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SUSCEPTIBILITY TO ANTIFUNGAL AGENTS OF *CANDIDA* SPP. FROM BLOOD AND FECES COLLECTED IN NOVI SAD IN 3-YEAR PERIOD (2008-2010)

ABSTRACT: Candidemia is an important emerging nosocomial infection in patients with risk factors. *Candida* species from nonsterile sites can give insight into the characteristics of strains that may cause invasive disease.

The aim of this study was to evaluate antifungal susceptibility of *Candida* blood and fecal isolates in Novi Sad, Vojvodina.

During a 3-year period (2008 to 2010), 424 isolates of *Candida* spp. were collected, 30 bloodstream isolates and 394 strains from fecal samples. *In vitro* susceptibility of these isolates to five antifungal agents was established using commercial ATB FUNGUS 3 (Bio-Mérieux).

Predominant species was *Candida albicans* (6 isolates from blood and 269 from feces). Resistance to one or more antifungal agents was less common in *Candida albicans* (3.63%) than in other species (24.83%). Resistance to itraconazole was the most commonly found in both groups of isolates, 9.64% strains from feces and 20% from blood samples. Twelve isolates were multiply resistant, usually to fluconazole, itraconazole, and voriconazole. Resistance to amphotericine B was extremely rare.

Although resistance to antimycotics of *Candida* spp. is rare at present, continued surveillance of antifungal susceptibility is necessary in order to monitor trends, and to choose the right empiric therapy.

KEY WORDS: antifungal susceptibility, candidemia, *Candida* spp.

INTRODUCTION

Candidemia is an important emerging nosocomial infection in patients with risk factors. In the last two decades an increase of bloodstream infections caused by *Candida* species (*Candida* spp.) has been documented, with a consequent rise in related mortality and prolonged hospitalizations (T u l u m o g l u S. et al., 2009). Risk factors for development of candidemia are: immunocompromised host, immunosuppressive therapy and neutropenia, intensive care

units patients, central venous catheters, parenteral nutrition, and the long-term use of broad-spectrum antibacterial drugs (T a k a k u r a S. et al., 2006).

Candida spp. at multiple nonsterile sites often suggests colonization but these strains also may cause invasive disease. *Candida* spp. from nonsterile sites can also give insight into the characteristics of strains that cause the disease (T a s i c S. et al., 2008).

The variability in the susceptibility of clinical isolates to antifungal drugs emphasizes the importance of performing species identification and antifungal susceptibility testing (M i t r o v i c S. M. et al., 2007).

There are data which show that *Candida albicans* (*C. albicans*) maintains excellent susceptibility to all antifungal agents, but many non-albicans species have increased resistance to antifungal drugs over the past decades. (T u l u m o g l u S. et al., 2009)

There are differences in species distribution and antifungal susceptibility profiles and it is important to obtain such information for each geographic area (M u e l l e r F. M. et al., 2000)

The aim of this study was to assess the antifungal susceptibility profiles in both strains of *Candida* spp. associated with systemic disease and fecal isolates.

MATERIAL AND METHODS

In the Center for Microbiology of Institute for Public Health of Vojvodina during a 3-year period, from January 2008 to December 2010, a total of 424 isolates of *Candida* spp. were collected, 30 bloodstream isolates and 394 strains from fecal samples.

Blood samples were collected in sterile conditions into 30 ml culture medium for BacT/Alert- BioMérieux. The bottles which were positive after incubation at 37º C for 2-5 days were transferred to blood agar and Sabouraud Dextrose Agar and incubated 24 hours at 35ºC, and next 24 hours at 25ºC.

Fecal samples were inoculated to Sabouraud Dextrose Agar and incubated 24 hours at 35ºC, and next 24 hours at 25ºC.

Colonies of *Candida* spp. were identified by examination of their microscopic and macroscopic features. *Candida albicans* was identified by the application of germination tube test in human serum. Species identification was confirmed by API 20C (BioMérieux) or Vitek2 (BioMérieux) for resistant blood isolates of *Candida* spp.

In vitro susceptibility of all isolates to five antifungal agents was established using commercial ATB FUNGUS 3 (BioMérieux, France) that enables the determination of the susceptibility to five antifungal agents under conditions similar to the reference method for micro-dilution according to EUCAST (European Committee on Antibiotic Susceptibility Testing) and CLSI (Clinical and Laboratory Standards Institute) recommendations. The results obtained give a MIC (Minimal inhibitory concentration) for amphotericin B (AMB), fluconazole (FCA), itraconazole (ITR), voriconazole (VRC) and/or

classify the strain as Sensitive (S), Intermediate (I) or Resistant (R) flucytosine (5FC).

The interpretative breakpoints were proposed by the manufacturer of the test. Fluconazole MICs ≤ 8.0 mg/L were considered susceptible and ≥ 64 mg/L were considered resistant, with the exception of *C. krusei*, which is considered inherently resistant to fluconazole, regardless of the MIC value. Isolates showing itraconazole MICs ≤ 0.125 mg/L and voriconazole ≤ 1 mg/L were classified as susceptible and those with MICs ≥ 1 mg/L for itraconazole and ≥ 4 mg/L for voriconazole as resistant. Although interpretative breakpoints for amphotericine B have not yet been established, isolates showing MIC of ≥ 2 mg/L suggest resistance.

RESULTS

From 30 blood isolates and 394 fecal isolates predominant species was *Candida albicans*, 269 from fecal samples and 6 strains from blood (in hemocultures *Candida* spp. was more common). Resistance to one or more antifungal agents was less common in *Candida albicans* (3.63%) than in other *Candida* species (24.83%).

From total of 269 fecal isolates of *Candida albicans* 259 (96.28%) were susceptible to all antimicrobial drugs tested. Ten isolates (3.72%) had one or more resistance markers. One resistance marker had 4 strains, they were resistant to itraconazole, one was resistant to fluconazole and itraconazole, and five isolates had 3 markers and were resistant to fluconazole, itraconazole, and voriconazole. In *Candida* spp. (non-albicans) 24.80% had resistance markers, 24 (19.20%) strains with one marker, 1 (0.80%) with 2 markers, 5 (4.00%) with 3 markers and 1 (0.80%) strain with 4 resistance markers (Table 1).

Resistance to itraconazole was the most commonly found in both groups of fecal isolates, 3.72% of *Candida albicans* strains and 22.40% of *Candida* spp.

Tab. 1 – Resistance types of *Candida* isolates from feces (comparative data for *Candida albicans* and *Candida* spp.)

Number of resistance markers	Resistance types of *Candida albicans*	Number (%) of isolates	Number of resistance markers	Resistance types of *Candida* spp.	Number (%) of isolates
0		259 (96.28%)	0		94 (75.20%)
1	ITR	4 (1.48%)	1	FCA ITR VRC	2 (1.6%) 21 (16.8%) 1 (0.80%)
2	FCA, ITR	1 (0.37%)	2	FCA, ITR	1 (0.80%)
3	FCA, ITR, VRC	5(1.85%)	3	FCA, ITR, VRC AMB, FCA, ITR	4 (3.20%) 1 (0.80%)
			4	AMB, FCA, ITR, VRC	1 (0.80%)
Total number		269 (100%)	Total number		125 (100%)

Resistance to fluconazole in *Candida albicans* was established in 2.23 % and 7.20% of *Candida* spp. Resistance to voriconazole was found in 1.85% *C. albicans* and 4.80% *Candida* spp. No resistance to amphotericin B was found in *Candida albicans* and in *Candida* spp., it was rare (1.60%).

From the blood isolates 24 (80%) were sensitive to all drugs tested (all strains of *Candida albicans* were completely sensitive) and from 20% resistant isolates of *Candida* spp., 3 were identified as *C. parapsilosis*-1 isolate and *C. tropicalis* -2 isolates (10%), had 1 resistance marker and were resistant to itraconazole; 2 isolates of *C. kruzei* (6.66%) had 2 markers – fluconazole and itraconazole; and 1 *C. tropicalis* (3.33%) was resistant to 3 antimycotics – fluconazole, itraconazole and voriconazole (Table 2).

Tab. 2 – Resistance types of *Candida* isolates from blood (comparative data for *Candida albicans* and *Candida* spp.)

Number of resistance markers	Resistance types of *Candida albicans*	Number of isolates	Number of resistance markers	Resistance types of *Candida* spp.	Number of isolates
0		6	0		18
1			1	ITR	3
2			2	FCA, ITR	2
3			3	FCA, ITR, VRC	1
Total number		6	Total number		24

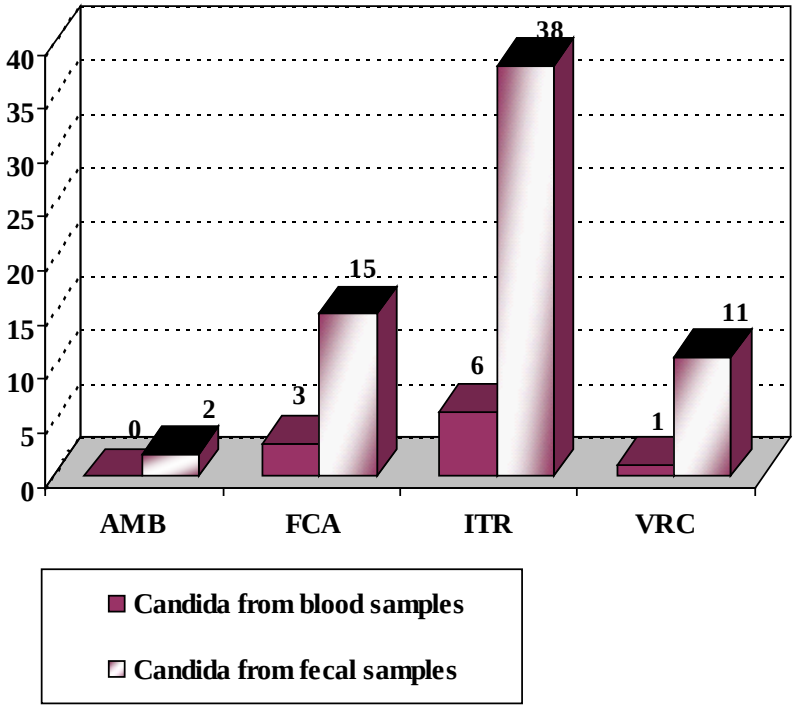
Candida albicans remains the most susceptible. All the isolates were susceptible to flucytosine and in isolates from blood no resistance to amphotericine B was found, and in fecal isolates it was rare. Cross-resistance to azoles was found in the total of 16 isolates (3.77%), and it was more common in *Candida* spp. (6.71%) than in *C. albicans* (1.81%).

Twelve isolates were multiple resistant.

Resistance to itraconazole was dominant in both fecal and blood isolates, 38 (9.64%) strains from fecal samples and 6 (20%) strains from blood were resistant to that drug (Graph 1). Isolates resistant to itraconazole were more often found in blood than in feces, the difference is statistically significant ($p<0.01$).

Non-albicans *Candida* species were significantly ($p<0.05$) more frequently resistant to itraconazole and fluconazole than *Candida albicans.* To itraconazole were resistant 10 from 275 (3.63%) *C. albicans* and 33 from 149 *Candida* spp. (22.14%) ($p<0.05$). Resistance to fluconazole was less common in both groups of isolates, 6 from 275 *C. albicans* (2.18%) and 12 from 149 *Candida* spp. (8.05%) ($p<0.05$). Overall resistance to itraconazole in 424 isolates was 10.37% of isolates, to fluconazole 4.24% and to voriconazole 2.83%.

Graph 1. Total number of resistant *Candida* isolates from blood and feces



DISCUSSION

Candida spp. is a member of physiological flora of the human skin and mucosal membranes. The prevalence of *Candida* colonization of the gastrointestinal tract in healthy people is very high (T a s i c S. et al. 2008). There is a balance between *Candida* spp. of normal flora and immune defense mechanisms, when this balance is disturbed colonization usually results in infection (B a b i c M, H u k i c M. 2010). Rates of candidemia have increased over the past few decades causing the morbidity and mortality for immunocompromised patients, such as those with cancer or AIDS (S a f d a r A. et al., 2002). This analysis included clinical isolates from bloodstream and from feces, as the most *Candida* infections arise from the hosts' endogenous microflora.

Our susceptibility results are similar to other studies: resistance is uncommon among *C. albicans,* and there is higher level of reduced susceptibility among non-albicans *Candida* spp.

All the isolates studied were susceptible to flucytosine, and decreased susceptibility to amphotericin B was found only in fecal isolates of non-albicans strains (1.60%) and resistance of all isolates tested was 0.47% that is consistent with reports from Spain, Turkey, and Mexico (F l o r e z C. et al., 2008, T u l u m o g l u S. et al. 2009, G o n z a l e s GM. et al. 2008).

The occurrence of azole cross-resistance in clinical *C. albicans* isolates has been demonstrated by German authors (M u e l l e r F, M. et al., 2000) in children with HIV (human immunodeficiency virus). In our study, cross-resistance to azoles was found in 16 isolates (3.77%), and it was more common in *Candida* spp. (6.71%) than in *C. albicans* (1.81%). In a study from Glasgow, UK, none of the blood culture isolates was resistant to either fluconazole or itraconazole (K e n n e d y H. F. et al., 2006).

Resistance to fluconazole in this study was found in 4.24% of all isolates tested, very similar to 4.1% found in Spain (F l o r e z C. et al., 2009) and less common than 15% seen in Portugal (C o s t a - d e - O l i v i e r a S. et al., 2008).

Overall resistance to itraconazole in 424 isolates was 10.37% of isolates; it was more common in *Candida* spp. (22.14%) than in *C. albicans* (3.63%). These results are higher than in Spain and Turkey (F l o r e z C. et al., 2009, T u l u m o g l u S.) but lower than 24.7% in Lithuania (S k r o d e n i e n e E. et al., 2006), 27.6% in Venezuela (P a n i z o M. M. et al., 2009) and 43.3% in Mexico (G o n z a l e s G. M. et al., 2008).

Overall resistance to voriconazole in this investigation was low (2.83%) especially in C. *albicans* (1.81%) which is consistent to the studies in which this drug displayed potent antifungal activity (T o r t o r a n o A. M. et al., 2006 and Q u i n d o s G. et al., 2008).

In conclusion, this study shows that in Novi Sad, Vojvodina, resistant isolates were found both in *C. albicans* and in *Candida* spp. and reduced susceptibility was documented in blood as well as in fecal isolates.

Reduced susceptibility to azoles was relatively rare; most commonly found in all isolates was the resistance to itraconazole. Isolates resistant to itraconazole were more often found in blood than in feces, the difference is statistically significant. Non-albicans *Candida* species were significantly more frequently resistant to itraconazole and fluconazole than *Candida albicans*.

Voriconazole and amphotericine B were found to be very active against all species of *Candida*. Hence, these agents should be used in empirical treatment for candidemia rather than fluconazole and itraconazole.

Although resistance to antifungal agents of *Candida* spp. is rare at present, continued surveillance of antifungal susceptibility is necessary in order to monitor trends, and to choose the right empiric therapy.

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ОСЕТЉИВОСТ НА АНТИМИКОТИКЕ ИЗОЛАТА *CANDIDA* SPP. ИЗ КРВИ И ФЕЦЕСА ПРИКЉУЧЕНИХ У НОВОМ САДУ У ТРОГОДИШЊЕМ ПЕРИОДУ (2008-2010)

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Кандидемија је значајна и све чешћа нозокомијална инфекција код пацијената са факторима ризика. *Candida* врста пореклом из нестерилних подручја може да укаже на карактеристике сојева који узрокују инвазивну болест.

Циљ овог испитивања био је да се утврди осетљивост сојева *Candida* из крви и фецеса изолованих у Новом Саду, у Војводини.

У току трогодишњег периода, од 2008. до 2010. године, прикупљено је 424 изолата *Candida* spp., 30 изолата из крви и 394 сојева пореклом из узорака фецеса. Осетљивост ових изолата *in vitro* на пет антимикотика утврђено је коришћењем комерцијалног теста *ATB FUNGUS 3* (*BioMérieux*).

Најчешћа врста била је *Candida albicans* (6 изолата из крви и 269 из фецеса). Резистенција на један или више антимикотика била је ређа код *Candida albicans* (3.63%) у односу на друге non-albicans врсте (24.83%). Резистенција је најчешће била изражена на итраконазол код обе испитиване групе, утврђена је код 9.64% сојева пореклом из фецеса и 20% изолата из крви. Мултипла резистенција на три или више антимикотика доказана је код дванаест изолата, углавном на флуконазол, итраконазол и вориконазол. Резистенција на амфотерицин Б била је врло ретка.

Иако је резистенција *Candida* spp. на антимикотике код нас засад ретка појава, потребно је континуирано праћење и истраживање њихове осетљивости у циљу праћења трендова у појави резистентних изолата, али и због избора праве емпиријске терапије.