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Performance of Chromogenic *Candida* Lab-Agar® Medium in Presumptive Identification of *Candida* Species from Clinical Samples at Sourô Sanou University Hospital of Bobo-Dioulasso, Burkina Faso

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Abstract: Introduction: The incidence of *Candida* infections is increasing worldwide. In clinical laboratories of resource-constrained countries, *Candida* speciation is commonly limited to germ tube tests and culture onto a chromogenic medium. In this study, we evaluated the diagnostic performance of Chromogenic *Candida* Lab-Agar® (CCL) in identifying *Candida* species from clinical samples. **Methods**: We evaluated the diagnostic performance of CCL with 83 yeast isolates collected from 73 clinical samples at the laboratory department of Sourô Sanou University Hospital of Bobo-Dioulasso, Burkina Faso. Clinical specimens included vaginal swabs, urine, and blood cultures. After preliminary isolation on Sabouraud chloramphenicol agar, yeast isolates were inoculated onto the CCL medium and incubated at 35 °C for 48 h. Matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF MS) and ribosomal DNA internal transcribed spacer (ITS) sequencing

were used as reference methods. **Results**: Among yeast species, *Candida albicans* was the most prevalent (43.4%), followed by *C. krusei* (13.3%), *C. glabrata* (12.0%), *C. kefyr* (8.4%), and *C. tropicalis* (7.2%). The overall agreement rate of CCL was 56.6% and varied across *Candida* species; it was 94.4% for *C. albicans*, 50% for *C. glabrata*, 18.2% for *C. krusei,* and 33.3% for *C. tropicalis*. **Conclusions**: This study showed that CCL had moderate accuracy in identifying *Candida* at the species level from clinical specimens in a routine laboratory in Burkina Faso. The misidentification of non-*albicans* species may expose patients to inadequate antifungal treatment. Therefore, identifying yeast in a routine based on CCL is not enough and should be associated with more accurate methods.

Keywords: *Candida* species; chromogenic medium; performance; Bobo-Dioulasso

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1. Introduction

Candidiasis is an infection due to fungi of the *Candida* genus and constitutes a significant cause of morbidity and mortality in humans [1]. Candida albicans is the most common yeast identified in clinical samples. However, recent studies showed a rising proportion of non-*albicans Candida* species worldwide, including *C. glabrata*, *C. krusei*, *C. tropicalis*, and *C. parapsilosis* [1–5]. Therefore, candidiasis management is more and more challenging due to the geographical diversity of *Candida* species and their variable profile of susceptibility against antifungal agents [6–10]. The identification of *Candida* at the species level in clinical samples is, therefore, a prerequisite for the therapeutic decisions and guidance of local strategies in the prophylaxis and empirical treatment of candidiasis [11].

In most clinical laboratories in resource-limited settings, the diagnosis of *Candida* species relies upon germ tube testing, culture on chromogenic medium, or conventional biochemical testing. The chromogenic agar medium is one of the common methods used especially since it is cost-effective, easy to use, and allows the presumptive identification of the main pathogenic *Candida* species [12–14].

In the current study, we analysed the performance of Chromogenic *Candida* Lab-Agar® (BioMaxima Biocorp, Warsaw, Poland), used in routine practices in the laboratory of the Sourô Sanou University Hospital, for the identification of pathogenic yeast at the species level. MALDITOF-MS and DNA ITS sequencing were considered reference methods.

2. Methods

2.1. Strain Collection

We analysed a collection of 83 yeast strains isolated from 73 clinical samples at the laboratory department of Sourô Sanou University Hospital in Bobo-Dioulasso, Burkina Faso. Clinical specimens

were obtained during a five-month period (January to May 2021) and were from various clinical origins, including vaginal swabs (45), urine (27), and blood (1). Clinical samples were first streaked on Sabouraud Dextrose Agar containing chloramphenicol (SDA) and incubated at 35 °C for 48 h. Yeast-like colonies yielded on SDA were subsequently identified on the CCL medium.

2.2. Chromogenic Medium Identification

The chromogenic *Candida* lab-agar® medium (BioMaxima Biocorp, Warsaw, Poland) was prepared from dehydrated powder, in accordance with the manufacturer's instructions. Agar plates were stored at 2–8 °C and equilibrated at room temperature before use.

Yeast-like colonies on SDA were inoculated onto CCL plates by streaking them with sterile plastic loops. Inoculated plates were then incubated at 35 °C for 48 h. *Candida* species identification was performed based on the visual observation of the colour and morphology of the yeast colonies in accordance with the manufacturer's guidelines. Isolates showing atypical colours were considered undetermined species. Chromogenic *Candida* Lab-Agar® allowed the identification of four *Candida* significant species (*C. albicans, C. glabrata, C. krusei,* and *C. tropicalis*), as shown in Figure 1. After CCL identification, yeast isolates were aliquoted in distilled water and stored at −20 °C.

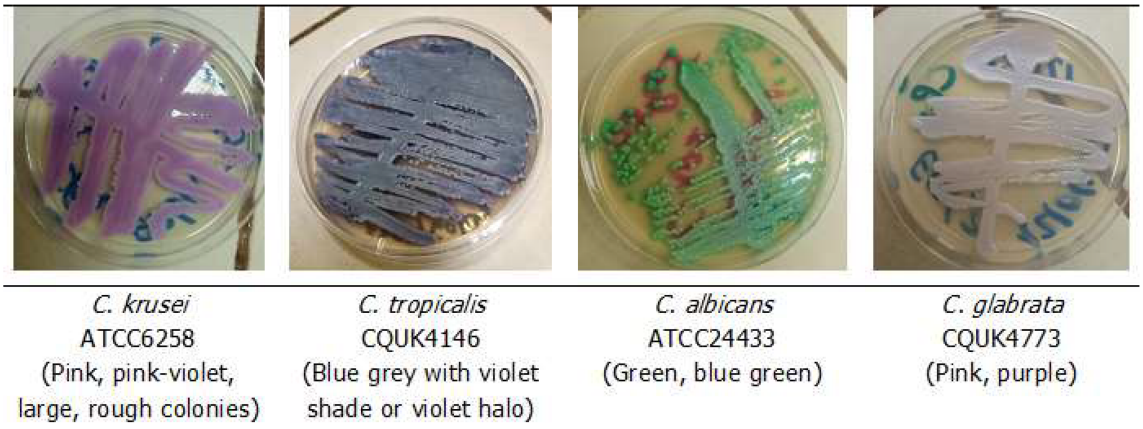

Figure 1: Colour and appearance of quality control strains on chromogenic Candida Lab-Agar® medium after 48 h of incubation at 35 °C.

2.3. Reference Methods

The yeast isolates were secondarily identified at the microbiology department of Cliniques Universitaires Saint-Luc, UCLouvain (Brussels, Belgium) using matrix-assisted laser desorption ionisation time of Flight (MALDI-TOF) mass spectrometry (Brucker Daltoniks, Bremen, Germany) and DNA sequencing.

MALDI-TOF-mass spectrometry identification: Fresh colonies were obtained from aliquots via culturing on Sabouraud chloramphenicol and a gentamicin dextrose agar plate (Dickinson, Sparks, MD, USA) and incubated for 24 h at 35 °C. A single colony was directly spotted onto the MALDI-TOF target plate and overlaid with 1 µL of 70% formic acid. As soon as the spot dried, 1 µL of a matrix (α-cyano-4-hydroxycinnamic acid) was applied and allowed to dry at room temperature. Then, the target plate was placed in the MALDITOF ionisation chamber. The spectra were analysed using the Microflex™ LT/SH smart system with MBT Compass IVD software plus the MBT IVD library. An identification score of 1.7 or above was accepted as a reliable result at the species level. Isolates showing lower scores were re-identified after the performance of the ethanol–formic acid extraction method in accordance with the manufacturer's recommendations. Isolates that failed to be identified via MALDI-TOF (score <1.7) were finally identified via DNA sequencing.

DNA sequencing: DNA extraction was carried out from lysed yeast aliquots using an automated DNA extractor Qiasymphony® SP (QIAGEN, Carlsbad, CA, USA). The internal transcribed spacer regions (ITS1-5.8s-ITS2) of ribosomal DNA were amplified via PCR using specific primers, ITS4 (5′ TCC-TCC-GCT-TAT-TGA-TAT-GC-3′) and ITS5 (5′-GGA-AGT-AAA-AGT-CGT-AAC-AAG-G-3′) [15]. The thermocycling program used was initial denaturation (at 95 °C for 10 min), 30 cycles of denaturation (at 95 °C for 30 s), annealing (at 60 °C for 30 s) and elongation (at 72 °C for 1 min), and then a stabilisation (at 72 °C for 7 min).

The PCR product was preliminarily run on 3% agarose gel and purified using the Illustra ExoProstar® kit (GE Healthcare UK). Sanger sequencing of the amplicons was performed using the Big Dye® Terminator v3.1 Cycling sequencing kit with the following conditions: initial denaturation (at 96 °C for 1 min), 25 cycles of denaturation (at 96 °C for 10 s), annealing (at 50 °C for 5 s), elongation (at 60 °C for 4 min), and then stabilisation at 10 °C for up to 24h. The sequences were purified through Sephadex® G50 DNA Grade and sequence analysis was performed on 3500xL Dx Genetic Analyzer® (Applied Biosystems, Foster City, CA, USA). The obtained sequence data were interpreted using bioinformatic software (Geneious Prime®, Version 2021.2.2) and fungal databases "http://www.mycobank.org, accessed on 07 September 2021)".

2.4. Data and Statistical Analysis

Identified strains on the CCL medium were categorised as *C. albicans*, *C. krusei*, *C. glabrata*, *C. tropicalis*, and undetermined (for atypical colours due to the presence of yeast species other than the four mentioned).

Chromogenic *Candida* Lab-Agar® diagnostic performance was determined through the calculation of the overall agreement between CCL and reference methods, and for each species, we determined the sensitivity, specificity, and positive and negative predictive value.

Calculations and statistical analysis were performed using Microsoft Excel (Microsoft corporation, Redmond, WA, USA).

2.5. Administrative Authorisation

Administrative authorisation was obtained from Sourô Sanou University Hospital authorities prior to yeast strain collection.

3. Results

3.1. Distribution of Yeast Species

The distribution of all 83 yeast isolates according to the clinical source is shown in Table 1. Reference methods allowed identification of 13 species belonging to three genera: *Candida*, *Cyberlindnera,* and *Trichosporon*. Among the yeast species, *C. albicans* was the most prevalent (43.37%). Overall, non-*albicans Candida* strains accounted for 51.81% of isolates, with a predominance of *C. krusei* (13.25%), *C. glabrata* (12.05%), *C. kefyr* (8.43%), and *C. tropicalis* (7.22%). The four *Candida* species identifiable on CCL represented 75.90% of the study sample. Two *Trichosporon* spp. were recovered from urine and genital swab. MALDITOF-MS allowed the speciation of all the strains except five, which were identified using DNA sequencing. The strains identified via DNA sequencing included *Meyerozyma caribbica* (anamorph *Candida fermentati)* and *Diutina mesorugosa* (anamorph *Candida mesorugosa)*.

Table 1: Distribution of 83 yeast isolates according to the species and clinical origins.

Yeast Species *	Overall		Genital		Urine		Blood	
	n = 83	%	n = 48	%	n = 33	%	n = 2	%
C. albicans	36	43.37	23	47.92	12	36.37	1	50.00
C. krusei	11	13.25	4	8.34	7	21.21	-	-
C. glabrata	10	12.05	8	16.67	2	6.06	-	-
C. tropicalis	6	7.22	2	4.17	4	12.12	-	-
C. kefyr	7	8.43	6	12.50	1	3.03	-	-
C. lusitaniae	2	2.41	1	2.08	1	3.03	-	-
C. rugosa	1	1.21	-	-	1	3.03	-	-
C. mesorugosa	2	2.41	1	2.08	1	3.03	-	-
C. guilliermondii	1	1.21	-	-	1	3.03	-	-
C. fermentati	3	3.61	1	2.08	1	3.03	1	50.00
Cy. fabianii	2	2.41	1	2.08	1	3.03	-	-
T. asahii	1	1.21	-	-	1	3.03	-	-
T. inkin	1	1.21	1	2.08	-	-	-	-

* Identification via MALDI-TOF MS and DNA sequencing. *C.*: *Candida; Cy.*: *Cyberlindnera*; and *T.*: *Trichosporon.*

3.2. Candida Species Identification on CCL Medium

The chromogenic *Candida* Lab-Agar® medium allowed the identification of 72/83 (86.7%) yeast isolates at the species level (Table 2). Almost all *C. albicans* isolates were correctly identified using the CCL medium except for two isolates. A green to blue-green colony colour on the CCL medium (specific to *C. albicans*) was also encountered with other species including *C. tropicalis* (n = 4), *C. glabrata* (n = 3), *Trichosporon* spp. (n = 2), *C. kefyr* (n = 2), *C. fermentati* (n = 1), or *C. krusei* (n = 1). Twelve isolates including *C. krusei* (n = 4), *C. kefyr* (n = 2), *Cyberlindnera fabianii* (n = 2), *C. fermentati* (n = 2), *C. guilliermondii* (n = 1), and *C. lusitaniae* (n = 1) were misidentified as *C. glabrata* on the CCL medium. Three isolates of *C. kefyr* and one of *C. glabrata* were incorrectly identified as *C. krusei* using the CCL medium.

Table 2: Identification of 83 yeast isolates via CCL in comparison with the reference methods.

Yeast Species *	CCL Identification					
	C. albicans	***C. glabrata***	***C. krusei***	***C. tropicalis***	**Undetermined**	**Total**
C. albicans	34	0	0	0	2	36
C. glabrata	3	5	1	0	1	10
C. krusei	1	4	2	0	4	11
C. tropicalis	4	0	0	2	0	6
C. kefyr	2	2	3	0	0	7
C. lusitaniae	0	1	0	0	1	2
C. rugosa	0	0	0	0	1	1
C. mesorugosa	0	0	0	0	2	2
C. guilliermondii	0	1	0	0	0	1
Cy. fabianii	0	2	0	0	0	2
C. fermentati	1	2	0	0	0	3
T. asahii	1	0	0	0	0	1
T. inkin	1	0	0	0	0	1
Total	47	17	6	2	11	83

* Identification via MALDI-TOF MS and DNA sequencing. *C.*: *Candida*; *Cy.*: *Cyberlindnera*; and *T.*: *Trichosporon.*

3.3. Chromogenic Candida Lab-Agar® Performance

The overall agreement rate between the CCL medium and reference methods was 56.63% (47/83). The performance of CCL varied among *Candida* species. Compared to the reference methods, the sensitivity and specificity of the CCL medium for the detection of *C. albicans* were 94.44% and 72.34%, respectively. Chromogenic *Candida* Lab-Agar® sensitivity values in the identification of non-*albicans Candida* species were 50%, 18.18%, and 33.33% for *C. glabrata, C. krusei,* and *C. tropicalis,* respectively (Table 3).

Table 3: Diagnostic performance of CCL in comparison to reference methods.

***Candida* Species ***	**No. of CCL Concordant**	**No. of CCL Discordant**	**Sensitivity (%)**	**Specificity (%)**	**PPV ** (%)**	**NPV *** (%)**
C. albicans (36)	34	13	94.44	72.34	72.34	94.44
C. glabrata (10)	5	12	50.00	83.56	29.41	92.42
C. krusei (11)	2	4	18.18	94.44	33.33	88.31
C. tropicalis (6)	2	0	33.33	100	100	95.06

* Identification via MALDI-TOF MS and DNA sequencing. ** Positive Predictive value; *** negative Predictive value.

4. Discussion

This study evaluated the diagnostic performance of Chromogenic *Candida* Lab-Agar® media (BioMaxima Biocorp, Warsaw, Poland) in identifying the most prevalent *Candida* spp. from clinical samples in routine laboratory work in Burkina Faso.

Several methods are used to identify *Candida* at the species level in clinical laboratories. Progressively, classic morphological and biochemical methods are being replaced by new ones based on molecular or proteomic approaches, allowing more rapid and more accurate identification [12,16]. For routine use in clinical microbiology laboratories, the choice of method for identifying yeast species depends on several factors including the affordability and reliability of the test, and the time consumed [17]. In most clinical laboratories of resource-rich countries, MALDI-TOF MS became the routine method and gold standard for bacterial and fungal identification due to its ease of use, rapidity, and accuracy [18,19]. However, the investment required for its equipment, software, and maintenance constrains its access to resource-limited settings, where other diagnostic tools such as the germ tube test, and subculture on chromogenic media are still the most commonly used methods in routine work. Indeed, chromogenic media are a simple, lower-cost method allowing the diagnosis of the major pathogenic Candida species [20]. On chromogenic media, Candida spp. are identified via the colour and appearance produced through the interaction of the chromogenic substrate and species-specific enzymes secreted by the yeast when growing on the medium. There are many commercially available chromogenic media for *Candida* identification, with variable performance depending on the manufacturer, strain origins, and experimental conditions. Overall, these media showed variable diagnostic performance with sensibilities and specificities ranging from 49.6 to 100% and 56.3 to 100%, respectively [21–25].

To the best of our knowledge, this study was the first of its kind of the analysis performed evaluating the diagnostic performance of the CCL medium in a routine clinical laboratory setting.

Our results showed a moderate performance of CCL (56.63%) for the identification of *Candida* species at the clinical laboratory of the Sourô Sanou University Hospital (Bobo-Dioulasso). Indeed, the sensitivity and specificity of the CCL medium in detecting *C. albicans* are good and concordant with the performances of other chromogenic media. In contrast, concerning non-*albicans Candida* species including *C. glabrata, C. tropicalis*, and *C. krusei,* the sensitivity of the CCL medium is inferior that in the data from the literature [22,26,27].

In routine practices, the difference in colony colours between *Candida* species was less striking than mentioned by the manufacturer. The expected species-specific colour may also be shown with other species. Indeed, a species-specific enzyme reacting with the chromogenic substrate might be produced by distinct yeast species [27–30]. Furthermore, the variation in enzyme production among strains of the same *Candida* species could cause misidentification, as reported by other authors [26,31].

In our study, *C. albicans* (43.37%) was the most common yeast encountered in all clinical sources. This result is consistent with previous reports from symptomatic patients in Burkina Faso and other parts of the world [5,32–36]. In this study, *Candida* non-*albicans* species together represented a greater proportion than *C. albicans* did, meaning that in Burkina Faso, almost half of suspected candidiasis cases are caused by non-*albicans* species. Indeed, there is a rising prevalence of candidiasis caused by non-*albicans Candida* species worldwide [37–39]. The use of accurate diagnostic methods (MALDITOF MS and ITS sequencing) allowed the identification of uncommon species, including *C. rugosa*, *C. mesorugosa*, *C. guilliermondii,* and *C. fermentati*, not described before at the SSUH of Bobo-Dioulasso.

In this context, the accurate identification of *Candida* spp. and yeast-like organisms such as *Trichosporon* spp. is crucial for candidiasis management due to their variable innate susceptibility to antifungal drugs and the different clinical interpretative breakpoints of antifungals among *Candida* spp. Indeed, *C. krusei* is naturally resistant to fluconazole, *C. glabrata* is susceptibly dose-dependent to fluconazole, and *Trichosporon* spp. are resistant to echinocandins [7,40]. Azoles are the most currently used drugs used in Burkina Faso for the treatment of candidiasis, and yeast misidentification might jeopardise treatment outcomes because of inadequate antifungal drug administration or inappropriate posology.

Due to the high cost of more accurate microbiological tools, laboratories in resource-constrained settings might use accessible methods such as CCL together with other techniques to improve the accuracy of the mycological information delivered to the clinicians [41].

Finally, our results highlight the importance of implementing an internal quality system that ensures good manufacturing and training in the reading of chromogenic media in Burkina Faso. The use of another diagnostic method in addition to CCL in routine work is recommended, specifically in cases of invasive candidiasis. The deployment of a mycology reference centre in Burkina Faso, equipped with more sophisticated methods, could be an efficient alternative to help the management of complex cases in fungal pathology in national health centres.

Limitations

Our study had some limitations. Firstly, the small size of the study sample was a limitation, although chromogenic medium-detectable species account for the majority. Therefore, the results of this study could not be extrapolated to the entire population of Bobo-Dioulasso. Secondly, the CCL medium was prepared and read in routine practice conditions of the clinical laboratory, reflecting the real-life performance of this method. Finally, some parameters such as the reader's experience could affect the performance of the test, as suggested by other authors [42].

5. Conclusions

In conclusion, this study assessed, for the first time, the performance of Chromogenic *Candida* Lab-Agar® for the identification of relevant clinical yeast isolates in the routine work of a clinical laboratory in Burkina Faso. The identification of pathogenic yeasts based on chromogenic media, in a resource-constrained setting, presents several limitations for identifying non-*albicans Candida* species (43.37% of misidentifications) that microbiologists and clinicians should consider. Around half of the yeast isolates of various clinical origins were *Candida* non-*albicans*, including uncommon species such as *C. fermentati, C. mesorugosa*, and *Trichosporon* spp. These facts underlie the

potential discrepancies in antifungal treatment outcomes in a setting where azoles remained the main antifungal agent. Our results indicate the need to add a complementary test in the case of a non-albicans isolate identified via the CCL medium, specifically in the case of invasive candidiasis.

Author Contributions: S.N.D., S.B. and H.R.-V conceived and planned the experiments. S.N.D., B.B., and I.M. (Isidore Mandy) carried out the laboratory analysis. H.R.-V. and S.B. supervised the experiments. S.N.D. drafted the manuscript. I.W.Y., A.A., O.D. and I.M. (Isabel Montesinos) provided critical feedback and contributed to the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interests.

Abbreviations

CCL	Chromogenic *Candida* Lab-Agar®
UCLouvain	Université Catholique de Louvain
ITS	Internal transcribed spacer
SDA	Sabouraud dextrose agar

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