Mycobacterium canettii is intrinsically resistant to both pyrazinamide and pyrazinoic acid

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Sir,

Pyrazinamide is part of the standard short-course regimen that enables the treatment of drug-susceptible tuberculosis (TB) within 6 months (in combination with isoniazid, rifampicin and ethambutol). It is the only first-line TB drug that is being considered as part of future regimens, which have just completed Phase 2A trials (pyrazinamide in combination with bedaquiline (also known as Sirturo, TMC207 or R207910), PA-824 or PA-824/moxifloxacin). These are designed to shorten the treatment duration for drug-susceptible TB, as well as multidrug-resistant and extensively drug-resistant TB, which is paramount to reduce both the further spread of TB and the significant direct and opportunity costs to patients. The ability of these regimens to treat TB effectively might be compromised when applied to members of the Mycobacterium tuberculosis complex, Mycobacterium caprae, Mycobacterium microti, Mycobacterium canettii and Mycobacterium pinnipedii (see the Methods section and Table S1, available as Supplementary data at JAC Online). The three M. canettii strains from our reference collection shared the aforementioned mutations with CIPT 140010059 and displayed pyrazinoic acid MICs of 200 mg/L compared with 50 mg/L for the pyrazinamide-susceptible H37Rv reference strain (Table 1), suggesting that the mutations in rpsA or pncA were responsible for its intrinsic resistance.

In contrast, we found that the four M. bovis isolates, which were intrinsically resistant to pyrazinamide, had pyrazinoc acid MICs that were lower or equal to the MIC for the H37Rv reference strain. This is in line with prior results that found that BCG is fully susceptible to pyrazinamide acid and becomes susceptible to pyrazinamide upon complementation of the mutated pncA (H57D) with a wild-type copy, thereby restoring the ability to convert pyrazinamide into pyrazinoic acid. Interestingly, the M. bovis strains harboured a non-synonymous rpsA mutation (A440T) that was shared with both M. caprae isolates in this study, as well as previously sequenced M. bovis isolates (AF2122/97 and 06-01518) and all BCG strains sequenced to date (China, Denmark 1331, Mexico 1931, Moreau RDJ, Pasteur 1173P2, Russia, Tice and Tokyo 172). However, none of the M. africanum, M. microti or M. pinnipedii isolates in this study had this particular mutation, suggesting it was a phylogenetically informative marker for the M. caprae/M. bovis/BCG group of animal-adapted strains, rather than a marker for pyrazinamide resistance.

We found a further non-synonymous mutation in rpsA (D37A) that did not correlate with pyrazinamide resistance in one M. tuberculosis Cameroon strain. Finally, we identified the known synonymous R212R mutation in rpsA, which had been
proposed as a marker for lineage 2 (East Asian) strains,\(^6\) whereas a second study had concluded that this was not a lineage-specific mutation.\(^8\) The fact that only the six Beijing isolates in our collection harboured this mutation supported the conclusion of the former study.

In the Republic of Djibouti, \(M.\) canettii is responsible for \(~9\%\) of active TB cases, which are clinically and radiographically indistinguishable from TB caused by other members of the MTBC.\(^10\) However, more data regarding its prevalence and mode of transmission are required. Our observation that \(M.\) canettii was resistant to both pyrazinamide and pyrazinoic acid, whereas \(M.\) bovis was only resistant to pyrazinamide due to an inactive pyrazinamidase as a result of the H57D mutation in \(pncA\), rather than the A440T mutation in \(rpsA\). It remains to be determined whether the mutations in \(rpsA\) account for the intrinsic resistance of \(M.\) canettii.

Supplementary data

Methods and Table S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References


Table 1. Overview of pyrazinamide susceptibility results, pyrazinamidase function, pyrazinoic acid MICs and \(pncA\) and \(rpsA\) sequence results for selected isolates (a complete list can be found in Table S1)

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Genotype</th>
<th>Pyrazinamide susceptibility result</th>
<th>Pyrazinamidase result</th>
<th>Pyrazinoic acid MIC (mg/L)</th>
<th>(pncA) (Rv2043c)</th>
<th>(rpsA) (Rv1630)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9679/00</td>
<td>(M.) tuberculosis H37Rv</td>
<td>S</td>
<td>+</td>
<td>50</td>
<td>wt</td>
<td>wt</td>
</tr>
<tr>
<td>9564/00</td>
<td>(M.) bovis ATCC</td>
<td>R</td>
<td>–</td>
<td>50</td>
<td>H57D</td>
<td>A440T</td>
</tr>
<tr>
<td>4258/00</td>
<td>(M.) bovis</td>
<td>R</td>
<td>–</td>
<td>25</td>
<td>H57D</td>
<td>A440T</td>
</tr>
<tr>
<td>751/01</td>
<td>(M.) bovis</td>
<td>R</td>
<td>–</td>
<td>50</td>
<td>H57D, C72G</td>
<td>A440T</td>
</tr>
<tr>
<td>7540/01</td>
<td>(M.) bovis</td>
<td>R</td>
<td>–</td>
<td>50</td>
<td>H57D</td>
<td>A440T</td>
</tr>
<tr>
<td>3151/08</td>
<td>(M.) canettii</td>
<td>R</td>
<td>+</td>
<td>200</td>
<td>A46A</td>
<td>T5A, P9P, T210A, E457E</td>
</tr>
</tbody>
</table>

S, susceptible; R, resistant; wt, wild-type.

\(M.\) canettii was resistant to both pyrazinamide and pyrazinoic acid, whereas \(M.\) bovis was only resistant to pyrazinamide due to an inactive pyrazinamidase as a result of the H57D mutation in \(pncA\), rather than the A440T mutation in \(rpsA\). It remains to be determined whether the mutations in \(rpsA\) account for the intrinsic resistance of \(M.\) canettii.

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Transparency declarations

None to declare.