Comparison of three methods of Mycobacterium tuberculosis complex spoligotype determination for clinical isolates, Lyon, France

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Declaration of Interests
The authors declare no competing interests.

All MTBC strains isolated from patients during routine practice at the mycobacteria laboratory of Lyon University Hospital, France, between November 2016 and December 2020 were included (n=597) to compare:

Hybridization to membrane-based spoligotyping: reference method
WGS-based spoligotyping (Illumina)

Among these MTBC strains, 133 were also analysed by hybridization to microbeads-based spoligotyping: Beamedex using Luminecx technology

Background
To tackle disease spreading: Epidemiological studies to investigate TB transmission chains
Spacer-oligonucleotide-typing or spoligotyping;
Genotyping assay for MTBC clinical isolates
Species identification among the MTBC

Here we compared the spoligotype profiles of MTBC clinical isolates by using different methods

Methods
All MTBC strains isolated from patients during routine practice at the mycobacteria laboratory of Lyon University Hospital, France, between November 2016 and December 2020 were included (n=597) to compare:

Hybridization to membrane-based spoligotyping: reference method
WGS-based spoligotyping (Illumina)

Species identification using spoligotype

Species identification using SNP calling

Results

Membrane-based vs WGS-based spoligotyping

<table>
<thead>
<tr>
<th>Discrepant spoligotypes</th>
<th>1 spacer</th>
<th>2 spacers</th>
<th>&gt;2 spacers</th>
</tr>
</thead>
<tbody>
<tr>
<td>84/597</td>
<td>74</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>14.2%</td>
<td>88.1%</td>
<td>11.9%</td>
<td>0</td>
</tr>
</tbody>
</table>

Overall concordance

Membrane-based vs WGS-based spoligotyping

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>28/133</td>
<td>22</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>21.4%</td>
<td>88.6%</td>
<td>10.7%</td>
<td>10.7%</td>
</tr>
</tbody>
</table>

Species identification

Membrane-based

Beamedex-based

Species identification

No discrepancy in species identification
29 Mtb isolates: not able to conduct identification based on spoligotyping
SNP calling allowed attribution of species

Discrepancy analysis

Redundant discrepancies: spacers not detected thought membrane-based spoligotyping and detected with Beamedex-based spoligotyping
→ More sensitive method, higher risks of cross-contaminations

Conclusion
WGS showed very few discrepancies compared to the hybridization-based assay for spoligotyping (including for species identification).
WGS provided added value in some cases of species identification.
Possibility of a smooth transition from the traditional to the in silico-based genotyping of MTBC isolates upon TB diagnosis and epidemiologic survey

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