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BRIEF REPORT

Verification, Analytical Sensitivity, Cost-effectiveness, and Comparison of 4 *Candida auris* Screening Methods

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In this verification study, we compare and contrast the performance characteristics of chromogenic agar culture, direct polymerase chain reaction (PCR), and broth enrichment followed by culture or PCR for the detection of *Candida auris* colonization. We find that culture and PCR both offer excellent performance, with broth enrichment offering little performance advantage given its cost.

Keywords. antifungal drug resistance; *Candida auris*; carrier state; cost-effectiveness analysis; diagnostic screening programs.

Candida auris is a rapidly emerging public health threat worldwide [1]. Many *C auris* isolates possess lower in vitro susceptibility to many antifungals, and some isolates within clades have the ability to become panresistant, underlying its 39% overall mortality rate [2, 3]. *C auris* colonizes the skin and mucosae and forms biofilm on medical devices, underlying its ability to cause nosocomial spread and outbreaks [4]. *C auris* can be difficult to identify with biochemical-based laboratory techniques [5] and to eradicate from health care environments because of its thermotolerance and poor disinfectant susceptibility [6, 7].

From 2019 to 2021, colonizations and clinical infections with *C auris* in the United States increased by >200% and 95%, respectively [3]. The proportion of contemporaneous isolates with echinocandin resistance increased >300% [3]. Choosing a sensitive screening method with fast turnaround times allows laboratories to support prompt identification of patients and institution of appropriate control measures.

The optimal testing method for *C auris* nosocomial screening is unclear. Centers for Disease Control and Prevention (CDC) guidance recommends using culture with chromogenic or salt/dulcitol agar, polymerase chain reaction (PCR), or salt/dulcitol enrichment broth [8]. Screening method choice balances analytical specifications, turnaround time, workflow, and cost. We performed a verification study of 4 *C auris* screening methods—chromogenic media culture, PCR, broth enrichment followed by chromogenic media culture (BE-CC), and broth enrichment followed by PCR (BE-PCR)—to determine test characteristics and laboratory cost-effectiveness.

METHODS

In this prospective verification study, we used simulated specimens spiked with *C auris* and non–*C auris* reference strains, the latter of which were chosen because they can be easily misidentified as *C auris* [4, 9]. The provincial reference laboratory supplied the isolates, which included CDC Antibiotic Resistance Isolate Bank strains (Supplementary Table 1). We obtained simulated specimens via convenience sampling of laboratory staff with bilateral axillary-groin ESwabs (COPAN Diagnostics) or from patient surveillance specimens that were *C auris* negative. We prepared 0.5 McFarland standard solutions and serially diluted them in liquid Amies broth to obtain 4 ten-fold dilutions. These dilutions were then spiked into individual simulated swabs to yield final concentrations of 1 to 1000 colony-forming units (CFU)/mL. Specimens with combinations of 1 *C auris* and 1 non–*C auris* isolate were spiked in a 1:1 ratio.

The primary study objective was verifying 4 *C auris* screening methods: direct specimen chromogenic media culture, direct specimen PCR, BE-CC, and BE-PCR. Secondary objectives included determining the test characteristics and laboratory cost-effectiveness of each method.

For direct chromogenic culture, we inoculated 10 µL onto CHROMagar Colorex Candida Plus agar plates (Micronostyx) using the Kiestra system (Becton Dickinson), where they were also incubated aerobically at 37 °C for 120 hours, with images acquired and reviewed every 24 hours. We extracted nucleic acid for PCR using the easyMAG system (bioMérieux). We adapted the CDC real-time PCR protocol [10], using the Luna Probe One-Step RT-qPCR Kit (New England Biosciences) on the CFX96 thermal cycler (BioRad). We used a modified dual-labeled target probe, 5′-/FAM/AAT CTT CGC GGT GGC GTT GCA TTC A/BHQ-1/-3′, with 16S rRNA primers and

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Table 1. Diagnostic Test Characteristics of 4 *Candida auris* Screening Methods

Test Characteristic	Chromogenic Agar Culture (n = 188)	Direct PCR (n = 222)	Broth Enrichment Followed by	
			Chromogenic Agar Culture (n = 63)	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 (90.7–100.0) 10 CFU/mL: 82.1 (66.5–92.5)	ND	ND
Diagnostic specificity, % (95% CI)	1000 CFU/mL: 100 (66.4–100) 100 CFU/mL: 94.7 (74.0–99.9)	100 CFU/mL: 100 (83.2–100.0) 10 CFU/mL: 100 (80.5–100.0)	ND	ND
Analytical sensitivity, CFU/mL (95% CI)[a]	256.34 (81.02–811.02)	27.11 (11.34–64.76)	16.32 (2.36–112.82)	2.75 (.63–12.06)
Analytical specificity, %	NA	100	NA	NA
95% time to pigment production, d (95% CI)[b]	4.26 (4.02–4.51)	NA	2.92 (—)[c]	NA
Approximate turnaround time, d	5	1	5	3

Standardized definitions from Clinical and Laboratory Standards Institute documents MM17 [11] and EP12 [12] were used for test characteristics.

Abbreviations: CFU, colony-forming units; NA, not applicable; ND, not determined; PCR, polymerase chain reaction.

[a]95% lower limit of detection.

[b]We defined a colony as characteristic of *C auris* when the colony color was pink with a surrounding diffusible blue halo.

[c]Probit regression did not converge; therefore, 95% CI is not reliably estimable.

probes as the internal control. For broth enrichment, we serially diluted spiked specimens in Oxoid *C auris* enrichment broth (Thermo Fisher) to create final organism concentrations of 0.005 to 500 CFU/mL, and we incubated them aerobically at 37 °C for 48 hours in a shaking incubator at 250 rpm, followed by PCR or chromogenic culture as previously described with 10 or 100 µL of broth, respectively.

We used Clinical and Laboratory Standards Institute MM17 [11] and EP12 [12] definitions for analytical sensitivity, analytical specificity, diagnostic sensitivity, and diagnostic specificity. We performed probit regression using the Clinical and Laboratory Standards Institute EP17A2 [13] method to determine the analytical sensitivity of PCR and chromogenic culture and the time to detection for chromogenic culture. For laboratory-specific cost-effectiveness analysis, we calculated the cost per case detected using sample positivity thresholds of 0.1%, 0.5%, 1%, 5%, and 10%. To define thresholds, we used point prevalence studies in Canada and New York State, which showed positivity rates of 0.4% and 5.0% among screened at-risk patients, respectively [14, 15].

We analyzed data using R version 4.3.0 (R Foundation for Statistical Computing). Where culture-based detection software errors caused missing data, we imputed values using last observation carry forward. All statistical tests were 2-sided with $P < .05$; 95% CIs were determined via exact methods. Institutional research ethics board approval was not required for this internal verification study.

RESULTS

Table 1 outlines the diagnostic test characteristics for *C auris* detection by chromogenic culture, PCR, BE-CC, and BE-PCR. For chromogenic culture, we spiked 188 simulated specimens with *C auris,* non–*C auris* yeast, or a combination thereof. Of those specimens at 10-fold dilutions of 1 to 1000 CFU/mL, 103 (54.7%) contained *C auris*; 73 (38.8%), non–*C auris* yeast; and 12 (6.3%), a combination of *C auris* and non–*C auris* isolates. We imputed data for 5 of 940 (0.53%) of the total time point observations for the 188 specimens tested.

We evaluated 222 simulated specimens containing *C auris*, non–*C auris* yeast, or a combination thereof using direct PCR. Of those specimens at 10-fold dilutions of 1 to 1000 CFU/mL, 112 (50.4%) contained *C auris*; 78 (35.1%), non–*C auris* yeast; and 30 (13.5%), a combination of *C auris* and non–*C auris* isolates. We noted no cross-reactivity with non–*C auris* isolates (Table 1).

For broth enrichment, we tested only *C auris*–simulated specimens at 10-fold dilutions from 0.005 to 500 CFU/mL and were thus unable to determine the diagnostic sensitivity, diagnostic specificity, or analytical specificity. For BE-CC and BE-PCR, we tested 63 and 64 specimens, respectively (Table 1).

To determine the base laboratory cost per test, we included consumables and medical laboratory technologist time in our calculations. We did not include equipment costs or patient time spent in isolation precautions, because the former depends on whether instrumentation is leased or purchased and the costs of the latter are not borne by the laboratory. We performed timed observations for each method, quantifying medical laboratory technologist time using the Canadian Institutes for Health Information's workload unit definition [16]. The cost per test was $9.43 for chromogenic culture and $11.27 for PCR; broth enrichment added an additional $20.27 per test. The cost-effectiveness analysis in cost per detected *C auris*

Table 2. Cost-effectiveness Analysis of Various Methods of *Candida auris* Screening: Estimated Laboratory Cost in US Dollars per Case Detected

	Percentage Positivity, US $				
Detection Method	0.1	0.5	1.0	5.0	10.0
Chromogenic agar culture	9430.00	1886.00	943.00	188.60	94.30
Direct PCR	11 270.00	2254.00	1127.00	225.40	112.70
Enrichment broth followed by					
Chromogenic agar culture	29 700.00	5940.00	2970.00	594.00	297.00
PCR	31 540.00	6308.00	3154.00	630.80	315.40

Cost-effectiveness analysis based on a cost per test that included laboratory consumables (reagents) and medical laboratory technologist time. Equipment cost was not included because costs incurred are dependent on lease or purchase and may vary significantly by laboratory platform used, nor was patient isolation time included as these costs are not borne by the laboratory.

Abbreviation: PCR, polymerase chain reaction.

case is outlined in Table 2. At a sample positivity threshold of 0.5%, the laboratory cost per case detected for chromogenic culture, PCR, BE-CC, and BE-PCR was $1886, $2254, $5940, and $6308, respectively.

DISCUSSION

C auris has emerged as a global public health threat because of its significant antifungal resistance potential, nosocomial transmission potential, and disinfectant resistance [1–4]. To respond to the challenges that *C auris* poses, microbiology laboratories must deploy rapid, accurate, and precise diagnostic methods. Without a clear criterion standard for *C auris* screening, laboratories may implement one of several methods based on local epidemiology, clinical needs, and cost [8]. In this verification study and cost-effectiveness analysis, we examined 4 *C auris* screening methods using spiked simulated specimens.

We found that chromogenic agar culture provides reliable identification at the lowest overall cost, with approximately 95% diagnostic sensitivity and specificity at a 100-CFU/mL threshold. Whereas some guidelines recommend 10-day culture incubation for primary specimens [17], our verification showed that direct specimen cultures may be reliably called "*C auris* positive" after incubation for 5 days, since 95% of cultures produced pigmentation characteristic of *C auris* by 4.26 days, where a positive colony is one possessing a pink color with diffusible blue pigmentation in the surrounding agar. Some *Candida* species, such as *C vulturna*, *C pseudohaemulonii*, and *C parapsilosis*, can cause false positives on this media [18–20]. While our verification panel did not include *C vulturna* and *C pseudohaemulonii,* our *C parapsilosis* isolates were reliably and distinctly identified. While PCR may require more medical laboratory technologist involvement and is associated with a higher base cost, it has the fastest turnaround time and improves the analytical sensitivity by approximately 1 $\log_{10}$ CFU/mL as compared with culture. Similarly, the use of an initial 48-hour broth enrichment step before culture or PCR resulted in an approximate 1 $\log_{10}$ CFU/mL improvement in analytical sensitivity when compared with direct culture or PCR; however, this adds incubation time, test complexity, and significant expense. Broth enrichment decreased the amount of incubation time to 3 days for culture plates to be reliably called negative.

Choosing an optimal screening method is complex. In a low-prevalence setting, chromogenic culture likely provides the best balance of cost, performance, and turnaround time. However, in a high-prevalence, point prevalence, or outbreak setting, PCR offers decreased turnaround time and multiplexing: at a hospital level, the ability to rapidly deisolate patients likely offsets higher costs. Broth enrichment offers performance improvements over direct methods, but these are marginal given its increased cost, workflow complexities, and delayed turnaround time. Our study's major limitation is that results reflect idealized performance characteristics that may differ when prospective patient specimens are used. Future work with prospective patient specimens and researching the bioburden of *C auris* colonization in patients will assist in selecting an optimal screening method.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Data availability. Data set and R code are available by written request to the corresponding author.

Institutional research ethics board approval statement. This internal validation study did not require research ethics board approval at our institution. It conforms to the standards currently applied in the country of origin.

Patient consent statement. This study does not include factors necessitating patient consent at our institution.

Potential conflicts of interest. All authors: No reported conflicts.

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