Determination of the Optimal Candida auris Screening Culture Procedure

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Background
- Candida auris colonization can cause life-threatening infections with mortality rates reported between 30-72%.1
- Over the past decade, increasing outbreaks of multidrug-resistant C. auris have impacted hospitals worldwide.1
- Canadian prevalence rates among hospitalized high-risk populations can reach up to 5.7%.2
- Although recommended for high-risk individuals, C. auris screening procedures are only established in 11% of Canadian labs surveyed.3
- Disparities exist in laboratory procedure recommendations for screening using culture methods.4

Objectives
- Determine the optimal screening culture procedure to obtain:
  - Highest recovery rate of C. auris;
  - Lowest breakthrough of other species;
  - Shortest turnaround time for identification;
  - Easiest implementation in clinical laboratories.

Materials and Methods
- Both provincial clinical isolates and CDC-FDA AR Isolate Bank panel isolates were selected as follows:
  - 11 unique C. auris isolates;
  - 23 non-aurs Candida isolates (3 per species):
    - C. albicans;
    - C. duboschaeumilongi;
    - C. lusitaniae;
    - C. haemulonii;
    - C. krusei;
    - C. guillermondii;
    - C. glabrata;
    - Kodamea ohmeri;
    - C. tropicalis;
- Isolates were inoculated into de-identified clinical nasal-axillary-groin-perineum swabs (Copan ESwab™ with liquid Amies).
- The final isolate concentration approached 10^6 CFU/mL.
- Serial experiments were performed to identify optimal culture parameters variables including:
  - Inoculum volume (30/150/300 µL);
  - Agar types (generously provided by BD and Thermofisher): BD BBL CHROMagar Candida™, ThermoFisher Brilliance Candida™, ThermoFisher custom salt/dulcitol agar, ThermoFisher Sheep Blood Agar (SBA), Thermofisher Inhibitory Mould Agar (IMA);
  - Incubation temperature (37/40/42°C);
  - Incubation time (1 to 4 days);
  - Direct agar inoculation vs. broth enrichment using ThermoFisher Auris Enrichment Broth;
  - For broth, daily inversion vs. continuous shaking (250 rpm).
- Colony counts and relative sizes were recorded daily for each culture parameter studied.
- The best parameters from each experiment were then used for the following experiment in a staggered approach, until the selected parameters were fully optimized and reproducible.

Results
- A. The first experiment compared 3 media using direct inoculation, 3 different inoculum volumes and incubation at 40°C 100% (1/1) recovery was only seen using 300 µL onto the salt/dulcitol agar between Day 5 and 7. Other agars had too high recovery of non-aurs species for 100% recovery Brilliance™ was unable to growth some C. auris isolates even without the presence of breakthrough.
- B. Experiment A was repeated using incubation at 42°C 100% (1/1) recovery was still only seen with the salt/dulcitol agar and as low as Day 3 of incubation. Although reduced, breakthrough was still too significant with other agars for optimal recovery.
- C. Using broth enrichment and shaker incubation at 40°C for either 1 or 2 days, only the salt/dulcitol agar provided 100% (1/1) recovery between Day 5 and 9. Breakthrough was too significant with other agars.
- D. Using broth enrichment and shaker incubation at 42°C for either 2, 3, or 7 days, the CHROMagar™ provided the quickest 100% (1/1) recovery at Day 4 with a 3-day enrichment. Salt/dulcitol agar and IMA also reached 100% (1/1) but more slowly (Day 5-10).
- E. When comparing broth enrichment using either continuous shaking or daily inversion, we see that inversion is insufficient to provide good recovery even with 4 days of enrichment. On the other hand, shaker incubation provides 100% (1/1) recovery after 3 days of enrichment. Breakthrough was only present on CHROMagar™.
- F. Both the direct-to-salt/dulcitol agar method and 3-day-broth-to-CHROMagar™ method had 100% (1/1) recovery at 37°C, although with more breakthrough than at higher temperatures. On salt/dulcitol agar, breakthrough appeared as pinpoints of growth undetectable by mass spectrometry (MALDI). On CHROMagar™, breakthrough of common species was easily distinguished by appearance.
- G. When using direct-to-salt/dulcitol agar, an inoculum volume of 30 µL can be sufficient to obtain 100% (1/1) recovery. With 2-day-broth-to-CHROMagar™, optimal recovery was only seen using 300 µL.

Conclusions and Future Directions
- CHROMagar™ requires broth enrichment with continuous shaking to reduce breakthrough of non-aurs isolates and thus improve recovery.
- Direct inoculation to salt/dulcitol agar is a satisfactory alternative for labs seeking streamlined workflows and/or automation compatibility.
- Breakthrough was minimal and easily handled using either approach.
- Turnaround time was 3 days using either methods, thereby facilitating timely infection prevention and control activities.
- Future experiments will include testing resistant C. auris strains on both methods as well as lot-to-lot variability for the custom salt/dulcitol agar.

References