Pitfalls in the use of MALDI TOF Mass Spectrometry for the Identification of problematic yeast isolates from a historical collection

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Background

The identification of yeast traditionally entails macroscopic/microscopic findings and biochemical testing. Recently, MALDI TOF MS has replaced traditional methods for identification as a proposed new standard. We performed identification of previously unidentifiable yeasts from a collection in the US.

Methods

- The Mycoses Study Group (MSG) collected 2,947 Candida isolates from 1911 patients as part of two US studies between 1995 and 1999.
- The identification of the isolates was done in 2002 using API 20 C aux with supplemented standard mycological and biochemical.
- 94 isolates could not be identified at that time. This was the sample used for this study.
- Isolates where tested by MALDI TOF MS following the methodology for the Bruker MALDI biotyper using a Formic acid method.

Results

In the first attempt, 65/94 (69%) isolates were identified. The remaining 29 samples were re-tested with a yield of 21/29 (72.4%) identified isolates. The remaining isolates had to be identified with another round of MALDI TOF and further biochemical testing. Quality control isolates were used in every testing batch. The score used was 1.7 for genus and 1.8 and above for genus and species.

MALDI TOF MS is superior to conventional methods for the majority of bacterial organism, however in the case of fungi, it does not differentiate between closely related species, and sometimes this can cause misidentification or no identification at all. The optimal cut off for the interpretation and validation of the score, in case of yeasts, it not clear yet. There is no guideline for validation and reporting of results. The extra step of extraction is considered by some as a pitfall in the technique, as it adds more time to the process, uses hazardous materials, and has a cost. Performance depends on the updates of the library software. In the case of fungi, this is cumbersome because of the constant changes of taxonomy and reclassification or disappearance of clinically known genera and species.

Table 1. Results of unidentified Yeast from 1995-1999 collection of The Mycoses Study Group (MSG)

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Frequency percentage</th>
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<tbody>
<tr>
<td>C. albicans</td>
<td>20 21%</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>22 23.40%</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>34 36.10%</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>8 8.50%</td>
</tr>
<tr>
<td>C. krusei</td>
<td>2 2.10%</td>
</tr>
<tr>
<td>C. orthopsilosis</td>
<td>3 3.10%</td>
</tr>
<tr>
<td>C. rivierensis</td>
<td>1 1.06%</td>
</tr>
<tr>
<td>C. kefyr</td>
<td>1 1.06%</td>
</tr>
<tr>
<td>Pichia norvegensiis</td>
<td>1 1.06%</td>
</tr>
<tr>
<td>C. lusitane</td>
<td>1 1.06%</td>
</tr>
<tr>
<td>Trichosporum spp.</td>
<td>1 1.06%</td>
</tr>
</tbody>
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Conclusions

- MALDI TOF MS is rapidly becoming a reference method for yeast identification.
- However, in a historical collection of yeast that could not be identified by conventional biochemical methods, it took up to 3 rounds of MALDI TOF MS with a yield of ~70% per round, and additional biochemical testing, for identification of all isolates.
- Continuing validation of MALDI TOF MS for identification of difficult yeast isolates is warranted.

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References


