



Available online at  
**ScienceDirect**  
www.sciencedirect.com

Elsevier Masson France  
**EM|consulte**  
www.em-consulte.com



SHORT COMMUNICATION/COURTE COMMUNICATION

# Comparison of two antifungal susceptibility testing of *Candida* sp. isolates using agar diffusion method: Neo-sensitabs<sup>®</sup> tablets and Bio-rad<sup>®</sup> disks



*Comparaison de deux méthodes d'antifongigramme sur milieu gélosé en utilisant les comprimés Neo-sensitabs<sup>®</sup> et les disques Bio-rad<sup>®</sup> pour tester la sensibilité des isolats de Candida sp.*

J. Uwingabiye<sup>\*</sup>, M. Iken, A.G. Zohoun, L. Boumhil, Z. Lemkhente, H. Naoui, M. Bouchrik, B. Lmimouni

Service de parasitologie-mycologie, faculté de médecine et de pharmacie, université Mohammed V, hôpital militaire d'instruction Mohammed V, Rabat, Morocco

Received 14 June 2015; received in revised form 17 August 2015; accepted 11 September 2015  
Available online 23 October 2015

## KEYWORDS

Antifungal susceptibility methods;  
Agar diffusion method;  
Concordance;  
*Candida*;  
Antifungal agents

## Summary

**Introduction.** – The aim of our study was to evaluate the concordance between the two antifungal susceptibility testing of *Candida* sp. isolates using agar diffusion method: Neo-Sensitabs<sup>®</sup> tablets and Bio-Rad<sup>®</sup> disks.

**Materials and methods.** – This is a prospective study conducted in the Laboratory of Parasitology and Mycology of the Mohammed V military teaching hospital from February to August 2012. Upon receiving blood cultures and peripheral sites samples, the identification of *Candida* isolates performed using routine phenotypic standard tests and the realization of the antifungal susceptibility was carried out on Neo-sensitabs<sup>®</sup> tablets and Bio-Rad<sup>®</sup> disks.

**Results.** – A total of 38 *Candida* strains were isolated: 15 *C. albicans* (39%), 13 *C. glabrata* (34%), 5 *C. tropicalis* (13%), 4 *C. krusei* (11%) and 1 *C. dubliniensis* (3%). There were no significant difference ( $P > 0.05$ ) in susceptibility rate between both methods for all antifungal agents tested except for 5-fluorocytosine. The concordance percentage between two methods was 100% for amphotericin B, 97.4% for fluconazole, 94.7% for voriconazole and 73% for 5-fluorocytosine.

<sup>\*</sup> Corresponding author.

E-mail address: [uwije2020@yahoo.fr](mailto:uwije2020@yahoo.fr) (J. Uwingabiye).

**MOTS CLÉS**

Méthodes de sensibilité aux antifongiques ;  
Méthode de diffusion en milieu gélosé ;  
Concordance ;  
*Candida* ;  
Antifongiques

**Conclusion.** — Both methods are easy to perform, rapid and cost effective. Our results showed the best agreement between the two methods for testing the susceptibility of *Candida* isolates to amphotericin B, fluconazole and voriconazole while for the 5-fluorocytosine, the concordance rate was low.

© 2015 Elsevier Masson SAS. All rights reserved.

**Résumé**

**Introduction.** — Le but de notre étude était d'évaluer la concordance entre les deux techniques d'antifongigramme en milieu gélosé avec les comprimés Neo-Sensitabs<sup>®</sup> et les disques Bio-rad<sup>®</sup> pour tester la sensibilité des isolats de *Candida* sp.

**Matériels et méthodes.** — Une étude prospective menée au laboratoire de parasitologie-mycologie de l'hôpital militaire d'instruction Mohammed V de février en août 2012. Dès la réception des hémocultures et des prélèvements des sites périphériques, l'identification des souches de *Candida* a été effectuée et la réalisation de l'antifongigramme a été faite en utilisant les comprimés Neo-sensitabs<sup>®</sup> et les disques Bio-rad<sup>®</sup>.

**Résultats.** — Au total, 38 souches de *Candida* ont été isolées : 15 *Candida albicans* (39 %), 13 *C. glabrata* (34 %), 5 *C. tropicalis* (13 %), 4 *C. krusei* (11 %) et 1 *C. dubliniensis* (3 %). Il n'y avait pas de différence significative ( $p > 0,05$ ) des niveaux de sensibilité entre les deux méthodes pour tous les antifongiques testés, sauf pour la 5-fluorocytosine. Le pourcentage de concordance entre les deux méthodes était de 100 % pour l'amphotéricine B, 97,4 % pour fluconazole, 94,7 % pour voriconazole et 73 % pour 5-fluorocytosine.

**Conclusion.** — Les deux méthodes testées sont faciles à réaliser, rapides et économiques. Nos résultats ont montré une très bonne concordance entre les deux méthodes pour tester la sensibilité des isolats de *Candida* à l'amphotéricine B, au fluconazole et au voriconazole tandis que pour la 5-fluorocytosine, le taux de concordance était faible.

© 2015 Elsevier Masson SAS. Tous droits réservés.

**Introduction**

*Candida* sp. represents the fourth or fifth agent found in nosocomial septicemia [6,13,14] with an attributable mortality rate ranging from 26 to 54% [15], this requires the use of in vitro susceptibility testing well standardized as part of guiding the clinician in the choice of the antifungal and its dosage. Intense efforts have been made to reach a consensus on antifungal susceptibility testing of yeast, and establishing repeatable and standardized methods for determining their sensitivity and correlation in vivo [2–4]. The reference method CLSI (Clinical and Laboratory Standards Institute, formerly NCCLS) for testing the antifungal susceptibility of yeasts (document M27-A2) has shown its limits: the difficulty of identifying strains of *Candida* with reduced susceptibility to amphotericin B, the phenomenon of residual growth observed for the azole and insufficient growth of certain *Candida* species [9,11]. Other methods inspired by the reference one have been developed for testing antifungal susceptibility [7,8]. The reference method M44-A has been proposed recently by CLSI to assess the sensitivity of yeasts using antifungal disk diffusion method [2]. Several studies on the comparison of antifungal susceptibility have been published and some authors compared the Neo-Sensitabs<sup>®</sup> method (Rosco Diagnostic, Denmark) to other methods [4,7,16]. To our knowledge, this study is the first that focuses specifically on the comparison of the Neo-sensitabs<sup>®</sup> method to that of Bio-Rad<sup>®</sup> (Bio-Rad Laboratories, Marnes-la-Coquette, France). The aim of this study was to evaluate the concordance between two antifungal susceptibility testing of

*Candida* isolates using agar diffusion method: Neo-Sensitabs<sup>®</sup> tablets and Bio-Rad<sup>®</sup> disks.

**Materials and methods**

This is a prospective observational study conducted in the Laboratory of Parasitology and Mycology of the Mohammed V military teaching hospital from February to August 2012. The isolates of *Candida* sp. obtained from blood cultures and peripheral sites samples of patients hospitalized in the intensive care units were included in this study. The culture was carried out on Sabouraud–chloramphenicol medium with and without actidione. Incubation of these media was done at 37 °C for 24 to 48 hours. The identification of *Candida* species was performed using standard phenotypic methods: morphological appearance of colonies, Candiselect 4<sup>®</sup> (Bio-Rad, France), filamentation test on serum and Auxacolor TM 2 (Bio-Rad, France). The study of the antifungal susceptibility of *Candida* isolates was carried out by the agar diffusion method using the Neo-sensitabs<sup>®</sup> tablets and Bio-Rad<sup>®</sup> disks. The four antifungal agents tested were: 5-fluorocytosine, fluconazole, voriconazole and amphotericin B. For the Neo-sensitabs<sup>®</sup> tablets method, the culture medium used was Mueller–Hinton agar supplemented with 2% glucose and 0.5 µg/mL of methylene blue [4,7,8]. For antifungal susceptibility disk Bio-rad<sup>®</sup>: 3 media were used: semi-synthetic medium for studying the activity of 5-fluorocytosine, casitone agar for polyenes and imidazoles [5] and Mueller–Hinton medium for testing triazoles [2]. The diameters of zones of inhibition were measured to the nearest

**Table 1** Interpretation table for both antifungal susceptibility testing.  
 Tableau d'interprétation pour les deux méthodes d'antifongigramme testées.

Antifungal agents	Neo-sensitabs <sup>®</sup>			Bio-rad <sup>®</sup>		
	Susceptible (mm)	Intermediate (mm)	Resistant (mm)	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
5-fluorocytosine 1 µg	≥ 20	12–19	≤ 11	20	20–10	10
Fluconazole 25 µg	≥ 19	18–15	≤ 14	≥ 19	18–15	≤ 14
Voriconazole 1 µg	≥ 17	16–14	≤ 13	≥ 17	16–14	≤ 13
Amphotericin B <sup>a</sup>	≥ 15	14–10	≤ 10	> 10	10	10

<sup>a</sup> The potency of Amphotericin B is 10 µg for Neo-sensitabs<sup>®</sup> tablets method and 100 µg for Bio-rad<sup>®</sup> disks method.

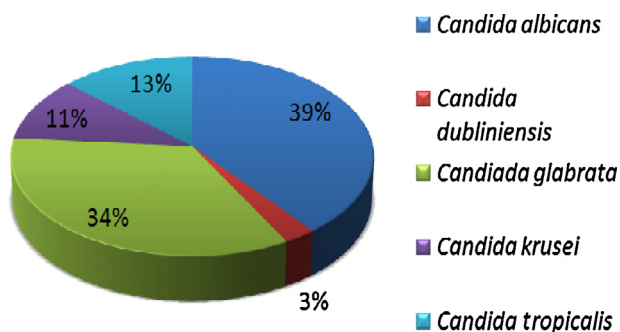
millimetre from the time when there was a significant reduction of growth after 24 to 48 hours of incubation. The results were interpreted according to the interpretation criteria provided by the manufacturers and M44-A guidelines [2] (Table 1). Both tests were considered concordant if the results obtained were identical and were designated discordant if the results of both tests were different from each other. The data were analyzed by SPSS Version 17.0 software. Chi<sup>2</sup> test or Fisher exact test was used to compare the difference in susceptibility rates between two methods for each antifungal. *P* values < 0.05 were considered significant.

## Results

During the study period, five different species of *Candida* were isolated: 15 *C. albicans* (39%), 13 *C. glabrata* (34%), 5 *C. tropicalis* (13%), 4 *C. krusei* (11%) and 1 *C. dubliniensis* (3%) (Fig. 1).

There were no significant difference (*P* > 0.05) in susceptibility rate between both methods for all antifungal agents tested except for 5-fluorocytosine (Table 2). The antifungal susceptibility rate based on the type of antifungal susceptibility for each antifungal was: amphotericin B (97.4% for Bio-rad<sup>®</sup> and Neo-Sensitabs<sup>®</sup>), voriconazole (78.9% for Bio-rad<sup>®</sup> and Neo-Sensitabs<sup>®</sup>), fluconazole (55.3% Bio-rad<sup>®</sup>, 50% Neo-Sensitabs<sup>®</sup>) and 5-fluorocytosine (34.2% Bio-rad<sup>®</sup>, Neo-Sensitabs<sup>®</sup> 7.9%) (Table 2).

The percentage agreement between the two methods was 100% for amphotericin B, 97.4% for fluconazole, 94.7% for voriconazole and 73% for 5-fluorocytosine (Table 3).



**Figure 1** The proportion of *Candida* species.  
Répartition des espèces de *Candida*.

## Discussion

Some progress has been achieved in recent years in the field of yeast antifungal susceptibility testing and previous studies on the comparison of antifungal susceptibility have been performed [4,7,8,12,16]. Our study was focused specifically on the comparison of the Neo-sensitabs<sup>®</sup> tablets technique to that of Bio-Rad<sup>®</sup> disks. The difference of susceptibility rate between the two methods was not statistically significant for all tested antifungal agents, except for 5-fluorocytosine and we observed an excellent agreement between the two methods for amphotericin B, fluconazole and voriconazole, but for the 5-fluorocytosine, the concordance rate was low. These methods were 100% concordant for susceptibility testing of *Candida* species to amphotericin B and in overall, this molecule was the most effective of all tested antifungals. Acquired resistance of *Candida* species to this molecule is exceptional [10,14]. Both methods were also 100% concordant for sensitivity testing of: *C. tropicalis*, *C. krusei*, and *C. dubliniensis* against voriconazole; *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. dubliniensis* against fluconazole; and *C. albicans* and *C. dubliniensis* against 5-fluorocytosine and they showed most mismatches with 5-fluorocytosine.

The Neo-sensitabs<sup>®</sup> tablets and Bio-rad<sup>®</sup> disks methods are used respectively by 8 and 2% of the centres which practice antifungal susceptibility testing in the French hospitals [1]. The Neo-sensitabs<sup>®</sup> method has been favorably compared to the reference methods: NCCLS and European Committee on Antimicrobial Susceptibility Testing (EUCAST) and it has been found that it can represent a cost effective and simple alternative to reference methods in clinical laboratories for testing the sensitivity of *Candida* species to antifungal agents [4,7,16]; in the study realized by Vandebossche et al. in Belgium, the correlation coefficient *r* between Neo-Sensitabs<sup>®</sup> and NCCLS methods was 0.873 [16]. Another study conducted in Spain in 2005, also showed a good correlation (*r* = 0.9) between EUCAST and Neo-Sensitabs<sup>®</sup> methods and the correlation coefficient was 0.93 for fluconazole, 0.90 for 5-fluorocytosine, 0.89 for amphotericin B and kétoconazole, and 0.88 for itraconazole [4]. The diameters of the inhibition zones of both tested methods are well defined and easy to read, which indicates that the two methods are very simple to use and the microbiologists need them for the routine antifungal susceptibility testing of *Candida* species specifically in developing countries because they are cheaper. The main disadvantage of

**Table 2** Susceptibility rates to antifungal agents of *Candida* isolates for two methods.  
Taux de sensibilité aux antifongiques des isolats de *Candida* sp pour les deux méthodes.

Antifungals	<i>C. albicans</i> n = 15	<i>C. glabrata</i> n = 13	<i>C. tropicalis</i> n = 5	<i>C. krusei</i> n = 4	<i>C. dubliniensis</i> n = 1	Total	P
<b>5-FLU</b>							
Bio-rad <sup>®</sup>	0	4	5	4	0	13	0.0049
	0%	30.8%	100%	100%	0%	34.2%	
Neo-sensitabs <sup>®</sup>	0	0	1	2	0	3	7.9%
	0%	0%	20%	50%	0%	7.9%	
<b>FLUC</b>							
Bio-rad <sup>®</sup>	0	12	5	0	0	21	0.64
	0%	92.3%	100.0%	0%	0%	55.3%	
Neo-sensitabs <sup>®</sup>	0	11	5	0	0	19	50.0%
	0%	84.6%	100.0%	0%	0%	50.0%	
<b>VCZ</b>							
Bio-rad <sup>®</sup>	7	13	5	4	1	30	1
	46.7%	100.0%	100.0%	100.0%	100.0%	78.9%	
Neo-sensitabs <sup>®</sup>	8	12	5	4	1	30	78.9%
	53.3%	92.3%	100.0%	100.0%	100.0%	78.9%	
<b>Am B</b>							
Bio-rad <sup>®</sup>	14	13	5	4	1	37	1
	93.3%	100.0%	100.0%	100.0%	100.0%	97.4%	
Neo-sensitabs <sup>®</sup>	14	13	5	4	1	37	97.4%
	93.3%	100.0%	100.0%	100.0%	100.0%	97.4%	

5-FLU: 5-fluorocytosine; FLUC: fluconazole; VCZ: voriconazole; AmB: amphotericin B.

**Table 3** The rate of concordance and discordance between the two methods for each tested antifungal.  
Taux de concordance et de discordance entre les 2 techniques pour chaque antifongique testé.

Antifungals	Concordance		Discordance	
	Number of concordance	%	Number of discordance	%
Amphotericin B	38	100	0	0
Fluconazole	37	97.4	1	2.6
Voriconazole	36	94.7	2	5.3
5-fluorocytosine	28	73	10	27

these methods resides in the absence of minimum inhibitory concentration values [4,7,16]. In addition, for the Bio-Rad<sup>®</sup> method, the diameters of interpretation zones are not yet available for all antifungal agents.

## Conclusion

The antifungal susceptibility testing is only one element among others to guide the clinician in the choice of the antifungal and its dosage. The dialogue between clinicians and mycologists is more essential than ever in this field. The mycologists need disk diffusion methods for routine clinical use because they are easy to perform, rapid and cost effective. Our results showed the best agreement between the two tested methods for testing the susceptibility of *Candida* isolates to amphotericin B, fluconazole and voriconazole while for the 5-fluorocytosine, the concordance rate was low.

## Disclosure of interest

The authors declare that they have no competing interest.

## References

- [1] Blanc V, Eloy O, Sanchez R. Pratique de l'antifongigramme dans 62 hôpitaux français. *J Mycol Med* 2005;15:197–210.
- [2] Clinical Laboratory Standard Institute/National Committee for Clinical Laboratory Standards. Method for antifungal disk diffusion susceptibility testing of yeasts: approved guideline. Document M44-A. Wayne, PA: Clinical and Laboratory Standard Institute; 2004.
- [3] Clinical Laboratory Standard Institute/National Committee for Clinical Laboratory Standards. Reference method for broth dilution susceptibility testing of yeasts: approved standard. 2nd Ed. Document M27-A2. Wayne, PA: Clinical and Laboratory Standard Institute; 2002.
- [4] Cuenca-Estrella M, Gomez-Lopez A, Mellado E, Rodriguez-Tudella JL. Correlation between the procedure for antifungal susceptibility testing for *Candida* sp. of the European Committee on Antibiotic Susceptibility Testing and four commercial techniques. *Clin Microbiol Infect* 2005;11:486–92.
- [5] Drouhet E, Barale T, Bastide J, Mallié M, Biava MF, Kures L, et al. Standardisation de l'antibiogramme antifongique. Rapport du groupe d'études de la Société française de mycologie médicale. *Bull Soc Myc Med* 1981;10:131–4.
- [6] Eloy O, Blanc V, Pina P, Gaudart A, Bressolle ML, Plainvert C, et al. Epidemiology of candidemia: results of a one-month French hospitals-based surveillance study in 2004. *Pathol Biol* 2006;54:523–30.
- [7] Espinel-Ingroff A, Canton A, Gibbs D, Wang A. Correlation of Neo-Sensitabs tablet diffusion assay results on three different agar media with broth microdilution M27-A2 and disk diffusion M44-A results for testing susceptibilities of *Candida* spp. and

- Cryptococcus neoformans* to amphotericin B, caspofungin, fluconazole, itraconazole, and voriconazole. J Clin Microbiol 2007;45:858–64.
- [8] Espinel-Ingroff A. Comparison of three commercial assays and a modified disk diffusion assay with two broth microdilution reference assay for testing zygomycetes, *Aspergillus* sp., *Candida* sp., and *Cryptococcus neoformans* with posaconazole and amphotericin B. J Clin Microbiol 2006;44:3616–22.
- [9] La Croix C, Dubach M, Feulhade M. Les échinocandines : une nouvelle classe d'antifongiques. [Echinocandins: a new class of antifungal agents]. Med Mal Infect 2003;33:183–91.
- [10] Kalkanci A, Berk E, Aykan B, Caglar K, Hizel K, Arman D, et al. Epidemiology and antifungal susceptibility of *Candida* species isolated from hospitalized patients. J Mycol Med 2007;17:16–20.
- [11] Peyron F, Favel A, Michel-Nguyen A, et al. Improved detection of amphotericin B-resistant isolates of *Candida lusitanae* by E-test. J Clin Microbiol 2001;39:339–42.
- [12] Rieu P, Eloy O, Bertout S, Mallié M, Bain P, Blanc V. Sensibilité comparée par CLSI, EUCAST, E-test et ATBW Fungus 2 des souches de *Candida* sp. isolées au cours d'une enquête épidémiologique sur les candidémies dans des hôpitaux non universitaires. J Mycol Med 2009;19:94–103.
- [13] Ruan SY, Hsueh PR. Invasive candidiasis: an overview from Taiwan. J Formos Med Assoc 2009;108:443–51.
- [14] Talarmin JP, Boutoille D, Tattevin P, Dargère S, Weinbreck P, Ansart S, et al. Épidémiologie des candidémies : étude observationnelle prospective d'un an dans l'Ouest de la France. Med Mal Infect 2009;39:877–85.
- [15] Toubas D. Épidémiologie des candidoses invasives. Rev Fr Lab 2013;450:27–36.
- [16] Vandenbossche I, Vaneechoutte M, Vandevenne M, De Baere T, Verschraegen G. Susceptibility testing of fluconazole by the NCCLS broth microdilution method, E-test, and disk diffusion for application in the routine laboratory. J Clin Microbiol 2002;40:918–21.