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In vitro Antifungal Susceptibility Profiles of Echinocandins and Triazoles for Clinical Opportunistic Candida isolates by E-test method.

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Abstract: ***Antifungal susceptibility testing has become an important tool for physicians faced with making difficult treatment decisions regarding treatment of patients with fungal infections. The Clinical Laboratory and Standards Institute (CLSI) has approved methods for testing of both yeast and moulds.*** *Antifungal susceptibility testing of Candidahas been standardized and refined and now may play a useful role in managing Candidainfections. Important new developments include validation of 24-h reading times for all antifungal agents and the establishment of species-specific epidemiological cutoff values (ECVs) for the systemically active antifungal agents and both common and uncommon species ofCandida. Standardization of* **in vitro** *susceptibility tests by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST), and current availability of reference methods constituted the major remarkable steps in the field.* ***We reviewed our antifungal susceptibility data for capsofungin, anidulafungin, voriconazole and posaconazole against*** **Candida** ***species and compared susceptibility patterns determined by the previous and recently revised CLSI antifungal breakpoints. With the new breakpoints, all C. albicans isolates and C.tropicalis were classified as susceptible to anidulafungin on MHA and while showed 63% and 66% of susceptibility on RPMI repectively according to revised CLSI M27 S4 breakpoints, whereas (36%) and (33%) of C. albicans and C.tropicalis isolates were found under susceptible dose dependent category on RPMI respectively. For capsofungin all, eleven (100%) C.albicans and three (100%) C.tropicalis were found to be susceptible on both the media and none of the tested isolates was categorized under resistance category. For posaconazole all eleven (100%) C.albicans were found to be susceptible on MHA media and none of the tested isolates was categorized under resistance category, although 54% were determined to show susceptibility and 45% showed resistance on RPMI to posaconazole. All the isolates that showed resistance to voriconazole were found to be susceptible to posaconazole. Among all four drugs activity were analysed by ANOVA: Single factor and students't' test Two-Sample Assuming Equal Variances was found to that capsofungin >anadulafungin>posaconazole >voriconazole aganist all 14 Candida isolates. Results obtained by the E-test method shows a > 71% correlation with those obtained by the AFST-EUCAST method. The agar-based E-test has been proposed as a more sensitive technology to discriminate strains of Candida species the role of microdilution methods seems to be restricted to reference laboratories because they are laborious. In addition, the micro broth format is not commonly used in clinical laboratories Disk and strip diffusion methodologies are simple, rapid, cost-effective and produce similar results to the reference methods for yeasts.The studies included in this thesis have contributed significantly to the understanding of the interplay between the Candida virulence, epidemiology and susceptibility and the importance of appropriate diagnostics and treatment choice.***

Keywords: In-vitro susceptibility testing, E-test, Candida, Triazoles, Echinocandin

I. INTRODUCTION

Candida albicans is a commensal yeast of the normal oral microbiota. However, several local and systemic factors can predispose to the development of oral candidiasis. Thus, conditions such as age extremes, immunodeficiency, endocrine disorders, radiotherapy, malignant diseases, xerostomia, denture wearing, poor oral hygiene and orthodontic treatment can be cited as predisposing factors [Espinel-Ingroff et.al 1998 andMarco 1998]. The increasing number of clinical isolates resistant to antifungal therapy, as well as the necessity of a guide to the selection and follow-up of the treatment led to a demand for susceptibility testing of fungi. For this purpose, the Clinical and Laboratorial Standards Institute (CLSI) approved a reference method for antifungal susceptibility testing of yeasts, the National Committee for Clinical and Laboratorial Standards (NCCLS) M-27 A2 document [Pfaller et.al 2011].

The E test [Spreghini et.al 2012] has been introduced as an easier testing procedure and an alternative for the NCCLS method [Spreghini et.al 2012]. The great advantage of E test is the simplicity of the methodology. Etest stable agar gradient susceptibility

testing method has been shown to be extremely flexible in testing a variety of fastidious and nonfastidious organisms, including bacteria, yeasts, and moulds [Ellis 2015]. The major perceived advantage of Etest for susceptibility testing of fungi is that laboratories wishing to test only one or two agents against an occasional yeast isolate may do so and generate quantitative MICs [Idelevichet.al 2014]. Numerous studies have now been published documenting that the performance of Etest is comparable with that of reference broth dilution testing of amphotericin B, fluconazole, itraconazole and ketoconazole. Notably, Etest may be the preferred method for detecting amphotericin B resistant strains of Candida spp. and Cryptococcus neoformans [Rathod et.al 2012]. The emergence of fluconazole-resistant Candida albicans and selection for inherently fluconazole-resistant Candida spp. has prompted the use of alternative agents for the treatment of invasive Candida infections. The alternatives include the echinocandins and the newer azoles, voriconazole, ravuconazole, and posaconazole. The azoles are inhibitors of the sterol 14-alpha-demethylase enzyme, blocking the production of the ergosterol component of the fungal cell membrane. Posaconazole, a triazole agent currently in clinical trials, is a more potent inhibitor of this enzyme than itraconazole and voriconazole in Aspergillus species and retains activity against the mutated enzyme responsible for resistance to fluconazole, itraconazole, and voriconazole in Candida [Manjunath et.al 2011]. It has shown activity superior to fluconazole and itraconazole against Candida spp. in previous in vitro surveys using the broth microdilution (MD) technique according to the CLSI method . There has been much research interest in agar based antifungal susceptibility via E-test (ET) and disk diffusion (DD) methods due to their relative ease and the lack of need for specialized equipment .

The aim of this study was to evaluate the in vitro susceptibility of C. albicans isolates to fluconazole, itraconazole, ketoconazole, voriconazole, posaconazole, amphotericin B, caspofungin, and anidulafungin using the Etest using CLSI guidelines.

II. MATERIALS AND METHODS

Test organisms. Fourteen clinical isolates of Candida species(11 Candida albicans, 3 Candida tropicalis) were selected for testing.

Antifungal agents. Etest strips containing posaconazole ,voriconazole, anidulafungin and capsofungin were supplied by Himedia .The concentration gradient for all four drugs on E -strips ranged from 0.002 to 32 mg ml. The strips were stored at 20'C until needed.

Media. The agar formulation used for the Etest was RPMI 1640 supplemented with 1.5% agar and 2% glucose w/o Sodium bicarbonate (RPG agar) and buffered with MOPS in accordance with the NCCLS M27-A3 method [Badiee et.al 2011] and Mueller Hinton Agar, 2% Glucose with Methylene blue recommended for testing performing Antifungal Disk Diffusion Susceptibility of yeasts in accordance with NCCLS M44A method [CLSI M27-S4 ,2005, NCCLS M27A2 ,2002].

Inoculum suspensions. Yeast inoculum suspensions were prepared as described in CLSI M27-A2 [Pfaller et.al 2011] and adjusted to match a 0.5 McFarland density standard resulting in an inoculum containing $1*10^6$ to $5*10^6$ yeast cells/ml.

Antifungal susceptibility testing method

Etest strips (AB BIODISK) containing voriconazole ,posaconazole, anidulafungin and capsofungin were purchased from Himedia. The concentration gradient for ranged from 0.002 to 32 mg ml. The strips were stored at 20'C until needed. Plates of 90-mm-diameter containing RPMI and MHA agar at a depth of 4.0 mm were used. The agar surface was inoculated by using a non-toxic swab dipped in a cell suspension adjusted spectrophotometrically to the turbidity of a 0.5 McFarland standard. After the excess moisture was absorbed into the agar and the surface was completely dry, Etest strips were applied to each plate. The plates were incubated at 35 'C and read at 24 and 48 h [Chen et.al 1996]. The MICs were read at the lowest concentration at which the border of the elliptical inhibition zone intercepted the scale on the strip. Any growth, such as microcolonies, throughout a discernible inhibition ellipse was ignored. When growth occurs along the entire strip i.e. no inhibition ellipse is seen, the MIC was reported as more than the highest value on the MIC scale. When the inhibition ellipse was below the strip i.e. the zone edge did not intersect the strip, the MIC was reported to be less than the lowest value on the MIC scale. MIC50 and MIC90 (the MIC at which 50% and 90% of the isolates are inhibited) were also calculated. [M44A2]

QC. Quality control (QC) was performed in accordance with NCCLS document M27-A3 using C. krusei ATCC 6258 and C. parapsilosis ATCC 22019 [Cantón et.al 2008].

Analysis of results. Etest MICs read at 24-48 h were compared to reference microdilution MICs read at 48 h. Since the Etest scale has a continuous gradient of concentrations, the MICs between twofold dilutions were raised to the next twofold level of the reference method for comparison [Perlin et.al 2007, CLSI. M27A3,2008]. Off-scale MICs at the upper limit were converted to the next higher concentration, and off-scale results at the lower limit were left unchanged.

Interpretation of results. The results were interpreted with revised clinical breakpoints for azoles and echinocandins, determined by CLSI broth dilution method, published by CLSI as CLSI M27-S4 in 2012and M27A3 in 2008 [Pfaller et.al 2012,]. The interpretative criteria used for susceptibility to all four drugs used in our study were depicted in the Table 1.

Interpretative criteria for Candida albicans and C.tropicalis as per (M27A3) for azoles and (M27S4) for Echinocandins			
Drugs	susceptible	Intermediate/Susceptible	Resistant
Voriconazole	<1	2	>4
Posaconazole	<1	2	>4
Anidulafungin	<0.25	0.5	>1
Capsofungin	<0.25	0.5	>1

Table 1. CLSI Breakpoints (BP) for Candida albicans and C.tropicalis

Data analysis Statistical analysis was done through the evaluation of students't' test Two-Sample Assuming Equal Variances and ANOVA: Single factor to compare results obtained by Etest based on CLSI M27A3 and M44P method.

III. RESULTS

Table 2 summarizes the in vitro susceptibilities of 14 Candida isolates to Azoles (Voriconazole, Posaconazole) and Echinocandins (Anidulafungin ,Capsofungin) as determined by the Etest method performed on two different media.

A. For Azoles-(Voriconazole and Posaconazole)

For voriconazole MIC values of 11 C. albicans strains were in the range of 0.032- 0.38 µg/mL on MHA and 0.032-0.5 µg/mL on RPMI agar. MIC values of 3 C. tropicalis strains were same 0.047 µg/mL on MHA and on RPMI agar. (Table 2, Fig 1-4).

For posaconazole MIC values of 11 C. albicans strains were in the range of 0.032- 0.25 µg/mL on MHA and 0.023-0.25 µg/mL on RPMI agar. MIC values of 3 C. tropicalis strains were in the range of 0.008-0.125 µg/mL on MHA and 0.032-0.064 µg/mL on RPMI agar.

Five (45%) and 4(36%) ofC. albicans isolates were classified as susceptible to voriconazole on MHA and RPMI repectively according to revised CLSI M27 S4 breakpoints whereas six (54%)and seven (63%) isolates were found to be resistant on MHA and RPMI respectively.

For posaconazole all eleven (100%) C. albicans were found to be susceptible on MHA media and none of the tested isolates was categorized under resistance category, although 54% were determined to show susceptibility and 45% showed resistance on RPMI to posaconazole (Table 3). All the isolates that showed resistance to voriconazole were found to be susceptible to posaconazole. Thus we conclude that posaconazole is more effective than voriconazole and showed better results on MHA agar because the strains which showed resistance on RPMI agar for posaconazole falls under susceptible category on MHA.

For C.tropicalis ,33% isolates showed susceptibility while 66% showed resistance on MHA and the reverse is seen on RPMI, whereas all three isolates showed showed 100% susceptibility on both media, again posaconazole is considered to be the better than voriconazoleforC. tropicalis.

Isolate No.	Candida species	ANTIFUNGAL DRUGS															
		VORICONAZOLE				POSACONAZOLE				ANIDULAFUNGIN				CAPSOFUNGIN			
		MHA	IC	RPMI	IC	MHA	IC	RPMI	IC	MHA	IC	RPMI	IC	MHA	IC	RPMI	IC
C1	Candida albicans	0.032	S	0.032	S	0.032	S	0.023	S	0.002	S	0.002	S	0.002	S	0.002	S
C2	Candida albicans	0.125	S	0.125	S	0.19	S	0.25	S	0.002	S	0.002	S	0.002	S	0.002	S
C3	Candida albicans	0.032	S	0.032	S	0.094	S	0.064	S	0.002	S	0.002	S	0.002	S	0.002	S
C4	Candida albicans	0.38	S	NI	R	0.19	S	NI	R	0.002	S	0.002	S	0.002	S	0.002	S
C5	Candida albicans	NI	R	NI	R	0.094	S	NI	R	0.002	S	0.38	I	0.002	S	0.002	S
C6	Candida albicans	0.125	S	0.5	S	0.25	S	NI	R	0.002	S	1	I	0.002	S	0.125	S
C7	Candida albicans	NI	R	NI	R	0.016	S	NI	R	0.002	S	0.002	S	0.002	S	0.002	S
C8	Candida albicans	NI	R	NI	R	0.125	S	0.064	S	0.002	S	0.002	S	0.002	S	0.002	S
C9	Candida albicans	NI	R	NI	R	0.19	S	0.047	S	0.002	S	0.75	I	0.002	S	0.002	S
C10	Candida albicans	NI	R	NI	R	0.125	S	0.064	S	0.002	S	0.002	S	0.002	S	0.002	S
C11	Candida tropicalis	NI	R	0.047	S	0.125	S	0.064	S	0.002	S	0.002	S	0.002	S	0.002	S
C12	Candida tropicalis	0.047	S	NI	R	0.125	S	0.032	S	0.002	S	0.002	S	0.002	S	0.002	S
C13	Candida tropicalis	NI	R	0.047	S	0.008	S	0.047	S	0.002	S	0.75	I	0.002	S	0.002	S
C14	Candida albicans	NI	R	NI	R	0.094	S	NI	R	0.002	S	1	I	0.002	S	0.002	S
C15	Candida parapsilosis ATCC 22019	0.032		0.032		0.064		0.047		0.002		ND		ND		ND	
C16	Candida Krusei ATCC 6258	ND		ND		ND		ND		ND		0.002		0.002		0.002	

TABLE 2. MIC VALUES OF 15 CLINICAL ISOLATES OF *CANDIDA* SPECIES AGAINST FOUR ANTIFUNGAL DRUG ON MHA (CLSI M44P) AND RPMI(CLSI M27A3) MEDIA
(Abbreviation: S- Sensitive; SDD- Susceptible Dose dependent; I-Intermediate; R –Resistant ,MHA -Mueller Hinton Agar, 2% Glucose with Methylene blue, RPMI- RPMI 1640 Agar w/ MOPS & 2% Glucose w/o Sodium bicarbonate)

DRUGS	VORICONAZOLE			POSACONAZOLE			ANIDULAFUNGIN			CAPSOFUNGIN		
CATEGORY	S	SDD	R	S	SDD	R	S	SDD/I	R	S	SDD/I	R
Candida albicans (11)												
MHA %	45% (5)	0	54 % (6)	100% (11)	0	0	100% (11)	0	0	100% (11)	0	0
RPMI %	36 % (4)	0	63 % (7)	54 % (6)	0	45% (5)	63 % (7)	36 % (4)	0	100% (11)	0	0
Candida tropicalis (3)												
MHA %	33% (1)	0	66 %(2)	100% (3)	0	0	100% (3)	0	0	100% (3)	0	0
RPMI %	66 %(2)	0	33% (1)	100% (3)	0	0	66 % (2)	33% (1)	0	100% (3)	0	0

TABLE 3. SUSCEPTIBILITY STATUS OF 15 CLINICAL ISOLATES OF CANDIDA SPECIES AGAINST FOUR ANTIFUNGAL DRUGS DEPENDENDING ON CLSI M27-S4 INTERPREATIVE BREAKPOINTS
(Abbreviation: S- Sensitive; SDD- Susceptible Dose dependent; I-Intermediate; R –Resistant ,MHA -Mueller Hinton Agar, 2% Glucose with Methylene blue, RPMI- RPMI 1640 Agar w/ MOPS & 2% Glucose w/o Sodium bicarbonate)

B. For Echinocandin (Anidulafungin and Capsofungin)

For anadulafungin MIC values of 11 C. albicans strains were in the range of >0.002 µg/mL on MHA and 0.002-1 µg/mL on RPMI agar. MIC values of 3 C. tropicalis strains were same 0.002 µg/mL on MHA and 0.008-0.75 µg/mL on RPMI agar.

For capsofungin MIC values of 11 C. albicans strains andfor 3C. tropicalis strains were same >0.002 µg/mL on both the media (Table 2, Fig 1-4).

All C. albicans isolates and C.tropicalis were classified as susceptible to anidulafungin on MHA and while showed 63% and 66% of susceptibility on RPMI repectively according to revised CLSI M27 S4 breakpoints, whereas (36%) and (33%) of C. albicans and C.tropicalis isolates were found under susceptible dose dependent category on RPMI respectively. For capsofungin all, eleven (100%) C.albicans and three (100%) C.tropicalis were found to be susceptible on both the media and none of the tested isolates was categorized under resistance category (Table 3). All the Candida isolates that showed SDD to anadulafungin were found to be susceptible to capsofungin. Thus we conclude that capsofungin is more effective than anadulafungin and showed better results on MHA agar because the strains which showed SDD on RPMI agar for anadulafungin falls under susceptible category on MHA.

The MICs (minimum inhibitory concentration) at which 50% (MIC50) and 90% (MIC90) of the isolates were inhibited were determined for each drug. On MHA and RPMI agar all Candida species showed 100% susceptibility for echinocandins drug i.e MIC90 and MIC50 was found 0.002 µg/mL respectively while for azoles there was no MIC50 nor MIC 90 because different isolates showed different values.

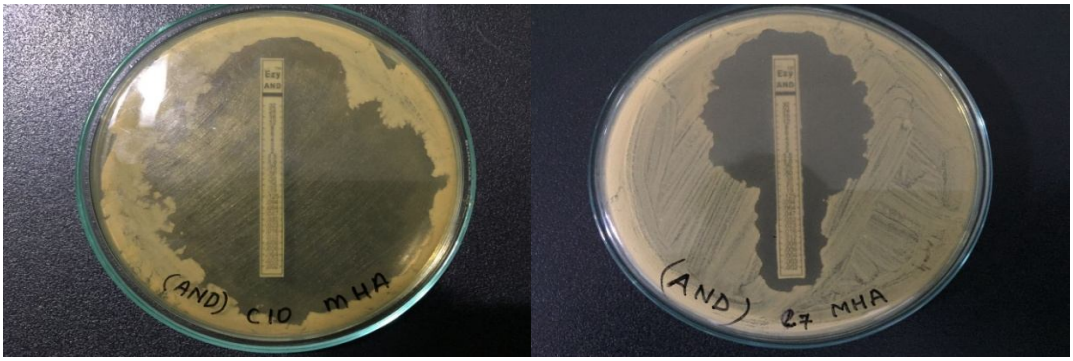

Fig 1. Zone of inhibition of Anidulafungin Ezy MIC™ strip (EM122) for *Candida* isolate no. C10 and C7 on MHA medium

Fig 2 Zone of inhibition of posaconazole Ezy MIC™ strip (EM120) for *Candida* isolate no. C1 on MHA and ATCC *C.parapsilosis* on RPMI medium

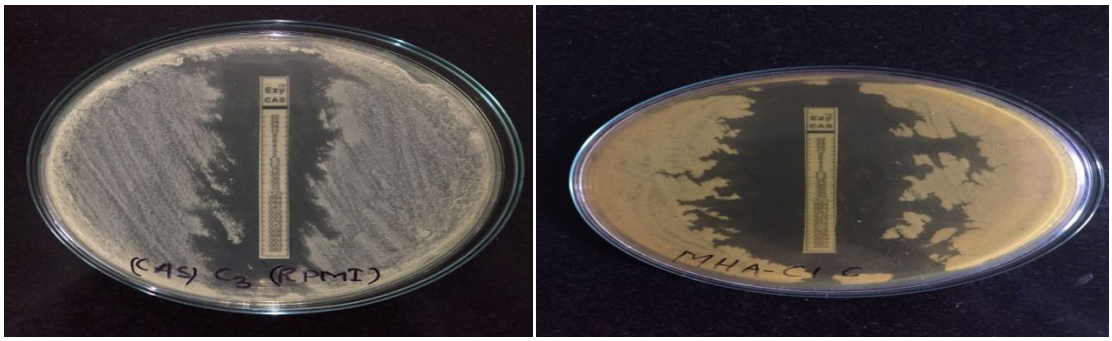

Fig 3. Zone of inhibition of Capsofungin Ezy MIC™ strip (EM199) for *Candida* isolate no. C1 on MHA and C3 on RPMI medium

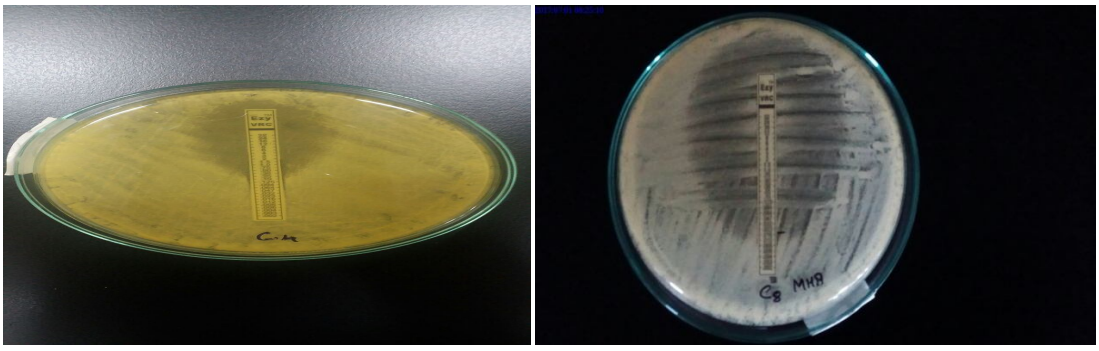

Fig 4. Zone of inhibition of Voriconazole Ezy MIC™ strip (EM086) for Candida isolate no. C4 on RPMI and C8 on MHA medium

C. Statistical Analysis of The Results

A comparison between the results obtained by Etest based on CLSI M27A3 and M44P method was performed. The comparison between both the media were represented in terms of level of significance using students't' test Two-Sample Assuming Equal Variances. After descriptive analysis 't' statistical value (P(T<=t) one-tail) for voriconazole and Capsofungin is more than 0.05, i.e U1=U2, therefore there is no significant difference between both the media and they provide same results (MIC value) for the above drug. For posaconazole and anadulafungin 't' statistical value; (P(T<=t) one-tail) is less than 0.05,i.e U1≠U2 ,therefore there is significant difference between both the media and MHA is better than RPMI agar.

Comparison of all the four drugs on MHA and RPMI agar was analysed using ANOVA: Single factor and students't' test Two-Sample Assuming Equal Variances and it could be concluded that all the four drugs showed significantly different results on each media and echinocandins are more effective than azoles. Among all four drugs activity of capsofungin >anadulafungin>posaconazole >voriconazole aganist all 14 Candida isolates.`

IV. DISCUSSION

In this study, we found that all Candida isolates were susceptible to capsofungin on both MHA and RPMI agar with MIC >0.002ug/mL and only 36% of the C. albicans and 33% of C.tropicalis isolates showed SDD category on RPMI agar while rest all showed susceptibility to anidulafungin with MIC90 > 0.002ug/mL .Messer et al.[Espinel-Ingroff et.al 1998] have measured the MIC range for capsofungin as 0.12 - 2 mg/L in an international surveillance study . On the other hand, Santhanam et al. [Espinel-Ingroff et.al 1996] have documented capsofungin MICs ranging from 0.25 to 16 mg/L in Malaysia. In contrast to our findings, Faria-Ramos et al. 2014 have documented the rate of anidulafungin resistance as 4% in C. albicans isolates. Resistance to ca-spofungin among C. albicans isolates has been reported by previous researchers. Although none of our isolates was found to be resistant to caspofungin, based on the new CLSI criteria, only 4 among C.albicans and 1 of C.tropicalis isolates were classified as showing SDD for anidulafungin.

Caspofungin, a member of a novel echinocandin family, is a potent fungicidal agent against all strains of Candida. Caspofungin resistance in Candida species is rare. This is probably due to limited use owing to high cost of echinocandin therapy especially in developing countries [Szekely et.al 1990]. But, in the face of increasing azole resistance [Pfaller et al 2000], use of echinocandins, namely caspofungin is expected to increase in the near future. Hence, knowledge about the caspofungin susceptibility pattern in the region will allow better patient management. In our study 100% (14/14) Candida species were caspofungin susceptible.

In each experiment, the MIC values of the quality control strains fell within the established ranges published for both media .Generally, the MIC values for all two echinocandins were low and below the susceptibility breakpoint, regardless of the method used. As shown by other authors [Canton et. al 2008, Morris et.al 2009] the MIC values for AND (geometric mean MIC EUCAST/CLSI, 0.16/0.22 μg · mL−1) and MCF (geometric mean MIC EUCAST/CLSI, 0.13/0.14 μg · mL−1) were lower those for CSP (geometric mean MIC EUCAST/CLSI, 0.29/0.33 μg · mL−1) by both assay, suggesting that they have superior in vitro potency. These data are consistent with those reported previously [Koehling et al 2014, pfaller 2008] and document the excellent potency and spectrum of echinocandins against most Candida spp. Given the mechanism of action that is shared among the echinocandins approved in 2002 is to inhibit the 1, 3-β d-glucan as an integral part of the fungal cell wall.

Posaconazole has a good in vitro activity profile against many yeast and filamentous fungi with low resistant isolates percentages**.** Resistance percentages for posaconazole observed in this study was 45% in C.albicans only on RPMI agar. Using the same agar diffusion method and microdilution methods, resistance percentages for fluconazole (10%), itraconazole (18%) and amphotericin B (2-3%) reported for another authors, show the high activity of posaconazole against clinical yeasts isolates [Rodriguez-Tudela et.al 2007, pfaller et.al 2003] addition, our results agree with those obtained by microdilution methods showing posaconazole ranges of activity between 0.03-0.125 mg/L for most isolates. Posaconazole is a third generation triazole antifungal agents designed to improve clinical profiles of fluconazole or itraconazole against Candida and Aspergillus spp.Posaconazole mode of action is directly based in the inhibition of lanosterol 14-α-demethylase activity1, resulting in a high in vitro activity against a wide spectrum of pathogenic yeast-like and filamentous fungi and also protozoans .

In our study, 54% and 63% C.albicans showed reistance on MHA and RPMI agar while 66% and 33% of the C. tropicalis RPMI agar for voriconazole. In another study reported by the same authors, the resistance rate of C. albicans to triazoles was 59.2% [Quindós et.al 2000]. The resistance rates to azoles in our study were also higher than those reported in a previous study conducted in a region west of Turkey between 2008 and 2009 [Espinel-Ingroff et.al 1999]. Alterations on genes encoding the target enzymes of these drugs (beta 1-3 D-glucan synthase for echinocandins (FKS) and 14 alpha sterol demethylase for azoles (ERG11) or up

regulation of multidrug efflux transporters also for azoles (ABC [ATP-binding cassette]/MFS [major facilitator superfamily]) have been blamed for the Candida spp. resistance to antifungal agent.

Numerous azole resistance mechanisms have been described, such as the induction of CDR and MDR genes-encoded efflux pumps, overexpression of 14-α demethylase, modification of the target enzyme structure, alteration of the ergosterol synthesis pathway, reduction of fungal membrane permeability, etc. Induction of the CDR gene-encoded efflux pump and modification of target enzyme structure can result in triazole resistance in C. albicans strains, whereas induction of the MDR gene-encoded efflux pump is only responsible for fluconazole resistance.

The results of this study provide the first documentation of the applicability of the Etest stable agar gradient method for determining the in vitro susceptibilities of Candida species to the triazole (voriconazole and posaconazole) and echinocandin (anidulafungin and capsofungin). We found that MHA with methylene blue and 2% glucose supported optimal growth of all species tested and provided excellent agreement with the MICs obtained with the broth microdilution method .(Table 2). As was seen with fluconazole , the problem of trailing end points due to partial inhibition of growth by azoles was minimized with the use of MHA agar and adherence to specific criteria for reading Etest MICs as described in the Etest package insert and technical guide for yeasts .Although RPMI agar with glucose (2% final concentration) did not perform as well as MHA, both media supported the growth of most of the test isolates and RPMI agar performed reasonably well compared to the reference method (Table 2). E-test however, is a relatively cheap and easy to perform alternative for caspofungin susceptibility testing [Espinel-Ingroff et al 1998].

Results obtained by the E-test method shows a > 71% correlation with those obtained by the AFST-EUCAST method. In both methods, the CLSI and EUCAST AFST, the agar-based E-test has been proposed as a more sensitive technology to discriminate strains of Candida species with fks mutations from wild-type (WT) strains by virtue of much higher MIC results observed in mutant strain. Considering Cryptococcus, the overall agreement level using the E-test MICs and the EUCAST AFST-MICs seems to be higher for voriconazole, fluconazole, itraconazole and flucytosine, than for amphotericin B, which has the lowest level of agreement. Regarding filamentous fungi, the agreement is higher for itraconazole than for amphotericin B, and the E-test method showed a good correlation with the CLSI M38-AFST one to detect Aspergillus resistance. Systematic comparisons between MIC results from reference laboratories and routine results obtained using commercially available methods could be more representative than the current practice to perform quality control with a specific set of reagents using a limited number of isolates [Koehling et.al 2014,pfaller et.al 2008]

V. CONCLUSION

In conclusion, E test method could be considered an alternative to trial routine susceptibility testing due to its simplicity. However, it cannot be considered, at this moment, a substitute for NCCLS reference method, since a complete agreement between both methodologies has not been reached, as demonstrated by the present study and corroboratedby others presented in literature. Moreover, studies on the correlation of in vitro antifungal susceptibility testing and clinical response to these drugs are essentially important. The use of Etest for direct susceptibility testing for Candida species has already been reported as a rapid antifungal susceptibility testing tool that could provide results in 24 to 48 h.

Continuous surveillance of antifungal susceptibilities in clinical isolates of Candida species at the national and international levels is required in order to control the spread of resistance and provide effective strategies for the prophylaxis and treatment of humans with fungal infections. However, the reason for the resistance trend of antifungal agents is unclear. This problem may be resolved by further studies on multiple resistance mechanisms in combination with continuous surveillance and extensive clinical evaluations.

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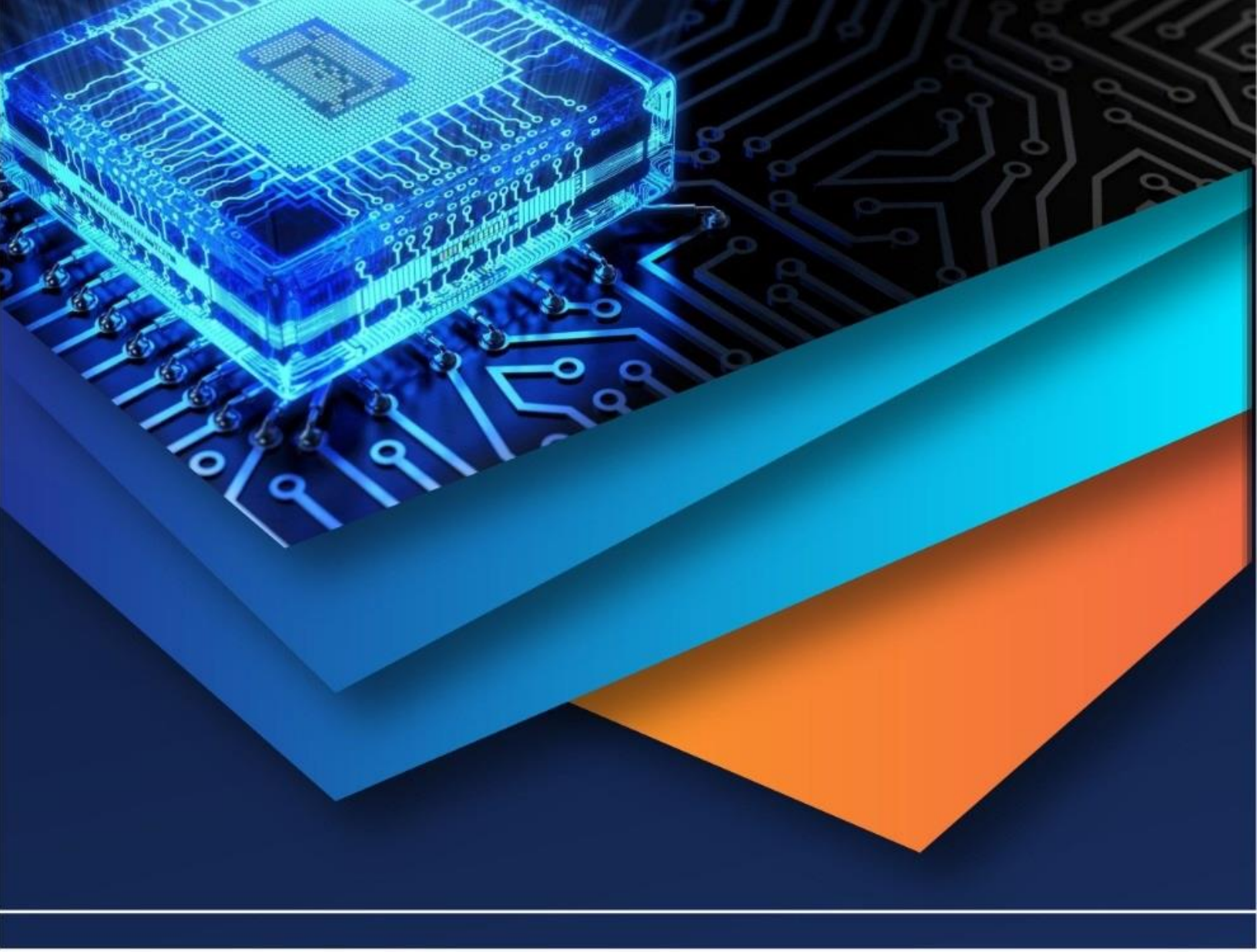

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