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IN VITRO SUSCEPTIBILITY OF *Candida* Spp. ISOLATED FROM CLINICAL SPECIMENS AGAINST SOME ANTIFUNGAL AGENTS

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Abstract

We evaluated the in vitro activity of ketoconazole (KET), fluconazole (FLU), itraconazole (ITRA), amphotericin B (AmpB), flucytosine (FCU) in comparison to voriconazole (VOR) against Candida species isolated from blood cultures.

The most common species of identified Candida were C. albicans (71), followed by C. parapsilosis (16), C. glabrata (12) C. tropicalis (9), C. kefyr (8) and one of C. lustaniae, C. spherical, C. sake, C. lambria respectively. NCCLS M 27-A method was used to evaluate the activity of KET, FLU, ITRA, AmpB, FCU and VOR. The MICs of the strains were evaluated by RPMI 1640 medium with microdilution method.

There were no isolates of tested Candida spp. resistant to KET, ITRA, FCU, AmpB and VOR. Only two of C. albicans isolates were resistant to FLU (MIC; ≥32µg/ml). Intermediate resistant appeared in 17.5% isolates to KET and FLU, 8.3% isolates to ITRA. Among 120 Candida isolates were found highly susceptible to KET (MIC;≤8µg/ml) followed by VOR (MIC;≤0.03µg/ml), ITRA (MIC;≤0.125µg/ml) FCU (MIC;≤4µg/ml) AmpB (MIC;< 1µg/ml), and FLU (MIC; ≤8µg/ml) so FCU, AmpB and VOR were each more active than KET, FLU, and ITRA.

Key Words: *Antifungal susceptibility; In vitro activity; Candida spp.*

Klinik Örneklerden İzole Edilen Candida Türlerine Karşı Bazı Antifungal Ajanların Duyarlılıklarının Araştırılması

Kan kültürlerinden izole edilen Candida türlerine karşı ketokonazol (KET), flukonazol (FLU), itrakonazol (ITRA), amfoterisin B (AmpB) ve flusitozin (FCU)'in in vitro duyarlılıkları vorikonazol (VOR) ile karşılaştırılarak değerlendirilmiştir.

En sık identifiye edilen Candida türleri sırasıyla C. albicans (71), C. parapsilosis (16), C. glabrata (12), C. tropicalis (9), C. kefyr (8) ve birer adet C. lustaniae, C. spherical, C. sake ve C. lambria'dır. KET, FLU, ITRA, Amp B, FCU ve VOR'un in vitro aktivitelerinin değerlendirilmesinde NCCLS M 27-A metodu kullanılmıştır. MİK değerleri RPMI 1640 besiyeri kullanılarak mikrodilüsyon yöntemi ile değerlendirilmiştir.

Test edilen Candida türleri; KET, ITRA, FCU, AmpB ve VOR'a dirençli değildir. Sadece iki C. albicans izolatı FLU(MIC;≥32µg/ml)'e dirençlidir. Suşların; KET ve FLU için %17.5, ITRA için %8.3'ünde orta duyarlılık görülmüştür. 120 Candida izolatı KET (MİK;≤8µg/ml), VOR (MİK;≤0.03µg/m), ITRA (MİK;≤0.125µg/ml), FCU (MİK;≤4µg/ml), AmpB (MİK;< 1µg/ml) ve FLU (MİK;≤8µg/ml) için yüksek derecede duyarlı bulunmuştur. FCU, AmpB ve VOR; KET, FLU ve ITRA'dan daha aktiftir.

Anahtar Kelimeler: *Antifungal duyarlılık, In vitro aktivite, Candida spp.*

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INTRODUCTION

In the past decade infections caused by yeast have increased significantly. Currently available antifungal drugs can have troublesome side effects, are ineffective against some fungi, and lead to development of resistance. Antifungal drug resistance has become an important issue for a variety of fungal infection. Also, *Candida* species have varying degrees of susceptibility to common antifungal agents (1).

Susceptibility testing is most helpful in dealing with infection due to *Candida* spp. If the patient has been treated previously with antifungal agents, the possibility of microbiological resistance must be considered (2).

In the present study, blood samples of *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. lustaniae, C. spherical, C. sake,* and *C. lambria* were investigated in case of antifungal susceptibility to ketoconazole, fluconazole, itraconazole, amphotericin B, flucytosine, and voriconazole.

EXPERIMENTAL

Organisms

A total of 120 isolates were identified by germ tube production and sugar assimilation tests and confirmed by standard biochemical testing with the API 20C system (API; bioMerieux Vitek, Inc., Hazelwood, MO, USA). All isolates were kept in 50% glycerol stock at –70°C.

Agents

Standard antifungal powders of ketoconazole (Bilim), fluconazole (Pfizer), itraconazole (ITRA), amphotericin B (Sigma), flucytosine (Sigma), and voriconazole (Pfizer) were obtained from the manufacturers. Stock solutions were dissolved in dimethylsulphoxide (ketoconazole, amphotericin B, voriconazole, itraconazole), and in water (fluconazole, flucytosine).

Inoculums

All *Candida* isolates were subcultured in Sabouraud dextrose agar plates and incubated at 35^0C for 24 hours to 48 hours prior to antifungal susceptibility testing and culture suspensions were prepared through the guideline of NCCLS M27-A(3). The inoculum suspension was prepared by the spectrophotometric method of inoculum preparation and with a final culture suspension of $2x10^3$ cfu/ml.

Antifungal Susceptibility Testing

All antifungal agents were in RPMI 1640 medium (Sigma) buffered to pH: 7.0 with 0.165 M morpholinopropansulfonic acid (MOPS) buffer (Sigma) and dispensed into each well of 96-well microdilution trays. The final concentrations of the antifungal agents were 64 to 0.03µg/ml for all antifungal agents. It is conducted previously described by Özçelik et al (4).

RESULTS

A total of 120 isolates were analyzed for their susceptibilities to ketoconazole (KET), fluconazole (FLU), itraconazole (ITRA), amphotericin B (AmpB), flucytosine (FCU) in comparison to voriconazole (VOR). *C. albicans* is the most common species, accounting for 59.16% of isolates. There were no *C. albicans* isolates resistant to KET, ITRA, FCU, AmpB and VOR (Table1). There were slight differences in the susceptibility patterns of 40.83% non-*albicans Candida* spp. isolates.

The table 1 shows that; all *Candida* isolates had range of MICs to KET (µg/ml) ≤0.03-32, FLU (µg/ml) ≤0.03-64, ITRA ≤0.03-0.5, FCU (µg/ml), ≤0.03-2, AmpB (µg/ml) ≤0.03-0.5 and VOR (µg/ml) ≤0.03. Only two of *C. albicans* isolates were resistant to FLU (MIC; ≥32µg/ml).

14 isolates of *C. albicans,* 1 isolate of *C. glabrata,* 6 isolates of *C. tropicalis* showed intermediate resistance to ketoconazole and fluconazole (MIC; 16-32 µg/ml), also 6 isolates of *C. albicans*, 1 isolates of *C. glabrata* and 3 isolates *C. tropicalis* to ITRA (MIC; 0.25-0.50 µg/ml).

Table 1. Minimum Inhibitory Concentrations (MICs) of *Candida* species to some antifungal agents.

		KET	FLU	ITRA	FCU	AmpB	VOR
C. albicans (71)	S	≤ 0.03-4 (57)	≤ 0.03-4 (55)	≤ 0.03-0.125 (65)	≤ 0.03-2 (71)	≤ 0.03-0.5 (71)	≤0.03 (71)
	I	16-32 (14)	16-32 (14)	0.25-0.5 (6)	-	-	-
	R	-	≥32(2)	-	-	-	-
C. parapsilosis (16)	S	≤ 0.03-4 (16)	0.25-4 (16)	≤ 0.03-4 (6)	≤ 0.03-0.5 (16)	≤ 0.03-0.5 (16)	≤0.03 (16)
	I/R	-	-	-	-	-	-
C. glabrata (12)	S	0.25-4 (11)	1-4 (11)	0.125-0.25 (11)	≤ 0.03 –0.5 (12)	≤0.03 –0.06 (12)	≤0.03(12)
	I	32 (1)	32 (1)	0.25 (1)	-	-	-
	R	-	-	-	-	-	-
C. tropicalis (9)	S	2 (3)	4 (3)	0.03-0.125 (6)	≤0.03-2 (9)	≤0.03–0.06 (9)	≤0.03 (9)
	I	16-32 (6)	16-32 (6)	0.25-0.5 (3)	-	-	-
	R	-	-	-	-	-	-
C. kefyr (8)	S	≤0.03 -4 (8)	≤0.03 -4 (8)	-≤0.03-0.125 (8)	≤0.03-0.125 (8)	≤0.03 (8)	≤0.03 (8)
	I/R	-	-	-	-	-	-
C. lustania (1)	S	2	1	≤0.03	≤0.03	≤0.03	≤0.03
	I/ R	-	-	-	-	-	-
C. spherical (1)	S	0.5	0.25	0.03	0.5	≤0.03	≤0.03
	I/R	-	-	-	-	-	-
C. sake (1)	S	32	32	0.5	≤0.03	0.5	≤0.03
	I/R	-	-	-	-	-	-
C. lambria (1)	S	2	2	0.125	≤0.03	≤0.03	≤0.03
	I/R	-	-	-	-	-	-
C. albicans ATCC 10231		≤0.03	0.06	≤0.03	≤0.03	≤0.03	≤0.03

KET: Ketoconazole; FLU: Fluconazole; ITRA: Itraconazole; Amp B: Amphotericin B; FCU: Flucytosine; VOR: Voriconazole. S: Susceptible; I: Intermediate susceptibility; R: Resistant.

DISCUSSION

The incidence and diversity of fungal infections have increased considerably over recent decades. This reflects the rising numbers of patients at risk of fungal infection. Studies have shown *C. albicans* to be the most common cause of *Candida* infection, with a prevalence of about 50%. Other important species of non-*albicans* include *C. parapsilosis*, *C. krusei*, *C. lustaniae*, and *C. glabrata* (5, 6).

In our study, *C. albicans* account for 59.16% of the total number of *Candida* spp. isolates, whereas non-*albicans* spp. were less frequent 40.83%. These are in agreement with those of previous reporters found that *C. albicans* was the most common isolate (5-7). Also *C. parapsilosis*, *C. glabrata*, and *C. tropicalis* were the second most common isolates pathogen from blood cultures. One of the isolates other of important species *C. lustaniae, C. spherical, C. sake, C. lambria* were also tested.

Recently, newer antifungal agents with broader antifungal activity, fever harmful affects and minimal resistance have become available. However, less effectiveness to antifungal agents and resistance arise have reported.

Rex, J.H. et al. reported that AmpB resistance appears uncommon isolates of *C. albicans*, *C. parapsilosis*, and *C. tropicalis*. Isolates of *C. lustaniae* most often demonstrate AmpB resistance (2). Also, Yang, Y.L. et al. have reported that *C. lustaniae* is relatively resistant to AmpB (1). In our study *C. lustaniae* was determined susceptible to all tested antifungal agents.

In the present study of 71 *C. albicans* isolates were observed intermediate susceptibility in 14 isolates to KET (MICs; 16-32µg/ml), 14 isolates to FLU (MICs; 16-32µg/ml), 6 isolates to ITRA (MICs; 0.03-0.5µg/ml). Except of two isolates resist to FLU (MIC; 32µg/ml) among all these *C. albicans* isolates were susceptible to KET (MICs; ≤0.03-4µg/ml), ITRA (MICs; ≤0.03-0.125µg/ml), FCU (MICs; ≤0.03-2µg/ml), AmpB (MICs; ≤0.03-0.5µg/ml), and VOR (MICs; ≤0.03µg/ml).

Girmena C. et al. have reported the widespread use of fluconazole has been accompanied by rising incidence of resistant *C. albicans* isolates especially AIDS patients (8). Our study in agreement with Pfaller, M.A. et al. that have reported as triazole voriconazole were active than that of fluconazole and itraconazole (9). Although the MICs of voriconazole susceptible in *C. albicans* isolates were intermediate susceptible to FLU.

Some of non-*albicans* isolates such as *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, and *C. krusei* associated with a decreased antifungal susceptibility to azoles (9). In our study all non-*albicans* isolates as *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, *C. kefyr*, *C. lustaniae, C. spherical, C. sake, C. lambria* were susceptible to tested antifungal agents. These entire species one of *C. sake* was intermediate susceptible to KET (MIC; ≥32µg/ml) and FLU (MIC; ≥32µg/ml).

C. lusitaniae is relatively resistant to AmpB. *C. krusei* and *C. glabrata* are less susceptible to FLU than are other *Candida* species. Although *C. tropicalis* is less commonly isolated from clinical specimens than is *C. albicans*, it is one of the most common non-*albicans Candida* species and it is always associated with diseases. It is reported by Yang, Y.L. et al. that *C. krusei* and *C. glabrata* are less susceptible to FLU than that of other *Candida* species (1).

Repeated exposure to FLU, even in short courses may results in the replacement of susceptible species such as *C. glabrata*. This resistance may be due to long-term intermittent or continuous

treatment with FLU (10-12). Among non-*albicans* species, *C. tropicalis* is considerably clinically important because it develops FLU resistance rapidly and the rate of resistance to FLU of clinical *C. tropicalis* is increasing (1).

C. tropicalis was initially regarded as a species susceptible to FLU and Amp B, displaying disclosed susceptibility to KET (13). Although, in our previous study one isolates of *C. tropicalis* was resist to KET, in the current study all antifungal agents were susceptible in *C. tropicalis*. *C. parapsilosis* isolates observed susceptible to tested antifungal agents (14). These results are in agreement previously published by Pfaller, M.A. et al. (9).

Rex, J.H. et al. reported that *C. glabrata* often has reduced susceptibility to both azoles and Amp B (2). I the present study of 12 *C. glabrata* isolates one of them were intermediate susceptible to KET, FLU, and ITRA. The results of these isolates were susceptible to KET, FLU, ITRA, FCU, Amp B, and VOR.

CONCLUSION

In conclusion, even each of these *Candida* species tested in this study may not allow one to make sufficient conclusion regarding their susceptibility to any of the antifungal agents, it gives local information. Continued observation and testing of species by NCCLS standardized methods, will provide clinically useful information. In summary, the evidence for the spectrum and potency of antifungal agents may be diverse for geographically collected of *Candida* spp.

In our study excellent activity against all *Candida* spp. with ketoconazole, amphotericin B, flucytosine and voriconazole have been observed. These results aspects that the antifungal agents would be effective in clinical use.

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