



Performance of Two Novel Chromogenic Media for the Identification of Multidrug-Resistant *Candida auris* Compared with Other Commercially Available Formulations

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ABSTRACT Non-*albicans* *Candida* species are emerging in the nosocomial environment, with the multidrug-resistant (MDR) species *Candida auris* being the most notorious example. Consequently, rapid and accurate species identification has become essential. The objective of this study was to evaluate five commercially available chromogenic media for the presumptive identification of *C. auris*. Two novel chromogenic formulations, CHROMagar *Candida* Plus (CHROMagar) and HiCrome *C. auris* MDR selective agar (HiMedia), and three reference media, CandiSelect (Bio-Rad), CHROMagar *Candida* (CHROMagar), and Chromatic *Candida* (Liofilchem), were inoculated with a collection of 9 genetically diverse *C. auris* strains and 35 strains from closely related comparator species. After 48 h of incubation, the media were evaluated for their ability to detect and identify *C. auris*. All media had the same limitations in the differentiation of the more common species *Candida dubliniensis* and *Candida glabrata*. Only on CHROMagar *Candida* Plus did *C. auris* colonies develop a species-specific coloration. Nevertheless, the closely related pathogenic species *Candida pseudohaemulonii* and *Candida vulturna* developed a similar appearance as *C. auris* on this medium. CHROMagar *Candida* Plus was shown to be superior in the detection and identification of *C. auris*, with 100% inclusivity for *C. auris* compared to 0% and 33% for the reference media and HiCrome *C. auris* MDR selective agar, respectively. Although *C. vulturna* and *C. pseudohaemulonii* can cause false positives, CHROMagar *Candida* Plus was shown to be a valuable addition to the plethora of mostly molecular methods for *C. auris* detection and identification.

KEYWORDS *Candida*, chromogenic media, selective media, rapid identification, *Candida auris*, coinfection

Candida species are the most frequently encountered fungi in hospital settings. *Candida* yeasts are among the top four of nosocomial bloodstream pathogens and cause more than 400,000 bloodstream infections annually (1). Therefore, *Candida* contributes significantly to morbidity and mortality (2, 3). Although *Candida albicans* is still the main cause of candidiasis, a shift is taking place. An increasing amount of non-*C. albicans* *Candida* species is causing life-threatening invasive infections (4, 5). One of the main contributors to this epidemiological shift is the unprecedented emergence of the multidrug-resistant (MDR) species *Candida auris*. This yeast was first described in 2009 as a novel member of the *Candida haemulonii* species complex (6, 7). *C. auris* has rapidly become widespread since its first description, causing difficult to control nosocomial outbreaks around the world (8, 9). Whole-genome sequencing of clinical *C. auris*

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isolates revealed four closely related but unique clades predominate on different continents (East Asian, South Asian, South African, and South American) (8, 10). In South Africa, *C. auris* already causes 14% of all reported candidemia cases (11). The shift from generally antifungal-susceptible *C. albicans* to more resistant species, like *C. auris*, calls for rapid and reliable identification in order to provide the right treatment on time (5, 12).

To detect pathogenic yeasts in polymicrobial clinical samples properly, selective media are crucial. However, conventional selective media, like Sabouraud dextrose agar and malt extract agar, do not differentiate between species. Chromogenic agars, conversely, allow for the identification of *Candida* species in polymicrobial samples due to generation of a species-specific color (13–15). As a result, they not only allow for rapid identification of single species but are also a great tool to detect the increasing number of yeast-yeast coinfections (16, 17). Currently available chromogenic media enable the identification of four of the common *Candida* pathogens, including *C. albicans*, *Candida tropicalis*, *Nakaseomyces glabrata* (syn. *Candida glabrata*), and *Pichia kudriavzevii* (syn. *Candida krusei*) (13, 14, 18). However, most chromogenic media do not differentiate emerging *Candida* species, including *C. auris*. Other diagnostic technologies like matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), PCR, and internal transcribed spacer (ITS) sequencing are available to reliably identify *C. auris* and its siblings (19–21) but require expertise and costly apparatus. In contrast, chromogenic media allow for rapid direct identification just by the color and morphology of the colony, usually within 24 to 48 h of incubation.

Here, we have evaluated and compared five commercially available chromogenic media, including two novel formulations specifically developed to differentiate *C. auris*. The performances of these five media were tested using a panel of genetically diverse *C. auris* strains and closely related comparator species. Only CHROMagar *Candida* Plus reliably differentiated *C. auris* from most of its sibling species and the common *Candida* pathogens. Exceptions with a similar phenotype on this novel chromogenic medium included *Candida vulturna* and *Candida pseudohaemulonii*.

MATERIALS AND METHODS

Media. Two novel chromogenic media specifically designed to detect *C. auris*, CHROMagar *Candida* Plus (CCP) (bioTRADING, Mijdrecht, The Netherlands) and HiCrome *C. auris* MDR selective agar (HAMA) (HiMedia, Mumbai, India), were evaluated for their ability to differentiate *C. auris* from its close relatives and the major clinical *Candida* species. Three other commercially available chromogenic media, CandiSelect (CS) (Bio-Rad, Veenendaal, The Netherlands), CHROMagar *Candida* (CAC) (bioTRADING), and Chromatic *Candida* (CC) (Liofilchem, Roseto degli Abruzzi, Italy), were used as a reference for specificity of the tested yeasts.

Fungal strains, identification, and culture. Nine *C. auris* strains were selected, as follows: two strains per clade, representing the four clades that are specific to each geographic region and one strain representing the potential fifth clade. Strains came from reputable international culture collections, including our own Centraalbureau voor Schimmelcultures (CBS) yeast collection located at the Westerdijk Fungal Biodiversity Institute (Utrecht, The Netherlands), and included *C. auris* CDC-AR0387 and CDC-AR0388 (clade I), CBS10913 and CBS12373 (clade II), CDC-AR0383 and CDC-AR0384 (clade III), CDC-AR0385 and CDC-AR0386 (clade IV), and CDC-AR1097 (potential clade V, Iran). To evaluate the specificity for *C. auris* of the tested (improved) chromogenic agars, the phenotype of these nine *C. auris* strains was compared to that of closely related yeast species ($n=35$). The selected comparator species included a wide range of *Candida* yeasts belonging to the *Metschnikowia* clade and, in particular, members of the *C. haemulonii* species complex that contains *C. auris*'s closest relatives as follows: *Candida akabanensis* ($n=2$), *Candida citri* ($n=1$), *Candida duobushaemulonii* ($n=3$), *Candida ecuadorensis* ($n=1$), *Candida fructus* ($n=1$), *Candida haemulonii* ($n=3$), *Candida haemulonii* var. *vulnera* ($n=3$), *Candida intermedia* ($n=2$), *Candida kutaonensis* ($n=1$), *Clavispora lusitanae* ($n=6$), *Clavispora opuntiae* ($n=2$), *Candida phyllophila* ($n=1$), *Candida pseudofloscolorum* ($n=1$), *Candida pseudohaemulonii* ($n=2$), *Candida pseudointermedia* ($n=2$), *Candida sharkiensis* ($n=1$), *Candida vitiphila* ($n=1$), and *Candida vulturna* ($n=2$). Additionally, one strain of the major clinical *Candida* species, *C. albicans*, *Candida dubliniensis*, *C. tropicalis*, *Nakaseomyces glabrata* (syn. *C. glabrata*), and *Pichia kudriavzevii* (syn. *C. krusei*) were included (22). All strains were subjected to ITS sequencing to confirm species identity (21, 23).

Strains were cultured on 2% glucose, 0.5% yeast extract, 1% peptone, and 1.5% agar (GYPA) at 25°C before testing. The ability of the tested chromogenic media to differentiate *C. auris* from its close relatives and the common clinical *Candida* species was evaluated as described previously (24) with minor modifications. In brief, cells were suspended in phosphate-buffered saline (PBS; without Ca, Mg, pH 7.3

to 7.5) (Lonza, Basel, Switzerland) to make aqueous suspensions for the nine *C. auris* strains and 40 comparator strains listed above. Subsequently, 2- μ l cell suspensions were spotted onto the chromogenic media plates and incubated for 48 h at 35°C (CCP, CS, CAC, CC) and 42°C (HAMA) as recommended by the manufacturers. To check for organism viability, the same suspensions were spotted in parallel onto GYPA plates, which were incubated for 48 h at 35°C and 30°C (as some of the species included did not grow well at 35°C).

To assess the ability of the tested chromogenic media to detect *C. auris* from coinfections with other yeasts, mixtures containing 2.0×10^3 cells/ml of *C. auris* CBS10913 plus major clinical *Candida* species or members of the *C. haemulonii* species complex were prepared 1:1 in PBS. One hundred microliters of the resulting mixtures, containing ~200 cells, was plated onto the different chromogenic media. Pure cultures were used as a control. Plates were incubated at 35°C for 48 h unless stated otherwise.

Data availability. Sequence data was deposited into GenBank under accession numbers [MT974606](#) to [MT974688](#).

RESULTS

After 48 h of incubation at 35°C on the reference chromogenic media (CS, CC, CAC), *C. auris* colonies appeared pale cream to light blue (CS) or beige to pink with pale borders (CC, CAC) (Fig. 1A to C, spots 33 to 41). With the CS, CC, and CAC media, it was not possible to distinguish *C. auris* from other closely related pathogenic siblings (*C. haemulonii* [spots 27, 28, 29], *C. duobushaemulonii* [spots 24, 25, 26], *C. pseudohaemulonii* [spots 44, 45], *C. haemulonii* var. *vulnera* [spots 30, 31, 32], and *C. vulturna* [spots 42, 43]). In addition, when compared to most other closely related yeast species tested, *C. auris* colonies displayed a similar phenotype on CS, CC, and CAC media as well. Only *Clavispora opuntiae* (spots 2, 3) produced distinctive coloration on all three media, being violet on CS and light to dark blue on CC and CAC, respectively. Since most chromogenic media are designed to detect the major clinical species, *C. albicans* (spot 46), *C. dubliniensis* (spot 47), *N. glabrata* (spot 48), *C. tropicalis* (spot 49), and *P. kudriavzevii* (spot 1), these yeasts all had a distinctive phenotype. Nonetheless, *C. albicans* and *C. dubliniensis* behaved similarly on all tested media, both producing green colonies on CC and CAC and purple on CS. Interestingly, *C. lusitaniae* (spots 10 to 15) showed a high intrastrain variability of colony phenotypes, especially on CAC medium, ranging from pale pink to dark purple. Because of this variability, some strains had a phenotype similar to *N. glabrata* on all three media. Similar to *C. lusitaniae*, the *C. haemulonii* strains tested also produced different phenotypes, with strain MC153 (spot 28) being the most distinctive.

The two novel chromogenic media evaluated in this study use different approaches for the isolation and differentiation of *C. auris*. CCP works like a traditional chromogenic medium, allowing for identification by color and colony morphology of the species. HAMA, conversely, inhibits growth of species other than multidrug-resistant *C. auris* and uses a chromogenic mixture to impart purple color to *C. auris* for identification. On CCP, all *C. auris* strains appeared pale cream to lavender with a distinctive blue halo surrounding the colony, independent of the clade they belong to (Fig. 1D, spots 33 to 41). *C. auris* could be easily differentiated from all species that were tested in parallel, except *C. vulturna* (spots 42, 43) and *C. pseudohaemulonii* (spots 44, 45). Both species are closely related to *C. auris* and produced similar colonies on the novel CCP medium.

Incubation of the yeast species at 42°C for 48 h on HAMA medium effectively inhibited the growth of all comparator species. Most species produced very faint colonies with a light to dark purple phenotype (Fig. 1E). Of the nine *C. auris* strains, only three showed good growth. Strains CDC-AR0385 and CDC-AR0386 (spot 35, 36), both belonging to the South American clade (clade IV, Venezuela), produced cream colonies with a pink center. Strain CDC-AR0388 (spot 38) of the South Asian clade (clade I, Pakistan) produced a colony with a different phenotype being much darker purple.

The performance data of the media tested in the spot assay (Fig. 1) are presented in Table 1. CCP showed the best capacity to discriminate between *C. auris* and most of the comparator yeast species (100% inclusivity), except *C. vulturna* and *C.*

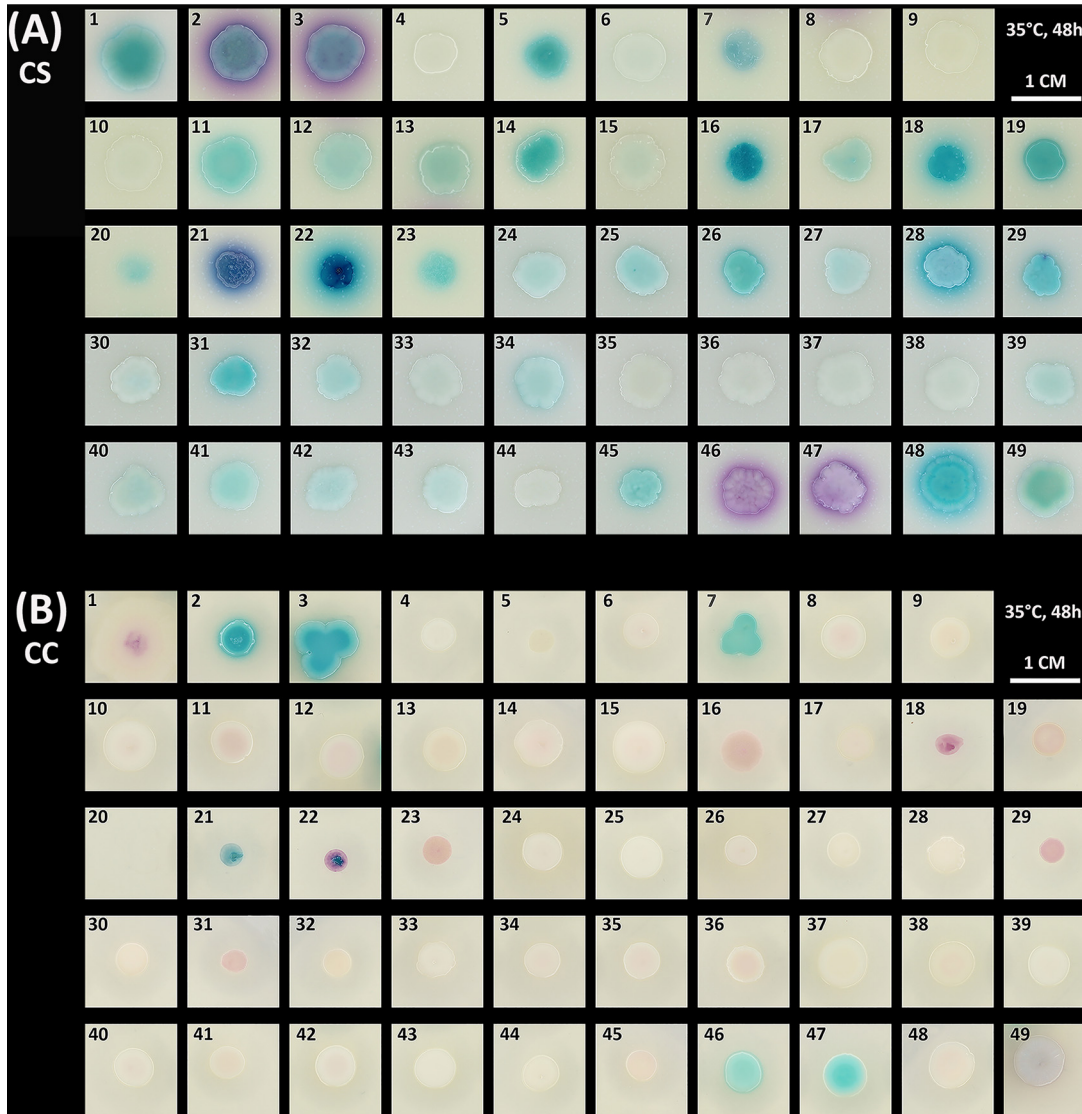


FIG 1 Appearance of *Candida auris* and comparator species on CandiSelect (CS) (A), Chromatic *Candida* (CC) (B), CHROMagar *Candida* (CAC) (C), CHROMagar *Candida* Plus (CCP) (D), and HiCrome *C. auris* MDR selective agar (HAMA) (E). Two microliters of aqueous suspension of each organism was spotted onto each medium. Plates were incubated at 35°C (CS, CAC, CC, CCP) and 42°C (HAMA) for 48 h. Tested yeast species were as follows: (1) *Pichia kudriavzevii* CBS2050 (syn. *Candida krusei*), (2) *Clavispora opuntiae* CBS11046, (3) *Clavispora opuntiae* CBS11164, (4) *Candida sharkiensis* CBS11368, (5) *Candida kutaonensis* CBS11388, (6) *Candida citri* CBS11858, (7) *Candida ecuadorensis* CBS12653, (8) *Candida phyllophila* CBS12671, (9) *Candida vitiphila* CBS12672, (10) *Clavispora lusitaniae* CBS5094, (11) *Clavispora lusitaniae* CBS4870, (12) *Clavispora lusitaniae* CBS11356, (13) *Clavispora lusitaniae* CBS5901, (14) *Clavispora lusitaniae* CBS1944, (15) *Clavispora lusitaniae* CBS2866, (16) *Candida intermedia* CBS2291, (17) *Candida intermedia* CBS5311, (18) *Candida pseudointermedia* CBS6918, (19) *Candida pseudointermedia* CBS2879, (20) *Clavispora fructus* CBS6380, (21) *Candida akabanensis* CBS5039, (22) *Candida akabanensis* CBS7878, (23) *Candida pseudoflooscolorum* CBS8584, (24) *Candida duobushaemulonii* CBS7800, (25) *Candida duobushaemulonii* CBS7799, (26) *Candida duobushaemulonii* CBS7098, (27) *Candida haemulonii* CBS6590, (28) *Candida haemulonii* MC153, (29) *Candida haemulonii* CBS12371, (30) *Candida haemulonii* var. *vulnera* CDC-AR0391, (31) *Candida haemulonii* var. *vulnera* CBS6332, (32) *Candida haemulonii* var. *vulnera* CBS12438, (33) *Candida auris* CDC-AR0383 (clade III), (34) *Candida auris* CDC-AR0384 (clade III), (35) *Candida auris* CDC-AR0385 (clade IV), (36) *Candida auris* CDC-AR0386 (clade IV), (37) *Candida auris* CDC-AR0387 (clade I), (38) *Candida auris* CDC-AR0388 (clade I), (39) *Candida auris* CBS10913 (clade II), (40) *Candida auris* CBS12373 (clade II), (41) *Candida auris* CDC-AR1097 (potential clade V), (42) *Candida vulturna* CBS14366, (43) *Candida vulturna* CBS15630, (44) *Candida pseudohaemulonii* CBS 10004, (45) *Candida pseudohaemulonii* CBS12370, (46) *Candida albicans* CBS562, (47) *Candida dubliniensis* CBS7987, (48) *Nakaseomyces glabrata* (syn. *Candida glabrata*) CBS138, and (49) *Candida tropicalis* CBS94.

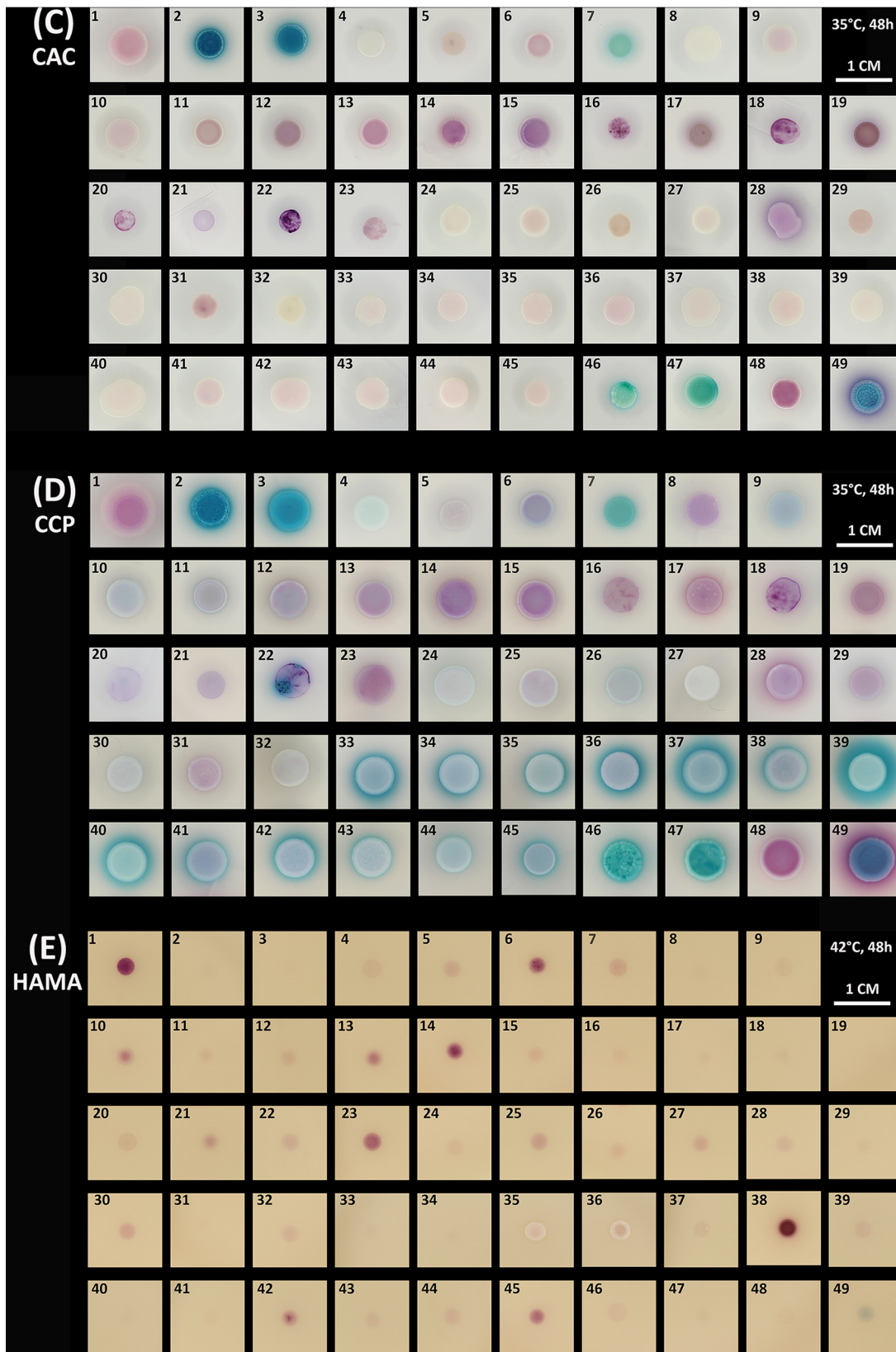


FIG 1 (Continued)

TABLE 1 Performance data of *Candida* chromogenic media after 48 h of incubation in spot assay

Strain and parameter	No. of samples using:				
	CS	CC	CAC	CCP	HAMA ^d
<i>Candida auris</i> (n = 9)					
True positive	0	0	0	9	3
False negative	9	9	9	0	6
Comparator strains (n = 40)					
Recovery	40	39	40	40	3
False positive (typical <i>Candida auris</i> morphology)	20	24	13	4	0
True negative	20	16	27	36	40
Accuracy (%) ^a	40.8	32.7	55.1	91.8	87.8
Exclusivity (%) ^b	50	40	67.5	90	100
Inclusivity (%) ^c	0.0	0.0	0.0	100.0	33.3

^aAccuracy (%) = (true positive + true negative) × 100 / total.

^bExclusivity (%) = true negative × 100 / (true negative + false positive).

^cInclusivity (%) = true positive × 100 / (true positive + false negative).

^dSelective medium inhibiting growth of species other than *C. auris* causing low recovery.

pseudohaemulonii (90% exclusivity). Therefore, we next evaluated the performance of this medium in the detection of *C. auris* from mixed microbial samples. To this end, suspensions of *C. auris* or *C. auris* mixed with other members of the *C. haemulonii* species complex (including *C. vulturna* and *C. pseudohaemulonii*) and the major clinical *Candida* species, *C. albicans*, *C. tropicalis*, and *N. glabrata*, were prepared in sterile saline. One hundred microliters of the resulting mixtures, containing approximately 200 cells, was inoculated on CCP and the other chromogenic media in parallel for comparison. After 48 h (the incubation time specified by the manufacturers), only CCP reliably discriminated *C. auris* from all comparator species (Fig. 2A). On CCP, distinctive colonies with blue haloes could be detected. This is in contrast to the reference media where *C. auris* appeared pale cream just like all other members of the *C. haemulonii* species complex (not shown). Although *C. pseudohaemulonii* produced a similar phenotype in the spot assay (Fig. 1), inoculated as a mixture, this species produced much smaller colonies compared to those of *C. auris* without the blue halo (Fig. 2A, 7). *C. vulturna*, in contrast, was harder to distinguish since colonies had the same size and morphology as *C. auris* (Fig. 2A, 8). Nonetheless, after 48 h, the blue haloes of *C. auris* colonies were much bigger and more intense. Only after further incubation for 72 h was *C. vulturna* able to produce similar haloes as *C. auris* (Fig. 2B, 1b). *C. pseudohaemulonii* colonies produced a blue halo as well af-

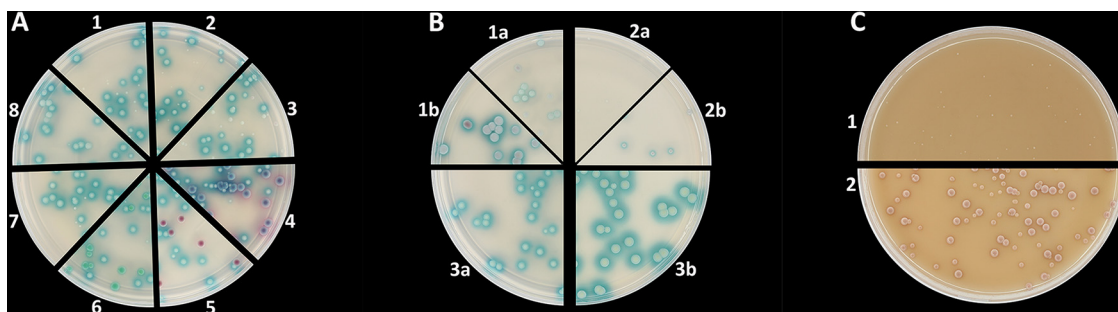


FIG 2 (A) Comparative detection of *Candida auris* from mixtures with other yeast on CHROMagar *Candida* Plus inoculated with mixtures of *C. auris* plus (1) *Candida haemulonii* var. *vulnera* CBS12438, (2) *Candida haemulonii* CBS6590, (3) *Candida duobushaemulonii* CBS7799, (4) *Candida tropicalis* CBS594, (5) *Nakaseomyces glabrata* (syn. *Candida glabrata*) CBS138, (6) *Candida albicans* CBS562, (7) *Candida pseudohaemulonii* CBS12370, and (8) *Candida vulturna* CBS14366 as described in Materials and Methods. (B) CHROMagar *Candida* Plus inoculated with pure suspensions of (1) *C. vulturna* CBS14366, (2) *C. pseudohaemulonii* CBS12370, and (3) *C. auris* CBS10913 (clade II) incubated at 35°C for (a) 48 h and (b) 72 h. (C) Recovery of *C. auris* CDC-AR0385 (clade I) on HiCrome *C. auris* MDR Selective Agar after (1) 48 h and (2) 96 h of incubation at 42°C.

ter 72 h, but still these colonies were much smaller and therefore easy to distinguish (Fig. 2B, 2b). On the HAMA medium, none of the comparator species grew after inoculation with pure suspensions (not shown). Since most *C. auris* strains also did not grow on this medium, strain CDC-AR0385 was used to plate out as a mixture. This strain produced very small white colonies after 48 h of growth at 42°C. Further incubation for 96 h was needed before colonies started to produce a pink to purple color (Fig. 2C).

DISCUSSION

Non-*albicans* *Candida* species are emerging in the nosocomial environment. Often, these species have a multidrug-resistant phenotype, with *C. auris* being the most notorious example. Consequently, rapid and accurate species identification has become essential in order to provide the optimal treatment. Chromogenic media are able to detect and identify *Candida* yeasts at species level in an easy and cost-effective way. In this study, we evaluated five commercially available chromogenic media for the detection and differentiation of *C. auris* from closely related comparator species and common clinical *Candida*.

The novel CHROMagar *Candida* Plus medium showed the best performance in the rapid identification of *C. auris* compared to that of the reference media, having the highest accuracy and inclusivity (Table 1). This result is in line with the study performed by Borman and colleagues who used 52 comparator species recovered from routine clinical samples to test the specificity of CCP for *C. auris* (24). In that study, only the rare species *Candida diddensiae* had a similar appearance. By using only comparator strains of closely related yeasts, we here identified two new clinically relevant species that have a phenotype similar to *C. auris* on CCP. Both species, *C. pseudohaemulonii* and *C. vulturna*, are pathogenic siblings of *C. auris* and part of the *C. haemulonii* species complex. They have been isolated from bloodstream infections and show resistance against amphotericin B (6, 25). Although *C. pseudohaemulonii* looked similar to *C. auris* in the spot assay, in mixed culture colonies, it was much smaller and produced the distinctive blue halo only after 72 h. This would make it less likely to misidentify *C. pseudohaemulonii* for *C. auris* when encountered in clinical samples. In contrast, *C. vulturna* colonies could only be differentiated from *C. auris* based on the size of the blue halo. However, as depicted by Fig. 1D, this halo size is strain dependent and probably not a reliable characteristic for differentiation. Therefore, *C. vulturna* is likely to cause false positives for *C. auris* when detected on the CCP medium. Complementary to this, the CCP medium showed similar problems as the reference media, with the identification of the more common clinical species *C. albicans*, *C. dubliniensis*, and *N. glabrata*. On all media, *C. albicans* and *C. dubliniensis* produced almost identical colony phenotypes. Likewise, *C. lusitanae* could be mistaken for *N. glabrata*. The low specificity of chromogenic media for these species has been reported before (14), and the new CCP medium could not solve this problem.

The HiCrome *C. auris* MDR selective agar showed very low sensitivity for *C. auris* compared to that of the CCP medium. Only three out of nine *C. auris* strains grew on this medium. This is probably because HAMA contains an inhibitory mixture, making it selective for multidrug-resistant yeasts. The composition and concentration of antifungals of the inhibitory mixture are not specified on the product data sheet though. Looking at the MIC metadata of the tested *C. auris* strains, all strains except CBS10913 have high MICs for one or more antifungals (26). However, only strains with fluconazole MIC values of $>256 \mu\text{g/ml}$ were able to grow, suggesting HAMA only selects *C. auris* strains with high fluconazole resistance. HAMA successfully inhibited growth of all comparator species, indicating it could be used for the detection of (fluconazole-resistant) *C. auris*. Nevertheless, HAMA does not allow for reliable identification due to its poor chromogenic performance. The lack of a specific, and even variable, color for *C. auris* makes additional methods, such as MALDI-TOF, to confirm identification necessary. This implies that the use of HAMA does not have any advantages over selective

media for *C. auris* that have been described previously (27, 28). Notably, all selective media need to be incubated at 42°C in order to inhibit the growth of most other clinical *Candida* species, except *C. auris* (7, 27, 28). Therefore, incubating the CCP medium at 42°C likely increases its selectivity for *C. auris*. Conversely, this would limit the detection of possible other clinically relevant yeasts in polymicrobial samples.

A possible limitation of this study is that we did not test the media with clinical samples from patients infected by *C. auris* or the other closely related clinically relevant species like *C. pseudohaemulonii* and *C. vulturna*. However, Bayona and coworkers recently confirmed the suitability of the CCP medium for the detection and identification of *C. auris* and other yeasts in the context of a hospital outbreak (29). Despite the lack of clinical samples, this study used a very broad panel of closely related yeasts with which *C. auris* can be misidentified by biochemical identification systems. This allowed for a thorough evaluation of the specificity of the tested chromogenic media.

In conclusion, the novel chromogenic medium CHROMagar *Candida* Plus was shown to be superior in the detection and identification of *C. auris* compared to the other chromogenic media tested. Nevertheless, *C. vulturna* and *C. pseudohaemulonii* produced a phenotype similar to *C. auris* on CCP. Both species have been reported to cause nosocomial infections and therefore could cause false positives. Moreover, the use of CCP medium could not overcome the limitations in the identification of *N. glabrata* and *C. dubliniensis*, previously reported for other chromogenic agars. However, despite some limitations, CCP showed to be a valuable addition to the plethora of mostly molecular methods for *C. auris* detection and identification.

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