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Verification, analytical sensitivity, cost-effectiveness, and comparison of four *Candida auris* screening methods

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Introduction

- *C. auris* is a rapidly emerging public health threat worldwide, with an overall mortality rate of 39% for invasive infection[1]
- It possesses intrinsic resistance to many antifungals and has the ability to become resistant to all current antifungals[2]
- *C. auris* can colonize mucosae, allowing nosocomial spread and outbreaks
- From 2019-2021, colonizations and infections in the United States increased by >200% and 95% respectively[3]
- Choice of a prompt *C. auris* identification method for screening specimens will support infection control efforts

- The optimal method to identify *C. auris* from screening specimens is unclear, US CDC guidance considers four methods without recommending one[4]
- Screening method choice is a laboratory decision, balancing analytical specifications, turnaround time, workflow implementation, and cost

Objectives

- **Primary**
  - Verification of direct chromogenic media culture, direct PCR, broth enrichment followed by chromogenic media culture, and broth enrichment followed by PCR
- **Secondary**
  - Determination of test characteristics for each verified method
  - Cost-effectiveness analysis

Methods

- Simulated specimens spiked with *C. auris* or non-*C. auris* reference strains supplied by the Public Health Ontario Laboratory
  - *Non-C.auris included: C. haemulonii, C. pseudohaemulonii, K. ohmeri, C. guiliermondii, C. krusei, C. parapsilosis, C. lusitaniae, S. cerevisiae*
- Convenience sampling method using bilateral axillary-groin ESwabs® (COPAN, Brescia, Italia), obtained either from laboratory staff or patient surveillance specimens negative for *C. auris*
- CLSI EP17A2 method[7] used to perform probit regression to determine the 95% limit of detection (analytical sensitivity) and time to pigment production

Chromogenic agar

- Incubated CHROMagar™ Colorex Candida Plus agar (Micronostyx, Ottawa, Canada) on the Kiestra system (Becton Dickinson, NJ, USA) aerobically at 37°C for 120 hours, with images acquired every 24 hours
- For time to pigment production, colony defined as characteristic of *C. auris* when the colony colour was pink with a surrounding diffusible blue halo

PCR

- Protocol adapted from the US Centers for Disease Control and Prevention[8]
- Modifications included: nucleic acid extraction using the easyMAG system (bioMérieux, France); Luna Probe One-Step RT-qPCR kit (New England Biosciences, MA, USA) use on the CFX96 thermal cycler (BioRad, CA, USA)
- 16S rRNA internal control, with modified dual-labelled probe: 5’ -/FAM/ AAT CTT CGC GGT GGC GTT GCA TTC A /BHQ-1/-3’

Broth enrichment

- Oxoid *C. auris* enrichment broth (Thermo Fisher, MA, USA) incubated aerobically at 37°C for 48 hours at 250rpm

Cost-effectiveness analysis

- Cost per test based on laboratory consumables (reagents) and medical laboratory technologist (MLT) time needed per test
- MLT time quantified by direct observation, using definition of one workload unit being equivalent to one minute of personnel time
- Cost per case detected determined using varying sample positivity thresholds from 0.1-10% based on North American point-prevalence studies

References

1. Chen *et al.* BMC Infect Dis. 2020;20(1):827.
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3. Lyman *et al.* Ann Intern Med 2023;176(4):489-95. Epub 20230321.
4. Centers for Disease Control and Prevention. 2023. Guidance for Detection of Colonization of *Candida auris.* Atlanta, GA.
5. CLSI. 2012. EP17A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. Wayne, PA.
6. Centers for Disease Control and Prevention. 2022. Real-Time PCR Based Identification of *Candida auris* using Applied Biosystems 7500 Fast Real-Time PCR Platform. Atlanta, GA.

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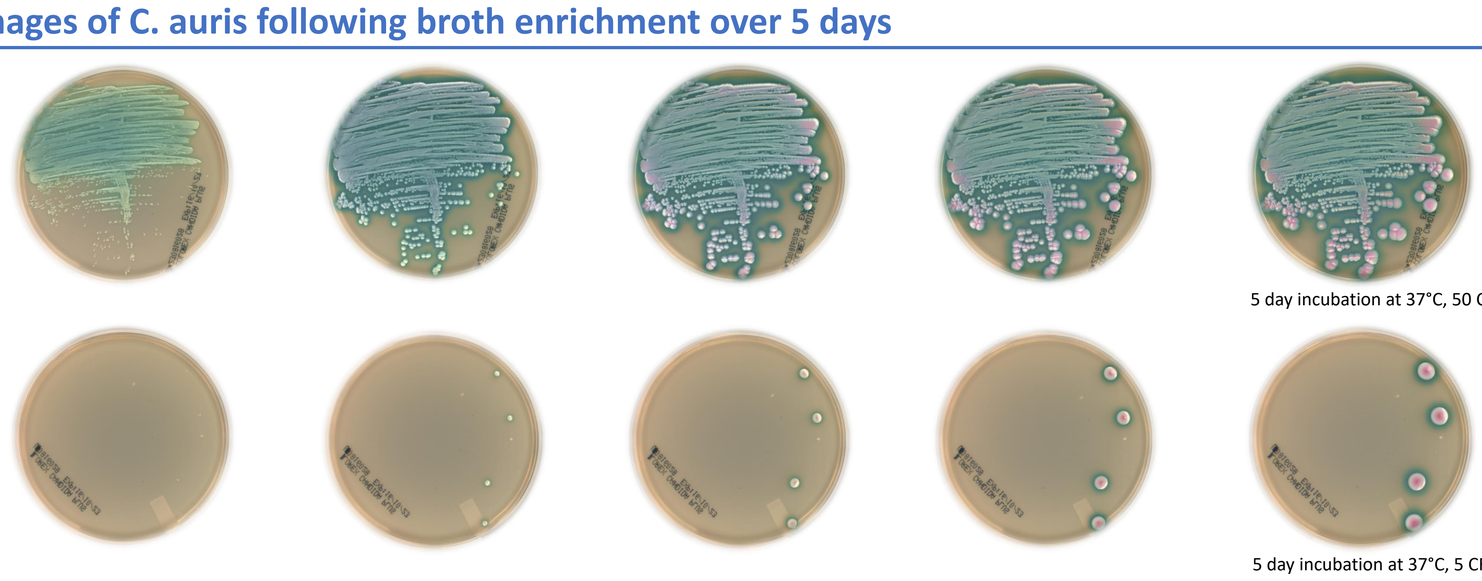
Results

- **Chromogenic culture**, *n*=188
  - *n*=103 (54.7%) were *C. auris* isolates, *n*=73 (38.8%) were non-*C. auris* yeast, *n*=12 (6.3%) contained a combination of *C. auris* and non-*C. auris*
  - Serial ten-fold dilutions tested from 1-1000 CFU/mL
  - Cultures plated directly from specimen can be reliably called “*C. auris* positive” after five days’ incubation
  - If positive colonies are only defined by diffusible pigment production, they may be reliably called positive after three days’ incubation
- **PCR**, *n*=222
  - *n*=112 (50.4%) were *C. auris* isolates, *n*=78 (35.1%) were non-*C. auris* yeast, *n*=30 (13.5%) contained a combination of *C. auris* and non-*C. auris*
  - Serial ten-fold dilutions tested from 1-1000 CFU/mL
- **Broth enrichment followed by chromogenic culture**, *n*=63
  - All isolates tested were *C. auris,* therefore unable to determine diagnostic sensitivity, diagnostic specificity, or analytical specificity
  - Serial ten-fold dilutions tested from 0.005-500 CFU/mL
  - When broth enrichment is used, the subsequent incubation of chromogenic culture plates can reliably be decreased to three days
- **Broth enrichment followed by PCR**, *n*=64
  - All isolates tested were *C. auris,* therefore unable to determine diagnostic sensitivity, diagnostic specificity, or analytical specificity
  - Serial ten-fold dilutions tested from 0.005-500 CFU/mL

Table 1: Summary of diagnostic test characteristics

Diagnostic test characteristic	Screening method			
	Chromogenic culture (*n*=188)	PCR (*n*=222)	Broth enrichment followed by chromogenic culture (*n*=63)	Broth enrichment followed by PCR (*n*=64)
Diagnostic sensitivity, % (95% CI)	*1000 CFU/mL,* 95.5 (77.2-99.9) *100 CFU/mL,* 96.7 (82.8-99.9)	*100 CFU/mL*, 100.0 (90.7-100) *10 CFU/mL*, 82.1 (66.5-92.5%)	Not determined	Not determined
Diagnostic specificity, % (95% CI)	*1000 CFU/mL,* 100 (66.4-100) *100 CFU/mL,* 94.7 (74.0-99.9)	*100 CFU/mL*, 100 (83.2-100) *10 CFU/mL*, 100 (80.5-100)	Not determined	Not determined
Analytical sensitivity, CFU/mL (95% CI)	256.34 (81.02-811.02)	27.11 (11.34-64.76)	16.32 (2.36-112.82)	2.75 (0.63-12.06)
Analytical specificity	NA	100%	NA	NA
95% time to pigment production, days (95% CI)	4.26 (4.02-4.51)	NA	2.92 (CI not estimable due to non-convergence)	NA
Turnaround time (days)	5	1	5	3

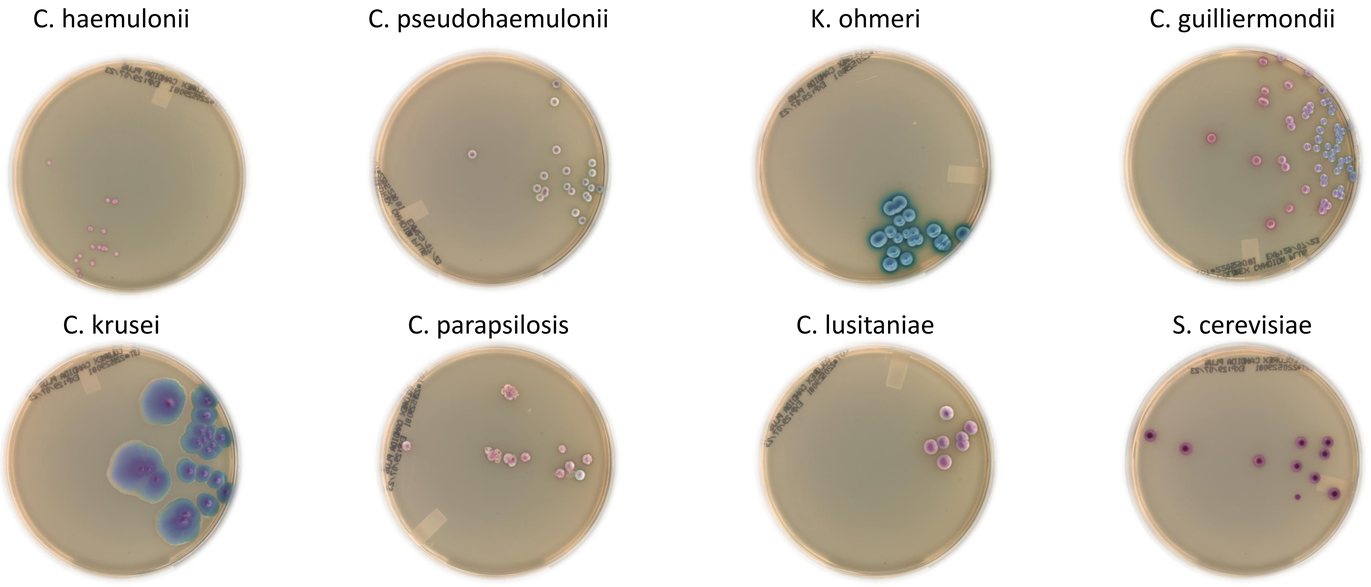
Images of C. auris following broth enrichment over 5 days



5 day incubation at 37°C, 50 CFU/mL

5 day incubation at 37°C, 5 CFU/mL

Images of non-C. auris at 5 days



5 day incubation at 37°C, 1000 CFU/mL

Cost per test

- Chromogenic culture: €8.62 / £7.48 / $9.43
- PCR: €10.30 / £8.94 / $11.27
- Broth enrichment: *additional* €18.52 / £16.08 / $20.27

Table 2: Cost-effectiveness analysis

Detection method	Percent positivity				
	0.1%	0.5%	1.0%	5.0%	10.0%
Chromogenic agar culture	€8617 £7480	€1723 £1496	€861 £748	€172 £149	€86 £74
PCR	€10298 £8939	€2079 £1787	€1029 £893	€207 £178	€102 £89
Enrichment broth followed by chromogenic culture	€27139 £23558	€5427 £4711	€2713 £2355	€542 £471	€271 £235
Enrichment broth followed by PCR	€28821 £25018	€5764 £5003	€2882 £2501	€576 £500	€288 £250

Conclusions

- Chromogenic culture is a reliable low-cost method, with ~95% diagnostic sensitivity and specificity at a threshold of 100 CFU/mL
- PCR has the fastest turnaround time despite greater MLT involvement and high cost, improving analytical sensitivity ~1$\log_{10}$ CFU/mL compared to culture
- Enrichment broth offers performance improvements over both PCR and culture alone but these are marginal when taken in context of cost, workflow, and increased turnaround time
- Future research should clarify the bioburden of *C. auris* colonized patients to further assist in determining the optimal screening method