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*Candida auris* is a multidrug-resistant yeast that causes a wide spectrum of infections, especially in intensive care settings. We investigated *C. auris* prevalence among 102 clinical isolates previously identified as *Candida haemulonii* or *Candida famata* by the Vitek 2 system. Internal transcribed spacer region (ITS) sequencing confirmed 88.2% of the isolates as *C. auris*, and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) easily separated all related species, viz., *C. auris* (*n* = 90), *C. haemulonii* (*n* = 6), *C. haemulonii* var. *vulnra* (*n* = 1), and *Candida duobushaemulonii* (*n* = 5). The *in vitro* antifungal susceptibility was determined using CLSI broth microdilution (CLSI-BMD), the Vitek 2 antifungal susceptibility test, and the Etest method. *C. auris* isolates revealed uniformly elevated fluconazole MICs (MIC₅₀ 64 µg/ml), and an alarming percentage of isolates (37%) exhibited elevated caspofungin MICs by CLSI-BMD. Notably, 34% of *C. auris* isolates had coexisting elevated MICs (≥2 µg/ml) for both fluconazole and caspofungin, and 10% of the isolates had elevated coexisting MICs (≥2 µg/ml) to two additional azoles, i.e., posaconazole and isavuconazole. In contrast to reduced amphotericin B MICs by CLSI-BMD/H9262, elevated MICs (>90), *C. haemulonii* is confirmed as a new species in 2009 (2). This pathogen was recently recognized as an emerging multiresistant yeast (MDR) in several outbreaks in Japan and other countries (2,19). *C. auris* misidentified as *C. haemulonii* by commercial identification systems, such as Vitek 2 and API20C-AUX, and exhibits a unique susceptibility profile (5–8). Notably, the potential of clonal transmission of *C. auris*, highly elevated MICs to fluconazole, and reduced susceptibility to voriconazole, caspofungin, and flucytosine are matters of serious concern (7–9). Therefore, accurate identification is important, because treatment strategies are often directed by species characterization of *Candida*. Further, a recent report of misidentified *C. auris* reports a high MIC to amphotericin B and caspofungin using the commercially available Vitek 2 automated system in a patient with pericarditis due to *C. auris* highlights issues of inappropriate treatment strategies if accurate susceptibility testing is not available (4).

In the recent era, molecular techniques and a growing database of fungal genome sequences have facilitated the reliable identification of phylogenetically related and phenotypically identical species. Herein, we investigated the prevalence of *C. auris* in a collection of clinical isolates previously phenotypically identified as *C. haemulonii/C. famata*, using internal transcribed spacer region (ITS) sequencing and evaluated matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and DNA sequencing and its antifungal susceptibility profile variability by Vitek 2, CLSI broth microdilution, and Etest method. J Clin Microbiol 53:1823–1830. doi:10.1128/JCM.00367-15.


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and data obtained by CLSI were compared with those obtained by the commercial Vitek 2 system and the Etest method.

(Part of this study was presented at the Interscience Conference on Antimicrobial Agents and Chemotherapy [54th ICAAC], M-1203, slide session, Washington, DC, USA [10].)

MATERIALS AND METHODS

Isolates. A total of 102 clinical isolates, from individual patients, previously identified as Candida haemulonii/C. famata by the Vitek 2 compact system (bioMérieux, Marcy l’Etoile, France) in 4 tertiary care hospitals in Delhi, North India, and a single center in Kochi, Kerala, South India, from 2010 to 2014 were included. Three centers were about 1,000-bed general hospitals, and the remaining 2 were a pediatric hospital and a transplant center. The pediatric hospital had neonatal intensive care units and surgical intensive care facilities. The isolates were mainly from patients with candidemia (blood; n = 78), and other specimens from invasive Candida infections included gangrenous tissue (n = 4), pleural fluid (n = 6), and peritoneal fluid (n = 7). Also, 7 isolates from urine (n = 4) and sputum (n = 3) specimens from immunocompromised patients were included. The control and type strains of three C. auris isolates from Korea (KCTC 17809, KCTC 17810) and Japan (DSM 21092T) and one isolate each of C. haemulonii strain CBS 7802 and C. duobushaemulonii strain CBS 7798T were also analyzed.

Phenotypic characterization. The isolates were identified by standard mycological procedures, including colony color on CHROMagar Candida medium (Difco, Becton Dickinson & Company, Baltimore, MD, USA) and morphology on rice Tween 80 agar. Growth patterns at different temperatures, 37°C, 42°C, and 45°C, were also observed (1). Additionally, the assimilation profile of all yeast isolates was done by commercially available API strips (ID32C; bioMérieux, Marcy l’Etoile, France), which were read and interpreted at 48 h.

Sequencing of ITS region. Genomic DNA was extracted from all test isolates along with reference strains as described by Xu et al. (11). DNA was amplified and sequenced using the ITS-1 (5′-TCTTGATTAGTGAAACC TTGCGG-3′) and ITS-4 (5′-TCTCCGTATATTGATAGC-3′) primers, which amplify the ITS region of the ribosomal subunit (8). Sequences were aligned, and GenBank Basic Local Alignment Search Tool (BLAST) searches were performed for species identification. For phylogenetic analyses, the ITS gene sequences of the C. auris, C. haemulonii, and C. duobushaemulonii isolates were aligned with the ClustalW program (version 1.82), and the final alignments were edited manually. A neighbor-joining (NJ) tree based on ITS gene sequences using 2,000 bootstrap replications was generated using MEGA version 5 (12). The sequences of the reference/type strains of C. auris from Japan (JCM 15448T) and Korea (KCTC 17809 and KCTC 17810), along with C. haemulonii (CBS 5150, Portugal; CBS 7801, United States), C. haemulonii var. vulnera (CNMCL-7462, Spain), and C. duobushaemulonii (CBS 7799, USA), were retrieved from GenBank and included for the analysis.

MALDI-TOF MS. The ethanol-formic acid extraction procedure was followed according to the manufacturer’s protocol for the identification of yeast isolates (13). The spectra were analyzed using the Flex Control 3.1 software (Bruker Daltonics, Inc., Billerica, MA, USA) and MALDI Biotyper OC version 3.1 (Bruker Daltonics, Bremen, Germany). Score values were analyzed as per manufacturer recommendations: a score of ≥2 indicated confidence to the species level, 1.7 to 1.99 indicated confidence to the genus level, and <1.7 indicated no identification.

MALDI data analysis. The MALDI Biotyper version 3 database contains spectra of 3 strains of C. auris, two from Korea (KCTC 17809 and KCTC 17810) and a type strain from Japan (DSM 21092T). For phylogenetic analysis, spectra of 90 C. auris isolates were added manually to the library for the creation of a species-oriented dendrogram in Biotyper as described previously for Aspergillus species (14). The mass spectra of each quadruplicate of the respective isolates with a score value of >2 were considered for dendrogram preparation. Additionally, available spectra of reference strains of C. auris from Japan (DSM 21092T) and Korea (KCTC 17809 and KCTC 17810) and of C. haemulonii (CBS 5149T and CBS 5150), C. duobushaemulonii (CBS 7799 and CBS 7800), and C. pseudohaemulonii (CBS 10004 and CBS 12453T) in the database were imported in the software for the analysis of the dendrogram. The dendrogram was generated by using the respective functionality of the MALDI Biotyper 3.1 offline client. The spectra of all the isolates tested were analyzed by a score-oriented dendrogram using an arbitrary distance level of 1,000 as the cutoff.

AST. (i) CLSI-BMD method. Antifungal susceptibility testing (AST) was carried out using the Clinical and Laboratory Standards Institute broth microdilution method (CLSI-BMD), following the M27-A3 guidelines (15). Antifungals tested were amphotericin B (AMB; Sigma, St. Louis, MO, USA), fluconazole (FLU; Pfizer, Groton, CT, USA), itraconazole (ITC; JTC Pharma, Hyderabad, India), voriconazole (VRC; Pfizer), posaconazole (POS; Merck, Whitehouse Station, NJ, USA), isavuconazole (ISA; Basilea Pharmaceutica, Basel, Switzerland), l bucystosine (5-FC; Sigma), caspofungin (CAS; Merck), micafungin (MFG; Astellas, Toyama, Japan), and anidulafungin (AFG; Pfizer). RPMI 1640 medium with glutamine without bicarbonate (Sigma) buffered to pH 7 with 0.165 mol/liter 3-N-morpholinepropanesulfonic acid (MOPS; Sigma) was used. Drug-free and yeast-free controls were included, and microtiter plates were incubated at 35°C and read visually after 24 h, as validated recently by Pfäffer et al. (16, 17). CLSI-recommended Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were used as quality control strains, and two reference strains of C. auris (KCTC 17809 and DSM 21092T) were also included. Excepting AMB, the MIC endpoints for all the antifungals were defined as the lowest drug concentration that caused 50% growth inhibition vis-à-vis the drug-free controls. The MIC for AMB was defined as the lowest concentration at which there was 100% inhibition of growth. The susceptibility for all the isolates was performed by two different personnel on two occasions, which revealed reproducible results.

(ii) Vitek 2 Compact system using an AST-Y507 card. Susceptibility was determined using an AST-Y507 card, which tests the MIC of 6 antifungals, i.e., FLU, 5-FC, VRC, AMB, CAS, and MFG. All the C. auris isolates were tested as per the manufacturer’s instructions. The time of incubation ranged from 18 to 24 h, based on the rate of growth in the drug-free control well, and the results were expressed as MICs in micrograms per milliliter.

(iii) Etest method. Further, the isolates which revealed >2-fold discrepancies in the antifungal MICs by the above-described two methods were also tested for susceptibility by Etest using Etest Technical Guide 4: Antifungal Susceptibility of Yeasts (AB Biodisk, bioMérieux, Solna, Sweden), as described previously (19, 20). The antifungals tested were AMB, CAS, and VRC. Briefly, the inoculum density of 0.5 × 10^6 to 2.5 × 10^6 cells/ml prepared for the CLSI-BMD test was used, and the test medium included RPMI 1640 with 1.5% agar supplemented with glucose (2%) and was buffered to pH 7.0 with MOPS. In addition, AMB was also tested on antibiotic medium 3 (AM3) agar plates. The plates were inoculated by dipping a sterile cotton swab into the inoculum and streaking it across the entire surface of the agar in three directions. The plates were dried for 15 min before the Etest strips (AB Biodisk) were applied and kept at 35°C and read visually after 24 h. The Etest MIC was defined as the drug concentration at which the border of the elliptical zone of complete inhibition intersected the scale on the antifungal test strip.

Statistical analysis. Statistical analyses were performed with SPSS version 20.0 (SPSS, Chicago, IL, USA). MIC values from the CLSI-BMD, Vitek 2, and Etest methods were assessed by using the Student t test (paired sample). The Etest MIC endpoints, which were in between the 2-fold dilution scale of the CLSI method, were rounded to the corresponding next upper 2-log dilution to simplify comparisons. The discrepancies among MIC endpoints of ≥2 dilutions (two wells) were used to calculate the essential agreement (EA).

FKS gene sequencing. Candida auris isolates with elevated CLSI-BMD MICs of CAS (MICs ≥ 1 μg/ml) were subjected to sequencing of the FKS1 and FKS2 genes. Considering that the genome sequence of C. auris is not yet available, the published mutations in the FKS gene of echinocandin-resistant Candida glabrata isolates were used to analyze the mutations in
the FKS gene of C. auris isolates by sequence homology (21, 22). Genomic DNA was amplified and sequenced for hot spot regions of both the genes. The primers were designed based on the C. glabrata FKS1 and FKS2 gene sequences (GenBank accession no. XM_446406 and XM_448401, respectively). The primers for FKS1 (FKS1HSF, 5’-CATTGCTATTTCCTCAG TCTAGC-3’) and FKS1HSS, 5’-CACAAGAAAAAACAGTTGTTGA-3’) and FKS2 (FKS2HSF, 5’-CTGTGACATTTCCTTCTG-3’) and FKS2HSS, 5’-TCAAGGAAGTAGAGGAAATCC-3’) were designed using Primer3 version 4 (http://primer3.ut.ee/). DNA sequences were analyzed with Sequencing Analysis software version 5.3.1 (Applied Biosystems). Consensus sequences were made using BioEdit software (version 7.0.5.3) and were aligned with hot spot FKS regions of reference C. glabrata (GenBank accession no. HM366439 for FKS1 and HM366442 for FKS2).

**Nucleotide sequence accession numbers.** The sequences determined in this study were deposited in GenBank under accession no. KF689009 to KF689022, KC692039 to KC692052, and KP862745 to KP862818.

**RESULTS**

Vitek 2 identified 100 isolates as C. haemulonii (88 with 91 to 98% identity and 12 with a low discrimination profile), whereas the remaining 2 isolates were identified as C. famata (93% identity). Of the 102 isolates, 88.2% (n = 90) were confirmed as C. auris by ITS sequencing. The remaining 12 isolates were identified as C. haemulonii (n = 6), C. haemulonii var. vulnera (n = 1), and C. duobushaemulonii (n = 5). All C. auris (n = 90) isolates showed smooth, white to cream-colored colonies on Sabouraud dextrose agar (SDA), whereas they developed a pink color on CHROMagar Candida medium. Microscopic examination showed ovoid to elongated budding yeast cells occurring singly or in pairs. No pseudohyphae were formed on rice Tween 80 agar after 4 to 8 days of incubation at 28°C. They grew well at 37°C and 42°C. In contrast, C. haemulonii and C. duobushaemulonii isolates revealed pseudohyphae with blastocandia and did not grow at 42°C. All the C. auris isolates were positive for assimilation of N-acetylglucosamine, succinate, and gluconate, whereas negative results were recorded for C. haemulonii and C. duobushaemulonii.

ITS sequences of the 90 isolates (GenBank accession no. KF689009 to KF689022, KC692039 to KC692052, KP862805, and KP862818) showed 99% homology (query coverage ranging from 98 to 100%) with C. auris isolates in GenBank (accession no. HE797773 and AB375772). However, the ITS sequences of 6 isolates (GenBank accession no. KP862806 to KP862811) showed 100% homology with C. haemulonii (GenBank accession no. KM014586 and JX459689), and 5 isolates (GenBank accession no. KP862813 to KP862817) showed 100% homology with C. duobushaemulonii (GenBank accession no. KM361511 and KJ476202). Also, a solitary isolate (VPCI 715/P/14; GenBank accession no. KP862812) showed 100% homology with C. haemulonii var. vulnera (GenBank accession no. JX459686). The ITS tree yielded 3 distinct clades with a good bootstrap value (99%) and enabled the differentiation of C. auris from C. haemulonii and C. duobushaemulonii strains (Fig. 1). All the Indian C. auris (n = 90) strains exhibited 99 to 100% sequence similarity among themselves. However, C. auris isolates from Japan and Korea formed a separate group away from Indian C. auris isolates in the same clade. The other species of the C. haemulonii complex were well differentiated in 2 separate clades, Candida duobushaemulonii (n = 5) formed clade 2, whereas Candida haemulonii (n = 6) and C. haemulonii var. vulnera (n = 1) formed two groups in clade 3.

**MALDI-TOF MS.** The MALDI-TOF MS mass spectra obtained for all C. auris isolates were evaluated against the original MALDI Biotyper OC version 3.1 database. The mean MALDI-TOF MS score of the tested C. auris isolates was 2.167 (range, 2.013 to 2.347). Of the 90 C. auris isolates, the majority (n = 77, 85.5%) were identified to the species level at the first attempt with a score value of >2. The remaining isolates (n = 13) were also identified as C. auris but with a score value of <2 and >1.7. These isolates were repeated and revealed high score values in the second attempt. All the C. haemulonii (n = 6) and C. duobushaemulonii (n = 5) strains were identified to the species level (score value, >2). Further, a solitary isolate of C. haemulonii was also identified to the variety level with a score value of >2 (C. haemulonii var. vulnera). The dendrogram clearly revealed separation of members of the Metschnikowiaceae clade in 4 phylogroups (Fig. 2). The mass spectra of the Indian C. auris isolates showed marked similarity, whereas the Japanese (n = 1) and Korean C. auris isolates (n = 2) exhibited variations in mass spectra among themselves and with those of Indian C. auris isolates, resulting in a separate cluster in C. auris (phylogroup 4). The dendrogram generated was in agreement with the phylogenetic NJ tree with ITS sequences.

**In vitro susceptibility and FKS mutation analysis.** The in vitro susceptibility data and the MIC distribution of C. auris isolates using different methods along with essential agreements between the tested methods are presented in Tables 1 and 2.

**CLSI-BMD.** FLU exhibited no activity against 89% (n = 80) of C. auris isolates (MIC of 16 to >64 μg/ml), whereas the remaining 10 isolates revealed a MIC of 4 μg/ml. Similarly, an elevated MIC (MIC90, 8 μg/ml) was noted for VRC. Notably, 58% of C. auris isolates (n = 52) showed VRC MICs of ≥1 μg/ml. In contrast, MIC90 values of POS (0.06 μg/ml) and ISA (0.25 μg/ml) were relatively low compared to that of VRC (Table 1). Also, 11% of C. auris isolates revealed MICs of ≥1 μg/ml for both POS and ISA, and a solitary isolate showed a MIC of ≥1 μg/ml only to ISA. All C. auris isolates showed reduced MICs to ITC (geometric mean [GM] MIC, 0.15 μg/ml). Furthermore, C. auris isolates had AMB MIC90 values of 1 μg/ml; however, 15.5% (n = 14) of the isolates revealed MICs of ≥2 μg/ml for AMB. Moreover, elevated GM MICs were observed for CAS (0.58 μg/ml) in comparison to MFG (0.11 μg/ml) and AFG (0.23 μg/ml). Notably, 37% (n = 33) of the C. auris isolates revealed MICs of ≥1 μg/ml to CAS. Also, all the echinocandins had no activity in 8% (n = 7) of the isolates, with MICs ranging from 4 to >8 μg/ml (Table 2). Further, 88% of C. auris isolates had reduced MICs to 5-FC (GM MIC, 0.4 μg/ml), whereas 11 isolates showed highly elevated MICs (≥32 μg/ml). In contrast to C. auris, all the C. haemulonii (n = 7) and C. duobushaemulonii (n = 5) isolates had markedly elevated AMB MICs ranging from 4 to 16 μg/ml. Also, variable FLU MICs were observed for C. haemulonii (MIC range, 2 to >64 μg/ml) and C. duobushaemulonii (MIC range, 1 to >16 μg/ml). However, reduced MICs of VRC (MIC range, 0.03 to 0.5 μg/ml) were noted for both the C. haemulonii and C. duobushaemulonii isolates, except a solitary isolate of C. haemulonii, which showed a MIC of 4 μg/ml. Moreover, both the C. haemulonii and C. duobushaemulonii isolates exhibited reduced GM MICs to ISA (0.027 μg/ml and 0.023 μg/ml), followed by POS (0.05 μg/ml and 0.11 μg/ml) and ITC (0.31 μg/ml). Also, in contrast to C. auris (GM MIC, 0.58 μg/ml), both C. haemulonii (GM MIC, 0.19 μg/ml) and C. duobushaemulonii (GM MIC, 0.14 μg/ml) showed reduced MICs to CAS. However, a wide MIC range (<0.125 to 64 μg/ml) for 5-FC was observed for C. haemulonii, while reduced MICs (GM MIC, 0.125 μg/ml) were found for C. duobushaemulonii (Table 1).
FKS gene sequencing of C. auris isolates with elevated caspofungin MICs (>1 μg/ml). Amplification of FKS1 and FKS2 regions generated amplicons of 391 bp and 460 bp, respectively. Mutations reported for caspofungin-resistant C. glabrata were not observed in the FKS1 and FKS2 regions of any of the tested C. auris strains.

Vitek 2. In contrast to low AMB MICs recorded by CLSI for C. auris, exceptionally elevated AMB MICs (CLSI MIC50 of 1 μg/ml compared to Vitek MIC50 of 8 μg/ml) were noted, which were statistically significant (P < 0.0001). Barring a solitary isolate of C. auris, all the other isolates had MICs of ≥8 μg/ml for AMB (Table 2). Also, the AMB MICs of C. haemulonii and C. duobushaemulonii ranged from 8 to 16 μg/ml, which was in concordance with CLSI. Vitek 2 MIC50 values of VRC (1 μg/ml), CAS (0.5 μg/ml), and MFG (0.125 μg/ml) of C. auris isolates were in 100% agreement with those by the CLSI method. Vitek 2 MIC50 values of FLU (32 μg/ml) and 5-FC (1 μg/ml) were within 2 dilutions by CLSI-BMD.

Etest. Similar to CLSI MICs, low MIC50 values of AMB for C. auris isolates were observed by Etest on AM3 medium (0.5 μg/ml) and on RPMI agar (1 μg/ml). Except a solitary isolate, all C. auris isolates showed MICs of ≤1 μg/ml for AMB. The MIC50 (1 μg/
ml) of VRC was similar to that by CLSI-BMD. In contrast, Etest AMB MICs were better differentiated than CLSI-BMD MICs, and Vitek showed a wide range from 0.002 to 4 μg/ml, which were in agreement with the CLSI-BMD MICs. Interestingly, 26 of these 33 isolates revealed MICs of 0.064 to 4 μg/ml by Vitek. The remaining 7 isolates had MICs of 4 μg/ml for CAS by CLSI. The essential agreement within ±2 dilutions of CLSI MICs.

**Agreement between methods.** The essential agreement within ±2 dilutions for the comparison of 24-hour CLSI-BMD with Vitek 2 and Etest results showed 10% and 81% for AMB, 90% and 79% for CAS, and 91% and 79% for VRC, respectively.

**DISCUSSION**

The present study highlights that Candida auris remains an unnoticed pathogen in routine microbiology laboratories in India, as 90% of the isolates characterized by commercial identification systems misidentify this yeast as C. haemulonii. In the past 5 years, Candida auris has emerged as a significant pathogen in tertiary care general hospitals and a pediatric center in north and south India, representing 8.6% to 30% of cases of candidemia (7, 8). The actual prevalence of C. auris in varied clinical settings in India is unexplored, as the majority of centers do not perform molecular or MALDI-TOF MS-based identification. In this work, a large number of C. auris isolates were tested for antifungal susceptibility with three methods which showed uniform fluconazole resistance and an alarming percentage of isolates (37%) exhibiting elevated caspofungin MICs by CLSI-BMD. Taken together, 10% of isolates showed highly elevated MICs to 4 antifungals (AMB, FLU, CAS, VRC) by the CLSI-BMD method. Notably, 34% of isolates had coexisting elevated MICs for two commonly used azoles, i.e., FLU and VRC (MICs of ≥2 μg/ml), and 10% of the isolates had elevated coexisting MICs (≥1 μg/ml) to two additional azoles, i.e., POS and ISA. Considering the frequent prevalence of MDR strains of C. auris in the intensive care units and other wards of 5 different hospitals in the present series, the accurate identification and antifungal susceptibility testing of this yeast is pertinent for guiding therapy and determining the prognosis in such settings. Also, accurate identification of the cryptic species C. auris is important in assessing the epidemiology and pathogenicity of the disease.

**TABLE 1 In vitro antifungal susceptibility profile of C. auris, C. haemulonii, and C. duobushaemulonii strains by the CLSI M27-A3 broth microdilution method**

<table>
<thead>
<tr>
<th>Species tested</th>
<th>MIC parameter</th>
<th>AMB</th>
<th>ITC</th>
<th>VRC</th>
<th>ISA</th>
<th>POS</th>
<th>FLU</th>
<th>5-FC</th>
<th>CAS</th>
<th>MFG</th>
<th>AFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. auris (n = 90 isolates)</td>
<td>GM</td>
<td>0.8</td>
<td>0.15</td>
<td>1.01</td>
<td>0.18</td>
<td>0.06</td>
<td>36</td>
<td>0.5</td>
<td>0.58</td>
<td>0.11</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>AMB</td>
<td>1</td>
<td>0.125</td>
<td>1</td>
<td>0.25</td>
<td>0.06</td>
<td>64</td>
<td>0.25</td>
<td>0.5</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>CAS</td>
<td>4</td>
<td>0.5</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>64</td>
<td>8</td>
<td>1</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>MIC range</td>
<td>0.125–8</td>
<td>&lt;0.03–2</td>
<td>&lt;0.03–16</td>
<td>&lt;0.015–4</td>
<td>&lt;0.015–8</td>
<td>4–64</td>
<td>&lt;0.125– &gt;64</td>
<td>0.125–8</td>
<td>&lt;0.015–8</td>
<td>&lt;0.015–8</td>
</tr>
<tr>
<td>C. haemulonii (n = 7 isolates)</td>
<td>GM</td>
<td>12.7</td>
<td>0.314</td>
<td>0.31</td>
<td>0.027</td>
<td>0.05</td>
<td>32</td>
<td>2.82</td>
<td>0.19</td>
<td>0.28</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>AMB</td>
<td>16</td>
<td>0.25</td>
<td>0.5</td>
<td>0.015</td>
<td>0.125</td>
<td>64</td>
<td>32</td>
<td>0.25</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>CAS</td>
<td>4</td>
<td>0.5</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>64</td>
<td>64</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MIC range</td>
<td>4–16</td>
<td>0.25–0.5</td>
<td>&lt;0.03–4</td>
<td>&lt;0.015–0.5</td>
<td>0.015–0.25</td>
<td>2–64</td>
<td>0.125–&gt;64</td>
<td>0.06–1</td>
<td>0.125–1</td>
<td>0.25–1</td>
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<tr>
<td>C. duobushaemulonii (n = 5 isolates)</td>
<td>GM</td>
<td>16</td>
<td>0.315</td>
<td>0.068</td>
<td>0.023</td>
<td>0.11</td>
<td>6.35</td>
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<td>0.14</td>
<td>0.35</td>
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<tr>
<td></td>
<td>AMB</td>
<td>16</td>
<td>0.25</td>
<td>0.06</td>
<td>0.015</td>
<td>0.125</td>
<td>8</td>
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<td>0.125</td>
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<tr>
<td></td>
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<td>0.66</td>
<td>0.25</td>
<td>0.16</td>
<td>0.125</td>
<td>0.25</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MIC range</td>
<td>&gt;16</td>
<td>0.25–0.5</td>
<td>&lt;0.03–0.125</td>
<td>&lt;0.015–0.06</td>
<td>0.03–0.125</td>
<td>1–16</td>
<td>&lt;0.125</td>
<td>0.06–0.25</td>
<td>0.125–1</td>
<td>0.5–1</td>
</tr>
</tbody>
</table>

**TABLE 2 Distribution of MICs of amphotericin B, caspofungin, and voriconazole obtained by 3 different methods for Candida auris (n = 90) strains**

<table>
<thead>
<tr>
<th>Drug testeda</th>
<th>Test method</th>
<th>No. of isolates at MIC (μg/ml)</th>
<th>MIC (μg/ml)</th>
<th>AMB</th>
<th>CAS</th>
<th>VRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMB</td>
<td>CLSI-BMD</td>
<td>&lt;0.03</td>
<td>0.03</td>
<td>0.06</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>16</td>
<td>23</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Vitek 2</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Etest</td>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CLSI-BMD</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Vitek 2</td>
<td></td>
<td></td>
<td></td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Etest</td>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>CLSI-BMD</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Vitek 2</td>
<td>3</td>
<td>5</td>
<td>12</td>
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<td></td>
<td>Etest</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>15</td>
</tr>
</tbody>
</table>

**a** AMB, amphotericin B; CAS, caspofungin; VRC, voriconazole.
caused by this underreported pathogen in different geographic areas. In the past 5 years, *C. auris* fungemia has been reported from South Africa, South Korea, Japan, and India (3, 5–8). All of the reports from these countries confer the major issue of notable elevated MICs for azoles and caspofungin in *C. auris* and its misidentification by phenotypic methods. The present study employed MALDI-TOF MS, a more robust diagnostic technique, for rapid identification. The strength of the present study is that it developed a comprehensive reference database built with a large number of molecularly characterized *C. auris* strains from different geographical regions of India to supplement the Bruker BioTyper library, which has a database of only 3 strains from Korea and Japan. Not only was interspecies differentiation well characterized, but also the mass spectra variation at the intraspecies level separated *C. auris* isolates from India. It is pertinent to mention here that, previously, Indian *C. auris* isolates have been reported to exhibit differences in biochemical profiles compared to the Japanese and the Korean *C. auris* isolates (7, 8). Similarly, genotypic variation among *C. auris* isolates from different geographical regions has also been observed with M13 fingerprinting and amplified fragment length polymorphism analysis (7). It is evident from the present study that the high resolution and discriminatory power of MALDI-TOF MS facilitate differentiation of closely related cryptic species within the Metschnikowiaceae clade (23), which has also been documented previously for Mucorales, particularly the *Lichtheimia* species (24).

Another issue of concern is the misleading highly elevated MICs of AMB with Vitek automated readings in all *C. auris* isolates tested. The overall EA between Vitek automated readings and the CLSI-BMD method for AMB was very low (10%). Notably, the reference CLSI-BMD method in the present series showed reduced AMB MICs in 84% of *C. auris* isolates. Similarly, low AMB MICs (0.25 to 1 μg/ml) by CLSI-BMD were reported for 20 *C. auris* isolates from South Korea by Shin et al. (25). However, these authors observed a high EA (100%) between the CLSI-BMD and Vitek method for AMB, which is in contrast with the observations in the present study. This deviation could be attributed to the low number of isolates tested (25). Major errors of azole susceptibility in 218 isolates of 5 *Candida* species using another commercial automated reading system (ATB FUNGUS 3) have been reported recently from China, resulting in pseudohigh rates of antifungal resistance (26). In fact, the erroneously elevated MICs by the Vitek 2 automated reading method not only may lead to inappropriate selection of antifungal therapy but also depict false rates of high antifungal resistance in epidemiological studies. Further, the percentage of *C. auris* isolates that showed elevated CAS MICs (≥ 1 μg/ml) by CLSI-BMD in the present series (37%) declined to 12% using the Etest. The lower Etest MIC values than CLSI-BMD MIC values for CAS have also been reported earlier for other *Candida* species (27). Recently, the performance of the CAS Etest based on the recently revised CLSI breakpoints for *Candida* isolates showed that 13.1% were misclassified as intermediate or resistant (28). Also, marked interlaboratory variation has been observed with both CLSI-BMD and the EUCAST method for CAS susceptibility (29). In order to investigate the resistance mechanism with respect to elevated CAS MICs, in the present series we attempted to sequence FKS hot spot regions in *C. auris* isolates using known FKS *C. glabrata* primers due to a lack of published genomic data for *C. auris*. Although none of the isolates with elevated CAS MICs harbored mutations reported for echinocandin-resistant *C. glabrata*, the possibility of other mutations not reported so far could not be ruled out. Future studies on complete genomic analysis are warranted to detect true antifungal resistance in this significant pathogen.

Finally, *C. auris* is emerging as a serious multidrug nosocomial pathogen in many centers in India, which could be reliably and rapidly identified by MALDI-TOF MS. Notwithstanding the fact that routine laboratories heavily rely on commercial systems for identification and antifungal susceptibility testing for yeasts, a cautionary approach is recommended for isolates showing elevated MICs with these systems.

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The authors alone are responsible for the content and writing of the paper.

**REFERENCES**


12. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likeli-


