1
Pavone et al. [17] (n:1)	Sensititre YeastOne	0.12	0.25	0.25	4	–	–	0.25	–	–	–
Lo Cascio et al. [18] (n:7)	Sensititre Yeast One	0.12–0.25	0.25–0.5	–	16	–	–	0.5–1	2	8	8
Buchta et al. [19] (n:6)	Sensititre Yeast One, Etest	–									
This study (n:8)	Sensititre Yeast One	1–2	0.12–0.25	0.25–0.5	4–32	0.12–32	–	0.5–1	1–2	–	1–4

FC: Flucytosine; VZ: Voriconazole; PZ: Posaconazole; FZ: Fluconazole; IZ: Itraconazole; ISVZ: Isavuconazole; Amp-B: Amphotericin-B; AND: Anidulafungin; CAS: Caspofungin; MIC: Micafungin.

species [23]. Studies have reported no significant difference between anidulafungin and micafungin, while MIC elevation has been observed in echinocandins. In addition, voriconazole and itraconazole have the lowest MIC values [1, 23]. Antifungal MIC values reported other studies involving *S. clavata* are shown in Table 3.

CONCLUSIONS

In conclusion, *S. clavata* causes relatively high mortality rates, particularly in immunosuppressed patients but few reports have been published concerning infections caused by *S. clavata* worldwide, and no clinical breakpoints for this species have yet been determined. To the best of our knowledge, this study was among the first publications presenting isolation, identification and antifungal susceptibilities of *S. clavata* from clinical specimens in Turkey. Moreover, it was salient that one isolate of *S. clavata* was isolated from a bile sample of a patient followed up with the diagnosis of cholelithiasis, while other isolates were from the samples of cancer patients, similar to literature.

High MIC range was determined in *S. clavata* isolates against antifungal agents such as fluconazole and flucytosine. The MIC values obtained against the other antifungal agents tested were found to be lower than these. However, higher MIC values seen in some isolates suggest that antifungal susceptibility tests should be performed before deciding antifungal therapy regimen. And also continuous monitoring of sensitivity rates and observation of regional differences will thus be useful guides in determining infection control and antifungal use policies.

Ethical approval: This study was approved by Karadeniz Technical University, Faculty of Medicine Scientific Research Ethics Committee (May 2018, No: 2018/80) and was in compliance with the guidelines of the Declaration of Helsinki.

Conflict of interest: The authors declare that they have no competing interests.

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