Antifungal Susceptibility Testing in HIV/AIDS Patients: a Comparison Between Automated Machine and Manual Method

Erni J. Nelwan[1], Evi Indrasanti[2], Robert Sinto[1], Farida Nurchaida[2], Rustadi Sosrosumihardjo[2]

[1] Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta, Indonesia.
[2] Department of Clinical Pathology, Faculty of Medicine Universitas Indonesia - Cipto mangunkusumo Hospital, Jakarta, Indonesia.

Corresponding Author:
Erni J. Nelwan, MD. Division of Tropical and Infectious Disease, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro 71, Jakarta 10430, Indonesia. email: ejnelwan@yahoo.com.

ABSTRAK

Tujuan: *untuk mengevaluasi kinerja Vitek2 compact mesin (Biomerieux Inc ver 04,02, Prancis) mengacu pada metode manual untuk menguji kepekaan ketahanan Candida pada pasien HIV/AIDS.* ***Metode:*** *kami melakukan uji perbandingan hasil pemeriksaan sensitifitas obat anti-jamur antara Vitek2 compact machine (Biomerieux Inc. ver 04.02, France) dengan metode manual. Kesepakatan kategorik antara hasil pemeriksaan dengan kedua metode tersebut dinilai sesuai dengan kriteria yang telah disepakati. Kami juga melakukan pengukuran waktu yang diperlukan untuk mendapatkan hasil dengan menggunakan kedua metode.* ***Hasil:*** *terdapat 137 isolat Candida yang terdiri atas 8 spesies Candida dengan C. albicans dan C. glabrata sebagai spesies yang terbanyak pertama (56,2%) dan kedua (15,3%), secara berturutan. Sebanyak 2,6% C. albicans resisten terhadap flukonazol pada pemeriksaan dengan metode manual namun tidak ditemukan resistensi terhadap flukonazol pada pemeriksaan dengan mesin Vitek2. Seluruh spesies C. krusei resisten terhadap flukonazol pada pemeriksaan dengan kedua metode tersebut. Pola resistensi C. glabrata terhadap flukonazol, vorikonazol, amfoterisin B secara berturut sebanyak 52,4%, 23,8%, 23,8% pada pemeriksaan dengan metode manual dibandingkan 9,5%, 9,5%, 4,8% pada mesin Vitek2. Waktu yang diperlukan untuk mendapatkan hasil uji dengan menggunakan mesin Vitek2 lebih singkat dibandingkan metode manual.* ***Kesimpulan:*** *terdapat kesepakatan kategorik yang baik antara hasil pemeriksaan dengan metode manual dan mesin Vitek2, kecuali pada spesies C. glabrata. Waktu yang diperlukan untuk mendapatkan hasil uji dengan menggunakan mesin Vitek2 lebih singkat dibandingkan metode manual.*

Kata kunci: *uji kepekaan anti-jamur, metode otomatis, metode manual, HIV.*

ABSTRACT

Aim: *to evaluate the performance of Vitek2 compact machine (Biomerieux Inc. ver 04.02, France) in reference to manual methods for susceptibility test for Candida resistance among HIV/AIDS patients.* ***Methods:*** *a comparison study to evaluate Vitek2 compact machine (Biomerieux Inc. ver 04.02, France) in reference to manual methods for susceptibility test for Candida resistance among HIV/AIDS patient was done. Categorical agreement between manual disc diffusion and Vitek2 machine was calculated using predefined criteria. Time to susceptibility result for automated and manual methods were measured.* ***Results:*** *there were 137 Candida isolates comprising eight Candida species with C.albicans and C. glabrata as the first (56.2%)*

and second (15.3%) most common species, respectively. For fluconazole drug, among the C. albicans, 2.6% was found resistant on manual disc diffusion methods and no resistant was determined by Vitek2 machine; whereas 100% C. krusei was identified as resistant on both methods. Resistant patterns for C. glabrata to fluconazole, voriconazole and amphotericin B were 52.4%, 23.8%, 23.8% vs. 9.5%, 9.5%, 4.8% respectively between manual diffusion disc methods and Vitek2 machine. Time to susceptibility result for automated methods compared to Vitex2 machine was shorter for all Candida species. ***Conclusion:*** *there is a good categorical agreement between manual disc diffusion and Vitek2 machine, except for C. glabrata for measuring the antifungal resistant. Time to susceptibility result for automated methods is shorter for all Candida species.*

Keywords: *antifungal susceptibility test, automated test, HIV, manual test.*

INTRODUCTION

During the last few decades, fungal infections due to *Candida* species is increasing, mostly among immunocompromised patients including HIV/AIDS.[1] Oropharyngeal candidiasis is the most common site of infection with the prevalence of as much as 80-95% in HIV/AIDS patients.[2,3] As a consequence of this, the use of antifungal in this population has also increased. The treatment guidelines for candidiasis include the administration of nystatin, azol derivates such as fluconazole, voriconazole, itraconazole or ketoconazole and amphotericin B.[4] In many cases, *Candida* infection is often treated empirically; this practice may further lead to the frequent use of antifungal drugs in the clinic. There is a need to understand the pattern of susceptibility to antifungal drugs since a decrease of sensitivity to Candida species was reported from several studies.[3,5] The use of conventional test for drug sensitivity with manual diffusion has already been routinely performed in the clinic. However, this method needs media preparation, antifungal discs and more time consumption for the final result. The availability of a rapidly provided automated method and ready to use media could yield faster results.

The Vitek2 system (BioMérieux Inc. ver 04.02, France) is an automated bacterial identification and susceptibility testing system that uses fluorescence-based technology.[6] The availability of Vitek2 Compact machine (Biomerieux) in our institution was used initially to identify bacteria pathogens only. Previous studies showed that this system could give reliable identification and susceptibility results with pure bacterial cultures.[7-9] A comparison study to evaluate the performance of Vitek2 compact machine (Biomerieux Inc. ver 04.02, France) in reference to manual methods for susceptibility test for *Candida* resistance among HIV/AIDS patients was done.

METHODS

One hundred and thirty seven Candida isolates were included in the study. Candida species were identified using chromogenic media (CHROMAgar, France). We classified yeast species according to the colour of each colony: green colonies were identified as *C. albicans*; blue colours were regarded as C. tropicalis, pink and purple as *C. krusei*, and light white-purple as *C. parapsilosis* or *C. glabrata*. Purification was conducted according to standard method. (Rex, JH-no 38) After being purified, isolates were plated onto Sabourraud Dextrose Agar (SDA) for further identification with Vitek2 machine (Biomerieux Inc. ver 04.02, France). Vitek2 machine identification was based on biochemical reaction and measured the capacity of using source of carbon, nitrogen and enzymatic activity of Vitek2 using YST reagent (Biomerieux, France).

Sensitivity tests with the manual disc method was conducted by measuring the diameter of inhibition zone according to CLSI M 44-A2, using three types of antifungal discs, fluconazole 25 µg (Oxoid, UK), voriconazole 1 µg (Oxoid, UK), and amphotericin 20 µg (Liofilchem, Italy). For Vitek2 machine, sensitivity test was based on turbidimetry using reagent card AST (Biomerieux, France) consisted of antifungal such as fluconazole (diluted to 1, 4, 8, 16 mg/L), voriconazole (diluted to 0.5, 1, 4, 8 mg/L) and

amphotericin B (diluted to 1, 4, 8, 16 mg/L).

Categorical agreement was classified as sensitive, intermediate/SDD and resistant after matching two different methods using manual diffusion method as a reference. Discordance between two methods was concluded as error in interpretation and categorized as very major error (VME) if found resistant on the automatic methods and sensitive on the manual methods. Major error (MaE) was concluded if found sensitive on the automatic methods and resistant on the manual methods. Minor error (MiE) was classified if SDD/I was found in the automatic method while sensitive/resistant in the manual method or the other way around.[6]

Candida albicans ATCC 14053 (Canada, USA) was used as a quality control (QC) standard strain for identification. C. parapsilosis ATCC 22019 (Canada, USA) was used as QC for sensitivity test. Antifungal disc and card AST for Vitek2 were tested with ATCC strains for seven times, before the tests were conducted. These procedures were repeated after the tests have been perfomed twenty times.

RESULTS

For identification test, both methods were comparable with control strains *C. albicans* ATCC 14053 (Canada, USA) as recommended by CLSI M35-A and Vitek2 manual. For sensitivity test, Vitek2 machine was comparable with *C. parapsilosis* ATCC 22019 (Canada, USA). The results were compared by entering data on excel sheets and statistical calculations were made and recorded.

There were 137 *Candida* isolates comprising eight *Candida* species with *C. albicans* as the most common species (56.2%) and *C. glabrata* as the second most common species (15.3%) (**Table 1**). For evaluation of resistance pattern, the *C. famata* and *C. magnoliae* were excluded because Vitek2 machine was not designed to detect these species. For fluconazole drug, among the *C. albicans*, 2.6% was found resistant on manual diffusion methods and no resistant was determined by Vitek2; whereas 100% resistant was identified for *C. krusei* on both methods. Resistance pattern for *C. glabrata* to fluconazole, voriconazole and amphotericin B was (52.4%, 23.8%, 23.8% vs. 9.5%, 9.5%, 4.8%) between manual disc diffusion method and Vitek2 machine. Detail on resistance pattern was shown in **Table 2**.

Table 1. Distribution of *Candida* isolates (N=137)

Species	n (%)
C. albicans	77 (56.2)
C. glabrata	21 (15.3)
C. tropicalis	19 (13.9)
C. krusei	9 (6.7)
C. parapsilosis	5 (3.6)
C. dubliniensis	4 (2.9)
C. famata	1 (0.76)
C. magnolia	1 (0.76)

Error interpretation between manual diffusion and Vitek2 for 77 isolates of *C. albicans* to fluconazole found four (5.2%) MiE and three (3.8%) MaE; 3 (3.8%) MaE and 3 (3.8%) MiE to voriconazole and one (1.2%) VME to amphotericin B. Total categorical agreement for *C. albicans* to fluconazole, voriconazole and amphotericin B was 90.9%, 92.2% and 98.7% respectively. For *C. glabrata* error interpretation among 21 isolates to fluconazole 9 (42.8%) MiE and 8 (38.1%) MaE, voriconazole 5 (23.8%) MaE and 1 (4.7%) MiE; to amphotericin B 2 (9.5%) MaE and 2 (2.9%) MiE. Total categorical agreement for C. glabrata to fluconazole, voriconazole and amphotericin B was 19.1%, 71.4% and 80.9%. Of 19 isolates of C. tropicalis error interpretation to fluconazole and voriconazole 1 (5.26%) MiE and 1 (5.26%) MaE and 1 (5.26%) MiE and 2 (10.5%) MiE for amphotericin B. Total categorical agreement was 89.5% for each studied drugs. The five isolates of *C. parapsilosis* for error interpretation resulted in 1 (20%) MiE only for amphotericin B, hence resulted in total categorical agreement of 100% for fluconazole, 100% for voriconazole and 80% for amphotericin B. Among the 4 isolates of *C. dubliniensis* the error interpretation was found 1 (25%) MiE to fluconazole only with total categorical agreement of 75% to fluconazole, 100% to voriconazole and 100% to amphotericin B. Total error interpretation to all Candida species between manual disc diffusion and Vitek2 for fluconazole showed no VME, 8.89%

Table 2. Error and categorical agreement of manual diffusion and Vitek2 machine

Candida species	Antifungal	Resistant (%)						VME	MaE	MIE	CA (%)
		Vitek2 machine			Manual diffusion						
		S	I/SDD	R	S	I/SDD	R				
C. albicans (77)	FCA	97.4	2.6	0	94.8	2.6	2.6	0	3	4	90.9
	VOR	97.4	1.3	1.3	96.1	1.3	2.6	0	3	3	92.2
	AMB	97.4	0	2.6	100	0	0	1	0	0	98.7
C. glabrata (21)	FCA	80.9	9.5	9.5	14.3	33.3	52.4	0	8	9	19.1
	VOR	90.5	0	9.5	66.7	9.5	23.8	0	5	1	71.4
	AMB	90.5	4.8	4.8	56.7	19.5	23.8	0	2	2	81
C. tropicalis (19)	FCA	100	0	0	89.5	5.3	5.3	0	1	1	89.5
	VOR	100	0	0	89.5	5.3	5.3	0	1	1	89.5
	AMB	100	0	0	94.7	0	5.3	0	0	2	89.5
C. krusei (9)	FCA	0	0	100	0	0	100	0	0	0	100
	VOR	100	0	0	88.9	11.1	0	0	0	1	88.9
	AMB	66.7	22.2	11.1	44.4	44.4	11.1	1	1	2	55.6
C. parapsilosis (5)	FCA	100	0	0	100	0	0	0	0	0	100
	VOR	100	0	0	100	0	0	0	0	0	100
	AMB	80	20	0	80	0	20	0	0	1	80
C. dubliniensis (4)	FCA	100	0	0	75	25	0	0	0	1	75
	VOR	100	0	0	100	0	0	0	0	0	100
	AMB	75	25	0	75	25	0	0	0	0	100

VME = very major error; MaE = major error; MiE = minor error; CA = categorical agreement; S = sensitive; I/SDD = intermediate/ susceptible dose dependent; R = resistant; FCA = fluconazole; VOR = voriconazole; AMB = amphotericin B

MaE and 11.11% MiE. Voriconazole reported no VME, 6.67% MaE and 4.44% MiE. Among the amphotericin B 1.46% VME, 2.22% MaE and 5.18% MiE were found (**Figure 1**).

Time to susceptibility result for manual method was 24 h for *C. albicans*, *C. dubliniensis* and *C. tropicalis*; and 48 hours for *C. glabrata*, *C. krusei* and *C. parapsilosis*. Using Vitek2, the average time to susceptibility result for *C. albicans* was 13.25 hours, *C. glabrata* 12.75 h, *C. dubliniensis* 14.75, *C. tropicalis* 12.25 h, *C. parapsilosis* 16.75 h, and *C. krusei* 18.75 h.

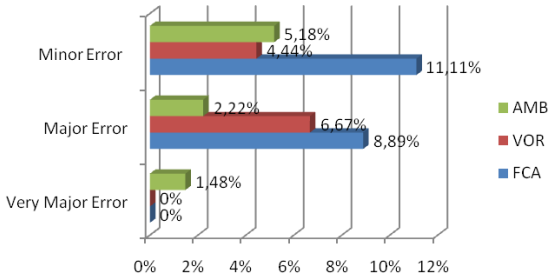

Figure 1. Total error interpretation (%) to all candida species between manual disc diffusion and Vitek2

DISCUSSION

The majority of *Candida* species that were included in our study was *C. albicans*, in resemblance with other reports of epidemiology of *Candida* infection in HIV/AIDS patients.[5,10,11] The cathegorical agreement between two methods as presented in **Table 2** showed that there was no VME for fluconazole and voriconazole. Very major error for amphotericin B was 1.46%, lower than the acceptable threshold allowed by FDA, i.e. 1.5%. Major errors for fluconazole, voriconazole and amphotericin B were 8.89%, 6.67%, 2.22% respectively. Only amphotericin B met the FDA acceptable threshold for major error, i.e. 3%. In addition, the minor errors for fluconazole, voriconazole and amphotericin B were 11.11%, 4.44%, 5.18%, respectively. Only voriconazole met the FDA acceptable threshold for minor error, i.e. 5%.

There was a good categorical agreement between manual disc diffusion and Vitek2, except for *C. glabrata* (**Table 3**). This good

Table 3. Categorical agreement for *C. albicans* of manual diffusion and Vitek2 machine in other studies.

Study	No. of isolate	Antifungal	Methods	Resistance (%)	CA (%)	Population
Pfaller, 2007[12]	198	FCA	Vitek2	1.5		All Candida specimens
			BMD	1.5	99.5	
		VOR	Vitek2	1.5		
			BMD	1.5	99	
Bargoeis, 2009[13]	84	FCA	Vitek2	1.2		
			Microdilution CLSI	1.2	100	Candidemia
			Etest	1.2	100	
		VOR	Vitek2	0		
			BMD	0	100	
			Etest	0	100	

CA = categorical agreement; FCA = fluconazole; VOR = voriconazole; AMB = amphotericin B; BMD = broth microdilution; CLSI = clinical & laboratory standards institute

Table 4. Categorical agreement for *C. glabrata* of manual diffusion and Vitek2 machine in other studies

Study	No. of isolate	Antifungal	Methods	CA (%)	Population
Pfaller, 2003[14]	235	FCA	BMD		All Candida specimens
			Etest (48 hours)	52.3	
			Manual disk (24 hours)	64.7	
		VOR	BMD		
			Etest (48 hours)	94.8	
			Manual disk (24 hours)	97.4	
Alexander, 2007[15]	38	FCA	BMD		Candidemia
			Etest (24 hours)	55	
			Sensititre	34	
		VOR	BMD		
			Etest (24 hours)	76	
			Sensititre	87	
Bourgeois, 2009[13]	56	FCA	Vitek2		Candidemia
			Microdilution CLSI	78.6	
			Etest (48 hours)	23.2	
		VOR	Vitek2		
			Microdilution CLSI	87.5	
			Etest (48 hours)	51.8	

CA = categorical agreement; FCA = fluconazole; VOR = voriconazole; AMB = amphotericin B; BMD = broth microdilution; CLSI = clinical & laboratory standards institute

categorical agreement for *C. albicans* species and fair categorical agreement for *C. glabrata* were similar with the result from previous studies as shown in **Table 4**. The major contributors of categorical disagreement for *C. glabrata* were MaE and MiE, with no VME. From the previous studies, the hypothesis for *C. glabrata* categorical disagreement was the presence of heteroresistance or selection within the specimen which further resulted in the presence of subpopulation resistance of the less sensitive group.[12-14] The latter will be found as a less clear zone during manual disc diffusion technical examination. The manual disc diffusion test could provide more accurate results for measuring resistance in homogeneous specimen

of *C. glabrata*.[15]

Using microdilution principles, Vitek2 provides antifungal and microcuvet in one ready-used kit. Resistance test can be done easily because the reagent and media have been standardized by the manufacturer. A lot of samples can be tested at a single time point; thus with a shorter time to susceptibility result compared with standard method; this method is suitable to be used in laboratory with high workload. However, this is not the fastest method available for having timely result.[16] Apart from the advantages in terms of cost analysis and applicability, manual method needs a meticulous expertise, especially in the interpretation of less clear zone during examination.

CONCLUSION

The result of this study can be used as a scientific justification for clinicians to perform antifungal specificity test for the majority of *Candida* species in HIV/AIDS patients with automated method (Vitek2), except for *C. glabrata*, due to its good categorical agreement compared to manual method.

REFERENCES

1. Lochart SR, Diekema DJ, Pfaller MA. The epidemiology of fungal infection. Clinical mycology. Churchill Livingstone: Elsevier; 2009. p. 1-12.
2. Tong K, Murtagh K, Lau C, Selfeldin R. The impact of esophageal candidiasis on hospital charges and costs across patient subgroups. Curr Med Res. 2008;24:167-74.
3. Enwuru CA, Ogunledun A, Idika N, et al. Fluconazole resistant opportunistic oro-pharyngeal *Candida* and non-Candida yeast-like isolates from HIV infected patients attending ARV clinics in Lagos, Nigeria. African Health Sciences. 2008;8:142-8.
4. Pappas PG, Rex KH, Sobel JD, et al. Guidelines for treatment of candidiasis. Clin Infect Dis. 2004;38:161-89.
5. Badiee P, Alborzi A, Davarpanah MA, Shakiba E. Distributions and antifungal susceptibility of *Candida* species from mucosal sites in HIV positive patients. Arch Iran Med. 2010;13:282-7.
6. Pfaller MA, Diekema DJ, Procop GW, Rinaldi MG. Multicenter comparison of the Vitek2 antifungal susceptibility test with the CLSI broth microdilution reference method for testing amphotericin B, flucytosine, and voriconazole against *Candida spp*. J Clin Microbiol. 2007;45:3522-8.
7. Barry AL, Pfaller MA, Brown SD, et al. Quality control limits for broth microdilution susceptibility tests of ten antifungal agents. J Clin Microbiol. 2000;28:3457–9.
8. Chen YC, Chang SC, Luh KT, Hsieh WC. Stable susceptibility of *Candida* bloodstream isolates to fluconazole despite increasing use during the past 10 years. J Antimicrob Chemother. 2002;52:71–7.
9. Espinel-Ingroff A, Barchiesi F, Cuenca-Estrella C, et al. Comparison of visual 24-hour and spectrophotometric 48-hour MICs to CLSI reference microdilution MICs of fluconazole, itraconazole, posaconazole, and voriconazole for *Candida spp*.: a collaborative study. J Clin Microbiol. 2005;43:4535–40.
10. Magaldi S, Mata S, Hartung C, Verde G, Deibis L, Roldan Y. In vitro susceptibility of 137 Candida sp. isolates from HIV positive patient to several antiungal drugs. Mycopathologia. 2000;149:63-8.
11. Hamza OJM, Matee MIN, Moshi MJ, Simon ENM, Mugusi F, Mikx FHM. Species distribution in vitro antifungal susceptibility of oral yeast isolates from Tanzanian HIV infected patient with primary and recurrent oropharyngeal candidiasis. BMC Microbiol. 2008;8:1-9.
12. Pfaller MA, Diekema DJ, Gibbs DL, et al. Results from the ARTEMIS DISK global antifungal surveillance study, 1997 to 2005: an 8.5-year analysis of susceptibilities of Candida species and other yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J Clin Microbiol. 2007;45:1735-45.
13. Bourgeois N, Dehandschoewercker L, Bertout S, Bousquet PJ, Rispail P, Lachaud L. Antifungal susceptibility of 205 Candida spp. isolated primarily during invasive candidiasis and comparison of the Vitek2 system with the CLSI broth microdilution and etest methods. J Clin Microbiol. 2010;48:154–61.
14. Pfaller MA, Diekema DJ, Boyken L, Messer SA, Tendolkar S, Hollis RJ. Evaluation of the etest and disk diffusion methods for determining susceptibilities of 235 bloodstream isolates of Candida glabrata to fluconazole and voriconazole. J Clin Microbiol. 2003;41:1875–80.
15. Alexander BD, Byrne TC, Smith KL, Hanson KE, Anstrom KJ. Comparative evaluation of Etest and sensititre yeast one panels against the clinical and laboratory standards institute M27-A2 reference broth microdilution method for testing *Candida* susceptibility to seven antifungal agents. J Clin Microbiol. 2007;45:698–6.
16. Junkinsa AD, Arbefeville SS, Howard WJ, Richter SS. Comparison of BD Phoenix AP workflow with Vitek2. J Clin Microbiol. 2010:48;1929-31.