COMPARISON BETWEEN FOUR USUAL METHODS OF IDENTIFICATION OF *Candida* SPECIES

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SUMMARY

Infection by *Candida* spp. is associated with high mortality rates, especially when treatment is not appropriate and/or not immediate. Therefore, it is necessary to correctly identify the genus and species of *Candida*. The aim of this study was to compare the identification of 89 samples of *Candida* spp. by the manual methods germ tube test, auxanogram and chromogenic medium in relation to the ID 32C automated method. The concordances between the methods in ascending order, measured by the Kappa index were: ID 32C with CHROMagar *Candida* ($\kappa = 0.38$), ID 32C with auxanogram ($\kappa = 0.59$) and ID 32C with germ tube ($\kappa = 0.9$). One of the species identified in this study was *C. tropicalis,* which demonstrated a sensitivity of 46.2%, a specificity of 95.2%, PPV of 80%, NPV of 81.1%, and an accuracy of 80.9% in tests performed with CHROMagar *Candida;* and a sensitivity of 76.9%, a specificity of 96.8%, PPV of 90.9%, NPV of 91%, and an accuracy of 91% in the auxanogram tests. Therefore, it is necessary to know the advantages and limitations of methods to choose the best combination between them for a fast and correct identification of *Candida* species.

KEYWORDS: *Candida*; Auxanogram; ID 32C; Chromogenic medium; Germ tube.

INTRODUCTION

In recent decades, new diagnostic and therapeutic resources have contributed to alter the natural history of infectious, degenerative and immunosuppressive diseases. Such resources, on one hand, contribute to increase the patients' survival rate, but on the other, can generate "damage" to the host immunity, which becomes a victim of its own colonizing microbiota or organisms until then considered of low pathogenicity[26]. Among the systemic infectious diseases which have been increasing, especially since 1980, we can mention the ones caused by yeast, especially of the genus *Candida*[13].

The most common systemic infection caused by that yeast is candidemia, associated with high mortality rates, especially when the antifungal treatment is not immediate or adequate[27]. *Candida albicans* is considered the main opportunistic pathogenic yeast for being the most frequently isolated species in humans. However, in recent decades, a significant increase in other species such as *C. tropicalis*, and *C. krusei*[13,32] has been observed.

The identification of these yeasts at the species level is necessary in order to help in selecting the appropriate antifungal agent for the treatment of serious infections caused by these yeasts[10]. Some of the currently used methods of identification of yeasts from the genus *Candida* are the germ tube test, manual and automated methods of assimilation of carbohydrates and nitrogen (auxanogram), chromogenic culture media, and molecular techniques[31].

Among the various molecular techniques, the best known are: multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), duplex PCR, restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), length heterogeneity polymerase chain reaction (LH-PCR), and microsatellites[4,15]. The molecular techniques are accurate, sensitive, specific and relatively simple, and have the potential to greatly decrease the time of identification of pathogens that grow slowly or are difficult to grow[9,11,22,35]. Unfortunately, these methods also have their limitations: 1) DNA isolated from fungi need to be of suitable quality[14]; 2) the potential contamination with clinically irrelevant fungal species; 3) they require technical skills and expensive equipments[7]; 4) they are not available in all laboratories[33].

Considering the need to quickly and accurately identify *Candida* species, not only for their relevance in epidemiological studies, but also to choose the appropriate antifungal treatment so as to reduce the high mortality rate caused by these yeasts, other parameters such as the fact that not all laboratories have automated or semi-automated systems, and molecular biology-dedicated departments, it is worth to use a manual method that is fast, reliable and has high sensitivity and specificity. Therefore, this study aimed to compare the identification of *Candida* species by ID 32C automated method (bioMérieux, France) with manual

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methods of auxanogram, the germ tube test and the chromogenic medium, CHROMagar *Candida* (*Candida* CHROMagar, France).

MATERIAL AND METHODS

***Candida* samples:** Eighty-nine (89) isolates of *Candida* spp. were obtained from 89 different patients admitted to the *Irmandade Santa Casa de Misericórdia de Porto Alegre* (ISCMPA) during the period of January to December, 2009. These samples were isolated from different biological specimens, predominantly derived from blood cultures (66 samples), and the remaining samples from urine, ascites, and synovial fluids.

These isolates were previously identified by the ID 32C automated method by the Laboratory of Clinical Mycology of ISCMPA, being this method considered the gold standard in this laboratory. The other tests were performed in the Laboratory of Mycology of the *Universidade Federal de Ciências da Saúde de Porto Alegre* (UFCSPA).

The ID 32C automated method: The positive cultures were analyzed macroscopically and microscopically and confirmed by the ID 32C automated method - ATB Expression (bioMérieux, France). This method consists of a single-use disposable plastic strip with 32 wells containing substrates for 29 assimilation tests (carbohydrates, organic acids, and amino acids), one susceptibility test (cycloheximide), one colorimetric test (esculin), and a negative control[29].

The yeast identification procedures were conducted in accordance with the manufacturer's instructions (ID 32C, bioMérieux, France). To perform this, recent colonies (24h) were used for the preparation of a standardized suspension of 2 McFarland (McF) in a densitometer (ATB 1550, bioMérieux, France). Five drops of this suspension was then dispensed to an ampule of C medium (Medium C, bioMérieux, France) provided by the manufacturer and homogenized to prepare an even dispersion of inoculum. After homogenizing, the inoculum suspension was used to inoculate the wells in the strip, the lid of the strip was replaced, and the system was incubated at 30 °C[29]. Then, following the manufacturer's instructions, the reading of the test was performed after 24h, 48h and 72h of incubation in the equipment ATB Expression.

Germ tube test: The germ tube is a continuous prolongation of the mother cell produced at the beginning of the filamentation process of the yeast *C. albicans* and is considered a transitional form between the yeast and the mycelium[13]. The germ tube walls are parallel and there are no constrictions at the junction with the blastoconide, thus differing from the pseudo-hyphae present in the other species of *Candida*[24].

The germ tube test, described by TASCHDJIAN in 1960[34], is a simple screening method for the distinction of *C. albicans* from other yeast species, through observation of germ tube formation. To perform the germ tube test, a suspension was made in a tube containing 0.5 mL of human serum with a colony of the yeast sample and incubated at 37 °C for up to three hours[34]. After microscopic observation, the samples were considered *C. albicans* when they formed germ tube and *Candida* spp. when they did not form germ tubes.

Chromogenic medium - CHROMagar *Candida*: The chromogenic medium CHROMagar *Candida* (CHROMagar *Candida*, France) is available for the isolation and presumptive identification of *C. albicans*,

based on the pigmentation of the developing colonies, which is due to different enzyme activities from *Candida* species. This medium shows different color colonies for *C. albicans* (green), *C. tropicalis* (metallic blue, with a pink halo), and *C. krusei* (pink with velvety appearance)[10].

The samples identified as *Candida* spp. after the germ tube tests performed, were seeded in the CHROMagar *Candida*. The tests were performed according to the manufacturer's instructions: after 48h of incubation at 30 ºC, the results were interpreted according to the color of the colony formed.

Auxanogram: carbohydrates assimilation by manual method: The carbohydrate assimilation is the ability of yeast to grow aerobically in the presence of a particular carbohydrate supplied as the only energy source. The test is performed in a nitrogen base medium containing only one carbohydrate. The positivity of the test is measured by observing the growth of yeast in the presence of the carbohydrate supplied[31].

Eight liquid media of *Yeast Nitrogen Base* (Himedia, Brazil) were prepared, according to the manufacturer's instructions, each containing one of the following carbohydrates: glucose (positive control), raffinose, galactose, lactose, maltose, trehalose, saccharose or xylose. After 0.5 mL of each medium were diluted into tubes containing 4.5 mL of sterile distilled water and 0.5 McF suspension of *Candida* sample. Following the kit instructions, the tubes were incubated at 25 °C for seven days and, when there was no growth observed during this period in samples containing xylose and galactose, they were incubated for a further 14 days due to possible delayed growths characteristic of these carbohydrates. The results were classified as negative, weakly negative, weakly positive and positive, according to the turbidity.

Auxanogram: nitrogen assimilation by manual method: The nitrogen assimilation is the ability of yeast to grow aerobically in the presence of particular nitrogen supplied as the only energy source. The test is performed in a carbon base medium containing just one particular nitrogen. The positivity of the test is measured by observing the growth of yeast in the presence of the nitrogen supplied[31].

Two liquid media of *Yeast Carbon Base* (Himedia, Brazil) were prepared according to the manufacturer's instructions: mycological peptone nitrogen compound (positive control) and potassium nitrate. Afterwards, 0.5 mL of each medium was diluted into tubes containing 4.5 mL of sterile distilled water and 0.5 McF suspension of *Candida* sample. The tubes were incubated at 25 °C for six days, following the kit instructions. The results were classified as positive when samples had growth in the medium with potassium nitrate.

From the observations of the results of the auxanogram tests (assimilation of carbohydrates and nitrogen) it was possible to identify the species of the samples.

Statistical analysis: The agreement between methods was analyzed by the Kappa (k) coefficient test, and the diagnostic performance of the manual methods (sensitivity, specificity, predictive values and accuracy) was compared to the ID 32C (gold standard). The ID 32C system was chosen because it has been proven to be accurate, with the advantage of having an extensive database[12] and being a universally validated method[11].

The Kappa agreement test was interpreted as follows: Poor, if $\kappa = 0$, Slight, if $\kappa = 0$ to 0.20, Considerable, if $\kappa = 0.21$ to 0.40, Moderate if $\kappa = 0.41$ to 0.60, Substantial, if $\kappa = 0.61$ to 0.80, or Excellent, if $\kappa = 0.81$ to 1. The program used for calculations was the SPSS 16.0.

Ethical guidelines: This study was approved by the Research Ethics Committee of UFCSPA, process number 852/11.

RESULTS

The frequencies of identification of the 89 samples by the different methods were described in Table 1. When it was not possible to determine the species of a sample, the result was classified as *Candida* spp.

In order to measure the agreement between the ID 32C with the manual methods, the samples were grouped into new groups containing only species that could be identified by both methods (Table 2). The agreement between the germ tube with the ID 32C, considering groups as *"albicans"* and *"non-albicans"*, was $\kappa = 0.9$. In CHROMagar *Candida*, the samples were grouped as *"albicans"*, *"krusei"*, *"tropicalis"* and "other species", and the result obtained was $\kappa = 0.38$. To calculate the correlation between the ID 32C and auxanogram, the samples were grouped as *"albicans"*, *"krusei"*, *"famata"*, *"tropicalis"*, *"parapsilosis"*, *"glabrata"* and *"guilliermondii"*, and the result was $\kappa = 0.59$.

The diagnostic performance of the manual methods and the

concordance between the methods in relation to the ID 32C were calculated for the two more frequent species, i.e., *C. albicans* and *C. tropicalis*. However, we could not calculate the performance of the germ tube test for *C. tropicalis*, since the test is not able to identify this species. The results for *C. albicans* were: germ tube test - 89.3% sensitivity, 96.7% specificity, 92.6% positive predictive value (PPV), 95.2% negative predictive value (NPV), 94.4% accuracy and $\kappa = 0.9$; CHROMagar *Candida* - 78.6% sensitivity, 88.5% specificity, 75.9% PPV, 90% NPV, 85.4% accuracy and $\kappa = 0.7$; Auxanogram - 71.4% sensitivity, 95.1% specificity, 87% PPV, 87.9% NPV, 87.6% accuracy and $\kappa = 0.6$ (Table 3). The results for *C. tropicalis* were: CHROMagar *Candida* - 46.2% sensitivity, 95.2% specificity, 80% PPV, 81.1% NPV, 80.9% accuracy and $\kappa = 0.5$; Auxanogram - 76.9% sensitivity, 96.8% specificity, 90.9% PPV, 91% NPV, 91% accuracy and $\kappa = 0.8$ (Table 4).

DISCUSSION

Among the 89 strains isolated from patients admitted to the ISCMPA from January to December 2009, the species *C. albicans* was the most prevalent (31.46%), followed by *C. tropicalis* (29.21%) and *C. parapsilosis* (21.35%), according to the ID 32C method. In the study by ANTUNES *et al*.[1], which identified 120 *Candida* isolates from patients admitted to the ISCMPA from August 2002 to August 2003, a prevalence of 48.3% of *C. albicans*, 25.8% of *C. parapsilosis* and 13.3% of *C. tropicalis* were found. Thus, a change was noticed in the frequency of species isolated from patients admitted to this hospital in

Table 1

Comparison of frequencies and percentages found in the results obtained by methods ID 32C, germ tube test, CHROMagar *Candida* and auxanogram

Specie	ID 32C	Germ tube test	CHROMagar *Candida*	Auxanogram
C. albicans	28 (31.46%)	27 (30.34%)	29 (32.58%)	23 (25.83%)
C. tropicalis	26 (29.21%)	0	15 (16.85%)	22 (24.72%)
C. parapsilosis	19 (21.35%)	0	0	13 (14.61%)
C. glabrata	5 (5.62%)	0	0	2 (2.25%)
C. pulcherria	3 (3.39%)	0	0	0
C.guilliermondii	2 (2.25%)	0	0	1 (1.12%)
C. dubliniensis	1 (1.12%)	0	0	0
C. famata	1 (1.12%)	0	0	2 (2.25%)
C. krusei	1 (1.12%)	0	21 (23.6%)	2(2.25%)
C. lambica	1 (1.12%)	0	0	0
C. lipolytica	1 (1.12%)	0	0	0
C. sake	1 (1.12%)	0	0	0
C. catenulata	0	0	0	2 (2.25%)
C. ciferri	0	0	0	1 (1.12%)
C. pelliculosa	0	0	0	4 (4.50%)
C. rugosa	0	0	0	4 (4.50%)
C. zeylanoides	0	0	0	1 (1.12%)
Candida spp.	0	62 (69.66%)	24 (26.97%)	12 (13.48%)
Total	89 (100%)	89 (100%)	89 (100%)	89 (100%)

Table 2
Agreement between the methods compared to the ID 32C. The samples were grouped into groups containing only species that could be identified by both methods

Groups	Germ tube		CHROMagar		Auxanogram	
	N	kappa	N	kappa	N	kappa
C. albicans	27		29		23	
C. famata	-		-		2	
C. glabrata	-		-		2	
C. guilliermondi	-		-		1	
C. krusei	-	0.9	21	0.38	2	0.59
C. parapsilosis	-		-		13	
C. tropicalis	-		15		22	
Other species	62		24		24	
Total	**89**		**89**		**89**	

recent years, reducing the number of *C. albicans* infections and increasing the number of infections caused by *Candida* non-*albicans* species such as *C. tropicalis*.

Although the *C. albicans* is the most common etiologic agent in candidiasis, other species have emerged as important opportunistic pathogens, such as *C. tropicalis*[28]. This has been shown in this work, since 68.54% of the samples were identified as *Candida* non-*albicans*, which is consistent with other studies showing that the majority of species isolated in this genus are classified as non-*albicans*[1, 21, 32].

There was similarity between the frequency of *C. albicans* identified by the methods ID 32C (31.46%), germ tube test (30.34%) and CHROMagar *Candida* (32.58%), however the auxanogram showed a lower frequency for this species (25.83%). There were differences in the identification of other species by CHROMagar *Candida*. The most prevalent species in this method was *C. albicans* (32.58%), followed by *C. krusei* and *C. tropicalis*, accounting for 23.6% and 16.85%, respectively. In contrast to the high frequency of samples identified as *C. krusei* by CHROMagar *Candida*, the ID 32C identified this species only in 1.12% of samples, while in auxanogram the frequency was 2.25%.

Table 3
Diagnostics performances (sensitivity, specificity, predictive value and accuracy) of manual methods and concordance between the methods in relation to the ID 32C, considering only the species *C. albicans*

C. albicans	ID 32C (+)	ID 32C (-)	Total	Sensibility (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Kappa
Germ tube (+)	25	2	27						
Germ tube (-)	3	59	62	89.3	96.7	92.6	95.2	94.4	0.9
Total	**28**	**61**	**89**						
CHROMagar (+)	22	7	29						
CHROMagar (-)	6	54	60	78.6	88.5	75.9	90.0	85.4	0.7
Total	**28**	**61**	**89**						
Auxanogram (+)	20	3	23						
Auxanogram (-)	8	58	66	71.4	95.1	87.0	87.9	87.6	0.6
Total	**28**	**61**	**89**						

Table 4
Diagnostics performances (sensitivity, specificity, predictive value and accuracy) of manual methods and concordance between the methods in relation to the ID 32C considering only the species *C. tropicalis*

C. tropicalis	ID 32C (+)	ID 32C (-)	Total	Sensibility (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Kappa
CHROMagar (+)	12	3	15						
CHROMagar (-)	14	60	74	46.2	95.2	80	81.1	80.9	0.5
Total	**26**	**63**	**89**						
Auxanogram (+)	20	2	22						
Auxanogram (-)	6	61	67	76.9	96.8	90.9	91.0	91.0	0.8
Total	**26**	**63**	**89**						

The ID 32C method was able to identify the species of all the isolates, while 69.66%, 26.97% and 13.48% of the samples tested by germ tube test, CHROMagar *Candida* and auxanogram, respectively, were not able to identify samples at the species level, being classified as *Candida* spp.

Considering the Kappa index, showed in Table 2, the classifications of the concordance between the methods were: considerable concordance between ID 32C and CHROMagar *Candida* (κ = 0.38), moderate concordance between ID 32C and auxanogram (κ = 0.59) and excellent concordance between ID 32C and germ tube test (κ = 0.9). Thus, it was observed that CHROMagar *Candida* is the test that agrees less with the ID 32C, and the best agreement was found between ID 32C and the germ tube test.

The agreement between the methods considering only the species *C. albicans* with other "non-*albicans*" (Table 3) was classified as moderate between ID 32C and auxanogram (κ = 0.60), substantial between ID 32C and CHROMagar *Candida* (κ = 0.7) and excellent between ID 32C and the germ tube test (κ = 0.9). Therefore, considering the species *C. albicans*, all methods have a satisfactory concordance.

When analyzing the results obtained in Table 3, it can be observed that the CHROMagar *Candida* showed low sensitivity (78.6%) and low PPV (75.9%) for the identification of *C. albicans*, i.e., only 78.6% of positive samples for this species were classified as *C. albicans* and the probability of success (correct identification) when the test is positive is 75.9%. The specificity was 88.5% with a NPV of 90%, indicating that the probability of the test to yield a negative result in the absence of disease is 88.5%, and the probability for the sample to be actually negative for *C. albicans* when the test result is negative is 90%. The accuracy found was 85.4%, i.e., 85.4% of the samples were correctly classified. The values found in this study differ from other studies, where sensitivity and specificity rates were found to be more than 90% for this species[2,3,8,10,20]. A recent example was shown in the study of DAEF *et al.*[8] in which the sensitivity and specificity of CHROMagar *Candida* were determined as 96.9% and 97.9% for *C. albicans*.

The auxanogram tests for identification of *C. albicans* showed a slightly higher accuracy than the CHROMagar *Candida* (87.6%). The sensitivity for the identification of *C. albicans* was lower compared to CHROMagar *Candida* (71.4%), but with a higher PPV (87%) and high specificity (95.1%), with a NPV of 87.9%. The lowest sensitivity of auxanogram for the identification of *C. albicans* is consistent with the lowest frequency found for this species compared to other methods.

Finally, the germ tube test has a very good diagnostic performance with high accuracy values: (94.4%), sensitivity (89.3%), specificity (96.7%), PPV (92.6%) and NPV (95.2%). All results found with the germ tube test were more significant than with the other two methods analyzed for the species *C. albicans*.

In relation to the species *C. tropicalis*, as shown in Table 4, we can observe that the diagnostic performance of auxanogram was more sensitive than the CHROMagar's one: 76.9% and 46.2%, respectively, however the specificities were similar (96.8% and 95.2%, respectively); the auxanogram also showed a higher predictive values compared to CHROMagar (PPV = 90.9% and 80%, respectively; NPV = 91% and 81.1%, respectively) and also higher accuracy (91% and 80.9%, respectively).

In our study, an important finding in the identification of the species *C. krusei* was that 14 of 21 samples (66.67%) identified as *C. krusei* by CHROMagar *Candida* were identified *as C. parapsilosis* by ID 32C and/or the auxanogram tests (data not shown). Such misclassification of species was also found in the study by ESTRADA-BARRAZA *et al.*[11]. In this study, it was not possible to calculate the diagnostic performance of the species *C. krusei,* since it was found in only one sample, according to the gold standard ID 32C. Thus, further studies should be performed. However, analyzing the study of BALLESTÉ *et al.*[2], they found a very low PPV (20%) for the identification of *C. krusei*, meaning that only 20% of the samples classified as positive by the CHROMagar were actually of this species, indicating that the test produces many false-positive results.

Another observation is that the species *C. parapsilosis* is a complex of three species (*C. parapsilosis, C. metapsilosis* and *C. orthopsilosis*), however, since the phenotypic differentiation of this complex is only made at the molecular level[23], we did not make the distinction among the three species of the complex.

Some laboratories have elected the ID 32C as the gold standard due to its large number of carbohydrate assimilation tests, reducing the use of complementary microscopic tests[11]. However, not all laboratories that provide services of mycological diagnostics have sufficient demand to maintain an expensive equipment like this.

The germ tube test is the gold standard of laboratories for the identification of *C. albicans*[5], because besides being a quick and simple technique, among all *Candida* species, this is the only method able to detect germ tube formation in the presence of human or animal serum when samples are incubated at 37 °C for two to four hours[17,27]. Moreover, as evidenced in this study, it has a high diagnostic performance for the identification of *C. albicans*. Despite the easy implementation and low cost, the germ tube test does not identify non-*albicans* species of *Candida* and cannot be considered absolute, because 5-10% of *C. albicans* do not form germ tubes[18,19]. In addition, there are reports indicating that other species of non-*albicans Candida*, such as *C. tropicalis* and *C. parapsilosis* can produce very similar structures[6].

The CHROMagar *Candida* has the advantages of being a cheap and quick method, able to detect the presence of mixed cultures[10]. However, only *C. albicans, C. tropicalis* and *C. krusei* can be observed[16,25,26,30], although some studies suggest that this medium allows the identification of the species *C. glabrata* and *C. dubliniensis*[16,25]. Also, in this study, we demonstrated that the CHROMagar was the test that agreed less with the test considered the gold standard, i.e., the ID 32C, besides presenting a low sensitivity for the species *C. tropicalis* and producing many false positive results for *C. krusei*.

In relation to the auxanogram, it has disadvantages such as being laborious, taking at least a week to get the results and being highly susceptible to contamination. However, an advantage observed in this study was its better diagnostic performance for the detection of *C. tropicalis* in relation to the CHROMagar.

Among the techniques analyzed, we conclude that the traditional germ tube test showed the highest sensitivity for the identification of *C. albicans*, however when the result is non-*albicans Candida,* additional tests are required. The ID 32C automated method is efficient in identifying

other species of *Candida.* However, the use of this method is limited by the high cost. The CHROMagar *Candida* has been previously reported as effective in the laboratory routine, however it showed the least concordance with the ID 32C, the gold standard in this study, besides a low sensitivity for the species *C. tropicalis.* The auxanogram tests have good accuracy, but are more time consuming than other methods.

A rapid and accurate identification of a patient with a *Candida* spp. infection will impact the mortality rates, the costs of treatment and the hospitalization time[10]. Thus, we conclude that the knowledge of the advantages and limitations of methods is necessary to choose the best combination of methods according to the size of the laboratory and to make a critical evaluation of the results obtained.

RESUMO

Comparação entre quatro métodos usuais de identificação de espécies de *Candida*

A infecção por *Candida* spp. está associada com alta mortalidade, principalmente quando o tratamento não é adequado, nem imediato. Assim, a correta identificação do gênero e espécie é necessária. O objetivo deste trabalho foi comparar 89 amostras de *Candida* spp. pelos métodos manuais prova do tubo germinativo, auxanograma e CHROMagar em relação ao método automatizado ID 32C. As concordâncias entre os métodos em ordem crescente, medidas pelo coeficiente de Kappa, foram: ID 32C com CHROMagar *Candida* ($\kappa = 0,38$), ID 32C com auxanograma ($\kappa = 0,59$) e ID 32C com tubo germinativo ($\kappa = 0,9$). Uma das espécies identificadas neste trabalho foi a *C. tropicalis*, que demonstrou uma sensibilidade de 46,2%, especificidade de 95,2%, VPP de 80%, VPN de 81,1% e acurácia de 80,9% nos testes com CHROMagar *Candida* e uma sensibilidade de 76,9%, especificidade de 96,8%, VPP de 90,9%, VPN de 91% e acurácia de 91% nos testes de auxanograma. Portanto, o conhecimento das vantagens e limitações dos métodos é necessário para a escolha da melhor combinação entre os mesmos visando uma rápida e correta identificação das espécies de *Candida*.

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