Utility of Chromagar Medium for Antifungal Susceptibility Testing of Candida Species Against Fluconazole and Voriconazole in Resource Constrained Settings

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Abstract

Due to cost and complexity, reference broth microdilution antifungal susceptibility method is not feasible in resource constraint setting. Therefore here is a need to develop a cost effective method which would help in simultaneous rapid identification of Candida species and performance of disc diffusion susceptibility testing. Comparison of results of disc diffusion susceptibility testing of Candida isolates against fluconazole and voriconazole was performed on CHROMagar and on MHA-GMB. Overall agreement between the two methods for fluconazole was 91.66% and for voriconazole was 93.33% and thus CHROMagar is found to help in simultaneous identification of Candida species as well as in antifungal susceptibility testing.

Introduction

Candidiasis is one of the commonly encountered problem in hospitalised patients. Candidaemia is the fourth common cause of blood stream infection.[1] Although previously Candida albicans accounted for majority cases of candidiasis, recently non-albicans Candida (NAC) species have been increasingly reported.[2,3] Less than 50% of all candidal blood isolates are C. albicans whereas Candida glabrata, Candida parapsilosis, Candida tropicalis, and other NAC account for rest of the candidial blood stream infection.[4]

Resistance to antifungal agents by candida species has been reported. Non albicans candida in particular Candida krusei and Candida glabrata demonstrate decreased susceptibility to fluconazole.[5,6,7]

There has been attempts and subsequent success in developing reliable methods for antifungal susceptibility testing of yeast. Standardised guidelines using microdilution methods (M27-A2) has been developed by clinical laboratory standard institute (CLSI), it is not being routinely used in majority of laboratories because of its complexity and cost.[8,9] Recently CLSI has given guidelines for antifungal susceptibility testing of yeast by disc diffusion method (M44-A). This disc diffusion testing is carried out for fluconazole and voriconazole using Muller Hilton Agar supplemented with 2% glucose and methylene blue(MH-GMB). This method is more practically feasible for majority of laboratories.[10]

A number of chromogenic media are available which helps in rapid yeast

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identification. These chromogenic media work on principle of reaction of chromogenic substrates with enzymes secreted by yeast producing colonies with various pigmentations. Colours of colonies are species specific thus allowing identification of organisms to the species level.[11, 12, 13]

Keeping in mind the emerging resistance among Candida species, it is but obvious for laboratories to perform the antifungal susceptibility testing of Candida species for timely treatment. In resource constrained settings, where laboratories cannot perform broth microdilution susceptibility method, disc diffusion is most feasible method for antifungal susceptibility testing of Candida species.[14]

However routine identification of Candida species and then performing disc diffusion testing in laboratories is time consuming. This results in delay in reporting of results to clinicians.

Therefore, there is need to develop the method which helps in simultaneous rapid identification of Candida species and performance of disc diffusion susceptibility testing which generates the result in timely manner.

With this aim, a prospective study was undertaken to perform disc diffusion susceptibility testing directly on CHROMagar and compare its results with antifungal susceptibility on MHA-GMB to assess the usefulness of CHROMagar for simultaneous identification and determination of antifungal susceptibility of Candida species to fluconazole and voriconazole.

Material and Methods

60 isolates of Candida species from blood culture were subjected to

1. Identification as per standard protocol and then disc diffusion antifungal susceptibility on MHA with 2% glucose and methylene blue
2. Direct antifungal susceptibility on CHROMagar.

All isolates were tested for antifungal susceptibility against fluconazole and voriconazole.

Antifungal susceptibility was carried out as follows:

Inoculum preparation:-Yeast cultures were subcultured twice onto Sabourauds Dextrose Agar (SDA) to ensure isolation of pure colonies. After 48 hours of incubation, colonies were suspended in 5 ml of sterile normal saline and adjusted to 0.5 McFarland standard. Then yeast suspension was vortexed on vortex machine for 5 minutes for proper mixing of colony and uniform suspension.

Antifungal agents

1. Fluconazole disc (25 µg)
2. Voriconazole disc (1 µg)

Susceptibility testing methods

Using sterile swabs, MHA-GMB agar and CHROMagar were inoculated to produce a confluent lawn of growth from prepared colony inoculums. Fluconazole disc and voriconazole disc were placed on both plates with centre to centre distance in discs ≥to 24 mm. Plates were inverted and incubated for 24 hrs at 35°C. Plates were read at 24 hrs. Zone of inhibition were read and interpretated CLSI M44-A. Zone diameter endpoints were read manually with caliper at 80% growth

inhibition. The interpretive criteria for fluconazole was susceptible (zone diameter of ≥ 19 mm); susceptible dose dependent (zone diameter of 15 to 18 mm) and resistant (zone diameter of ≤14 mm). Interpretative criteria used for voriconazole was susceptible (zone diameter of ≥ 17 mm); susceptible dose dependent (zone diameter of 14 to 16 mm) and resistant (zone diameter of ≤13 mm).

Statistical method: Categorical interpretative agreement was found out by the method of least squares.

Results

60 Candida species isolated from blood culture were included in the study. All Candida isolates were grown on CHROMagar with distinct colour and thus helped in identification of different candida species.

Of 60 candida species included, 27 were C.albicans and 33 were Non albicans Candida. The distribution of Non albicans Candida was as follows:

C. parapsilosis, 15; C. glabrata, 10; C. tropicalis, 6; C. krusei, 2.

Of the 27 C.albicans isolates 21 strains were susceptible, 3 were susceptible dose dependent and 3 were resistant to fluconazole on MHA-GMB agar while 19 strains were susceptible, 4 were susceptible dose dependent and 4 were resistant to fluconazole on CHROMagar Candida. 2 C.albicans strains which were susceptible to voriconazole on MHA-GMB were resistant on CHROMagar. Of 15 strains of C. parapsilosis, 13 were susceptible and 2 were resistant to fluconazole on MHA-GMB agar while 12 were susceptible, 1 was susceptible dose dependent and 2 were resistant to fluconazole on CHROMagar. All 15 strains were susceptible to voriconazole both on MHA-GMB agar and CHROMagar. 4 strains of C.glabrata were resistant to fluconazole on MHA-GMB agar and CHROMagar while 7 strains of C.glabrata were sensitive to Voriconazole on MHA-GMB agar and 6 strains were susceptible, 2 were susceptible dose dependent and 2 were resistant to voriconazole on CHROMagar. Of 6 strains of C.tropicalis, 5 were susceptible to fluconazole and all 6 were susceptible to voriconazole on MHA-GMB agar. 1 strain of C.tropicalis was susceptible dose dependent to fluconazole and 1 strain resistant to voriconazole on CHROMagar. 2 strains of C.krusei were susceptible to fluconazole and voriconazole on MHA-GMB agar as well as CHROMagar.

Table 1: Antifungal susceptibility of Candida strains to Fluconazole and Voriconazole on MHA-GMB and CHROMagar by disc diffusion method

Species (No. of isolates)	Method	Fluconazole (No. of isolates)			Voriconazole (No. of isolates)		
		S	SDD	R	S	SDD	R
C.albicans(27)	MH-GMB CHROM agar	21 19	3 4	3 4	27 25	0 0	0 2
C.parapsilosis (15)	MH-GMB CHROM agar	13 12	0 1	2 2	15 15	0 0	0 0
C.glabrata (10)	MH-GMB CHROM agar	4 4	2 2	4 4	7 6	1 2	2 2
C.tropicalis (6)	MH-GMB CHROM agar	5 4	0 1	1 1	6 5	0 0	0 1
C.krusei (2)	MH-GMB CHROM agar	2 2	0 0	0 0	2 2	0 0	0 0

S-Susceptible, SDD-Susceptible Dose Dependent, R-Resistant

Disc diffusion test performed on CHROMagar showed strong correlation for both fluconazole and voriconazole with the test performed on MH-GMB agar. Not much difference occurred between different species of Candida. Overall agreement between the two methods for fluconazole was 91.66% and for voriconazole was 93.33%.

Within different Candida species, categorical agreement for C.albicans was 88.89% and 92.59% for fluconazole and voriconazole respectively. Non albicans Candida showed 93.94% categorical agreement both for fluconazole and voriconazole.

Table 2: Categorical interpretative agreement for disc diffusion Between MHA-GMB and CHROMagar

Organisms (No of isolates)	Agents	Percentage Agreement	No. of Errors		
			Minor	Major	Very Major
C.albicans (27)	Fluconazole	88.89%	2(7.40%)	1(3.70%)	0
	Voriconazole	92.59%	0	2(7.40%)	0
C.parapsilosis (15)	Fluconazole	93.33%	1(6.67%)	0	0
	Voriconazole	100%	0	0	0
C.glabrata (10)	Fluconazole	100%	0	0	0
	Voriconazole	90%	1(10%)	0	0
C.tropicalis (6)	Fluconazole	83.33%	1 (16.67)	0	0
	Voriconazole	83.33%	0	1(16.67%)	0
C.krusei (2)	Fluconazole	100%	0	0	0
	Voriconazole	100%	0	0	0
Total (60)	Fluconazole	91.66%	4 (6.67%)	1(1.67%)	0
	Voriconazole	93.33%	1(1.67%)	3 (5%)	0
Non albicans Candida (33)	Fluconazole	93.94%	2 (6.06%)	0	0
	Voriconazole	93.94%	1 (3.03%)	1(3.03%)	0

The zone of inhibition (in millimeters) for fluconazole and voriconazole discs on MH-GMB agar were compared with respective zone diameter from CHROMagar at 24 hours.

Very major errors were classified as susceptible by disc diffusion taken from CHROMagar and resistant when cultured from MH-GMB agar. Major errors were classified as resistant by disc diffusion on CHROMagar and susceptible by MH-GMB agar. Minor errors occurred when the result of one isolate was susceptible or resistant and that of the parallel isolate was susceptible dose dependent.

In the present study, overall error on CHROMagar was 6.67% minor and 1.67% major error for fluconazole while 1.67% minor and 5% major error for voriconazole. Within different candida species, C.albicans showed 7.40% minor error and 11.10% major error. Non albicans Candida showed 9.09% minor error and only 3.03% major error. Not a single instance of very major error noticed in present study.

Discussion

The present study aims to find out the usefulness of CHROMagar for identification and determination of antifungal susceptibility of Candida species simultaneously.

In present study, all 60 Candida isolates gave distinct colour on CHROMagar and thus helped in identification of Candida species. Horvath et al[15] also reported similar finding. Grace L et al[11] reported 78% identification of Candida isolates. This might be due to direct subculture of positive blood culture bottle on CHROMagar. Lynn L et al[4] reported that C. albicans, C. glabrata, C. krusei, and C. tropicalis, were readily identifiable on CHROMagar. The use of CHROMagar considerably reduced not only the reporting time but also the laboratory costs as it allowed rapid identification of clinically important

Candida.[16,17]

Present study demonstrated that disc diffusion testing can be performed on CHROMagar using the CLSI M44-A method. Categorical agreement between MHA-GMB and CHROMagar was 92% for fluconazole and 94% for voriconazole. Michael Klevayet al[14] showed that categorical agreement between potato dextrose agar (PDA) and CHROMagar was 95% for fluconazole and 98% for voriconazole. In present study, there was not much difference in the categorical agreement between MHA-GMB and CHROMagar for C.albicans and Non albicans Candida.

We have reported 6.67% minor and 1.67% major error for fluconazole while 1.67% minor and 5% major error for voriconazole. The zone of inhibition is distinct in the conventional Kirby-Bauer method for bacterial antibiotic susceptibility testing while predominant reduction of growth is required for reading zone of inhibition in case of yeast antifungal susceptibility testing. Thus reading the zone of inhibition around the antifungal disc is quite difficult at times and subjective thus giving rise to minor errors.[10]

Many authors showed that there was 80% correlation between results of disc diffusion antifungal susceptibility testing and CLSI M27-A broth microdilution reference method.[18,19,20] This argue well for major error occurring in our study between the result of MHA-GMB and CHROMagar. However Grace L et al[11] proposed that major errors can be resolved by extending the incubation time to 48 hours. Further studies need to be conducted to establish the observation proposed by Grace L et al.

Therefore, performing disc diffusion testing on CHROMagar directly helps in identification of Candida to the species level as well as provide antifungal susceptibility pattern simultaneously. This will also provide valuable information to clinicians to decide appropriate antifungal therapy and thereby decrease patient morbidity and mortality in invasive yeast infections.

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Angiotensin receptor blocker in patients with ST segment elevation myocardial infarction with preserved left ventricular systolic function

Does treatment with angiotensin receptor blocker reduces the risk of cardiac death or myocardial infarction in patients with ST segment elevation myocardial infarction (STEM) and preserved left ventricular systolic function after primary percutaneous coronary intervention?

Treatment with angiotensin receptor blockers showed beneficial effects comparable with angiotensin converting enzyme (ACE) inhibitors in STEMI patients with preserved left ventricular systolic function.

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