First two fungemia cases caused by *Candida haemulonii var. vulnera* in China with emerged antifungal resistance

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*Candida haemulonii var. vulnera* is a rare variant of *C. haemulonii*, which has been previously reported to cause human infections. Owing to the close kinship between *C. haemulonii* sensu stricto and *C. haemulonii var. vulnera*, accurate identification of *C. haemulonii var. vulnera* relied on DNA sequencing assay targeting, for example, rDNA internal transcribed spacer (ITS) region. In this work, two strains of *C. haemulonii var. vulnera* were collected from the China Hospital Invasive Fungal Surveillance Net (CHIF-NET). The identification capacity of three matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and VITEK 2 YST ID biochemical methods were evaluated against ITS sequencing. In addition, antifungal susceptibility testing was performed using Sensititre YeastOne. Moreover, we comprehensively screened drug-resistant related genes by whole-genome sequencing. The two strains were not correctly identified to species variant level using MALDI-TOF MS and YST ID cards. Both strains were resistant to amphotericin B (minimum inhibitory concentration [MIC] > 2 µg/ml). Moreover, strain F4564 and F4584 exhibited high MIC to fluconazole (>256 µg/ml) and 5-flucytosine (>64 µg/ml), respectively, which were supposed to result from key amino acid substitutions Y132F and G307A.
in Erg11p and V58fs and G60K substitutions in Fur1p. The rare species C. haemulonii var. vulnera has emerged in China, and such drug-resistant fungal species that can cause invasive diseases require further close attention.

**KEYWORDS**

Candida haemulonii var. vulnera, antifungal susceptibility, ERG11, FUR1, whole-genome sequence, drug resistant mechanisms

**Introduction**

*Candida haemulonii var. vulnera* belongs to the *C. haemulonii* species complex, along with *C. haemulonii* sensu stricto and *C. duobushaemulonii* (Gade et al., 2020; Rodrigues et al., 2021; Ramos et al., 2022). Generally, *C. haemulonii* species complex isolates display high multidrug-resistant rates and transmission properties, which have attracted increased attention (Gade et al., 2020).

In 2012, *C. haemulonii var. vulnera* was reported for the first time by Cendejas-Bueno et al. They found four strains with low rDNA internal transcribed spacer (ITS) sequence identity to *C. haemulonii* sensu stricto type strain (~96%) (Cendejas-Bueno et al., 2012). Infections caused by *C. haemulonii var. vulnera* were later reported in Brazil, India, Argentina, and Peru (de Almeida et al., 2016; Kumar et al., 2016; Isla et al., 2017; Pérez-Lazo et al., 2021; Ramos et al., 2022). In addition, by retesting a set of previously collected strains, Rodrigues et al. found a *C. haemulonii var. vulnera* strain isolated in 2009, which became the earliest strain of the species variant discovered till now and its genome sequence was elucidated (Rodrigues et al., 2020). Moreover, antifungal resistance to azoles, echinocandins, and amphotericin B has been reported in *C. haemulonii var. vulnera* isolates (Cendejas-Bueno et al., 2012; Isla et al., 2017; Ramos et al., 2022).

In this study, we reported two fungemia cases caused by *Candida haemulonii var. vulnera* found from China Hospital Invasive Fungal Surveillance Net (CHIF-NET) study. Clinical characters, identification capacity of Vitek YST card and three matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) systems, isolates’ antifungal susceptibility phenotypes, and potential resistant mechanisms were illustrated. To our best knowledge, these were the first *Candida haemulonii var. vulnera* infection cases reported in China, including the first 5-flucytocine resistant strain discovered globally.

**Materials and methods**

**Ethics statement**

This study was approved by the Human Research Ethics Committee of the Peking Union Medical College Hospital (No. S–263). Written informed consent was obtained from all patients who participated in this study, which aimed to culture and study the isolates obtained from the patients.

**Microorganisms and identification**

From 2010 to 2017 (Table 1), two *C. haemulonii var. vulnera* isolates were collected from different hospitals in two provinces from the CHIF-NET study. The colony

**TABLE 1 Information for two Candida haemulonii var. vulnera isolates identified in this study.**

| Strain | F4564 | F4584 |
|--------|-------|-------|
| **General information** |       |       |
| Age/Gender | 88/Female | 12/Male |
| Year of isolation | 2015 | 2016 |
| Source of isolate | Blood | Blood |
| Clinical diagnosis | Pulmonary infection | Abdominal infection |
| Ward | ICU | General surgery |
| Location | Nanchang, China | Changsha, China |
| Identification (identity/score/confidence value) |       |       |
| ITS sequencing | *C. haemulonii var. vulnera* (100%) | *C. haemulonii var. vulnera* (100%) |
| Vitek 2 Compact | Low discrimination | C. haemulonii (94%) |
| Vitek MS | *C. haemulonii* (99.9) | *C. haemulonii* (99.9) |
| Autof-MS 1000 | *C. haemulonii* (9.505) | *C. haemulonii* (9.516) |
| Smart MS | No identification | No identification |
| **Antifungal susceptibility (µg/ml)** |       |       |
| Fluconazole | >256 | 16 |
| Voriconazole | 2 | 0.12 |
| Itraconazole | 0.25 | 0.25 |
| Posaconazole | 0.25 | 0.12 |
| 5-Flucytosine | <0.06 | >64 |
| Anidulafungin | 0.12 | 0.12 |
| Micafungin | 0.12 | 0.12 |
| Caspofungin | 0.12 | 0.12 |
| Amphotericin B | 4 | 4 |
| **Potential resistance mechanisms** |       |       |
| Erg11p | Y113F and G307A | WT |
| Fur1p | WT | V58fs and G60K |

*WT: wild type; fs: frameshift mutation; ICU: intensive care unit.*
FIGURE 1
Phenotypic characteristics of two C. haemulonii var. vulnera isolates on Sabouraud dextrose agar. The plate contains colonies with typical appearance of circular, smooth, and moist (A,B). Microscopic examination showed budding yeast cells with Gram’s staining (C,D). Scale bar = 10 µm (C).

DNA extraction and whole-genome sequencing

The whole genomic DNA of C. haemulonii var. vulnera was extracted as previously reported (Huang et al., 2022). The 350-bp DNA library was constructed using NEBNext Ultra II DNA Library Prep Kit for Illumina according to the manufacturer’s instructions. Library integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies). Sequencing was performed on an Illumina NovaSeq using the PE150 strategy (Beijing Novogene Bioinformatics Technology Co., Ltd.). The Illumina reads generated in this study were obtained from the National Center for Biotechnology Information (NCBI) under BioProject PRJNA890168.

Genome analysis

The C. haemulonii var. vulnera K1 (GenBank accession number GCA_012184645.1) genome was concatenated and used as a reference genome for read mapping. BWA 0.7.17, SSPACE, and bcftools 0.1.19 (Li and Durbin, 2009; Li et al., 2009) were used for single-nucleotide polymorphism (SNP) and insertion/deletion (indel) analysis, and snpEff 4.3 was used for SNP and indel function annotations (Cingolani et al., 2012). Mutations on antifungal-resistant related genes, including ERG11 and TAC1b for azoles, FUR1 for 5-flucytosine, FKS1 and FKS2 for echinocandins, ERG3 and ERG6 for amphotericin B, and ERG4 for other ergosterol pathway genes, were analyzed in detail (Arendrup and Patterson, 2017; Berkow and Lockhart, 2017).

Antifungal susceptibility testing

Antifungal susceptibility testing was performed using Sensititre YeastOne YO10 methodology (Thermo Scientific, Cleveland, OH, USA). Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were used as quality controls. As clinical breakpoints or epidemiological cutoff values against C. haemulonii var. vulnera have not been established, we used interpretation criteria for Candida species referring to CLSI M27-S3 guidelines (CLSI, 2008), including MIC value of ≥32 µg/mL as resistance to fluconazole ≥32 µg/mL as resistance to 5-flucytosine. In addition, MIC of ≥2 µg/mL was used for interpreting “resistance” to amphotericin B (Pfaller et al., 2012).

Review of Candida haemulonii var. vulnera cases reported

A literature review of previously reported C. haemulonii var. vulnera infections was done. A literature search was performed on 28 August 2022, using the following three databases: PubMed, Web of Science, and Embase. The terms “Candida haemulonii var. vulnera” were entered in the category of “Title/Abstract” in the PubMed Advanced Search Builder, and “TS (Candida haemulonii var. vulnera)” was entered into the Web of Science database. The search in Embase was

1 https://pubmed.ncbi.nlm.nih.gov
2 https://webofknowledge.com
3 https://www.embase.com
Results

Isolate information

The first case was an 88-year-old male patient who was admitted to the intensive care unit (ICU) with clinical diagnosis of pulmonary infection. F4564 was isolated from peripheral blood of this patient and initially misidentified as "Candida krusei" using CHROMagar chromogenic medium at local laboratory (Table 1). The second case was a 12-year-old boy who was admitted to the general surgical ward, and clinical diagnosis was abdominal infection. F4584 was isolated from peripheral blood of this patient and identified as "Candida spp." using CHROMagar chromogenic medium initially (Table 1).

Identification of Candida haemulonii var. vulnera using ITS sequencing, MALDI-TOF MS, and Vitek 2

The ITS sequences of the isolates exhibited 100% identity with the corresponding ITS sequences of the reference C. haemulonii var. vulnera CBS12439T isolates (GenBank accession number: JX459686.1). Furthermore, both clinical isolates were identified as C. haemulonii by the Autof MS 1000 and Vitek MS, but with "no identification" results by Smart MS. While using the Vitek 2 Compact system, one strain was identified with low discrimination and the other was identified as C. haemulonii (score = 94%) (Table 1). In phylogenetic tree generated by C. haemulonii complex, C. auris,
and *C. pseudoohaemulonii* ITS sequences, F4564 and F4584 were in the branch of *C. haemulonii var. vulnera* (Figure 2).

Genome analysis

We sequenced genomes of F4564 and F4584, and their total genome sizes were 13.51 Mb and 13.26 Mb, respectively, with an average GC content of 45% (N50 was 346,464 bp and 283,647 bp, and a number of assembled contigs were 270 and 262, comprising 95.7% and 95.6% of data according to BUSCO analysis, respectively).

Antifungal susceptibility

MIC values obtained for the nine antifungal agents against *C. haemulonii var. vulnera* isolates are shown in Table 1. F4564 was resistant to fluconazole with an MIC of >256 µg/ml. In addition, F4564 was classified as susceptible

| TABLE 2 Overview of published reports on antifungal susceptibility profiles of *Candida haemulonii var. vulnera*. |
|----------------------------------------------------------------------------------------------------------------|
| **No. isolates** | **Strain or isolate** | **Antifungals (µg/ml)** | **Method** |
| **[Reference]** | | | |

**N = 7 (Ramos et al., 2022)**

| MIC | LIPCh14 | FLU | VRC | ITC | POS | MCF | CAS | ANF | AMB | 5FC |
|-----|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC  |       | >64 | >16 | >16 | >6  | 0.06| 0.25| 0.125| 2   | 0.25|
|      | LIPCh18| >64 | >16 | >16 | >6  | 0.125| 1   | 0.125| 4   | 0.25|
|      | LIPCh20| >64 | >16 | >16 | >6  | 0.125| 1   | 0.25 | 8   | 0.25|
|      | LIPCh21| >64 | >16 | >16 | >6  | 0.125| 1   | 0.06 | 4   | 0.25|
|      | LIPCh24| 64  | 0.25| 0.25| 0.125| 0.125| 1   | 0.06 | 16  | 0.25|
|      | LIPCh25| >64 | >16 | >16 | >6  | 0.06 | 0.5 | 0.06 | 4   | 0.25|
|      | LIPCh37| >64 | >16 | >16 | >6  | 0.06 | 0.5 | 0.06 | 8   | <0.125|

**N = 3 (Ramos et al., 2015)**

| MIC | LIPCh5 | FLU | VRC | ITC | POS | MCF | CAS | ANF | AMB | 5FC |
|-----|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC  | 64     | 0.5 | 0.5 | ND  | ND  | 0.25| ND  | 2   | ND  | CLSI|
|      | LIPCh9 | 64  | 16  | 8   | ND  | 0.25| ND  | 2   | ND  | CLSI|
|      | LIPCh11| >64 | >16 | >16 | ND  | 0.5 | ND  | 4   | ND  | 0   |

**N = 2 (Rodrigues et al., 2020)**

| MIC | K1 | FLU | VRC | ITC | POS | MCF | CAS | ANF | AMB | 5FC |
|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC  | K2 | 8   | 0.25| 0.5 | 0.25| 0.12| 0.25| 0.06| 8   | <0.06|

**N = 4 (Cendejas-Bueno et al., 2012)**

| MIC | CNM-CL7239 | FLU | VRC | ITC | POS | MCF | CAS | ANF | AMB | 5FC |
|-----|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC  | CNM-CL7256 | >64 | >8  | >8  | >8  | >16 | >16 | 1   | <0.12| EUCAST|
|      | CNM-CL7073 | >64 | >8  | >8  | >8  | 0.12| 0.5 | 0.06| 1   | 0.25 |
|      | CNM-CL7462 | >64 | >8  | >8  | >8  | 0.06| >16 | <0.03| 1   | 0.5  |

**N = 1 (Pérez-Lazo et al., 2021)**

| MIC | CNM-CL7073 | FLU | VRC | ITC | POS | MCF | CAS | ANF | AMB | 5FC |
|-----|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC  |            | >64 | ND  | ND  | ND  | ND  | ND  | 0.06| 1   | ND   |

**N = 8 (de Almeida et al., 2016)**

| MIC | GM | FLU | VRC | ITC | POS | MCF | CAS | ANF | AMB | 5FC |
|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC  | 17.4 | 11.53| ND | ND | ND | 0.26 | 0.016 | 1   | ND   | CLSI |
|      | 64  | ND  | ND  | ND  | ND  | 0.5 | 0.03 | 2   | ND   | CLSI |

**N = 5 (Isla et al., 2017)**

| MIC | Mode | FLU | VRC | ITC | POS | MCF | CAS | ANF | AMB | 5FC |
|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC  | GM  | 2   | 0.03| 0.5 | 0.06| 0.12| 0.12| ND  | 4   | 0.12|
|      | 8   | 0.24| 0.33| 0.07| 0.14| 0.12| ND  | 3.48| 0.12|
|      | 2-128 | 0.03-8| 0.06-1| 0.0-0.5| 0.12-0.25| 0.006-0.25| ND  | 2-8  | 0.12 |
|      | 4   | 0.1 | 0.5 | 0.06| 0.12| 0.12| ND  | 4   | 0.12 |
|      | 128 | 8   | 1   | 0.25| 0.25| 0.25| ND  | 8   | 0.12 |

**N = 3 (Rodrigues et al., 2021)**

| MIC | FLU | VRC | ITC | POS | MCF | CAS | ANF | AMB | 5FC |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| MIC  | 16-32 | 0.06-0.12| ND | ND | 0.06-0.12| 0.06-0.12| 0.015| ND  | 0.25-1| CLSI|

FIC: Fluconazole; VRC: Voriconazole; ITC: Itraconazole; POS: Posaconazole; CAS: Caspofungin; ANF: Anidulafungin; MCF: Micafungin; AMB: Amphotericin B; 5FC: 5-Flucytosine; MIC: minimum inhibitory concentration; GM: geometric mean; MIC<sub>50</sub>: minimum inhibitory concentration able to inhibit 50% of all isolates tested; MIC<sub>90</sub> minimum inhibitory concentration able to inhibit 90% of all isolates tested.
dose-dependent to voriconazole (2 μg/ml) and itraconazole (0.25 μg/ml). F4684 was resistant to 5-flucytosine (≥4 μg/ml) and susceptible dose-dependent to itraconazole. In addition, both isolates had high MIC values of 4 μg/ml for amphotericin B, while neither strain was non-susceptible to posaconazole, caspofungin, anidulafungin, and micafungin (Table 1).

**Potential genomic variations contributed to antifungal resistance**

Compared with the reference genome K1, which is from an isolate susceptible to all antifungals except for amphotericin B, we found that F4564 has two previously reported key amino acid substitutions (Y132F and G307A) in Erg11p. Interestingly, we discovered two novel mutations in Fur1p (V58fs and G60K) in strain F4584 with high 5-flucytosine MIC. However, we did not find any key variation in sterol metabolism-related genes like ERG2, ERG3, and ERG6 that may result in amphotericin B resistance. In addition, all strains were susceptible to echinocandins and did not carry substitutions in Fks1p or Fks2p.

**Literature review**

We found eight articles in all reported antifungal susceptibility of C. haemulonii var. vulnera isolates. A number of strains exhibit high MICs to fluconazole or even all azoles (Cendejas-Bueno et al., 2012), but there was not any investigation on related resistance mechanisms. In addition, one study reported the emergence of pan-echinocandin-resistant C. haemulonii var. vulnera strains (Cendejas-Bueno et al., 2012). To date, there have not been any reports describing 5-flucytosine-resistant C. haemulonii var. vulnera strain (Table 2).

**Discussion**

*Candida haemulonii var. vulnera* was identified ever-first by ITS sequencing (Cendejas-Bueno et al., 2012). Although it has been involved in identification database of latest Vitek 2 YST ID system, the results from the current study and Rodrigues et al. (2020) still found YST ID card was not able to achieve a reliable identification between C. haemulonii sensu stricto and C. haemulonii var. vulnera. Furthermore, none of the current available MALDI-TOF MS systems could accurately identify C. haemulonii var. vulnera (Kathuria et al., 2015; Grenfell et al., 2016; Rodrigues et al., 2020), though Grenfell et al. described that some discriminatory protein peaks may be able to differentiate C. haemulonii sensu stricto and C. haemulonii var. vulnera in using FlexAnalysis and ClinProTools to analysis MALDI-TOF MS results (Grenfell et al., 2016). Therefore, DNA sequencing remained the only applicable methods for the identification of C. haemulonii var. vulnera. Interestingly, Cendejas-Bueno reported that C. haemulonii sensu stricto and C. haemulonii var. vulnera can be separated by ITS sequences, but these two species have identical RPB1, RPB2, and D1/D2 sequences. Therefore, these strategies could not be used for the identification of these two species (Cendejas-Bueno et al., 2012). To date, isolation of C. haemulonii var. vulnera has only been reported in Brazil, Argentina, and Peru. Due to the above-mentioned limitations of commercial identification systems, we reidentified all C. haemulonii complex isolates collected in CHIF-NET study by ITS sequencing and finally found two C. haemulonii var. vulnera strains among >80 cases.

Previously, fluconazole- and amphotericin B-resistant C. haemulonii var. vulnera have been discovered (Cendejas-Bueno et al., 2012; Ramos et al., 2015; de Almeida et al., 2016). Like findings in previous reports, one of the isolates we discovered was also resistant to fluconazole and amphotericin B. However, we also found a 5-flucytosine-resistant strain, and it is also cross-resistant to amphotericin B. Although 5-flucytosine resistance has been recognized in C. haemulonii sensu stricto and C. duobushaemulonii in China and other regions (Kathuria et al., 2015; Hou et al., 2016), this is the first 5-flucytosine-resistant C. haemulonii var. vulnera case characterized to date. Of note, both strains we discovered remained susceptible to all echinocandins.

Compared with C. haemulonii sensu stricto and C. duobushaemulonii that have been well characterized, there are very few studies on resistant mechanisms of C. haemulonii var. vulnera. Key amino acid substitutions in Erg11p and Fur1p have been noted as major reasons contributing to azole and 5-flucytosine resistance, respectively, in other C. haemulonii complex species (Gade et al., 2020) and Chen et al. (2022). In this study, it was found that the fluconazole-resistant C. haemulonii var. vulnera isolate carried Y132F and G307A mutations in Erg11p. Erg11p Y132F substitutions have been reported in a broad range of fluconazole-resistant Candida species including C. albicans, C. tropicalis, and C. haemulonii (Fan et al., 2019; Warrillow et al., 2019; Gade et al., 2020), while G307A has been reported in C. parapsilosis (Arastehfar et al., 2020; Binder et al., 2020). While in our 5-flucytosine-resistant C. haemulonii var. vulnera strain, a frameshift V58fs and a mutation G60K were found in Fur1p, which has not been recovered in any other Candida strains to our best knowledge. Literature review showed a high proportion of C. haemulonii var. vulnera isolates (13/17, 76.5%) were with reduced susceptibility to amphotericin B (≥2 μg/ml). However, resistant mechanisms to amphotericin B were not well understood, and in this study, we also failed to found any mutations in key genes may potentially contributed to amphotericin B resistance. As a major limitation, our study did not collect detailed medical records of these two cases; therefore, we were not able to further analyze the source of infections or assessing clinical risk factors of this species.
In conclusion, there is a potential threat posed by *C. haemulonii* var. *vulnera*, a highly antifungal-resistant fungal resistance with tests in *C. haemulonii* var. *vulnera* was supposed to be resulted from variations in Erg11p and Fur1p, respectively. Although invasive infection with *C. haemulonii* var. *vulnera* remained rare, further monitoring of this species is still warranted.

**Data availability statement**

The datasets presented in this study can be found in the online repositories. The names of the repository/repositories and accession number(s) can be found in the manuscript.

**Author contributions**

X-FC, XH, HZ, and X-MJ conceived and designed the experiments. L-PN and WC provided the isolates. X-FC and HZ performed experiments. X-FC and HZ analyzed the data and wrote the manuscript. MX, XH, and Y-CX revised the manuscript. All authors contributed to the manuscript and approved the submitted version.

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**References**

Arastehfar, A., Daneshnia, F., Hilmigiu-Polat, S., Fang, W., Yasar, M., Polat, F., et al. (2020). First report of candidemia clonal outbreak caused by emerging fluconazole-resistant *Candida parapsilosis* isolates harboring Y132F and/or Y132F+K143R in Turkey. *Antimicrob. Agents Chemother.* 64, e01001-20. doi: 10.1128/AAC.01001-20

Arendrup, M. C., and Patterson, T. F. (2017). Multidrug-resistant candida: Epidemiology, molecular mechanisms, and treatment. *J. Infect. Dis.* 216, S445–S451. doi: 10.1093/infdis/jix131

Berkow, E. L., and Lockhart, S. B. (2017). Fluconazole resistance in *Candida* species: A current perspective. *Infect. Drug Resist.* 10, 237–245. doi: 10.2147/IDR.S118892

Binder, U., Arastehfar, A., Schnegg, L., Horstnagl, C., Hilmigiu-Polat, S., Perlin, D. S., et al. (2020). Efficacy of LAMB against emerging azole- and multidrug-resistant *Candida parapsilosis* isolates in the *Galleria mellonella* model. *J. Fungi* (Basel, Switzerland) 6:377. doi: 10.3390/jf6040377

Cendejas-Bueno, E., Kolecka, A., Alastruey-Izquierdo, A., Theelen, B., Groenewald, M., Kostrewa, M., et al. (2012). Reclassification of the *Candida haemulonii* complex as *Candida haemulonii* (C. *haemulonii* group I), *C. duobushaemulonii* sp. nov. (C. *haemulonii* group II), and *C. haemulonii* var. *vulnera* var. nov.: Three multiresistant human pathogenic yeasts. *J. Clin. Microbiol.* 50, 3641–3651. doi: 10.1128/JCM.02248-12

Chen, X.-F., Zhang, H., Jia, X.-M., Cao, J., Li, L., Hu, X.-L., et al. (2022). Antifungal susceptibility profiles and drug resistance mechanisms of clinical *Candida duobushaemulonii* isolates from China. *Front. Microbiol.* doi: 10.3389/fmicb.2022.1001845

Cingolani, P., Platts, A., Wang le, L., Coon, M., Nguyen, T., Wang, L., et al. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)* 6, 80–92. doi: 10.4161/fly.19695

CLSI (2008). *Reference method for broth dilution antifungal susceptibility testing of yeasts, third informational supplement.* CLSI document M27-S3, 3rd Edn. Wayne, PA: Clinical and Laboratory Standards Institute.

de Almeida, J. N. Jr., Assy, J. G., Levin, A. S., Del Negro, G. M., Giudice, M. C., Tringoni, M. P., et al. (2016). *Candida haemulonii* Complex Species, Brazil, January 2010-March 2015. *Emerg. Infect. Dis.* 22, 561–563. doi: 10.3201/eid2203.151610

Fan, X., Xiao, M., Zhang, D., Huang, J. J., Wang, H., Hou, X., et al. (2019). Molecular mechanisms of azole resistance in *Candida tropicalis* isolates causing invasive candidiasis in China. *Clin. Microbiol. Infect.* 25, 885–891. doi: 10.1016/j.cmi.2018.11.007

Gade, L., Munoz, J. F., Sherb, M., Wagner, D., Berkow, E. L., Forberg, K., et al. (2020). Understanding the emergence of multidrug-resistant *Candida* using whole-genome sequencing to describe the population structure of *Candida haemulonii* species complex. *Front. Genet.* 11:554. doi: 10.3389/fgene.2020.00554

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**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Grenfell, R. C., Da Silva Junior, A. R., Del Negro, G. M. B., Munhoz, R. B., Gimenes, V. M. F., Assis, D. M., et al. (2016). Identification of Candida haemulonii complex species: Use of ClinProToolsTM to overcome limitations of theBruker BiotyperTM, VITEK MSTM IVD, and VITEK MSTM RUO databases. Front. Microbiol. 7:940. doi: 10.3389/fmicb.2016.00940

Hou, X., Xiao, M., Chen, S. C., Wang, H., Cheng, J. W., Chen, X. X., et al. (2016). Identification and antifungal susceptibility profiles of Candida haemulonii species complex clinical isolates from a multicenter study in China. J. Clin. Microbiol. 54, 2676–2680. doi: 10.1128/JCM.01492-16

Huang, J. J., Chen, X. F., Tsui, C. K. M., Pang, C. J., Hu, Z. D., Shi, Y., et al. (2022). Persistence of an epidemic cluster of Rhodotorula mucilaginosa in multiple geographic regions in China and the emergence of a 5-flucytosine resistant clone. Emerg. Microbes Infect. 11, 1079–1089. doi: 10.1080/22221751.2022.2059402

Isa, G., Taverna, C. G., Struz, W., Vivot, W., García-Effron, G., and Davel, G. (2017). Candida haemulonii sensu lato: Update of the determination of susceptibility profile in Argentina and literature review. Curr. Fungal Infect. Rep. 11, 203–208. doi: 10.1007/s12281-017-0300-y

Kathuria, S., Singh, P. K., Sharma, C., Prakash, A., Mabih, A., Kumar, A., et al. (2015). Multidrug-resistant Candida auris misidentified as Candida haemulonii: Characterization by matrix-assisted laser desorption ionization-time of flight mass spectrometry 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

Kumar, A., Prakash, A., Singh, A., Kumar, H., Hajen, F., Meis, J. F., et al. (2016). Candida haemulonii species complex: An emerging species in India and its genetic diversity assessed with multilocus sequence and amplified fragment-length polymorphism analyses. Emerg. Microbes Infect. 5:e49. doi: 10.1016/j.evim.2016.09.009

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1550. doi: 10.1093/molbev/msy096

Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760. doi: 10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009). The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079. doi: 10.1093/bioinformatics/btp152

Pérez-Lazo, G., Morales-Moreno, A., Soto-Febrío, F., Hidalgo, J. A., Neyra, E., and Bustamante, B. (2021). Liver abscess caused by Candida haemulonii var. vulnera. First case report in Peru. Rev. Iberoam Microl. 38, 138–140. doi: 10.1016/j.riam.2020.12.001

Pföller, M. A., Espinel-Ingroff, A., Canton, E., Castañeda, M., Cuenda-Estrella, M., Diekema, D. J., et al. (2012). Wild-type MIC distributions and epidemiological cutoff values for amphotericin B, flucytosine, and itraconazole and Candida spp. as determined by CLSI broth microdilution. J. Clin. Microbiol. 50, 2040–2046. doi: 10.1128/JCM.00248-12

Ramos, L. S., Figueiredo-Carvalho, M. H. G., Barbado, L. S., Zaccardi, M., Chaves, A. L. S., Zancopé-Oliveira, R. M., et al. (2015). Candida haemulonii species complex: Species identification and antifungal susceptibility profiles of clinical isolates from Brazil. J. Antimicrob. Chemother. 70, 111–115. doi: 10.1093/jac/dku321

Ramos, L. S., Figueiredo-Carvalho, M. H. G., Silva, I. N., Siqueira, N. I. M., Lima, J. C., Oliveira, S. S., et al. (2022). The threat called Candida haemulonii species complex in Rio de Janeiro State, Brazil: Focus on antifungal resistance and virulence attributes. J. Fungi (Basel, Switzerland) 8:574. doi: 10.3390/jf8060574

Rodríguez, D. K. B., Bordetti, I. X., García, R. A., Araujo, M. R., Rodrigues, J. S., Gimenes, V. M. F., et al. (2021). Antifungal susceptibility profile of Candida clinical isolates from 22 hospitals of São Paulo State, Brazil. Braz. J. Med. Biol. Res. 54:e10928. doi: 10.1590/1414-431X2020e10928

Rodríguez, L. S., Gazara, R. K., Passarelli-Araujo, H., Valengo, A. E., Pontes, P. V. M., Nunes-da-Fonseca, R., et al. (2020). First genome sequences of two multidrug-resistant Candida haemulonii var. vulncola isolates from pediatric patients with candidemia. Front. Microbiol. 11:1535. doi: 10.3389/fmicb.2020.01535

Warrillow, A. G., Nishimoto, A. T., Parker, J. E., Price, C. L., Flowers, S. A., Kelly, D. E., et al. (2019). The evolution of amole resistance in Candida albicans stereo 14a-pha-demethylase (CYP51) through incremental amino acid substitutions. Antimicrob. Agents Chemother. 63:e02586-18. doi: 10.1128/AAC.02586-18