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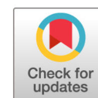

A Selective Medium for Isolation and Detection of *Candida auris*, an Emerging Pathogen

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ABSTRACT Identification of *Candida auris* is challenging and requires molecular or protein profiling-based approaches, availability of which is limited in many routine diagnostic laboratories, necessitating the development of a cost-effective, rapid, and reliable method of identification. The objective of this study was to develop a selective medium for *C. auris* identification. Eighteen *C. auris* and 30 non-*C. auris* yeasts were used for the standardization of the selective medium. Sodium chloride (10% to 13% concentration) and ferrous sulfate (8 mM to 15 mM) were added to yeast extract-peptone-dextrose (YPD) agar in various combinations followed by incubation at 37°C, 40°C, or 42°C for 2 to 3 days. For validation, 579 yeast isolates and 40 signal-positive Bactec blood culture (BC) broths were used. YPD agar comprising 12.5% NaCl and 9 mM ferrous sulfate incubated at 42°C for 48 h, named Selective Auris Medium (SAM), allowed selective growth of *C. auris*. A total of 95% (127/133) of *C. auris* isolates tested grew on the standardized media within 48 h, and the remaining 6 isolates grew after 72 h, whereas the growth of 446 non-*C. auris* yeast isolates was completely inhibited. The specificity and sensitivity of the test medium were both 100% after 72 h of incubation. The positive and negative predictive values were also noted to be 100% after 72 h of incubation. The formulated selective medium can be used for the detection and identification of *C. auris*. The SAM is inexpensive, can easily be prepared, and can be used as an alternative to molecular diagnostic tools in the clinical microbiology laboratory.

KEYWORDS *Candida auris*, invasive candidiasis, rapid identification, selective media

Following the global emergence of *Candida auris*, a clinical alert was issued by the Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA, highlighting it as a serious threat to public health (1). Since the first report in 2009 from Japan, *C. auris* infection has been reported from six continents (2, 3). Based on whole-genome sequencing, *C. auris* has been classified into four major geographical clades, i.e., clade I (South Asia), clade II (East Asia), clade III (South Africa), and clade IV (South America), and a possible fifth clade in Iran (4, 5). *C. auris* infection represents a formidable challenge in health care due to its propensity for rapid nosocomial spread, unknown environmental niche, and ability to form adherent biofilms on clinically important substrates (6–10). Other major issues include its resistance to various classes of antifungals and high mortality due to the infection, ranging from 30% to 70% (2, 11–13).

Despite the alarming rise in reports of *C. auris* infections over the past decade, the reported numbers represent perhaps an underestimation of the true incidence since the fungus is difficult to identify by conventional phenotypic and biochemical tests in routine clinical laboratory practice (14). The commercial microbial identification systems, such as Vitek-2 YST, API AUX 20C, BD Phoenix, and MicroScan, may misidentify *C. auris* (12, 15).

Accurate identification of *Candida* species is important to initiate appropriate

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TABLE 1 List of 18 *C. auris* isolates included in the standardization of the medium[a]

Sl no.	Isolate category	Isolation site	No. of isolates	MIC (μg/ml)
1	Fluconazole resistant	Blood	2	512 (both isolates)
		Hospital environment (ECG leads)	2	256 (both isolates)
		Patient's skin and groin	2	256 (both isolates)
2	Fluconazole susceptible	Blood	2	4 (both isolates)
		Hospital environment (temp probes)	2	0.5 (both isolates)
		Patient's skin and axilla	2	0.5 (both isolates)
3	Caspofungin resistant	Blood	1	16
		Blood	1	16
4	Caspofungin susceptible	Blood	1	0.125
		Blood	1	0.125
5	Reference strain (JCM 15448, Japan); fluconazole susceptible, caspofungin susceptible	Ear	1	1 0.5
6	Reference strain (CBS 12372, South Korea); fluconazole susceptible, caspofungin susceptible	Blood	1	2 0.5

[a]CBS, Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; ECG, echocardiogram; JCM, Japan Collection of Microorganisms; Sl, serial.

antifungal treatment and prevent its spread within the hospital. Various tests developed for rapid identification of *C. auris* use either DNA-based or matrix-assisted laser desorption ionization–time of flight (MALDI-TOF)-based protein profiling (2, 16–18). The majority of the clinical laboratories performing routine assays are not equipped with such advanced facilities. Further, sending the unidentified yeast to reference laboratories adds to the turnaround time. While major attention and efforts have focused on rapid detection using advanced molecular techniques, the development of an easy-to-prepare, low-cost, and yet reproducible and sensitive method for resource-limited settings is largely ignored. In this study, we attempted to develop a selective medium which could significantly reduce the time and cost associated with the identification of *C. auris* even in low-resource health care settings. The first phase of the study was physiological stress induction, and the result from this served as the concept behind formulating the selective medium.

MATERIALS AND METHODS

Isolates, culture, and antifungal susceptibility testing. For standardization, a total of 18 *C. auris* isolates, including 6 fluconazole-resistant, 6 fluconazole-susceptible, 2 caspofungin-resistant, 2 caspofungin-susceptible, and 2 reference isolates, were used (Table 1). To confirm resistance or susceptibility, we perform microbroth dilution antifungal susceptibility testing following Clinical and Laboratory Standards Institute (CLSI) M-60 guidelines (19). A fluconazole MIC of $\geq 32\,\mu$g/ml and a caspofungin MIC of $\geq 2\,\mu$g/ml were considered to represent resistance per the breakpoints proposed by the Centers for Disease Control and Prevention (CDC), Atlanta, GA (20).

Additionally, 30 clinical non-*C. auris* yeast isolates (1 isolate each of *Candida albicans*, *Candida tropicalis*, *Candida krusei* [syn. *Pichia kudriavzevii*], *Candida glabrata* [syn. *Nakaseomyces glabrata*], *Candida parapsilosis*, *Candida metapsilosis*, *Candida orthopsilosis*, *Candida guilliermondii* [syn. *Meyerozyma guilliermondii*], *Candida utilis* [syn. *Cyberlindnera jadinii*], *Candida lusitaniae* [syn. *Clavispora lusitaniae*], *Candida viswanathii*, *Candida rugosa* [syn. *Diutina rugosa*], *Candida pararugosa* [syn. *Wickerhamiella pararugosa*], *Candida kefyr* [syn. *Kluyveromyces marxianus*], *Candida ciferrii* [syn. *Trichomonascus ciferrii*], *Candida famata* [syn. *Debaryomyces hansenii*], *Kodamaea ohmeri*, *Wickerhamomyces anomalus*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans* var. *grubii*, *Cryptococcus neoformans* var. *gatti*, *Cryptococcus neoformans* var. *laurentii* [syn. *Papiliotrema laurentii*], *Geotrichum silvicola*, *Lodderomyces elongisporus*, *Cyberlindnera fabianii*, *Trichosporon asahii*, *Trichosporon dohaense*, *Candida haemulonii*, *Candida pseudohaemulonii*, and *Candida duobushaemulonii*) from the National Culture Collection for Pathogenic Fungi (NCCPF), Chandigarh, India, were included in the study to evaluate the specificity of test medium. *C. albicans* SC5314 was used as a control for the phenotypic stress studies. All strains were cultured fresh on yeast extract-peptone-dextrose (YPD) agar plates (HiMedia, India), and a single pure colony was inoculated into 20 ml YPD broth medium (HiMedia, India) and incubated at 30°C overnight (16 to 24 h) by shaking at a speed of 200 rpm. Cells from overnight cultures were washed twice with sterile normal saline solution, and the optical density at 600 nm (OD_{600}) was measured. The cells were diluted in YPD broth medium to obtain a cell count of $\sim 1 \times 10^6$ cells/ml, corresponding to an OD_{600} of 0.3. Those aliquots (with $\sim 1 \times 10^6$ cells/ml) were then

exponentially diluted from 10-fold to 1,000-fold, and 10 μl of each diluted cell suspension was spotted on YPD agar with different modifications depending on the stress being studied.

Stress induction. Initially, as a part of a pilot study, 53 *C. auris* isolates were spotted ($\sim 10^6$ cells/ml) onto YPD agar plates containing sodium chloride at concentrations ranging from 8% to 18% (with 0.5% increments). After 48 to 72 h of incubation, the majority of the isolates showed moderate to heavy growth until a 16% to 17% concentration of sodium chloride was reached (see Fig. S1 in the supplemental material). Similarly, metal stress induction was performed for 15 *C. auris* isolates by addition of ferrous sulfate to YPD agar at final concentrations ranging from 1 mM to 17 mM (with 0.5 mM increments). The *C. auris* isolates could tolerate 13 mM to 15 mM ferrous sulfate.

Dual stress at the various combinations of 10% to 13% sodium chloride (with 0.5% increments) and 8 mM to 15 mM ferrous sulfate (with 0.5 mM increments) was evaluated in the present study. All stock solutions except the sodium chloride solutions were freshly prepared immediately before use and were filtered by the use of 0.2-μm-pore-size sterile syringe filters (Acrodisc syringe filters with Supor membrane; Pall Corporation) before being added to the media. Sodium chloride was directly added to the YPD agar media before autoclaving. All plates were incubated at 37°C, 40°C, and 42°C for 2 to 3 days.

Salt, metal, and temperature tolerance. As the 18 *C. auris* isolates used during standardization were all clade I isolates, 2 *C. auris* clade II isolates (JCM 15448, Japan; CBS 12372, South Korea), 1 *C. auris* clade III isolate (CDC AR 0383), and 1 *C. auris* clade IV isolate (CDC AR 0385) were evaluated for salt, metal, and temperature tolerance. The levels of metal and salt tolerance were checked on YPD agar plates with the stress of 9 mM ferrous sulfate at 37°C or of sodium chloride (8% to 18% concentration) at 37°C and 42°C.

Growth kinetics. For growth curve analysis, a fresh overnight (16-to-24-h) culture consisting of $\sim 1 \times 10^6$ cells/ml cell suspension in YPD broth was used. Subsequently, 200 μl of that suspension was added to 96-well flat-bottomed plates (Nunclon surface; Nunc, Denmark) and incubated in a kinetic enzyme-linked immunosorbent assay (ELISA) microplate reader (Epoch 2 microplate reader; BioTek Instruments, Inc., USA) for 30 h at 35°C and 42°C separately with continuous orbital shaking. The reader was programmed to read the results at 600 nm at 30-min intervals. All experiments were performed in triplicate.

Preparation of standardized selective medium. On the basis of the results of the dual-stress induction, the best combination of stress factors yielding isolation of *C. auris* was optimized for the preparation of selective medium. Briefly, YPD broth was prepared per the manufacturer's directions, after which agar powder was added (Agar Ultra-pure; HiMedia, India) at a concentration of 2.5%. Sodium chloride was added to obtain a final concentration of 12.5%. The medium was autoclaved for 15 min at 121°C. A ferrous sulfate solution (1 M) was freshly prepared (no more than 10 to 15 min before addition to the autoclaved medium to avoid aerial oxidation) and sterilized by syringe filtration. Once the agar-containing medium cooled to 80°C, the ferrous sulfate solution (9 ml per liter) was added to obtain a final concentration of 9 mM. The medium was then mixed thoroughly (to avoid the necessity for vortex mixing) and manually poured into a sterile petri plate (90-mm diameter) to obtain a thickness of 5 to 6 mm. The plates were dried at room temperature followed by sterility checking using two plates from each batch incubated at 25°C and 37°C for 2 days.

Validation of the standardized selective medium. For validation of the selective medium, yeast isolates recovered from the clinical laboratory at the Department of Medical Microbiology, Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, and the isolates received from various centers across the country through June to November 2019 as a part of National Antimicrobial Resistance Surveillance (AMR) were included. An isolated yeast colony was touched with a sterile stainless-steel inoculation loop and streaked directly on the standardized selective medium. The minimum inoculum was picked and streaked to ensure the lack of visibility of that inoculum at the primary streaking area.

For signal-positive blood culture (BC) samples (maintained using BD Bactec blood culture vials [Bactec Plus Aerobic/F and Bactec Peds Plus/F, BD Diagnostics, Heidelberg, Germany]), one loopful of the broth was used as the inoculum. The isolates were identified by matrix-assisted laser desorption ionization–time of flight mass spectroscopy (Microflex LT Biotyper system [Bruker Daltonics, Bremen, Germany]) per a previously described protocol (18).

Genotypic characterization. Fluorescent amplified fragment length polymorphism (FAFLP) analysis was performed to determine the genetic similarity of strains used in the study. A total of 22 *C. auris* isolates, including 17 isolates from the present study (9 blood isolates, 6 colonization isolates [3 aggregative and 3 nonaggregative], and 2 environmental isolates [1 aggregative and 1 nonaggregative]) and 5 from other sources (1 *C. auris* clade I isolate [NCCPF 470146]; 2 clade II isolates; 1 isolate each from clades III and IV [as mentioned above]) were used. The FAFLP analysis was performed per the previously standardized protocol (21). Briefly, EcoRI and HindIII restriction enzymes (New England Biolabs, Ipswich, MA, USA) and corresponding adapters were used. Amplification was performed using preselective primers of EcoRI (5′GACTGCGTACCAGCTT-3′) and HindIII (5′GACTGCGTACCAATTC-3′). Both a 6-carboxyfluorescein (FAM)-labeled EcoRI primer with one selective residue (5′-GACTGCGTACCAGCTTT-3′) and a HindIII primer with two selective residues (5′-GACTGCGTACCAATTCAC-3′) were used. Capillary electrophoresis of the amplified products along with standard marker LIZ600 was performed in an ABI automated DNA sequencer (model 3500 Dx genetic analyzer; Applied Biosystems, USA). Typing data were imported to BioNumerics v 6.6 software (Applied Maths, Ghent, Belgium). The similarity coefficient was determined by Pearson correlation, with negative similarities clipped to zero. Cluster analysis was performed by the use of the unweighted pair group method using average linkages and BioNumerics software.

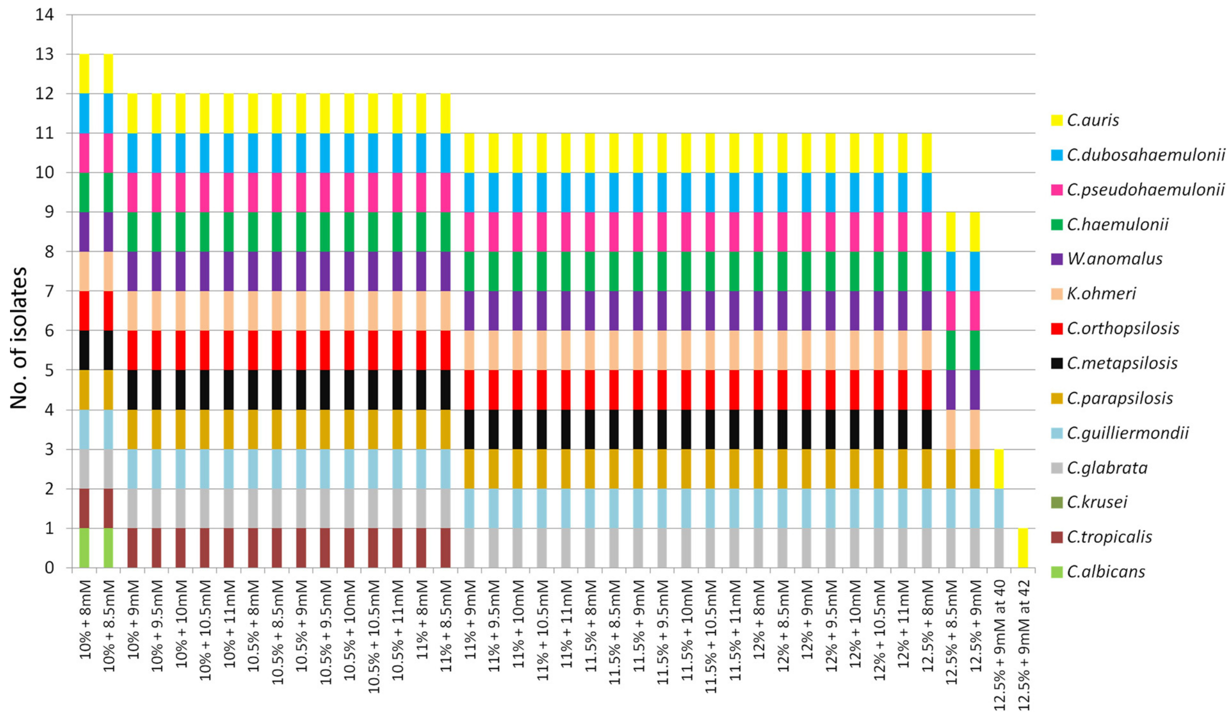

FIG 1 Results of dual-stress induction in *C. auris* and 13 common non-*C. auris* yeast isolates.

RESULTS

Single-stress and dual-stress induction. Only *C. auris* and five other *Candida* species (*C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, and *C. guilliermondii*) could grow on YPD medium at 42°C without salt and metal stress. Salt (12.5% sodium chloride) tolerance at 37°C after 24 h of incubation was noted in *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. guilliermondii*, *C. utilis*, *C. lusitaniae*, *C. viswanathii*, *C. pararugosa*, *C. kefyr*, *C. ciferrii*, *C. famata*, *S. cerevisiae*, *C. neoformans*, *C. gattii*, *C. laurentii*, *G. silvicola*, *L. elongisporus*, *C. fabianii*, *T. asahii*, *T. dohaense*, and 18 *C. auris* isolates. Metal (9 mM ferrous sulfate) tolerance at 37°C after 24 h of incubation was noted in *C. auris* and all non-*C. auris* yeast species (except *C. parapsilosis*). The members of clades I, II, III, and IV tolerated salt concentrations of 16%, 12%, 16%, and 16%, respectively. No differences in temperature and metal stress tolerance were observed among the clades. Tolerance at the stress levels represented by 12.5% sodium chloride and 9 mM ferrous sulfate as shown by growth at 42°C was seen only in *C. auris* (all four clades) except in two clade II isolates (Japanese *C. auris* JCM 15448 and South Korean *C. auris* 12372) and in no non-*auris* yeast isolates. Finally, YPD medium containing 12.5% sodium chloride and 9 mM ferrous sulfate, used with incubation at 42°C, was chosen as the selective medium (Selective Auris Medium [SAM]) for *C. auris* in the validation study. The control plates with YPD media and its modifications, maintained at different temperatures, are shown in Fig. S2 in the supplemental material. Representative images depicting the results of combined stress inductions in 13 common non-*C. auris* yeasts are presented in Fig. 1.

Growth kinetics. Growth curve analysis of one reference *C. auris* isolate each from clades I, II, III, and IV showed that the four clades had different growth patterns at 42°C. The doubling times for clades I, II, III, and IV at 42°C were 6.769 ± 0.388, 12.670 ± 0.337, 5.136 ± 0.868, and 3.426 ± 0.940 h, respectively (means $\pm$ standard deviations [SD]) (Fig. S3). The doubling time of the clade II isolate at 42°C was significantly higher than that seen with each of other three clade isolates (compared with clade I, $P = 0.0032$; compared with clade III, $P = 0.0019$; compared with clade IV, $P = 0.0026$).

Validation of selective medium. During validation, a total of 579 yeast isolates were evaluated on the SAM (Fig. 2).

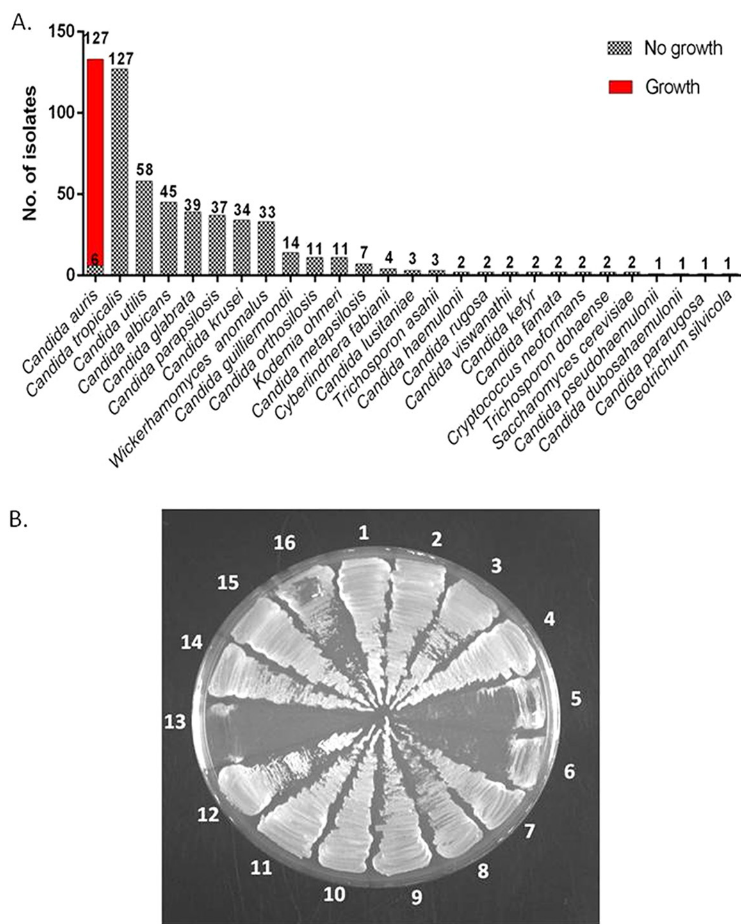

FIG 2 (A) Validation of results of the use of selective medium with *C. auris* and non-*C. auris* yeast species by growth at 48 h of incubation. (B) Directly streaked *C. auris* clinical isolates at positions 1 to 12 and positions 14 to 16, showing heavy confluent growth on the selective medium after 3 days of incubation. Position 13 represents non-*C. auris* yeast (*C. parapsilosis*) showing no growth.

Of 133 *C. auris* isolates (clinical, $n = 117$; environmental, $n = 8$; patient colonizer, $n = 8$) tested, 127 *C. auris* isolates grew on the selective medium plates, showing moderate to heavy growth after 48 h of incubation, whereas the remaining 6 isolates exhibited growth only after 72 h of incubation (Fig. 3). None of the other yeast isolates tested grew on this selective medium at 42°C even after incubation for 72 h. The specificity of the selective isolation of *C. auris* by the test medium was 100% (95% confidence interval [CI], 99.18% to 100%) whereas the sensitivity was 95.5% (95% CI, 90.44% to 98.33%) at 48 h, and specificity and sensitivity were 100% each at 72 h of incubation. The positive predictive value (PPV) for correct identification was 100%, with a negative predictive value of 98.67% (95% CI, 97.14% to 99.4%) at 48 h, and the values were 100% each at 72 h of incubation.

A total of 40 yeast-positive BD Bactec blood culture broths were inoculated onto the selective medium. Only one showed confluent growth on the selective medium, which was later confirmed as *C. auris* by MALDI-TOF mass spectrometry (MS). The results of selective medium validation for direct isolation of *C. auris* from yeast-positive blood culture vials are shown in Fig. 3.

FAFLP. FAFLP analyses revealed four distinct clusters (A, B, C, and D) (Fig. 4) with percent similarity of >74% and intercluster difference of <8%. The *C. auris* isolates in cluster B were clustered with the standard clade I isolate. The isolates were from different locations of India and from clinical samples (blood, urine, vascular tip), colonizing sites (oral, rectal, axilla), and environmental (bed surface) sources. The

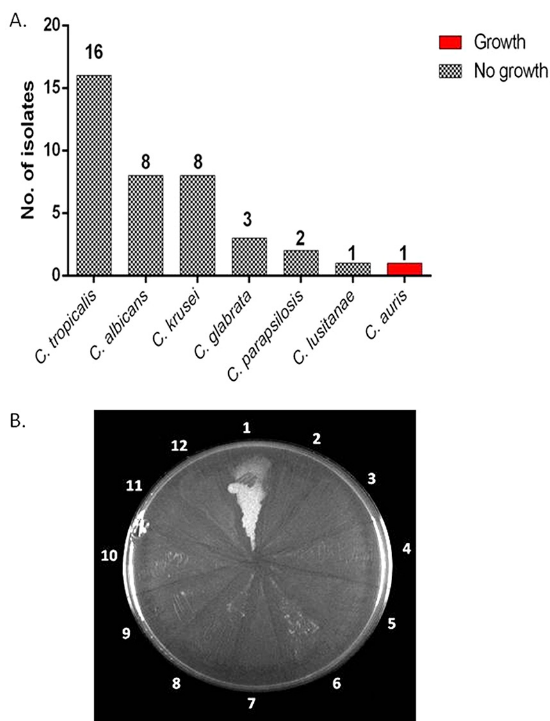

FIG 3 (A) Validation of results of the use of selective medium for isolation of *C. auris* directly from positive automated blood culture vials. (B) Confluent growth of *C. auris* obtained from direct inoculation from one *C. auris*-positive blood culture vial (position 1) after 48 h of incubation.

reference strains of clade II (JCM 15448 and CBS 12372), clade III (CDC-AR 0383), and clade IV (CDC-AR 0385) separated distinctly from each other, forming clusters A, C, and D, respectively.

DISCUSSION

In the present study, a simple, cost-effective, and sensitive selective medium was developed for the isolation and identification of *C. auris*. This medium was successfully validated in a large number of clinical yeast isolates, including *C. auris* from the four clades described above. Additionally, direct isolation of *C. auris* was performed from positive automated blood culture vials, with encouraging results.

The worldwide emergence of *C. auris* has emphasized the need for identifying *Candida* to the species level. This is essential for prompt initiation of appropriate antifungal therapy in the context of predictable species-specific susceptibility profiling of *Candida* species. Moreover, the CDC (Atlanta, GA) advises species-level identification of *Candida* species isolated from clinical sample, especially when *C. auris* infection or colonization has been detected in any health care facility or when a patient had had an overnight hospital stay in a country with *C. auris* transmission in the previous 6 months (2).

Identification of *C. auris* by conventional phenotypic methods is challenging, and the results are often inconclusive (22). On commercial chromogenic media such as CHROMagar *Candida*, BBL CHROMagar *Candida*, CAN2 chromogenic, *Candida* ID, and Brilliance *Candida* agar, the colony color of *C. auris* ranges from beige and pink to dark purple, making it difficult to differentiate from other yeasts, including *C. parapsilosis*, whose colonies appear similar in color (23, 24). Commercial identification systems also misidentify or fail to identify this pathogen (23). *C. auris* isolates are mistakenly identified by commercial systems as *C. famata*, *Candida sake*, *Rhodotorula glutinis*, and *S. cerevisiae* by API 20C AUX (bioMérieux); as *C. haemulonii*, *C. duobushaemulonii*, *C. famata*, and *C. glabrata* by the older version of Vitek 2 (bioMérieux); as *C. albicans*, *C.*

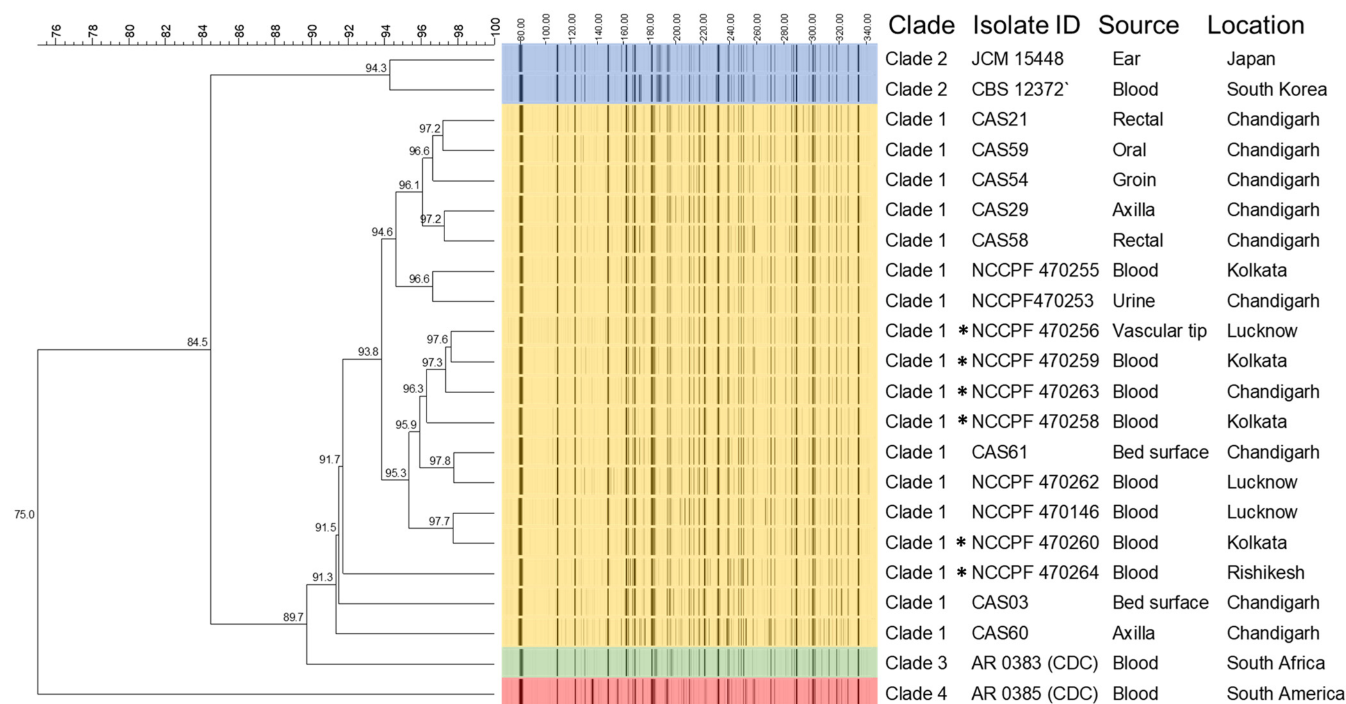

FIG 4 Fluorescent amplified fragment length polymorphism (FAFLP) analysis of 22 isolates of *C. auris* (9 clinical isolates [NCCPF], 6 colonizing isolates [Chemical Abstracts Service {CAS} no. 21, 29, and 54, aggregative; CAS no. 58, 59, and 60, nonaggregative], 2 environmental isolates [CAS 03, aggregative; CAS 61, nonaggregative], 1 *C. auris* clade I isolate [NCCPF 470146], 2 *C. auris* clade II isolates [JCM 15448, Japan; CBS 12372, South Korea], 1 *C. auris* clade III isolate [AR 0383, CDC], and 1 *C. auris* clade IV isolate [AR 0385, CDC]). *, six isolates which grew after prolonged incubation (72 h) on standardized medium.

parapsilosis, *C. tropicalis*, *C. famata*, *C. lusitaniae*, *C. guilliermondii*, and *Candida catenulate* by MicroScan (Beckman Coulter); and as *C. haemulonii*, *C. parapsilosis*, and *C. catenulata* by BD Phoenix (Becton, Dickinson) (12, 23–28).

Identification by ribosomal DNA (rDNA) sequencing, *C. auris*-specific PCR/quantitative PCR (qPCR), or MALDI-TOF MS is accurate. However, such advanced molecular methods are not available at the majority of routine clinical laboratories, especially in resource-limited settings (29). This highlights the need for adopting a more practical solution for *C. auris* identification in those developing countries (30).

Supplemented or modified media have been used previously for the screening of *C. auris* with limited success (23, 31, 32). A previous study claimed that *C. auris* could be distinguished from the *C. haemulonii* species complex on CHROMagar *Candida* medium supplemented with Pal's agar (24). While *C. auris* forms creamy white colonies on this medium when incubated at 37°C, *C. haemulonii* complex forms light pink colonies with growth inhibition at 42°C. However, variations between different clades of *C. auris* have been seen with this medium, limiting its applicability.

In another study, Welsh et al. developed an enrichment broth procedure based on the ability of *C. auris* to survive under high-salt and high-temperature conditions, facilitating its isolation (31). They observed that among all *Candida* isolates tested, only *C. auris* grew at a temperature of 40°C and salinity of 10% (wt/vol) in Sabouraud's or yeast nitrogen base broth in the presence of dulcitol or mannitol (31). In the present study, a 12.5% sodium chloride concentration and a high (42°C) incubation temperature were not enough to inhibit a few non-*C. auris* yeast species when used individually. However, a combination of 12.5% sodium chloride with 9 mM ferrous sulfate added to YPD and incubation at 42°C inhibited all common non-*C. auris* yeast isolates. Tolerance of high salt stress and metal stress in *C. auris* might be due to higher expression of the *Hog1* gene than has been seen with other species (33). We did not find selective inhibition of *C. glabrata* and *C. guilliermondii* in the presence of high salinity, metal stress, and high temperature. However, on

raising the temperature to 42°C, we were able to overcome this hindrance, resulting in a highly specific medium for the selective isolation of *C. auris*. Similarly to our findings, Welsh et al. reported observable growth of *C. glabrata* in 10% salt-containing Sabouraud's broth whereas growth of all other *Candida* species, including *C. haemulonii* complex, was inhibited.

We also isolated *C. auris* on this medium directly from a signal-positive Bactec culture BC broth in one confirmed case of infection. This could enable rapid identification from the blood culture broth, but future studies with a large number of samples are needed. This selective medium may cost ~22 to 25 Indian rupees (INR) (approximately 0.5 U.S. dollars) per plate, which is less expensive than any of the other chromogenic or supplemented media described earlier. Simple inexpensive chemicals, an easy preparation method, and direct inoculation (streaking) of isolated culture would make this medium user-friendly.

In the present study, all of the *C. auris* isolates except the Japanese isolate (*C. auris* JCM 15448) and the South Korean isolate (*C. auris* 12372) showed growth on SAM. The differences in the characteristics of the strains in terms of origin or clade might represent the possible cause of this growth inhibition. All the Indian isolates of *C. auris* and reference isolates of clade III and clade IV grew well on this medium. To assess the reasons for the inability of the two clade II *C. auris* isolates to grow on the standardized medium, we employed growth kinetics, temperature tolerance (42°C), and metal stress (9 mM $FeSO_4$) assays. *C. auris* clade II isolates showed significantly higher doubling times at 42°C than the other 3 clades, which could possibly be one of the reasons for its absence of growth on this selective medium when additional stresses were added. Those two clade II isolates were able to grow at a maximum level of salt tolerance of 12%. Hence, in the regions where clade II is circulating more extensively, we suggest reducing the salt concentration and prolonging the period of incubation at 42°C or lower. Though, in view of clade-wise variations in the expression levels of stress response pathway genes, the lower level of expression of the *Hog1* gene in clade II isolates may be one of the reasons for the lack of growth described above, the hypothesis needs further confirmation. Nonetheless, the applicability of this medium in the global scenario needs to be validated using a greater number of *C. auris* isolates from different geographic locations. Another minor limitation of this medium is its short shelf life (2 to 4 days, at 4°C) due to gradual oxidation and precipitation of iron, necessitating fresh preparation before use.

Conclusion. We successfully developed a selective medium, Selective Auris Medium (SAM), for the rapid detection and reliable identification of *C. auris*. This could be especially useful in diagnostic settings where costly molecular tests are not available. Due to its high sensitivity and specificity, this medium can be applied for the routine screening of suspected *C. auris* isolates. It could be particularly useful in regions with a preponderance of clades I, III, and IV of *C. auris*. For those regions with a predominance of clade II, the same medium can be used with a lower salt concentration and longer period of incubation at 42°C or lower. A study on the utility of this medium for direct isolation of *C. auris* from a signal-positive commercial isolation system is warranted.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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We declare no conflict of interest.

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REFERENCES

1. Centers for Disease Control and Prevention (CDC). 2019. Global emergence of invasive infections caused by the multidrug-resistant yeast Candida auris. https://www.cdc.gov/fungal/diseases/candidiasis/candida-auris-alert.html. Accessed 20 December 2019.
2. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, Colombo AL, Calvo B, Cuomo CA, Desjardins CA, Berkow EL, Castanheira M, Magobo RE, Jabeen K, Asghar RJ, Meis JF, Jackson B, Chiller T, Litvintseva AP. 2017. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin Infect Dis 64:134–140. https://doi.org/10.1093/cid/ciw691.
3. CDC. Tracking Candida auris. 2019. https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Ffungal%2Fdiseases%2Fcandidiasis%2Ftracking-c-auris.html#world. Accessed 7 December 2019.
4. Chow NA, De Groot T, Badali H, Abastabar M, Chiller TM, Meis JF. 2019. Potential fifth clade of *Candida auris*, Iran, 2018. Emerg Infect Dis 25:1780–1781. https://doi.org/10.3201/eid2509.190686.
5. Abastabar M, Haghani I, Ahangarkani F, Rezai MS, Taghizadeh Armaki M, Roodgari S, Kiakojuri K, Al-Hatmi AMS, Meis JF, Badali H. 2019. Candida auris otomycosis in Iran and review of recent literature. Mycoses 62:101–105. https://doi.org/10.1111/myc.12886.
6. Chowdhary A, Sharma C, Meis JF. 2017. *Candida auris*: a rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathog 13:e1006290. https://doi.org/10.1371/journal.ppat.1006290.
7. Lone SA, Ahmad A. 2019. Candida auris-the growing menace to global health. Mycoses 62:620–637. https://doi.org/10.1111/myc.12904.
8. Sherry L, Ramage G, Kean R, Borman A, Johnson EM, Richardson MD, Rautemaa-Richardson R. 2017. Biofilm-forming capability of highly virulent, multidrug-resistant Candida auris. Emerg Infect Dis 23:328–331. https://doi.org/10.3201/eid2302.161320.
9. Biswal M, Rudramurthy SM, Jain N, Shamanth AS, Sharma D, Jain K, Yaddanapudi LN, Chakrabarti A. 2017. Controlling a possible outbreak of Candida auris infection: lessons learnt from multiple interventions. J Hosp Infect 97:363–370. https://doi.org/10.1016/j.jhin.2017.09.009.
10. Singh R, Kaur M, Chakrabarti A, Shankarnarayan SA, Rudramurthy SM. 2019. Biofilm formation by Candida auris isolated from colonising sites and candidemia cases. Mycoses 62:706–709. https://doi.org/10.1111/myc.12947.
11. Osei Sekyere J. 2018. *Candida auris*: a systematic review and meta-analysis of current updates on an emerging multidrug-resistant pathogen. Microbiologyopen 7:e00578. https://doi.org/10.1002/mbo3.578.
12. Jeffery-Smith A, Taori SK, Schelenz S, Jeffery K, Johnson EM, Borman A, Candida auris Incident Management Team, Manuel R, Brown CS. 2018. *Candida auris*: a review of the literature. Clin Microbiol Rev 31:e00029-17. https://doi.org/10.1128/CMR.00029-17.
13. Rudramurthy SM, Chakrabarti A, Paul RA, Sood P, Kaur H, Capoor MR, Kindo AJ, Marak RSK, Arora A, Sardana R, Das S, Chhina D, Patel A, Xess I, Tarai B, Singh P, Ghosh A. 2017. Candida auris candidaemia in Indian ICUs: analysis of risk factors. J Antimicrob Chemother 72:1794–1801. https://doi.org/10.1093/jac/dkx034.
14. Cortegiani A, Misseri G, Giarratano A, Bassetti M, Eyre D. 2019. The global challenge of *Candida auris* in the intensive care unit. Crit Care 23:150. https://doi.org/10.1186/s13054-019-2449-y.
15. Lockhart SR, Jackson BR, Vallabhaneni S, Ostrosky-Zeichner L, Pappas PG, Chiller T. 2017. Thinking beyond the common Candida species: need for species-level identification of Candida due to the emergence of multidrug-resistant Candida auris. J Clin Microbiol 55:3324–3327. https://doi.org/10.1128/JCM.01355-17.
16. Sharma C, Kumar N, Pandey R, Meis JF, Chowdhary A. 2016. Whole genome sequencing of emerging multidrug resistant *Candida auris* isolates in India demonstrates low genetic variation. New Microbes New Infect 13:77–82. https://doi.org/10.1016/j.nmni.2016.07.003.
17. Lockhart SR, Berkow EL, Chow N, Welsh RM. 2017. *Candida auris* for the clinical microbiology laboratory: not your grandfather's *Candida* species. Clin Microbiol Newsl 39:99–103. https://doi.org/10.1016/j.clinmicnews.2017.06.003.
18. Ghosh AK, Paul S, Sood P, Rudramurthy SM, Rajbanshi A, Jillwin TJ, Chakrabarti A. 2015. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for the rapid identification of yeasts causing bloodstream infections. Clin Microbiol Infect 21:372–378. https://doi.org/10.1016/j.cmi.2014.11.009.
19. Clinical and Laboratory Standards Institute. 2019. M60 reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard, 2nd ed. https://clsi.org/standards/products/microbiology/documents/m60/.
20. CDC. 2019. Candida auris: antifungal susceptibility testing and interpretation. https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html.
21. Chakrabarti A, Rudramurthy SM, Kale P, Hariprasath P, Dhaliwal M, Singhi S, Rao KLN. 2014. Epidemiological study of a large cluster of fungaemia cases due to *Kodamaea ohmeri* in an Indian tertiary care centre. Clin Microbiol Infect 20:O83–O89. https://doi.org/10.1111/1469-0691.12337.
22. Caceres DH, Forsberg K, Welsh RM, Sexton DJ, Lockhart SR, Jackson BR, Chiller T. 2019. *Candida auris*: a review of recommendations for detection and control in healthcare settings. J Fungi (Basel) 5:111. https://doi.org/10.3390/jof5040111.
23. Mizusawa M, Miller H, Green R, Lee R, Durante M, Perkins R, Hewitt C, Simner PJ, Carroll KC, Hayden RT, Zhang SX. 2017. Can multidrug-resistant *Candida auris* be reliably identified in clinical microbiology laboratories? J Clin Microbiol 55:638–640. https://doi.org/10.1128/JCM.02202-16.
24. Kumar A, Sachu A, Mohan K, Vinod V, Dinesh K, Karim S. 2017. Diferenciación sencilla y de bajo coste de *Candida auris* del complejo *Candida haemulonii* con el empleo del medio CHROMagar Candida enriquecido con medio de Pal. Rev Iberoam Micol 34:109–111. https://doi.org/10.1016/j.riam.2016.11.004.
25. Spivak ES, Hanson KE. 2017. *Candida auris*: an emerging fungal pathogen. J Clin Microbiol 56:e01588-17. https://doi.org/10.1128/JCM.01588-17.
26. Pekard-Amenitsch S, Schriebl A, Posawetz W, Willinger B, Kölli B, Buzina W. 2018. Isolation of *Candida auris* from ear of otherwise healthy patient. Emerg Infect Dis 24:1596–1597. https://doi.org/10.3201/eid2408.180495.
27. Bidaud AL, Chowdhary A, Dannaoui E. 2018. *Candida auris*: an emerging drug resistant yeast – a mini-review. J Mycol Med 28:568–573. https://doi.org/10.1016/j.mycmed.2018.06.007.
28. Girard V, Mailler S, Chetry M, Vidal C, Durand G, van Belkum A, Colombo AL, Hagen F, Meis JF, Chowdhary A. 2016. Identification and typing of the emerging pathogen *Candida auris* by matrix-assisted laser desorption ionisation time of flight mass spectrometry. Mycoses 59:535–538. https://doi.org/10.1111/myc.12519.
29. Chindamporn A, Chakrabarti A, Li R, Sun P-L, Tan B-H, Chua M, Wahyuningsih R, Patel A, Liu Z, Chen Y-C, Chayakulkeeree M. 2018. Survey of laboratory practices for diagnosis of fungal infection in seven Asian countries: an Asia Fungal Working Group (AFWG) initiative. Med Mycol 56:416–425. https://doi.org/10.1093/mmy/myx066.
30. Durante AJ, Maloney MH, Leung VH, Razeq JH, Banach DB. 2018. Challenges in identifying *Candida auris* in hospital clinical laboratories: a need for hospital and public health laboratory collaboration in rapid identification of an emerging pathogen. Infect Control Hosp Epidemiol 39:1015–1016. https://doi.org/10.1017/ice.2018.133.
31. Welsh RM, Bentz ML, Shams A, Houston H, Lyons A, Rose LJ, Litvintseva AP. 2017. Survival, persistence, and isolation of the emerging multidrug-resistant pathogenic yeast *Candida auris* on a plastic health care surface. J Clin Microbiol 55:2996–3005. https://doi.org/10.1128/JCM.00921-17.
32. S2 Media. 2018. 5067: salt Sabouraud dulcitol broth (SSDB) package insert. S2 Media, Spokane, WA. https://www.inciensa.sa.cr/inciensa/centros_referencia/cursos_cnrb/taller%20regional/diagnostico%20y%20psa%20de%20candida/lab%20protocols/Medio%20selectivo%20C.%20auris.pdf.
33. Day AM, McNiff MM, da Silva Dantas A, Gow NAR, Quinn J. 2018. *Hog1* regulates stress tolerance and virulence in the emerging fungal pathogen *Candida auris*. mSphere 3:e00506-18. https://doi.org/10.1128/mSphere.00506-18.