Evaluation and Validation of Optimal Laboratory Screening Methods using a Custom Dulcitol Agar and Commercially Available Chromogenic Agars in detecting *Candida auris* growth

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INTRODUCTION

Candida auris is an emerging multidrug resistant yeast that causes severe invasive infections and nosocomial outbreaks[1], with mortality rates ranging from 30-72%[2]. During the COVID-19 pandemic, *C. auris* outbreaks have been reported in healthcare facilities around the globe[3,4,5,6], including nosocomial outbreaks of pan-resistant *C. auris* in the United States [7]. In Canada, 26 cases of *C .auris* have been reported as of January 2021[8], with the first reported outbreak in a community healthcare facility in the spring of 2018[8].

C. auris is considered to be a notable threat to global health because a) multidrug resistance is common, limiting treatment options, b) its ability to colonize on skin and environmental surfaces allows it to spread between patients in healthcare settings, and c) it can be misidentified by commonly available laboratory identification methods[1]. As such, developing a reliable and timely method of identifying *C. auris* is needed for its management worldwide.

Chromogenic agars are useful in differentiating different *Candida* species from each other; the novel chromogenic medium Colorex Candida Plus claims to allow for specific differentiation of *C. auris* from other *Candida* species which is unique compared to conventional mycological media and other chromogenic media[9,10,11]. The unique ability for *C. auris* to assimilate dulcitol, unlike other common *Candida* species[12,13], makes dulcitol useful in selective broth[13] and gives it potential for use as a selective agar. PCR is known to be a rapid and sensitive screening method for *C. auris*[14].

This study aims to determine the optimal screening procedure that can reliably identify *C. auris* with the shortest turn-around time, by evaluating and comparing screening culture procedures using a custom dulcitol agar (CA), CHROMagar™ Candida (BD, Phoenix) (BD), and Colorex Candida Plus (Micronostyx, Ottawa) (M), and molecular methods using polymerase chain reaction testing.

METHODS

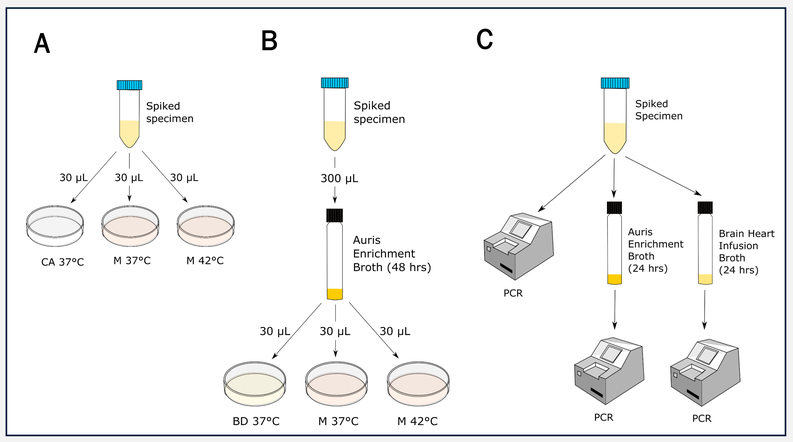

Figure 1: Direct-to-agar (A), AEB-enriched (B), and PCR (C) screening methods

- 11 susceptible and 9 multidrug resistant *C. auris* isolates, representative of the East Asian, South Asian, South American, and African clades were tested.
- A 0.5 McFarland in saline was prepared from each isolate and inoculated into fresh nasal-axillary-groin-perineum-rectal swabs (Copan ESwab™ with liquid Amies) pooled from randomly selected patients, to achieve a final concentration of 10^4 CFU/mL.
- For direct-to-agar screening methods (Figure 1a), 30 µL of spiked specimen were inoculated directly onto DA and M plates in triplicate. All DA plates were incubated in O_2 at 37°C, and the M plates were incubated in O_2 at 37°C and 42°C.
- For broth-enriched culture screening methods (Figure 1b), 300 µL of spiked specimen were inoculated into Auris Enrichment Broth (Thermo Fisher Scientific, Nepean) (AEB) and incubated for 48 hrs at 37°C in O_2. After 48 hrs, 30 µL of AEB cultures were inoculated onto M and BD plates in triplicate. All BD plates were incubated in O_2 at 37°C, and the M plates were incubated in O_2 at 37°C and 42°C.
- Growth of *C. auris* was checked daily, and colony counts, colour, and sizes were recorded for each culture parameter studied. MALDI-TOF MS (MALDI) (Vitek MS, bioMérieux) was used to verify the identity of all visibly distinct colony types.

METHODS (continued)

- For PCR screening methods (Figure 1c), spiked specimen was directly tested for *C. auris* using PCR, or incubated for 24 hrs at 37°C in AEB or Brain Heart Infusion (BHI broth) before testing using BioGX C. auris research-use-only PCR using EasyMag (bioMérieux) extraction

RESULTS

Method	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Proportion confirmed as C auris by MALDI
Culture-based, Direct-to-Agar Screening Procedures								
Direct M 37°C	25.00%	**100.00%**	100.00%	100.00%	100.00%	100.00%	100.00%	**100.00%**
Direct DA37°C	0.00%	0.00%	95.00%	**100.00%**	100.00%	100.00%	100.00%	95.00%
Direct M 42°C	0.00%	35.00%	85.00%	95.00%	95.00%	95.00%	95.00%	80.00%
Culture-based, AEB-Enriched Screening Procedures								
AEB-enriched BD 37°C	Broth		**100.00%**	100.00%	100.00%	100.00%	100.00%	**100.00%**
AEB-enriched M 37°C	Broth		**100.00%**	100.00%	100.00%	100.00%	100.00%	**100.00%**
AEB-enriched M 42°C	Broth		90.00%	**100.00%**	100.00%	100.00%	100.00%	90.00%
PCR Screening Procedures								
Direct PCR	**100.00%**							
AEB-enriched PCR 37°C	Broth	**100.00%**						
BHI-enriched PCR 37°C	Broth	70.00%						

Table 1: Proportion of presumptive positives, and MALDI-TOF MS identification from each set of culture parameters.

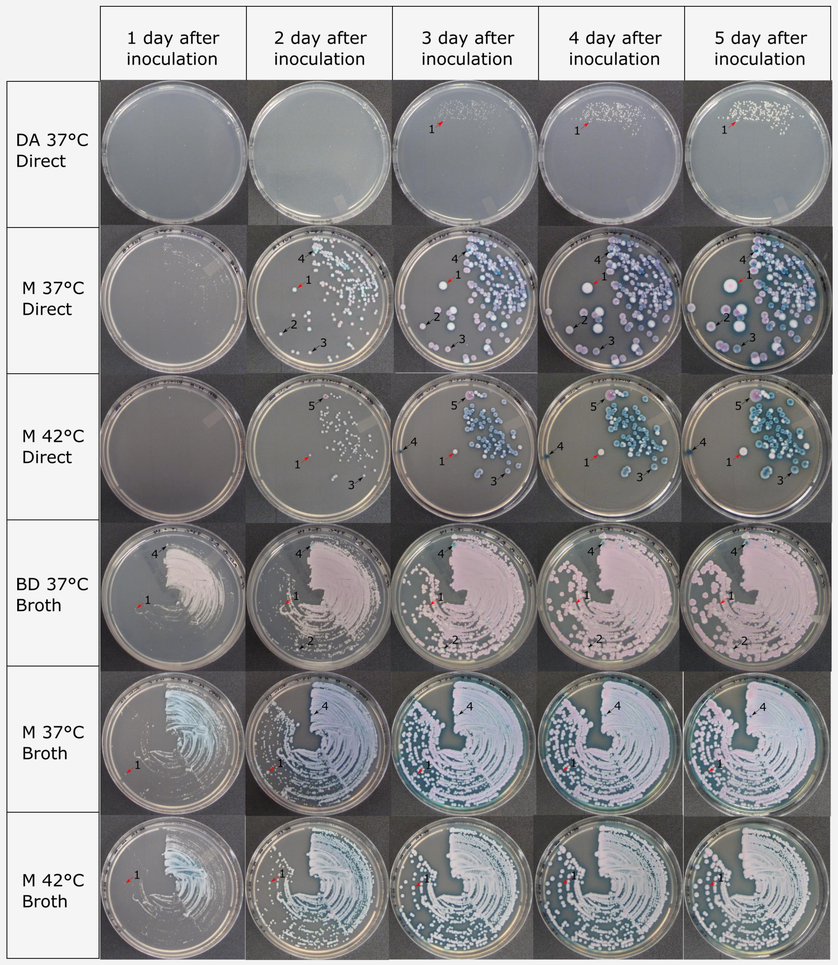

Figure 2: Growth of spiked specimen samples at each set of culture parameters over 5 days. Samples contain *C. auris* (1) and background non-*auris Candida* isolates: *C. parapsilosis* (2), *C. lusitaniae* (3), *C. albicans* (4), and *C. krusei* (5).

RESULTS (continued)

Direct-to-agar culture on DA at 37°C: *C. auris* grew as small white colonies. Colonies first appeared as tiny white pinpoint colonies on day 3, which could be identified using MALDI if several were used. Identification by MALDI using single colonies was possible after day 4. Growth of non-*auris* isolates were suppressed completely.

Direct-to-agar culture on M at 37°C: *C. auris* first appeared as tiny white pinpoint colonies on day 1. On day 2, *C. auris* was morphologically distinct, appearing as small white colonies with a characteristic blue halo diffused in the surrounding agar. Suspected colonies had enough cells for identification with MALDI. On day 4 and beyond, colonies became light pink but retained the blue halo.

Direct-to-agar culture on M at 42°C: *C. auris* appeared as tiny white pinpoint colonies on day 1. On day 2, only some colonies were morphologically distinct, appearing white with a less prominent blue halo, while most appeared pink with no halo; all colonies had enough cells to perform MALDI. After day 3, *C. auris* colonies became were morphologically distinct with blue halos. These colonies were dryer than the ones grown on M at 37°C, making them more challenging to identify using MALDI.

Broth-enriched culture on BD at 37°C: On all days, *C. auris* growth dominated, appearing as pink colonies. There were no challenges in identification using MALDI.

Broth-enriched culture on M at 37°C: On all days, *C. auris* growth dominated, first appearing as blue or white colonies with a blue halo. 3 days after inoculation, some colonies turned light pink but retained the blue halo. There were no challenges in identification using MALDI.

Broth-enriched culture on M at 42°C: On all days, *C. auris* growth dominated, and appeared as white colonies with blue halos. These colonies were dryer than the ones grown on M at 37°C, making them more challenging to identify using MALDI.

CONCLUSIONS AND FUTURE DIRECTIONS

- Direct inoculation on Colorex Candida Plus incubated at 37°C results in growth of morphologically distinct *C. auris* colonies within two days of inoculation, which can be reliably identified using MALDI-TOF MS. It is the optimal culture-based screening method with the minimal turn-around time
- Broth-enriched cultures minimized breakthrough of non-auris isolates, but require a 3-day turn-around time. Ability to identify *C. auris* from broth-enriched cultures inoculated to either CHROMagar™ Candida or Colorex Candida Plus are indistinguishable
- Direct PCR from specimen had the lowest turn-around time amongst all tested screening methods, and PCR from AEB-enriched specimen also had a relatively low turnaround time.
- Assessing the limit of detection of these screening methods will be important in determining the potential benefit of AEB enrichment

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