



Original Article

CHROMagar™ Candida Plus: A novel chromogenic agar that permits the rapid identification of *Candida auris*

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Abstract

Candida auris is a serious nosocomial health risk, with widespread outbreaks in hospitals worldwide. Successful management of such outbreaks has depended upon intensive screening of patients to identify those that are colonized and the subsequent isolation or cohorting of affected patients to prevent onward transmission. Here we describe the evaluation of a novel chromogenic agar, CHROMagar™ Candida Plus, for the specific identification of *Candida auris* isolates from patient samples. *Candida auris* colonies on CHROMagar™ Candida Plus are pale cream with a distinctive blue halo that diffuses into the surrounding agar. Of over 50 different species of *Candida* and related genera that were cultured in parallel, only the vanishingly rare species *Candida diddensiae* gave a similar appearance. Moreover, both the rate of growth and number of colonies of *C. auris* recovered from swabs of pure and mixed *Candida* species were substantially increased on CHROMagar™ Candida Plus agar when compared with growth on the traditional mycological isolation medium, Sabouraud dextrose agar. Taken together, the present data suggest that CHROMagar™ Candida Plus agar is an excellent alternative to current conventional mycological media for the screening of patients who are potentially colonized/infected with *Candida auris*, can be reliably used to identify this emerging fungal pathogen, and should be tested in a clinical setting.

Lay Abstract

Candida auris is a novel pathogenic yeast that has been associated with large hospital outbreaks across several continents. Affected patients become colonized, predominantly on the skin, with large quantities of *C. auris* which they then shed into the hospital environment. Identification of *C. auris* is challenging using routine laboratory methods, and time consuming when patients are colonized with a mixture of different *Candida* species. Here we demonstrate that a novel chromogenic agar, CHROMagar™ Candida Plus, permits the rapid differentiation of *C. auris* from a wide range of other yeast species and is potentially ideally suited to screening of patients that are suspected of being colonized or infected with this medically important yeast.

Key words: chromogenic agar, *Candida auris*, identification, isolation media, CHROMagar.

Introduction

Candida auris, a novel member of the *Candida haemulonii* complex, was described from discharge from a human external ear canal in Japan in 2009,¹ an association with chronic otitis media that was confirmed the same year in South Korean studies.² *C. auris* has subsequently been reported from numerous

different clinical manifestations, ranging from colonization and superficial mucosal infections to deep-seated infections and candidemia.^{3–6} Today, *C. auris* is accepted to be an emergent nosocomial pathogen with evidence of clonal inter- and intrahospital transmission and has become widespread across several Asian countries, South America, and South Africa,^{3–11} with additional

outbreaks in several European countries and large parts of the USA (reviewed in ¹²). Genome sequence comparisons of *C. auris* isolates from different geographical regions revealed that closely related, but distinct clonal lineages predominate on different continents,^{2–5,8,13} and that isolates from these four lineages have seeded subsequent outbreaks across the world.^{12,14–17}

Most hospital outbreaks have been characterized by large numbers of superficially colonized patients and smaller numbers of patients who progress to develop disseminated infection.^{10,12,15–17} A number of reports have highlighted the importance of enhanced infection control measures coupled with exhaustive screening of patients to identify those who are colonized and their subsequent isolation to prevent onward transmission as being the cornerstones for successful management of such outbreaks.^{10,12,15–17} However, while *C. auris* can readily be recovered from swabs of superficial skin sites in colonized patients (reviewed in ¹²), subsequent identification and differentiation of *C. auris* isolates from other *Candida* species and related genera is more problematic. Currently available chromogenic media do not differentiate *C. auris* from many other common *Candida* species, and *C. auris* is frequently misidentified as other related species when conventional identification methodologies are employed.^{2,5,11,12,18} Although MALDI-TOF MS reliably and rapidly identifies *C. auris* isolates,^{5,12,19} it becomes extremely onerous if it has to be applied to the numerous individual colonies recovered from sampling of superficial sites from potentially colonized patients. In an attempt to circumvent these issues, a variety of *Candida auris*-specific polymerase chain reaction (PCR) tests have been developed,^{20–24} together with specific media/culture conditions that are selective for *C. auris*.²⁵ However, these approaches are still time-consuming and require mycological or molecular expertise. Here, we have evaluated the performance of a novel chromogenic agar developed to specifically facilitate the identification of *Candida auris*. CHROMagar Candida Plus improves the recovery of *C. auris* when compared to conventional isolation media, and reliably differentiates this emerging pathogen from most other common yeast species.

Methods

Fungal strains, identification, and culture

The current study employed 10 *C. auris* strains chosen to represent the four main global clonal lineages. Strains were from reputable international culture collections or isolates identified at the UK National Mycology Reference Laboratory (MRL): *Candida auris* NCPF 8977 (Clade III; South Africa); *Candida auris* MRL 206 (Clade III; South Africa); *Candida auris* NCPF 8971 (Clade I; India); *Candida auris* MRL 213 (Clade I; India); *Candida auris* CBS 12373 (Clade II; Japan); *Candida auris* NCPF 13029 (Clade II; Type strain; Japan); *Candida auris* CDC B16565 (Clade IV; Colombia); *Candida auris* CDC B13108 (Clade IV; Panama); *Candida auris* I-24 (Clade IV; Israel); *Candida auris* I-172 (Clade IV; Israel). An additional 52 comparator

species recovered from routine clinical samples and stored at the MRL were included. These encompassed a wide range of *Candida* (and former *Candida*) species: *Candida albicans*; *Candida boidinii*; *Candida diddensiae*; *Candida digboiensis*; *Candida dubliniensis*; *Candida duobushaemulonii*; *Candida haemulonii*; *Candida metapsilosis*; *Candida mucifera*; *Candida norvegica*; *Candida orthopsilosis*; *Candida parapsilosis*; *Candida picinguabensis*; *Candida tropicalis*; *Clavispora (Candida) lusitaniae*; *Cyberlindnera (Candida) fabianii*; *Debaryomyces hansenii (Candida famata)*; *Diutina (Candida) blankii*; *Diutina (Candida) catenulata*; *Kluyveromyces marxianus (Candida kefir)*; *Meyerozyma caribbica (Candida fermentati)*; *Meyerozyma (Candida) guilliermondii*; *Nakaseomyces (Candida) bracarensis*; *Nakaseomyces (Candida) glabrata*; *Pichia cactophila (Candida inconspicua)*; *Pichia kudriavzevii (Candida krusei)*; *Pichia occidentalis (Candida sorbosa)*; *Starmera (Candida) stellimalicola*; *Starmerella (Candida) sorbosivorans* and *Wickerhamomyces anomalus (Candida pelliculosa)*. Additional non-*Candida* yeasts that are frequently isolated from clinical samples or have known associations with skin and superficial sites were also included: *Apiotrichum (Trichosporon) montevidense*; *Cryptococcus neoformans*; *Cystobasidium (Rhodotorula) slooffiae*; *Hanseniaspora guilliermondii (Kloeckera apis)*; *Kazachstania servazzii*; *Lodderomyces elongisporus*; *Metschnikowia pulcherrima*; *Milleromyces farinosa*; *Naganishia (Cryptococcus) albidosimilis*; *Naganishia albida (Cryptococcus albidus)*; *Naganishia (Cryptococcus) diffluens*; *Rhodotorula glutinis*; *Rhodotorula mucilaginosa*; *Saccharomyces cerevisiae*; *Saprochaete capitata (Geotrichum capitatum)*; *Trichomonascus ciferrii*; *Trichosporon asahii*; *Trichosporon inkin*; *Trichosporon lactis*; *Trichosporon* sp. and *Wickerhamomyces onychis*. Finally, we also included the algae *Prototheca* sp., which often resembles yeast colonies in culture. The identity of all isolates was verified by MALDI-TOF MS and rDNA sequencing, exactly as described previously.¹⁹

All isolates were subcultured on Sabouraud dextrose agar with chloramphenicol (SABC; Oxoid Ltd, Basingstoke, UK) for 48 h at 30°C prior to testing. To evaluate the capacity of CHROMagarTM Candida Plus plates to serve as a primary isolation medium, serial dilutions of *Candida auris* NCPF 8971 or mixtures of *C. auris* NCPF 8971 plus closely related yeast species or those often found as commensal organisms on the skin (*S. cerevisiae*, *C. haemulonii*, *T. inkin*, or *C. parapsilosis*) were prepared in 5 ml volumes of sterile saline and plated using a sterile cotton swab dipped once into the suspension and spread in parallel onto SABC and CHROMagarTM Candida Plus agar plates. Plates were incubated for 60 h at 35°C, and colony counts were recorded at both 36 and 60 h. To assess the ability of CHROMagarTM Candida Plus to differentiate *C. auris* from other common and rare yeast species encountered in clinical specimens, aqueous suspensions corresponding to approximately 0.1 McFarland standard were prepared for the 10 *C. auris* test isolates and 52 comparator species listed above, and 1 µl volumes of each resulting suspension were spotted in a

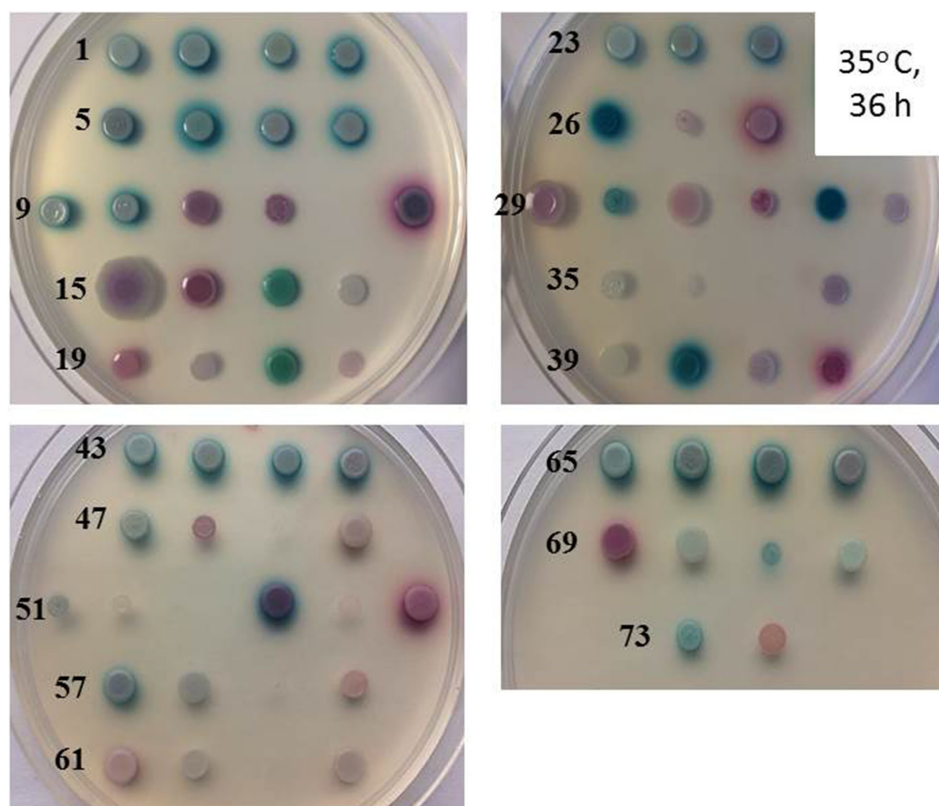


Figure 1. Appearance of *Candida auris* and 52 comparator yeast species on CHROMagar™ Candida Plus medium. One microliter of aqueous suspensions of each organism was spotted onto CHROMagar™ Candida Plus plates which were then incubated at 35°C for 36h. Organisms tested were: 1 = *Candida auris* NCPF 8977 (Clade III); 2 = *Candida auris* MRL 206 (Clade III); 3 = *Candida auris* NCPF 8971 (Clade I); 4 = *Candida auris* MRL 213(Clade I); 5 = *Candida auris* CBS 12373 (Clade II); 6 = *Candida auris* MRL 209 (Clade II; Type); 7 = *Candida auris* CDC B16565(Clade IV; Colombia); 8 = *Candida auris* CDC B13108 (Clade IV; Panama); 9 = *Candida auris* I-24 (Clade IV; Israel); 10 = *Candida auris* I-172 (Clade IV; Israel); 11 = *Clavispora (Candida) lusitaniae*; 12 = *Saccharomyces cerevisiae*; 13 = *Debaryomyces hansenii (Candida famata)*; 14 = *Candida tropicalis*; 15 = *Pichia kudriavzevii (Candida krusei)*; 16 = *Nakaseomyces (Candida) glabrata*; 17 = *Candida albicans*; 18 = *Candida parapsilosis*; 19 = *Meyerozyma caribbica (Candida fermentati)*; 20 = *Cryptococcus neoformans*; 21 = *Candida dubliniensis*; 22 = *Candida haemulonii*; 23 = *Candida auris* NCPF 8977 (Clade III); 24 = *Candida auris* NCPF 8971 (Clade I); 25 = *Candida auris* CBS 12373 (Clade II); 26 = *Trichosporon inkin*; 27 = *Rhodotorula mucilaginosa*; 28 = *Meyerozyma (Candida) guilliermondii*; 29 = *Kluyveromyces marxianus (Candida kefyri)*; 30 = *Trichosporon asahii*; 31 = *Pichia occidentalis*; 32 = *Cyberlindnera (Candida) fabianii*; 33 = *Lodderomyces elongisporus*; 34 = *Candida duobushaemulonii*; 35 = *Naganishia albida (Cryptococcus albidus)*; 36 = *Candida haemulonii*; 37 = *Naganishia (Cryptococcus) diffluens*; 38 = *Diutina (Candida) catenulata*; 39 = *Pichia cactophila (Candida inconspicua)*; 40 = *Trichosporon* sp.; 41 = *Saprochaete capitata (Geotrichum capitatum)*; 42 = *Hanseniaspora guilliermondii*; 43 = *Candida auris* NCPF 8977 (Clade III); 44 = *Candida auris* NCPF 8971 (Clade I); 45 = *Candida auris* CBS 12373 (Clade II); 46 = *Candida auris* CDC B16565(Clade IV; Colombia); 47 = *Candida metapsilosis*; 48 = *Wickerhamomyces anomalus (Candida pelliculosa)*; 49 = *Prototheca* sp.; 50 = *Candida orthopsilosis*; 51 = *Apiotrichum montevidense*; 52 = *Kazachstania servazzii*; 53 = *Naganishia albidosimilis*; 54 = *Diutina (Candida) blankii*; 55 = *Candida boidinii*; 56 = *Nakaseomyces (Candida) braccarensis*; 57 = *Candida diddensiae*; 58 = *Candida digboiensis*; 59 = *Candida norvegica*; 60 = *Candida pinguabensis*; 61 = *Milleroyza farinosa*; 62 = *Starmerella sorbosivorans*; 63 = *Cystobasidium slooffiae*; 64 = *Metschnikowia pulcherrima*; 65 = *Candida auris* NCPF 8977 (Clade III); 66 = *Candida auris* NCPF 8971 (Clade I); 67 = *Candida auris* CBS 12373 (Clade II); 68 = *Candida auris* CDC B16565(Clade IV; Colombia); 69 = *Wickerhamomyces onychis*; 70 = *Starmera stellimalicola*; 71 = *Trichosporon lactis*; 72 = *Trichomonascus ciferrii*; 73 = *Candida mucifera*; 74 = *Rhodotorula glutinis*.

grid fashion onto CHROMagar™ Candida Plus plates, which were then incubated for 36 h at 35°C (incubation conditions specified by the manufacturer). As a positive control for organism viability, suspensions were also spotted in parallel, in the same order, onto SABC plates, which were incubated for 36 h at the lower temperature of 30°C (as some of the yeast species included in the comparison grow poorly at 35°C).

Results

Candida auris colonies on CHROMagar™ Candida Plus agar after 36 h growth at 35°C appear pale cream, with a distinc-

tive blue halo in the surrounding agar (Figs. 1 and 2) irrespective of the clonal lineage to which they belong (Fig. 1, compare spots 1–10). *C. auris* colonies were easily distinguished from those of the members of the closely related *C. haemulonii* complex (*C. haemulonii* [spots 22 and 36] and *C. duobushaemulonii* [spot 34]) and those other yeast species with which it is frequently confused when conventional biochemical identification approaches are employed: *Clavispora (Candida) lusitaniae* (spot 11), *Saccharomyces cerevisiae* (spot 12), and *C. parapsilosis* (spot 18). A variety of *Trichosporon* species (spots 26, 30, 40, and 71), *Lodderomyces elongisporus* (spot 33), *Candida blankii* (spot 54), and *C. mucifera* (spot 73) also produced colonies on

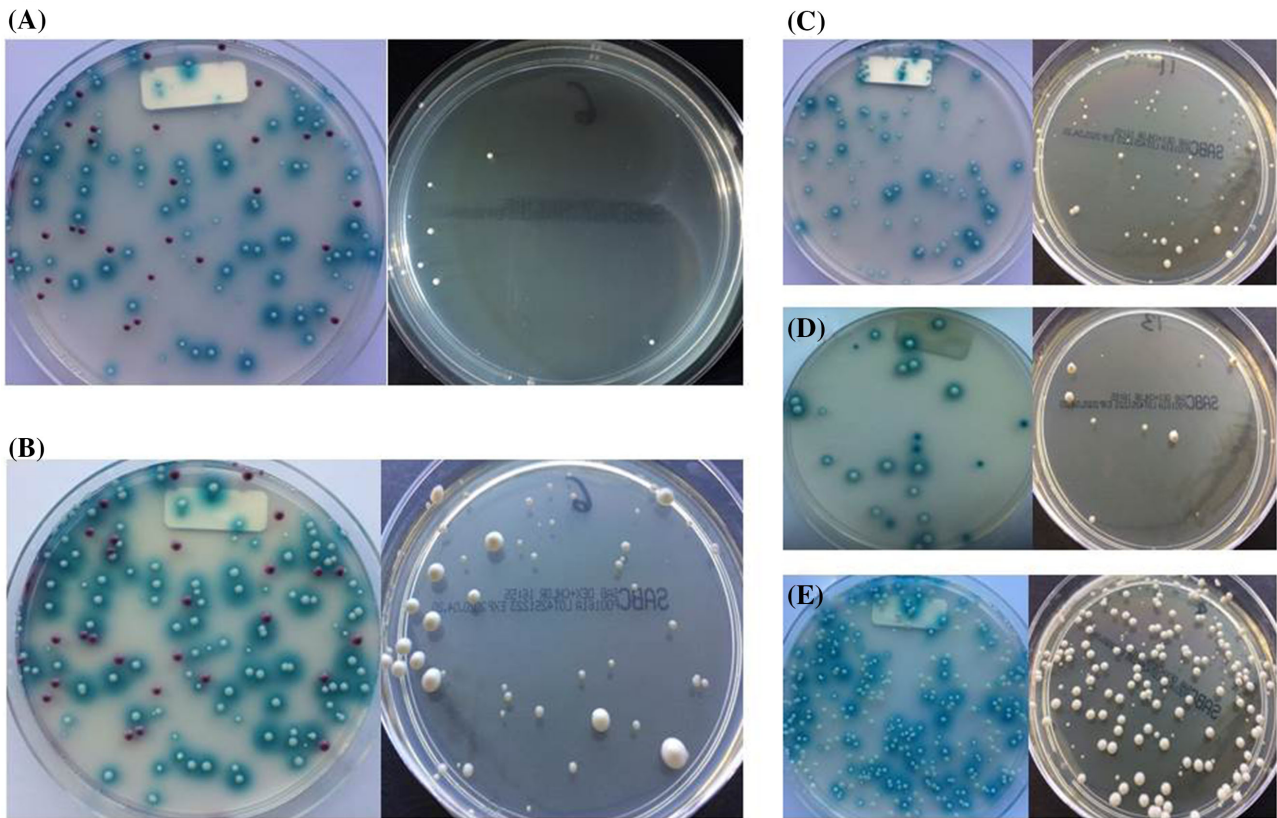


Figure 2. Comparative recovery of *Candida auris* and other yeast species from mixtures on CHROMagar™ Candida Plus agar and Sabouraud dextrose agar with chloramphenicol. Mixtures of *Candida auris* plus *Saccharomyces cerevisiae* (Panels A and B), *Candida auris* plus *Candida haemulonii* (Panel C), *Candida auris* plus *Trichosporon inkin* (Panel D), or *Candida auris* plus *Candida parapsilosis* (Panel E), were prepared in saline as described in Materials and Methods and plated using sterile swabs onto CHROMagar™ Candida plus agar (left-hand side of each panel) and SABC agar (right-hand side of each panel). Plates were incubated at 35°C for 36 h (Panels A, plus C [left-hand side] and E [left-hand side]), or 60 h (Panels B, and D plus C [right-hand side] and E [right-hand side]).

CHROMagar™ Candida Plus with faint to strong blue halos surrounding the colonies, but in all cases the colonies themselves were also various shades of blue and could thus be distinguished from the cream colonies of *C. auris*. A variety of other yeast species grew poorly or not at all on this novel agar after 36 h at 35°C, including *Debaryomyces hansenii* (*C. famata*) (spot 13) with which *C. auris* is also frequently confused, *Naganishia diffluens* (spot 37) and *N. albidosimilis* (spot 53), *Kazachstania servazzii* (spot 52), *C. norvegica* (spot 59), and *Cystobasidium slooffiae* (spot 63). The poor growth of many of these species at temperatures above 30°C has been noted previously.²⁶ Of the 52 unrelated species of *Candida* and other yeast genera that were tested in parallel, only *Candida diddensiae* (spot 57) behaved similarly to *C. auris* on this novel chromogenic agar. At least in the United Kingdom, *C. diddensiae* is encountered extremely rarely in clinical specimens, with only two isolates of this rare yeast that had been referred from a single UK center identified at the UK MRL in the previous 10 years (unpublished data).

Given the capacity of CHROMagar™ Candida Plus medium to discriminate between *C. auris* and a wide array of other yeast species, we next evaluated the utility of this novel agar to serve as a primary isolation medium. To this end, suspensions of *C. auris*,

or *C. auris* mixed with *S. cerevisiae*, *C. haemulonii*, *C. parapsilosis* or *T. inkin*, were prepared in sterile saline. To mimic the swabbing approach used to screen potentially colonized patients, sterile swabs were dipped once in the different suspensions and then used to inoculate CHROMagar™ Candida Plus plates and SABC plates in parallel (Table 1 and Fig. 2), and all inoculated plates were incubated at 35°C. Cultures were evaluated after 36 h (the incubation time specified by the manufacturer) and again after 60 h. When suspensions of pure *Candida auris* were tested, significant numbers of distinctive colonies with blue haloes could be detected on CHROMagar™ Candida Plus as early as 36 h postinoculation, despite there being no visible growth on the corresponding SABC plates incubated under the same conditions (Table 1). Significant numbers of *C. auris* colonies were evident on SABC plates after 60 h incubation, but even with this extended incubation time, better recovery of *C. auris* (as measured by cfu/plate) was achieved with CHROMagar™ Candida Plus medium.

Very similar results were obtained using mixed suspensions containing *C. auris* and a second unrelated yeast species (Table 1; Fig. 2). After 36 h incubation, significant numbers of distinctive *C. auris* colonies (blue halo) and *S. cerevisiae*

Table 1. Comparison of CHROMagar™ Candida Plus medium and SABC agar for the recovery of *Candida auris* and other yeast species.

Organism / Mixture rested	Colony count				% Yield increase*
	36 h 35°C		60 h 35°C		
	SABC	CHROM	SABC	CHROM	
<i>Candida auris</i> 1	0	160	115	183	139 / 159
<i>Candida auris</i> 2	0	55	51	75	108 / 147
<i>Candida auris</i> 3	0	47	25	56	188 / 224
<i>Candida auris</i> 4	0	16	17	20	94 / 118
<i>Candida auris</i> 5	0	13	6	15	217 / 250
<i>Candida auris</i> + <i>Saccharomyces cerevisiae</i>	11	69 Blue 27 Purple	21 Small 15 Large	87 Blue 27 Purple	329 / 414 180 / 180
<i>Candida auris</i> + <i>Candida parapsilosis</i>	148	110 Blue 158 White	74 Small 145 Large	ND ND	149 / ND 109 / ND
<i>Candida auris</i> + <i>Candida haemulonii</i>	0	95 Blue 100 White	111	ND ND	NA / NA NA / NA
<i>Candida auris</i> + <i>Trichosporon inkin</i>	4	13 Blue 9 "White"	9 Small 4 Large	18 Blue 9 Small dark blue	144 / 200 225 / 225

Colony counts (in total cfu/plate) are given for various suspensions of *C. auris* (*C. auris* 1–5) and mixtures of *C. auris* and *S. cerevisiae*, *C. parapsilosis*, *C. haemulonii*, or *T. inkin* incubated on CHROMagar™ Candida Plus (CHROM) and Sabouraud dextrose agar with chloramphenicol (SABC) for 36 h and 60 h at 35°C. ND = not done.

*% yield increase was calculated as cfu per plate CHROMagar™ Candida Plus 36 h vs SABC 60 h / cfu per plate CHROMagar™ Candida Plus 60 h vs SABC 60 h.

Borman et al.¹⁴, Table 1.

colonies (mauve) were visible on CHROMagar™ Candida Plus plates (Fig. 2A, left-hand panel), but only a small number of colonies (all corresponding to *S. cerevisiae*) were present on the equivalent SABC plates (Fig. 2A, right-hand panel). After incubation of these same plates for a total of 60 h (Fig. 2B), a number of smaller colonies (all corresponding to *C. auris*) were detected on the SABC plates in addition to the much larger *S. cerevisiae* colonies. However, recovery of both organisms was significantly greater CHROMagar™ Candida Plus plates than SABC medium (as judged by total cfu; Table 1). Panels C, D, and E of Figure 2 depict the results of the equivalent experiments performed with mixtures of *C. auris* with *C. haemulonii*, *T. inkin*, and *C. parapsilosis*, respectively. In all cases, *C. auris* colonies appeared significantly earlier, and in greater numbers, on CHROMagar™ Candida Plus plates as compared to SABC medium (Fig. 2, Table 1). In each experiment, *C. auris* colonies were only apparent after 60 h incubation on SABC medium and were much smaller than the colonies of the other species in the mixture, whereas the distinctive colonies of both species in each mixture were apparent in larger numbers after only 36 h incubation on CHROMagar™ Candida Plus plates.

Discussion

CHROMagar™ Candida Plus medium appears to be well suited for the rapid isolation and identification of *Candida auris* directly from screening samples from patients suspected of be-

ing colonized with this emerging nosocomial pathogen in hospitals with ongoing or novel outbreaks. It is easy to use, does not require mycological or molecular expertise, and it permits recovery of *C. auris* more efficiently than the standard isolation medium employed here. One potential limitation of the current study is that CHROMagar™ Candida Plus plates were not tested directly with swabs collected from patients suspected of being colonized with *C. auris*. However, given the impressive performance of this novel chromogenic medium in the current study with swabs designed to mimic clinical samples, we believe that CHROMagar™ Candida Plus merits further evaluation in the clinical setting. The addition of this novel chromogenic agar to the plethora of existing methods for *C. auris* identification/detection is likely to be extremely timely in light of the current pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/coronavirus disease 2019 (COVID-19), and the high numbers of critically ill patients requiring long intensive treatment unit (ITU) stays worldwide. Past experience of hospital centres with *C. auris* outbreaks would suggest that COVID-19 patients with long residency times in crowded high dependency units would appear to be prime candidates to develop *C. auris* colonization or infection.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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