Cryptococcus laurentii is one of the very rare non-neoforms Cryptococcus species that cause human infections (1–3). The clinical presentation of C. laurentii is similar to that of C. neoforms, but the cryptococcal antigen test is often negative (4), and the organism exhibits decreased fluconazole susceptibility (5, 6). Therefore, accurate identification of the species is essential for treatment drug selection.

China Hospital Invasive Fungal Surveillance Net (CHIF-NET) is a nationwide surveillance program for invasive fungal diseases (IFDs) in China (7). During a recent 5-year study period (2009 to 2014), 9,673 yeast isolates were collected, with 42 (0.4%) isolates initially identified as C. laurentii by Vitek 2 at participating hospitals. This unexpectedly high prevalence of C. laurentii than previously reported (e.g., ARTEMIS [1997 to 2007], 0.04%; SENTRY, 0%) (3, 8–10) prompted us to investigate further.

The 42 putative C. laurentii isolates originated from 16 different hospitals, and the identity of the isolates was confirmed at the coordinating lab, with testing staff blinded to previous Vitek 2 and sequencing results. Repeated Vitek 2 system testing at the central laboratory still misidentified 24 of the 42 isolates (57.1%) as C. laurentii, including C. neoforms (n = 14 [58.3%]), Candida spp. (n = 9 [37.5%]), and Pseudomyza sp. (n = 1 [3.8%]) (Table 1; also, see Table S1 in the supplemental material). Furthermore, the Vitek 2 system misidentified one C. parapsilosis sensu stricto isolate as Candida famata and one C. metapsilosis strain as C. parapsilosis (see Table S1 in the supplemental material). In comparison to the Vitek 2 system, the API 20C method correctly identified 35 (83.3%) isolates to species level but misidentified four isolates.
(9.5%) and gave three “no identification” results (7.1%). No isolates were misidentified as *C. laurentii* by this system.

Compared to the gold standard, Vitek MS and Bruker Biotyper correctly identified 34 (81.0%) and 38 (90.5%) isolates but failed to identify eight (19.0%) and four (9.5%) isolates, respectively (Table 1; also, see Table S1 in the supplemental material). Notably, none of the 42 isolates were misidentified by the MALDI-TOF MS systems. However, neither Vitek MS nor Bruker Biotyper system correctly identified the two *C. laurentii* isolates.

The MSP dendrogram (Fig. 1) indicated that the spectra of the two genuine *C. laurentii* isolates were distant from the reference spectra in the Bruker database. When the mass spectra data of the two *C. laurentii* isolates were added to the local fingerprint data-base, the Bruker system was able to correctly identify the two *C. laurentii* isolates and no misidentification occurred for the remaining 40 isolates.

The routine laboratory identification of yeasts in China, like in other developing countries, still relies largely on conventional methods, including commercial biochemical systems, of which Vitek 2 is the most used (Table 1). It is widely recognized that commercial biochemical systems have limited accuracy in identifying rare yeast species (accuracy, 50 to 65%) (14–16). The present study further confirms these findings, as the Vitek 2 system was highly unreliable in the identification of *C. laurentii*. Moreover, discrepant identification results were noted between local hospitals and the central laboratory using

### Table 1

<table>
<thead>
<tr>
<th>Identification method</th>
<th>No. (%) of hospitals where the method is routinely used</th>
<th>% of results&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Correct identification</td>
<td>Misidentification</td>
<td>No identification</td>
<td></td>
</tr>
<tr>
<td>rDNA sequencing</td>
<td>2 (3.3)</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Vitek 2</td>
<td>44 (72.1)</td>
<td>28.6</td>
<td>61.9</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Chromogenic medium</td>
<td>41 (67.2)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>API 20C</td>
<td>13 (21.3)</td>
<td>83.3</td>
<td>9.5</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>ATB ID32C</td>
<td>5 (8.2)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Vitek MS</td>
<td>3 (4.9)</td>
<td>81.0</td>
<td>0.0</td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td>Bruker Biotyper</td>
<td>2 (3.3)</td>
<td>90.5</td>
<td>0.0</td>
<td>9.5</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Results were based on testing at the central laboratory. NA, not applicable; ND, not done.

![FIG 1 MSP dendrogram constructed from mass spectra of the *C. laurentii* clinical isolates confirmed by ITS sequencing in the present study (strains 11HX143 and 11HX243 [asterisks]) and reference spectra of *C. laurentii* and other *Cryptococcus* species provided in the Bruker database.](http://jcm.asm.org/ on October 16, 2017 by guest)
the Vitek 2 system (16/42 [38.1%]), suggesting problems with interlaboratory reproducibility of results for rare yeast species, as previously reported (17).

MALDI-TOF MS has revolutionized the laboratory diagnosis of IFDs (12, 18–20). However, its application in China (Table 1) and other resource-poor countries has been limited by the initial high equipment cost (18). Unfortunately, the MALDI-TOF MS systems did not show a markedly better performance in this study, mainly owing to deficiencies in their mass fingerprint databases for rare yeast species (see Table S1 in the supplemental material) (12, 18). Therefore, ITS rDNA sequencing is still necessary as a supplementary confirmatory test for these rare yeasts (12, 20). Remarkably, due to notable differences between the spectra of the two clinical isolates and reference spectra in the current database, both systems failed to identify the two genuine C. lauritii isolates, although the organism is within the systems’ identification databases (Fig. 1). Therefore, it is important for MALDI-TOF MS databases to have spectra representing different strains of the same species for wide identification coverage (12, 18).

In conclusion, our findings suggest that the real incidence of C. lauritii in IFDs in China is still low. The identification of rare yeast species such as C. lauritii, as well as other easily misidentified species, poses a great challenge to clinical laboratories, not only because of the limited identification accuracy of commonly used biochemical methods such as Vitek 2, but also owing to deficiencies in MALDI-TOF MS fingerprint databases. ITS and D1D2 rDNA sequencing methods remain the most reliable means of confirmation. When uncommon yeast species are reported using automated systems, heightened clinical suspicion is warranted (21).

ACKNOWLEDGMENTS

We thank all the laboratories participating in the CHIF-NET study for making this large project possible. We declare that we have no conflicts of interest.

REFERENCES


