



Tools for Detecting a “Superbug”: Updates on *Candida auris* Testing

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ABSTRACT *Candida auris* is an emerging yeast species that has the unique characteristics of patient skin colonization and rapid transmission within health care facilities and the ability to rapidly develop antifungal resistance. When *C. auris* first started to appear in clinical microbiology laboratories, it could be identified only by using DNA sequencing. In the decade since its first identification outside of Japan, there have been many improvements in the detection of *C. auris*. These include the expansion of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) databases to include *C. auris*, the development of both laboratory-developed tests and commercially available kits for its detection, and special CHROMagar for identification from laboratory specimens. Here we discuss the current tools and resources that are available for *C. auris* identification and detection.

KEYWORDS *Candida auris*, CHROMagar, antifungal susceptibility testing, fungal, testing

Candida auris is a relatively new species of yeast, first identified in 2009, that has quickly spread across the world (1, 2). There are three features that make *C. auris* unique from other species of *Candida*: (i) antifungal resistance is the norm for *C. auris* rather than the exception (3–5), (ii) rather than primarily colonizing the gut, *C. auris* colonizes the skin, anterior nares, and other body sites of asymptomatic carriers, and (iii) *C. auris* is transmitted easily between patients in health care settings. Some patients can be asymptotically colonized with *C. auris* for long periods of time, and these colonized patients contribute to environmental contamination and transmission within health care settings. The transmissibility of *C. auris* and the alarming statistic that 5 to 10% of colonized patients subsequently develop bloodstream infections make *C. auris* a serious public health problem (6). To complicate matters further, an overwhelming percentage of isolates, up to 99% in some jurisdictions, are resistant to at least one commonly used antimicrobial, and isolates have been identified that are resistant to azoles, echinocandins, and amphotericin B, severely limiting treatment options using approved therapy (7, 8).

Candida auris has now been identified in 28 U.S. states and will likely continue to spread within those states and to new areas over time (Centers for Disease Control and Prevention [CDC]; <https://www.cdc.gov/fungal/candida-auris/tracking-c-auris.html>). Health care transmission of *C. auris* tends to disproportionately impact high-acuity long-term-care facilities and individuals with chronic illness, history of other resistant pathogens, and invasive medical devices, including mechanical ventilation, tracheostomies, feeding tubes, and urinary catheters (9). *C. auris* is associated with high mortality, but because infected or colonized individuals often have poor health at baseline, the attributable mortality of *C. auris* is not clear (6). Colonized or infected individuals shed *C. auris* into their immediate environment, where it can persist for long periods of time on health care surfaces, including shared medical equipment, potentially spreading to others and causing health care outbreaks (5). Prompt identification of individuals infected or colonized with *C. auris* is

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critical for guiding infection control measures, including isolation precautions and thorough cleaning and disinfection of their surroundings to prevent spread. Infection control guidance can be found on the CDC website (<https://www.cdc.gov/fungal/candida-auris/c-auris-infection-control.html>), and similar recommendations have been published by the Australasian Society for Infectious Diseases and the International Society for Antimicrobial Chemotherapy (10, 11). The CDC has also issued additional guidance for working with *C. auris* in a clinical microbiology laboratory. The guidance can be found at <https://www.cdc.gov/fungal/candida-auris/c-auris-lab-safety.html>.

Because *C. auris* was a newly identified species when it began emerging in multiple countries across the world, it did not appear in any identification platform databases (12). To make matters worse, its biochemical assimilation profile was very similar to that of two closely related species, *Candida haemulonii* and *Candida duobushaemulonii*, causing a great deal of misidentification (13). At least one laboratory system identified *C. auris* isolates as *Saccharomyces cerevisiae*, and those laboratories with early versions of MALDI-TOF MS either identified it as *C. haemulonii* or did not get an identification at all (12, 13). Complicating the identification of *C. auris* is the fact that there are five different clades (including a few cases from clade V so far identified only in Iran) and the clades have both different assimilation profiles and slight differences in their rDNA sequences (2, 14).

BIOCHEMISTRY-BASED IDENTIFICATION OF CANDIDA AURIS

Many commercial identification platforms use biochemical assimilation and fermentation patterns to identify bacteria and fungi. This poses a problem for the identification of *C. auris*, as the assimilation and fermentation patterns are similar to those of other closely related species of yeast. Complicating the problem, some identification systems make an identification to species based on the closest match to assimilation and fermentation pattern by percent match, rather than based on a perfect match. While this may work for the 90 to 95% of *Candida* isolates that comprise the six most frequently identified species (*C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei*, and *C. lusitanae*), it can cause inaccurate identification of some of the other 5 to 10% of *Candida* species. Rather than giving “no identification,” they will make an erroneous identification. This limitation caused many of the first isolates of *C. auris* to be incorrectly identified as *Rhodotorula glutinis*, *Saccharomyces cerevisiae*, or *Candida sake* when the API 20C AUX or API ID 32C system was used or as *Candida haemulonii* when the Vitek 2 system was used (12, 15, 16). Efforts to incorporate *C. auris* into these databases have led to some incremental improvements. For example, although an update to the Vitek 2 database (version 8.01) enabled accurate identification of *C. auris* clade IV isolates, this update was not sufficient to consistently identify isolates from clade I or III (17). Vitek 2 has since released version 9.01, but an evaluation for improved inclusivity has not yet been published. Other identification platforms, such as the MicroScan Walkaway and the BD Phoenix, have not yet added *C. auris* to their databases and continue to provide erroneous identification (Table 1) (12, 18). For these reasons, the CDC has created algorithms to indicate when additional follow-up may be needed for a variety of commonly used biochemistry-based identification systems (https://www.cdc.gov/fungal/candida-auris/pdf/Testing-algorithm_by-Method_508.pdf).

MASS SPECTROMETRY-BASED IDENTIFICATION

As it was a new species and was not represented in the databases, initial attempts to identify *C. auris* based on matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) were unsuccessful. Following the continual isolation and spread of *C. auris* across many countries, the commercial manufacturers of MALDI-TOF MS added it first to their research use only (RUO) databases and then subsequently to their FDA-cleared databases (19, 20). Isolates from all four of the major *C. auris* clades can now be correctly identified with the Vitek MS system (bioMérieux, Durham, NC) by using its FDA-cleared IVD v3.2 or RUO Saramis v4.14 or newer

TABLE 1 Methods for identification or isolation of *Candida auris*

Test type and details	Notes ^a	Reference(s)
Culture		
Original enrichment broth	Valuable reference method for diagnostic development	30
Chromogenic medium	Aids visual identification to the species level of the common <i>Candida</i> spp.	24, 26, 27
Other differential media	Use of Pal's medium, ferrous sulfate, and crystal violet	25, 28, 29
Biochemical tests		
API 20C AUX	Cannot currently identify <i>C. auris</i> ; see CDC follow-up algorithm	12, 15, 16
API ID 32C	Cannot currently identify <i>C. auris</i> ; see CDC follow-up algorithm	12
BD Phoenix	Cannot currently identify <i>C. auris</i> ; see CDC follow-up algorithm	12
MicroScan	Cannot currently identify <i>C. auris</i> ; see CDC follow-up algorithm	12
RapID yeast plus	Cannot currently identify <i>C. auris</i> ; see CDC follow-up algorithm	
Vitek 2 YST	Can ID some but not all <i>C. auris</i> ; see CDC follow-up algorithm	17
MALDI-TOF MS		
Bruker Biotyper 2.0 Microflex LT	FDA approved for isolate ID with CA System library (v4)	20
bioMérieux Vitek MS	FDA approved for isolate ID with IVD library v3.2	19
Blood culture, molecular		
BioFire BCID2	FDA approved for positive blood culture	
GenMark Dx ePlex BCID-FP panel	FDA approved for positive blood culture	58
RT-PCR		
TaqMan chemistry	Most common LDT for colonization screening in U.S. PHL	41, 52
SYBR green chemistry	Evaluated for skin and anterior nares	39, 42
Commercial RT-PCR kits		
AurisID, OLM Diagnostics	CE-IVD reagents for <i>C. auris</i> RT-PCR	47
BioGX <i>Candida auris</i>	RUO reagents supporting RT-PCR and extraction on BD Max platform	
Fungiplex <i>Candida auris</i>	RUO reagents for <i>C. auris</i> RT-PCR	47
Other		
LAMP	Unique molecular method for <i>C. auris</i> detection	40
T2MR <i>C. auris</i>	RUO test for <i>C. auris</i> using T2 magnetic resonance technology	50
Conventional PCR with GPI target	<i>C. auris</i> specific and multiplex tests feasible in low-resource settings	36–38

^aID, identification; LDT, laboratory-developed test; RUO, research use only; PHL, public health laboratories; CE-IVD, *in vitro* diagnostic approved for sale in the European Union; RT-PCR, real-time PCR.

databases and the Biotyper 2.0 Microflex LT spectrometer (Bruker Daltonics, Inc., Billerica, MA) by using the CA system library (version claim 4) or RUO version 2014 (5627) or newer databases.

IDENTIFICATION OF *C. AURIS* USING AGAR

Chromogenic medium has been a staple diagnostic tool for *Candida* species identification for a few decades (21). *Candida* species are identified based on colony color and, in the case of *C. krusei*, colony texture. There are many different commercially available formulations, but the majority claim identification for only *C. albicans*, *C. tropicalis*, *C. krusei*, and, depending on the formulation, *C. glabrata* (21–23). Most chromogenic medium formulations are not capable of reliably distinguishing *C. auris*, as colonies can appear cream, pink, red, or purple and resemble the majority of other species besides the four mentioned above (<https://www.cdc.gov/fungal/candida-auris/identification.html>) (24, 25). Recently, two new formulations of chromogenic media have been developed specifically for the additional identification of *C. auris*, CHROMagar *Candida* plus (CHROMagar, France) (Fig. 1) and HiCrome *C. auris* MDR selective agar (HiMedia, Mumbai, India) (24, 26, 27). When tested side by side against 49 *Candida* isolates including representatives from all four major *C. auris* clades, only CHROMagar *Candida* plus correctly distinguished all the *C. auris* isolates (24). However, with CHROMagar *Candida* plus, there were false-positive identifications with the closely related species *Candida vulturna* and *Candida pseudohaemulonii*. For this reason, colonies suspected of being *C. auris* by using colony color on chromogenic media should be further confirmed by sequencing or MALDI-TOF MS (24).



FIG 1 *Candida auris* after 48 h of growth on CHROMagar Candida plus showing light blue colonies with a blue halo around the colonies. The combination of the color and the halo are distinct for *C. auris* (also see reference 22).

The use of Pal's medium in combination with CHROMagar has been suggested as a way to distinguish between the closely related species *C. auris* and species in the *C. haemulonii* species complex, but this option is useful only when an identification has been narrowed down to these closely related species (28).

Two other noncommercial selective agars for *C. auris* have been recently developed (25, 29). The first medium uses high NaCl (12.5%) and ferrous sulfate in combination with elevated temperatures to allow only the differential growth of *C. auris*. This medium can be used to isolate *C. auris* when directly plated from blood culture as well (25). The second medium, developed by Ibrahim et al., is an alternative enrichment broth that uses 10% NaCl, mannitol, and crystal violet to differentially select for *C. auris* growth (29, 30). Both media will need to be validated against more *Candida* species, including closely related species in the *C. haemulonii* complex.

MOLECULAR IDENTIFICATION

DNA sequencing played a major role first in the initial discovery that *C. auris* was a new species and then in the correct identification of *C. auris* and its delineation from the closely related species in the *C. haemulonii* species complex (15, 31, 32). Identification can be accomplished by PCR amplification of the D1/D2 region of the 28S sequence or the full internal transcribed spacer of the ribosomal cistron (1). Unfortunately, DNA sequencing is not available in most clinical microbiology laboratories, and the majority of sequencing is performed in reference laboratories.

Conventional PCR, using species-specific primers and/or restriction length polymorphism analysis, for the identification of *Candida* species has been in use for decades (33–35). Due to the availability of both cheaper and easier to use commercial methods, this methodology is not widely used outside of research or reference laboratories and has not been commercialized. However, in resource-limited settings, conventional PCR can be used to identify specific emerging species such as *C. auris* (36–38). The methodology can be incorporated into an algorithm in which chromogenic medium is used to screen isolates for possible *C. auris* and traditional PCR is used to confirm the identity.

A number of real-time PCR assays and a loop-mediated isothermal amplification (LAMP) assay for *C. auris* have now been developed using many different DNA extraction methods, enzyme chemistries, primer targets, and thermocycler platforms (36, 39–46). Each of these methods has been optimized for use in the laboratory in which they

were developed, but they provide a variety of options for clinical laboratories that have the ability to incorporate laboratory-developed tests. Commercially available kits that include premixed primers and enzymes are also now available, such as the AurisID (OLM Diagnostics, Newcastle Upon Tyne, United Kingdom) and the Fungiplex (Bruker, Bremen, Germany), both of which can be used for detection from surveillance specimens or directly from blood, and the BioGX *Candida auris* kit (BioGX, Birmingham, AL) for the BD Max (Becton, Dickinson and Company, Franklin Lakes, NJ) (47–49). Other unique molecular tests include the T2 magnetic resonance (MR) assay, which was originally developed for blood specimens but has also been adapted for colonization screening (50). The T2Cauris panel is currently being marketed for research use only and has not been cleared for diagnostic use. In the United States, the FDA has approved both the GenMark Dx ePlex BCID-FP panel and BioFire's BCID 2 panel for the detection of *C. auris* in positive blood cultures.

DETECTION OF COLONIZATION

Another aspect of laboratory testing for *C. auris* is the use of screening to determine whether patients may be colonized with *C. auris*. As stated above, *C. auris* primarily colonizes the skin, is shed into the health care environment, where it contaminates surfaces including shared medical equipment, and is easily transferred between patients. For this reason, appropriate infection control precautions should be used for patients colonized or infected with *C. auris* to prevent further spread. Screening using skin swabs is useful for identifying asymptomatically colonized individuals and is often used for patients at high risk for acquiring *C. auris*, including health care contacts of known cases, patients with domestic or international health care in an area with *C. auris*, or patients with current or previous stays at long-term-care facilities. Some health care facilities will screen individuals upon admission or conduct point prevalence surveys in which all patients in the facility or a specific unit are screened. There are several laboratory-developed methods for screening patients for *C. auris* colonization, including both a conventional broth enrichment method and a number of real-time PCR methods. Rapid and easy methods for colonization screening are important, because results are used to guide infection control measures, e.g., whether a patient requires isolation precautions.

Broth culture was the first method developed for screening for *C. auris* colonization (30). Sample collection involves bilateral swabbing of the axilla and groin of patients to be screened, followed by enrichment for *C. auris* in Sabouraud broth containing 10% salt and dulcitol (30). A brief protocol for this methodology has been published (51), but the CDC has made a standard operating procedure for the method from start to finish available online (<https://www.cdc.gov/fungal/lab-professionals/pdf/c-auris-colonization-screening-508.pdf>). Additional guidance for swabbing patients can be found at <https://www.cdc.gov/fungal/candida-auris/c-auris-patient-swab.html>.

At the time of this writing, there was no FDA-approved test specific for *C. auris* colonization swab specimen screening, an existing gap in diagnostics. Because the public health response is oriented around colonization screening for early detection and containment, colonization screening demands the highest testing volumes by far and capacity is often limited. In the United States, most public health laboratories capable of *C. auris* colonization screening have validated a version of a TaqMan real-time PCR (RT-PCR) assay first developed by the Wadsworth Center in New York, run on either the ABI 7500 thermocycler or the BD Max platform (41, 52).

Current public health surveillance testing in the United States is managed through the Antimicrobial Resistance Laboratory Network (AR Lab Network) (<https://www.cdc.gov/drugresistance/laboratories.html>) and coordinated through state and jurisdictional public health laboratories. There are some clinical and private laboratories that perform targeted colonization screening of newly admitted patients, but wide-scale surveillance testing and large point prevalence surveys are largely performed through the AR Lab Network. *Candida auris* is a reportable disease in the United States, and

positive cases should be reported through state and jurisdictional public health laboratories but can also be coordinated through notification at candidaauris@cdc.gov.

SUSCEPTIBILITY TESTING

One of the more worrisome attributes of *C. auris* is the percentage of isolates that are antimicrobial resistant (53). There are no breakpoints for any antimicrobials against *C. auris*. While MIC values can be generated using commercially available susceptibility testing platforms, there are no interpretive criteria. As there are no clinical trials of currently available antimicrobials against *C. auris*, there are not likely to be established breakpoints in the near future either. Many laboratories refer to the tentative breakpoints that were established by the CDC based on MIC distributions, animal models of infection, and molecular identification of resistance mechanisms (<https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html>) (2, 54). While these tentative breakpoints may assist clinicians with MIC interpretation, they are not endorsed by the Clinical and Laboratory Standards Institute (CLSI), and these interpretations should not be included in patient reports.

There are additional concerns with susceptibility and *C. auris*. A high number of isolates exhibit the Eagle effect when testing using broth microdilution against caspofungin (13, 55, 56). If other echinocandins are not tested simultaneously with caspofungin, this could lead to the major error of predicting echinocandin resistance in a susceptible isolate. In addition, amphotericin B testing can be problematic. As the CLSI has not established amphotericin B breakpoints for any fungal species, most laboratories use a value of $\geq 2 \mu\text{g/mL}$ as the breakpoint for resistance to amphotericin B for all *Candida* species. Using broth microdilution, the majority of *Candida* isolates have MIC values of 0.25 to 1 $\mu\text{g/mL}$. If an isolate is read just 1 or 2 dilutions higher at 2 $\mu\text{g/mL}$, the two readings are in essential agreement, within the ± 2 dilution standard deviation, and still in categorical disagreement. Since reading is subjective and MIC values can fluctuate within the standard deviation, there are challenges with performing amphotericin B susceptibility testing. The use of gradient diffusion increases the range of obtainable MIC values, but since it is also a subjectively read test, isolates hovering between resistant and susceptible are difficult to interpret.

TESTING STRATEGIES

In a resource reference laboratory, MALDI-TOF MS or DNA sequencing should be used first for the identification of isolates that are suspected to be *Candida auris*. If neither of these options are available, the Vitek 2 system is the next most accurate way to identify isolates of *C. auris*, but the accuracy is not as high as for MALDI-TOF MS or sequencing, and some clades may be identified as members of the *C. haemulonii* species complex. Those isolates may have to be characterized further. When none of these options are available, CHROMagar Candida plus can be used to identify isolates by color. The specificity of this agar is not 100%, so for isolates that have a preliminary identification of *C. auris*, a species-specific PCR or RT-PCR assay should be used to confirm the identity. If none of these options are available, the isolate should be forwarded to a reference laboratory for identification.

ADDITIONAL TOOLS

Isolates of *C. auris* from all five clades for validation or study have been made available for free from the CDC AR Isolate Bank. The AR Isolate Bank and all available isolates can be found at <https://www.cdc.gov/drugresistance/resistance-bank/index.html>.

Whole-genome sequencing (WGS) is quickly becoming a useful tool for outbreak investigation and surveillance. While WGS is more difficult with a eukaryote like *C. auris*, there are already several labs that have incorporated it into their surveillance. To that end, the CDC has made a benchmark data set of 23 *C. auris* genomes available, and the directions for finding them and using them can be found in a report by Welsh et al. (57). In addition, there are publicly available data analysis tools (<https://github.com/CDCgov/mycosnp>),

and the CDC has established a *C. auris* umbrella project on the NCBI website (NCBI accession no. [PRJNA642852](https://www.ncbi.nlm.nih.gov/PRJNA642852)).

SUMMARY

As more laboratories have focused on the identification of *C. auris*, the number of available tools for detection and identification has increased. However, not all laboratories can implement a laboratory-developed test, and many do not have access to MALDI-TOF MS or a Vitek 2 system. The number of alternative commercially available tests is still quite limited, which leaves definitive identification of *C. auris* as a send-out test in many facilities. An especially glaring deficiency is the lack of a point-of-care test for detection of *C. auris* colonization. This would be an important tool for the identification of colonized patients, which plays an important role in the implementation of infection control practices, especially in cities where *C. auris* has become endemic. *Candida auris* continues to spread across the United States and across the world, and it has become a notifiable disease in many U.S. states and municipalities. While we may only be able to slow the spread, more tools for its detection and identification will allow us to combat it at the point of individual patient care.

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