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Short Communication

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Epidemiological data and antifungal susceptibility in invasive fungal infections - a Romanian infectious diseases tertiary hospital's experience. Preliminary report

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Abstract

***Introduction**: Invasive fungal infections have stood as an important research subject for the past 20 years, being considered as a crucial effect of advancing healthcare services. Low identification rates of invasive fungal infections in blood cultures and low sensibility of biomarkers determine empiric treatments which lead to a change in epidemiological data and antifungal susceptibility.*

***The aim**: The epidemiological evaluation of invasive fungal infections and the assessment of antifungal resistance related to this condition.*

***Methods and material**: An "antifungal stewardship" retrospective study was developed between January 2010 and April 2016. An epidemiological analysis was performed on 79 cases with proven invasive fungal infections in bloodstream, catheter, and cerebrospinal fluid. We considered: age, gender, HIV status, place of residence, and first option in medical practice of antifungal treatment. The laboratory analysis was performed by the Microbiology Laboratory at "Prof. Dr. Matei Bals" National Institute for Infectious Diseases, Bucharest. Minimum inhibitory concentrations (MIC's) of 15 isolates were identified using colorimetric micro broth dilution panel YEASTONE®YO10 and compared with susceptibilities obtained by VITEK2®C system. Candida parapsilosis ATCC 22019 was used as reference.*

***Results**: The incidence of invasive fungal infections was 3.7 on 1000 hospitalized patients. The age of the study population ranged between 12 and 83 years, and most were male (59%). The majority of subjects were from an urban area (84%), and 27% of them were HIV positive. The results obtained in VITEK2C® were similar with those from YEASTONE® YO10 for fluconazole, voriconazole, amphotericin B (100%), without any minor, major or very major errors. The fluconazole was the first option of treatment, followed by voriconazole, caspofungin, anidulafungin. In 37% of cases the first treatment option was replaced with a secondary antifungal therapy accordingly with antifungal breakpoints obtained by Vitek ®.*

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***Conclusions**: No rates of resistance to fluconazole, amphotericin B, voriconazole were obtained. Fluconazole was the major first line antifungal therapy.*

***Keywords**: invasive fungal infections, antifungal stewardship, VITEK2®, YEASTONE®YO10*

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Introduction

Resistance to antibiotics and antifungals represents a bigger threat than the financial crisis in 2008. Predicted estimations announce that 10,000,000 people can die due to this phenomenon with major impact for demography, economics (10 trillion USD), and healthcare [1]. There is a worldwide scientific dedication to analyze epidemiological data for each country, area, hospital, and department in order to control this phenomenon known as 'antifungal or antibiotic stewardship'. Especially in IFI, where the late and low identification rates of invasive fungal infections (IFI) in blood cultures, debatable specificity and sensibility of biomarkers and the high mortality of these infections, determined medical practitioners to administrate the empirical therapy along with prophylactic usage on patients at risk. This overuse of empirically and prophylactic treatments generate resistance and species distribution changes. The most common fungal infections of hospitalized patients are invasive candidiasis. Of all Candida species, 15 are involved in pathogenicity. Around 90% of invasive infections are determined by *C. albicans*, *C. glabrata*, *C. tropicalis*,*C. parapsilosis*, and *C. krusei* [2] with a shift from *C.albicans* in favor of non-albicans in recent years, due to high presence in intensive care patients predicted to be between 0.5 and 10% [3]. The lowest presence is in patients with neoplasm and transplant of hematopoietic stem cells from 0.15% to 1.55% [4]. Some antifungals do not have efficiency against some species, such as *C.krusei* to fluconazole. Globally, researchers are facing a risk of 33% high resistance to fluconazole and also to echinocandins (for example anidulafungin, percentage of resistance is close to 6.4%) [5]. *C.glabrata* has a high potential of resistance to echinocandins and was detected predominantly in the bloodstream of patients with infections associated with healthcare (HAI). Resistance was detected in a percentage of 5.1% for caspofungin, 3.8% to anidulafungin, 3.2% to micafungin, 7.7% for fluconazole, 5.1% for posaconazole, and 6.4% for voriconazole [6]. In Romania, clear evaluations regarding susceptibility are limited, excepting one multicenter study regarding susceptibility pattern of 551 species from bloodstream (BSI), superficial and deep-seated fungal infections which identified a resistance of 10.2% to fluconazole and 2.5% to voriconazole globally, and 4% in BSI [7]. This constituted the rationale to develop an analysis of antifungal susceptibilities in Romania for IFI. In order to identify susceptibility to antifungals we can use options such as automatic systems (VITEK 2 C®) or visual identifications compared with a scale like YEASTONE®, E-TEST®. Vitek 2 C® represents an automatic system of identification of germs and susceptibility offering the "breakpoints" associated with minimum inhibitory concentration (MIC). Studies showed that IFI with *C. albicans* having a value of MIC over 2 mg/L had a high rate of mortality. This means that it is important to see the level for our isolates in each center [8].

Material and method

In order to evaluate the susceptibility of antifungals and options for antifungal agents we retrospectively analyzed all the isolates on fungal bloodstream infections (BSI), catheter colonization and cerebrospinal fluid infections detected by VITEK 2 assessed at the microbiology laboratory in the National Institute for Infectious

Diseases "Prof. Dr. Matei Bals "Bucharest, from January 2010 to April 2016. The isolates responsible for invasive fungal infections, grown in Bact/Allert systems were identified and tested to antifungals panel of VITEK2® correlated with EUCAST. Those isolates were stored in a strain collection bank. In 2016 those isolates were cultivated on Sabouraud agar and incubated 24 to 48 hours at 35ºC. Small colonies were passed subsequently on YEASTONE, a colorimetric technology which represents micro broth dilutions used to detect susceptibility to *Candida, Aspergillus, Cryptococcus* and other fungi with rapid growth (Instructions for Use *Thermo Scientific SensititreYeastOne® (SYO) Susceptibility Plates,* 2015) [9]. As reference strain *C. parapsilosis* ATCC 22019 was used. The option was for SYO, YO10 type due to its structure of antifungals present on the Romanian pharmaceutical market (excepting Amphotericin B and 5-Flucytozine). Plate inoculation was done accordingly to the manufacturer's instructions. The interpretation was done visually. We identified within the microbiology lab files the susceptibility to antifungals offered by VITEK2® and compared to YEASTONE ® in order to track errors (minor, major, very major error).

Results

We evaluated 79 isolates identified in proven IFI and only 15 isolates were recovered after passing on Sabouraud medium from the 2010-2016 collection bank. The rate of incidence of IFI was evaluated at 3.7 on 1,000 hospitalized patients. Regarding epidemiological descriptive data, this study evaluated age, gender, HIV status. The age in this study (medium age) was 41 years and the extreme ages were 12 years old and 83 years old. Male gender was predominant with 59%. Most of the patients came from from urban areas. The rate of HIV infection in this IFI study group was 27%.

In the present study 15 isolates grown on Sabouraud, transferred on YEASTONE® YO10-MIC's were incubated at 35°C and the results were visually read after the legend provided by the producer. All the selected samples had a positive control marker. Regarding the accuracy of the method, no errors were noticed.

The comparison of the values of MIC's of the same isolates obtained on YEASTONE and MIC´s obtained by automated system VITEK2C® (breakpoints) are presented in tables I and table II.

The range of MIC's on YEASTONE® YO10 varied as follows: fluconazole between ≤ 0.06 mcg/ml and 0.25 mcg/ml, voriconazole ≤ 0.008 mcg/ml and 0.25 mcg/ml, posaconazole 0.015 mcg/ml and 1 mcg/ml, itraconazole ≤ 0.015 mcg/ml and 0.50 mcg/ml, caspofungin between 0.015 mcg/ml and 0.50 mcg/ml, anidulafungin 0.015 mg/ml and 2 mg/ml, micafungin ≤ 0.008mcg/ml and 2 mcg/ml, amphotericin B between ≤ 0.12 mcg/ml and 0.5 mcg/ml, 5 –flucytozine 0.25 mcg/ml to 8 mcg/ml.

The range of MIC's identified on VITEK2® (breakpoints) varied as follows: fluconazole between ≤ 1 mcg/ml and 2 mcg/ml, voriconazole ≤ 0.12 mcg/ml, caspofungin between ≤ 0.25 mcg/ml and 1 mcg/ml, micafungin ≤ 0.06 mcg/ml and 0.50 mcg/ml, amphotericin B between ≤ 0.25 mcg/ml and 1 mcg/ml.

This present study compared the MIC from tables I and II generated by YEASTONE® with EUCAST standards (8.0 version, November 2015) and versus CLSI standards (2012 version) and obtained –S - susceptible, I - intermediary, R - resistant.

The susceptibilities were compared in table III, table IV and table V with susceptibilities offered by VITEK 2C system. No errors were noticed.

The first options for antifungal treatment, proportionally, were represented by fluconazole,

Table I. MIC's values in vitro of *Candida* spp. - YEASTONE Y010 versus VITEK2C® (part I)

Isolate/MIC mcg/ml	MF		CAS		FZ		PZ	
	YEA-STONE Y010	VITEK2C®	YEA-STONE Y010	VITEK2C®	YEA-STONE Y010	VITEK2C®	YEA-STONE Y010	VITEK2C®
1. *C. albicans*	0.015	≤ 0.06	0.06		≤ 0.06	≤ 1	0.03	-
2. *C. albicans*	0.015	≤ 0.06	0.06	≤ 0.25	0.12	≤ 1	0.015	-
3. *C. albicans*	0.015		0.06		≤ 0.06	≤ 1	0.015	-
4. *C. albicans*	0.015	≤ 0.06	0.015	≤ 0.25	≤ 0.06	≤ 1	0.015	-
5. *C. albicans*	0.015	≤ 0.06	0.12	≤ 0.25	≤ 0.06	≤ 1	1	-
6. *C. albicans*	0.015	≤ 0.06	0.12	≤ 0.25	≤ 0.06	≤ 1	0.50	-
7. *C. albicans*	0.008	≤ 0.06	0.06	≤ 0.25	0.12	≤ 1	0.015	-
8. *C. albicans*	0.015	≤ 0.06	0.03	≤ 0.25	0.12	≤ 1	0.015	-
9. *C. albicans*	≤ 0.008		0.06		≤ 0.06	≤ 1	0.015	-
10. *C. parapsilosis* ATCC 2209	1	-	0.50	-	0.25	-	0.06	-
11. *C.parapsilosis*	0.50	0.50	0.25		≤ 0.06	≤ 1	0.06	-
12. *C.parapsilosis*	0.50	0.50	0.25	1	≤ 0.06	≤ 1	0.03	-
13. *C.parapsilosis*	2		0.50		≤ 0.06	2	0.03	-
14. *C. kefyr*	0.12		0.06		≤ 0.06	2	0.03	-
15. *C.lusitaniae*	0.06		0.06		≤ 0.06	≤ 1	0.015	-
16. *C.dubliniensis*	0.03	≤ 0.06	0.06	≤ 0.25	≤ 0.06	≤ 1	0.03	-

MF-micafungin; CAS-caspofungin; FZ-fluconazole; PZ-posaconazole.

Table II. MIC's values in vitro of *Candida* spp. - YEASTONE Y010 versus VITEK2C® (part II)

Isolate/MIC mcg/ml	VOR		IZ		FC		AND		AB	
	YEASTONE Y010	VITEK2C®	YEASTONE Y010	VITEK2C®	YEASTONE Y010	VITEK2C®	YEASTONE Y010	VITEK2C®	YEASTONE Y010	VITEK2C®
1. C. albicans	≤ 0.008	≤ 0.12	0.06	-	0.50		0.12	-	0.5	≤ 1
2. C. albicans	≤ 0.008	≤ 0.12	0.03	-	0.25	≤ 1	0.06	-	0.5	1
3. C. albicans	≤ 0.008	≤ 0.12	≤ 0.015	-	0.25		0.06	-	0.25	1
4. C. albicans	≤ 0.008	≤ 0.12	≤ 0.015	-	0.25	≤ 1	0.06	-	0.25	≤ 0.25
5. C. albicans	0.25	≤ 0.12	0.50	-	8	≤ 1	0.06	-	0.5	1
6. C. albicans	0.12	≤ 0.12	0.25	-	8	≤ 1	0.06	-	0.5	0.50
7. C. albicans	≤ 0.008	≤ 0.12	0.03	-	0.25	≤ 1	0.015	-	0.5	1
8. C. albicans	≤ 0.008	≤ 0.12	0.03	-	0.50	≤ 1	0.06	-	0.25	1
9. C. albicans	≤ 0.008	≤ 0.12	0.03	-	0.25		0.06	-	0.5	0.50
10. C. parapsilosis ATCC 2209	0.03	-	0.12	-	2	-	1	-	0.5	-
11. C.parapsilosis	0.015	≤ 0.12	0.06	-	0.5		0.5	-	0.5	0.5
12. C.parapsilosis	≤ 0.008	≤ 0.12	0.03	-	0.5	≤ 1	0.5	-	≤ 0.12	≤ 0.25
13. C.parapsilosis	0.03	≤ 0.12	0.03	-	2		2	-	0.5	0.50
14. C. kefyr	0.015		0.03	-	0.50		0.25	-	0.5	1
15. C.lusitaniae	≤ 0.008		0.03	-	0.25		0.12	-	0.25	0.50
16. C.dubliniensis	≤ 0.008	≤ 0.12	0.06	-	0.25	≤ 1	0.12	-	0.25	1

VOR- voriconazole; IZ- itraconazole; FC-5-flucytozine; AND- anidulafungin; AMB-amphotericin B

Table III. Categorically agreement (CA) for *Candida* spp. susceptibility to FLUCONAZOLE

Fungus vs Fluconazole	YEASTONE®				VITEK2C®				Correspondence	Errors		
	T	S	I	R	T	S	I	R		Minor	Major	Very major
C. albicans	8	8	0	0	8	8	0	0	8 (100%)	0	0	0
C. *parapsilosis*	3	3	0	0	3	3	0	0	3 (100%)	0	0	0
C. glabrata	1	1	0	0	1	1	0	0	1 (100%)	0	0	0
C. kefyr	1	1	0	0	1	1	0	0	1 (100%)	0	0	0
C. lusitaniae	1	1	0	0	1	1	0	0	1 (100%)	0	0	0
C. dubliniensis	1	1	0	0	1	1	0	0	1 (100%)	0	0	0
Global	15	15	0	0	15	15	0	0	15 (100%)	0	0	0

T – total number of species ; S – number of species with susceptibility ; I – number of species with intermediary; R – number of species with resistance to antifungals.

Table IV. Categorically agreement (CA) for *Candida* spp. susceptibility to AMPHOTERICIN B

Fungus vs Amphotericin B	YEASTONE®				VITEK2C®				Correspondence	Errors		
	T	S	I	R	T	S	I	R		Minor	Major	Very major
C. albicans	8	8	0	0	8	8	0	0	8 (100%)	0	0	0
C. parapsilosis	3	3	0	0	3	3	0	0	3 (100%)	0	0	0
C. glabrata	1	1	0	0	1	1	0	0	1 (100%)	0	0	0
Global	12	12	0	0	12	12	0	0	12 (100%)	0	0	0

T – total number of species ; S – number of species with susceptibility ; I – number of species with intermediary susceptibility; R – number of species with resistance to antifungals.

For ***C. kefyr, C. lusitaniae, C. dubliniensis*** it has been no data on EUCAST and CLSI.

Table V. Categorically agreement (CA) for *Candida* spp. susceptibility to VORICONAZOLE

Fungus vs Voriconazole	YEASTONE®				VITEK2C®				Correspondence	Errors		
	T	S	I	R	T	S	I	R		Minor	Major	Very major
C. albicans	8	8	0	0	8	8	0	0	8 (100%)	0	0	0
C. parapsilosis	3	3	0	0	3	3	0	0	3 (100%)	0	0	0
C. glabrata	1	1	0	0	1	1	0	0	1 (100%)	0	0	0
Global	12	12	0	0	12	12	0	0	12 (100%)	0	0	0

T – total number of species; S – number of species with susceptibility; I – number of species with intermediary susceptibility; R – number of species with resistance to antifungals.

For ***C.kefyr***, ***C.lusitaniae ,C. dubliniensis*** – no data on EUCAST and CLSI were available to compare.

voriconazole, echinocandins (caspofungin and anidulafungin) were de-escaladed based on the susceptibility to antifungals generated by VITEK2C® in 37% of cases.

Discussions

Regarding identifications of isolates and the study importance, being a retrospective study, one of the limitations revolves around the low

number of isolates tested on micro broth dilutions (only 15) in comparison with 79 obtained from sterile sites on Vitek2C®, namely that some did not cultivate after passing form bank to Sabouraud agar. Another limitation is that some species of Candida were not identified on VITEK 2C®(5.45%). VITEK system can cause errors – for example 4 cases (8 isolates from blood cultures) of *C.auris*, first cases identified in Europe, a species alerted via CDC alerting system, resistant to fluconazole and voriconazole, with susceptibility conserved to posaconazole, itraconazole, echinocandines și amphotericin B identified using ITS rDNA was considered by VITEK technology as *C.lusitaniae, C.haemulonii* while 6 isolates remained unidentified [10]. Using ITS "internal transcribed spacer" of ribosomal DNA some species identified on VITEK can be reconsidered and those unidentified can be precisely identified as Merseguel et *al.* identified 300 species in IFI [11]. It is possible that some isolates be misidentified as literature presents.

For susceptibility data, some studies identified differences between MIC's versus Clinical and Laboratory Standards (CLSI) breakpoints established in 2012 - 96.5% in *C. albicans*, 85.8% in *C. tropicalis* and 92.1% in *C.parapsilosis* according to the revised CBP's fluconazole susceptibility [12]. Sensititre YEASTONE compared with CLSI standards modified in 2012 regarding susceptibility to echinocandins generated only 1% errors [13]. Sensititre YEASTONE a microbroth dilution method used in clinical practice [14] obtained values of susceptibility in percentages of 98.7% in *C. albicans*, 92.5% in *C.glabrata* 92.3% in the *C. parapsilosis* complex, 96.1% in *C. tropicalis* ,and 100% in *C. Guillermondii* [15] for voriconazole and fluconazole this proving the accuracy of the method. Vijgen *et al.* identified in 2011 a concordance of 78.4%, 84.6% and 90.8% for fluconazole, voriconazole and amphotericin B between Vitek 2 and Sensititre YeastOne (SYO) [16]. Farina *et al.* in 2011, in a cooperation microbiology project which evaluated susceptibilities of 70 isolates of *Candida* on VITEK2 System and Sensititre YeastOne® to amphotericin B, voriconazol, fluconazol, flucytosine showed the results which credited VITEK system with a concordance for amphotericin B, fluconazole, voriconazole and 5-flucytozine (from 81.4% to 88.6%).The researchers recommend VITEK with the mention to readjust the breakpoints [17].

In the present study, obtaining the MICs of *Candida* spp. was essential for epidemiological data and the errors between VITEK® MIC's and MIC's obtained by YEASTONE with 0 errors were crucial for clinicians' confidence and also for understanding the limits of the actual systems and potential of new technologies.

YEASTONE is correlated 100% with VITEK2® in terms of susceptibility. No antifungal resistance acquired events were reported in comparison with one study from Italy also using the YEASTONE panel where the fluconazole resistance on *Candida spp.* decreases to 5.4% in 2016 from 24.9% in a survey from 2009 [18]. Because no resistance to fluconazole was noticed in the susceptibility data we consider that first choice of fluconazole in medical practice the proper option when candidemia is suspected even if guidelines recommend echinocandins for candidemia [2, 19]. Susceptibility to azoles, echinocandins, and amphotericin B in Romanian species involved in infectious diseases IFI is preserved in comparison with a 6 years' analysis in Switzerland (FUNGINOS project) where resistant isolates were mentioned [20].

Conclusions

The incidence in proven candidemia was 3.7 on 1,000 hospitalized patients. In these IFI patients, HIV infection was present in 27% of patients, predominantly males from urban areas with age limits between 12-83 years old. All our

YEASTONE susceptibility determined data (S –susceptible, I-intermediary, R-resistant) corresponded 100% to VITEK2® technology with no antifungal acquired resistance even on identification VITEK had some limitations. No resistance to any antifungal was noticed. Proportionally, the first option of treatment was fluconazole, followed by voriconazole and caspofungin, anidulafungin.

Conflict of interest

The authors declare that they have no conflict of interest.

Abbreviations

BSI- Bloodstream Infections
CDC-Centers for Disease Control and Prevention
CLSI-Clinical and Laboratory Standards Institute
EUCAST-European Committee on Antimicrobial Susceptibility Testing
FUNGINOS- Fungal Infection Network of Switzerland
HAI-Hospital acquired infections
MIC-Minimum Inhibitory Concentration
ICU-Intensive Care Unit
IFI-Invasive Fungal Infections
ITS -Internal Transcribed Spacer
SDD-sensibility dose dependent
SYO-Sensititre YEASTONE

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