General review

Methods for identification of Candida auris, the yeast of global public health concern: A review

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A B S T R A C T

Candida auris has recently emerged as a fungus able to cause severe infections, especially bloodstream infections with high mortality rates. This multi-drug-resistant yeast has the capacity of persistence on environmental surfaces, and has been reported to cause hospital-acquired infections. The development of faster and inexpensive tools for identification is critical to controlling, preventing and establishing early diagnosis of this emerging pathogen. Identification of C. auris by use of conventional laboratory methods is challenging, and it is commonly misidentified as other Candida species. Less expensive, reliable DNA-based tests have been used for identifying C. auris in environmental and clinical samples. Matrix-assisted laser desorption ionization–time of flight mass spectrometry is also a useful tool for identification of cultured isolates. This review provides a succinct overview of the available methods for identification of C. auris with particular emphasis on their relative advantages and drawbacks.

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1. Introduction

Candida auris was isolated and described from the external ear canal of a patient in Japan in 2009 [1]. Subsequently, over the past 10 years, several cases of invasive fungal infections and episodes of nosocomial fungemia associated with C. auris have been reported from Kuwait, India, South Africa, United Kingdom and other countries, spanning a total of five continents [2–6]. Besides, it has quickly become a growing public health threat throughout the world due to its resistance to various antifungal drug classes, including azoles (particularly fluconazole and voriconazole), polyenes (amphotericin B), echinocandins and reduced susceptibility to the pyrimidine analogue flucytosine [2,6–9].

Based on phylogenetic analysis of C. auris strains isolated from different continents (East/South Asia, Africa and South America), four distinct clades of C. auris have been identified, which appear to have emerged independently [10]. Since C. auris has the ability to live and persist on environmental surfaces, it can be transmitted

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between patients in healthcare settings [11,12] and cause healthcare-associated outbreaks characterized by a notably high mortality rate [5,10,13]. Therefore, rapid and accurate identification of C. auris is critical to prevention of further spread [10,13–15].

A major challenge to timely diagnosis and appropriate treatment of C. auris infections is the failure of conventional and commercially available biochemical methods to identify this species [15,16]. Even when using commercial biochemical systems that enables identification to species level, C. auris might be misidentified as another Candida species or non-Candida yeast species [16,17]. Accordingly, various DNA- and protein-based methods have been developed with different results [18–23], some of which have been validated for detection of C. auris in environmental and clinical specimens [18,20]; others have been applied to identification of isolated colonies [24].

Currently, Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and PCR are the most efficient and commonly used diagnostic techniques for accurate identification of C. auris; nevertheless, each method has its own pros and cons. For laboratories using biochemical methods, it is of great importance to be aware of the common errors of various commercially available methods and to cover these shortcomings by the use of other approaches. Here, we shortly review various approaches including phenotypic and biochemical as well as DNA- and protein-based methods for identification of C. auris, with particular insight into their advantages and drawbacks.

2. Identification methods

2.1. Phenotypic and biochemical identification

C. auris appears as ovoid to elongate, single, paired and/or grouped, budding yeast cells. This species is unable to form germ tube in serum or chlamydoconidia in corn-meal agar, and rarely forms pseudohyphae, which appears to be dependent on specific conditions such as the presence of NaCl [25–28]. The yeast grows well at 37 to 42 °C, but not at 45 °C; can assimilate N-acetylglucosamine, succinate, and gluconate as carbon sources, and fails to grow in the presence of 0.01% or 0.1% cycloheximide [2–4,25,29,30]. On Sabouraud dextrose/glucose agar, C. auris forms white- to cream-colored colonies [27,30,31], while on commercial chromogenic media, including CHROMagar Candida, BBL CHROM- Magar Candida, CAN2 chromogenic, Candida ID and Brilliance Candida Garr, it appears as beige, pink, pale rose or pale purple colonies [26,28,31–33]. Regarding the non-specific phenotypical features of C. auris, differentiation of this pathogen from other Candida species is difficult [27]. Nevertheless, it had been claimed that C. auris could be distinguished from the Candida haemulonii species complex on CHROMagar Candida medium supplemented with Pal’s agar. When this medium is incubated at 37 °C, C. auris forms creamy white colonies, while yeasts of the C. haemulonii complex appear as light pink colonies. Furthermore, at 42 °C, members of the C. haemulonii complex do not grow on this medium, whereas C. auris exhibits confluent growth [32]. However, as these findings are not based on a global representation of C. auris isolates, variations between different clades of C. auris may limit the application of this method.

Commercially available biochemical tests are widely used. A major drawback of these systems is the similarity of assimilation patterns between C. auris and other Candida or even non-Candida species, resulting in misidentification. This shortcoming has been reported for several commercial brands. Table 1 summarizes the errors in identification of C. auris by various biochemical platforms.

### Table 1

<table>
<thead>
<tr>
<th>Commercial biochemical systems</th>
<th>Misidentifies Candida auris as</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>API 20C</td>
<td>C. famata, C. sake, Saccharomyces kluveri, Rhodotorula glutinis, Saccharomyces cerevisiae</td>
<td>[2,39–41]</td>
</tr>
<tr>
<td>API 32C</td>
<td>C. sake</td>
<td>[33,42]</td>
</tr>
<tr>
<td>MicroScan Walkaway</td>
<td>C. albicans</td>
<td>[43,47]</td>
</tr>
<tr>
<td>MicroScan AutoSCAN</td>
<td>C. tropicalis</td>
<td>[43,47]</td>
</tr>
<tr>
<td>BD Phoenix</td>
<td>C. haemulonii, C. catenulata, C. albicans, C. tropicalis</td>
<td>[26,30,38,42,43]</td>
</tr>
<tr>
<td>Remel Rapid YEAST Plus</td>
<td>C. parapsilosis</td>
<td>[35,48]</td>
</tr>
<tr>
<td>Auxa color 2 system</td>
<td>S. cerevisae</td>
<td>[8,26,49]</td>
</tr>
</tbody>
</table>

### 2.2. MALDI-TOF MS

MALDI-TOF MS is a specialized proteomic-based technique, which could be a rapid and accurate identification method based on the comparison of the generated spectra for each sample with the reference database [50]. MALDI-TOF MS can differentiate C. auris from other Candida species if the reference database contains the necessary information; if not, no identification or misidentification will be the result [16]. Therefore, updated versions of MALDI-TOF MS databases are essential for identification of C. auris.

The Vitek MS MALDI-TOF system combines two software platforms including the Vitek MS in vitro diagnosis (IVD) and Vitek MS research use only (RUO) libraries. To date, rapid identification of C. auris has been performed by two MALDI-TOF platforms, including MALDI Biotyper (Bruker-Daltonics) and Vitek MS (bioMérieux) using an updated research use only (RUO) library [26]. However, Grenfell et al. reported higher accuracy and efficiency of the Bruker Biotyper over the Vitek 2 MS system in the identification of C. auris isolates, even with an upgraded RUO library.

### Table 2

<table>
<thead>
<tr>
<th>MALDI-TOF system (manufacturer)</th>
<th>Errors in identification of C. auris</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALDI Biotyper (Bruker-Daltonics)</td>
<td>Misidentified as Neisseria meningitidis serogroup A and Pseudomonas rhinosphaerae</td>
<td>[53]</td>
</tr>
<tr>
<td>Vitek MS (bioMérieux)</td>
<td>Misidentified as C. albicans and C. haemulonii</td>
<td>[53]</td>
</tr>
<tr>
<td>Vitek MS IVD (bioMérieux)</td>
<td>NI of six C. auris and isolates as C. parapsilosis and C. haemulonii</td>
<td>[49]</td>
</tr>
<tr>
<td>Vitek MS versions 2.0 and 4.1 (bioMérieux)</td>
<td>NI of three clades of C. auris (South Asian, East Asian and South African clades)</td>
<td>[54]</td>
</tr>
<tr>
<td>Vitek MS PLUS V2.0 (bioMérieux)</td>
<td>NI of 22 C. auris isolates</td>
<td>[23]</td>
</tr>
</tbody>
</table>

NI: no identification (Candida auris is not in the database).
### Table 3
Overview of published molecular assays for identification of *Candida auris*.

<table>
<thead>
<tr>
<th>Method</th>
<th>Molecular target</th>
<th>Main details</th>
<th>Shortcoming(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific end-point PCR</td>
<td>ITS1-5.8S-ITS2</td>
<td>A <em>C. auris</em>-specific primer set</td>
<td>Limited number of controls, Does not identify closely related species</td>
<td>[34]</td>
</tr>
<tr>
<td>Specific end-point PCR</td>
<td>GPI protein-encoding genes</td>
<td>Two <em>C. auris</em>-specific primer sets in a colony-PCR format</td>
<td>One of the primer sets failed to amplify DNA of some <em>C. auris</em> isolates</td>
<td>[23]</td>
</tr>
<tr>
<td>Tetraplex end-point PCR</td>
<td>26S rDNA</td>
<td>Using a universal forward primer and species-specific reverse primers resulting in different PCR product sizes for amplification of <em>C. auris</em>, <em>C. haemulonii</em>, <em>C. dubushaemulonii</em>, <em>C. pseudohaemulonii</em></td>
<td>Not mentioned</td>
<td>[20]</td>
</tr>
<tr>
<td>Multiplex end-point PCR</td>
<td>ITS 1-5.8S-ITS2</td>
<td>A multiplex PCR for identification of <em>C. auris</em> and <em>C. haemulonii</em></td>
<td>Unable to differentiate <em>C. haemulonii</em> from <em>C. haemulonii</em> var. vulnera</td>
<td>[61]</td>
</tr>
<tr>
<td>YEAST PANEL multiplex PCR assay</td>
<td>Several targets for different species; 21 sets of primer for 21 species</td>
<td>A three-step multiplex PCR (7 species in each step) targeting various clinically important members of <em>Candida</em>, <em>Trichosporon</em>, <em>Rhodotorula</em>, <em>Cryptococcus</em>, and <em>Gnetrichum</em> including <em>C. auris</em> which is identified in the first step</td>
<td>Misidentified <em>Meyerozyma caribbica</em> as <em>M. guilliermondii</em> and <em>C. zeylanoides</em> as <em>M. guilliermondii</em>; <em>C. glabrata</em>-specific primers did not identify <em>C. nivariensis</em> and <em>C. bracarensis</em> identified <em>C. albicans</em> and <em>C. africana</em> as <em>C. albicans</em> complex; Identified <em>C. orthopsilosis</em>, <em>C. metapsilosis</em> and <em>C. parapsilosis</em> as <em>C. parapsilosis</em> complex</td>
<td>[62]</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td>ITS1-5.8S-ITS2</td>
<td>Could differentiate <em>C. auris</em> from other related species</td>
<td>Similar RFLP patterns for <em>C. auris</em> and <em>C. catenulata</em></td>
<td>[24]</td>
</tr>
<tr>
<td>Real-time PCR</td>
<td>ITS2-rDNA</td>
<td>A TaqMan-based real-time PCR assay Highly sensitive (1 CFU/PCR reaction), specific and reproducible</td>
<td>Limited number of controls, Not mentioned</td>
<td>[19]</td>
</tr>
<tr>
<td>PCR and real-time PCR assays</td>
<td>5.8S-ITS2-2BS of rDNA</td>
<td>Including two different assays: a sensitive and specific <em>C. auris</em>-specific end-point PCR, and a real-time PCR (melting curve analysis assay) using primers specific for <em>C. auris</em> and related species Controls: human genomic DNA and 21 yeast/mould species</td>
<td>Not mentioned</td>
<td>[63]</td>
</tr>
<tr>
<td>SYBR Green qPCR assay</td>
<td>5.8S-ITS2-2BS</td>
<td>Real-time PCR assay (melting curve analysis) Evaluated for detection of <em>C. auris</em> in a set of positive (48) and negative (55) skin swabs in comparison to culture as a gold standard</td>
<td>Due to the internal control, quantitative data could not be interpreted from the threshold cycle</td>
<td>[64]</td>
</tr>
<tr>
<td>Multiplex real-time qPCR</td>
<td>Four targets within 18S–28S rDNA region</td>
<td>A multiplex real-time PCR assay (melting curve analysis) for identification of <em>C. auris</em>, <em>C. haemulonii</em>, <em>C. dubushaemulonii</em> and <em>C. pseudohaemulonii</em> Successfully tested on extracted DNA and pure colony of the target species Successfully identified the target species in spiked serum samples Controls: human genomic DNA and 72 yeast and mould species</td>
<td>Not mentioned</td>
<td>[65]</td>
</tr>
<tr>
<td>GPS™ MONODOSE CanAur dtec-qPCR</td>
<td>Species specific primers and probe</td>
<td>Based on dehydrated ready-to-use tubes (only extracted DNA needs to be added) Successfully validated on 143 <em>C. auris</em> and non-<em>auris</em> strains based on the UNE/EN ISO/IEC 17025:2005 and French Standard NF T90-471:2010 guidelines Sensitivity, specificity (inclusiveness/exclusiveness), reliability (reproducibility/repeatability) and limits of detection and quantification were all acceptable and standard curve for calibration was successfully validated</td>
<td>Not mentioned</td>
<td>[66]</td>
</tr>
</tbody>
</table>
database [51]. The Vitek MS IVD as well as Bruker Biotype IVD libraries have been used in some studies but did not enable accurate identification, probably due to the lack of C. auris in the databases applied [16,31,49,51,52]. Table 2 summarizes the errors related to the identification of C. auris by different MALDI-TOF systems. The RUO Saramis Version 4.14 database, the Saccharomyces database upgrade for Vitek-MS and also Bruker's 6903 MSP databases for Biotype are required to obtain a higher likelihood of accurate identification of C. auris [30,40]. The Bruker Biotyper database 3.1 contains spectra of three C. auris strains, including two from Korea and one from Japan [17].

In 2018, Bao et al. developed a novel MALDI-TOF database, “CMdb”, to enable rapid and accurate identification of C. auris. The CMdb was created using one in house clinical C. auris isolate and the internationally collected yeasts by the CDC. The new CMdb database contains 22 mean spectrum projections from C. auris and four other related species including C. haemulonii, C. duobushaemulonii, C. krusei, and Kodamaea ohmeri. The authors found that all 23 clinical isolates of C. auris included as well as 10 CDC strains (100%) were correctly identified using the CMdb database. In comparison, only 39% of C. auris isolates were correctly identified by the RUO database. In addition, the use of EPdbs, a database combining the RUO and the CMdb, results in accurate identification of C. auris isolates [21]. Other new designed tools such as CDC's MicrobeNet may also correctly identify C. auris. C. auris strains included in the MicrobeNet database represent all the four clades of this species and could accurately identify C. auris isolates by the Bruker Biotype [40]. Recently, Bruker and bioMérieux began releasing the first CE-IVD and FDA-approved libraries for C. auris identification. The latest Biotype library update, IVD library, includes additional reference spectra, resulting in good identification scores. As both improved RUO libraries and clinically validated CE-IVD/FDA-approved libraries have been released recently, timely implementation of system updates is required for reliable identification [55].

Extraction method is another factor influencing the result of MALDI-TOF MS-based identification. Mizusawa et al. observed that using a direct extraction method in the Vitek MS system, all C. auris isolates were correctly identified, whereas the use of the direct on-plate extraction method in the Bruker MS system only identified 50% of the C. auris isolates. Other studies have reported on a partial extraction method, which is less laborious than full-plate extraction, and which achieves accurate C. auris identification by the Bruker MS system [56,57]. Hence, for accurate identification by Bruker MS system, the full-length or partial extraction method is required [16]. This finding is in disagreement with Desoubeaux et al. who observed that the full-tube extraction for MALDI-TOF MS was not better than direct on-plate extraction [33]. Kwon et al. used two MALDI-TOF MS systems including Biotype and Vitek MS for identification of 61 C. auris isolates. Results indicated that the Biotype with a RUO library correctly identified 75.4% (46/61) of the isolates after initial full-tube extraction, whereas after additive full-tube extraction, 83.6% (51/61) of the isolates were correctly identified. Moreover, the Vitek MS system correctly identified 93.4% (57/61) of the C. auris isolates after direct on-plate extraction, while the Vitek MS system equipped with a new IVD library (Vitek IVD 3.2), correctly identified 96.7% of the isolates after direct on-plate extraction [58].

Currently, MALDI-TOF MS is one of the most efficient identification tools for accurate detection of C. auris by decreasing the turnaround time to < 3 h as compared with conventional as well as DNA-based techniques [59]. However, this method requires high-cost equipment, which may not be available in many microbiology laboratories, neither especially in developing countries [16].

2.3. Molecular identification

Despite the limited number of morphological and biochemical characteristics that can be used for identification, C. auris could be correctly identified using sequencing of entire internal transcribed spacers (ITS) or the large subunit-D1-D2 region of ribosomal DNA (rDNA) [30,60]. Nevertheless, as DNA sequencing is a time-consuming and expensive method and not available in all diagnostic labs, its applicability may be limited, at least in developing countries [40]. To fill this gap, researchers have designed various sequencing-independent DNA-based methods. Some of the methods have been validated only for differentiation of C. auris from closely related species based on isolated yeast colonies, while others could be used for detection of C. auris in environmental or clinical samples. Table 3 shows the molecular methods developed for detection/identification of C. auris.

As shown in Table 3, there is a variety of molecular assays with different formats and applications. Therefore, it might be difficult to select the most appropriate method. An important factor in this regard is the instrumental requirements and the cost of each test. Generally, these methods can be classified into three groups: The first group consists of methods based on end-point PCR. These methods range from C. auris-specific single-plex PCR [23,34] to multiplex PCR's simultaneously identifying C. auris and closely related species [20,61], or even non-related species [62]. This group is the most affordable group for laboratories in developing countries because of the relatively limited instrumental requirements. The second group is based on real-time PCR, which may only detect C. auris [19,64] or C. auris and related species [63,65]. Although these methods are highly sensitive and some of them are successfully validated for direct detection of C. auris in clinical and environmental samples, their higher costs and needs...
for more sophisticated devices in comparison to conventional PCR are limiting their applicability in resource-limited countries. The third group includes methods based on other technologies such as PCR-RFLP [24], loop-mediated isothermal amplification (LAMP) [18], and the T2 magnetic resonance system [67], among which LAMP has been successfully validated for detection of C. auris in clinical and environmental samples. It worth mentioning that some of these methods such as GPS™ MONODOSE CanAvir detection PCR [66] are commercially available. Application of this method can decrease the turnaround time, as it uses dehydrated ready-to-use tubes, and for which only template DNA needs to be added. Meanwhile, the commercial methods cannot be used in all laboratories due to high financial costs. 

3. Conclusion

Accurate identification of C. auris is important in medical mycology reference laboratories. Due to shortcomings of various commercial assays, application of supplementary methods appears essential. When working on fungi isolated in culture, MALDI-TOF MS based on updated databases can successfully identify C. auris. In developing countries in which application of MALDI-TOF MS is not affordable due to financial constraints, this method could be replaced by DNA-based assays. Different C. auris specific end-point or multiplex PCR assays as well as PCR-RFLP have been developed for this purpose. Of these, those that enable simultaneous identification of C. auris and closely related species appears superior. For epidemiological surveys using skin or environmental samples, several real-time PCR assays, loop-mediated isothermal amplification and the T2 magnetic resonance assay have been introduced with promising results. Further work should be done to obtain internationally approved methods for detection and identification of C. auris.

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Disclosure of interest

The authors declare that they have no competing interest.

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